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STERNAL ODORS AS CUES FOR SOCIAL DISCRIMINATION BY FEMALE VIRGINIA OPOSSUMS, *DIDELPHIS VIRGINIANA*

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The role of secretions of sternal scent glands in social recognition was examined in captive adult female Virginia opossums, *Didelphis virginiana*. Five of six females directed significantly more investigative behavior toward plastic disks artificially scent marked with sternal odors from male conspecifics than toward those marked with sternal odors from females when odors were presented in paired male-female combinations. None investigated female odors more than those of males. Estrous status did not appear to influence responses to sternal odors. Subjects also demonstrated the ability to discriminate between individual odors of females presented in pairs, even though females lack the specialized sternal glands characteristic of males. No evidence was found for an ability of female opossums to discriminate among odors of individual males.

Key words: *Didelphis*, scent glands, olfactory communication

Sexually dimorphic scent glands are common in the Marsupialia (Müller-Schwarze, 1983; Russell, 1985), including the New World family Didelphidae; however, olfactory communication in marsupial social systems generally is not well understood. Sternal or suprasternal glands have been described for several species of didelphid opossums (*Didelphis virginiana*—Holmes, 1987; Holmes Meisner, 1986; *Monodelphis domestica*—Fadem and Schwartz, 1986; *Marmosa robinsoni*—Boggs, 1969), but their possible role in coordinating social behavior and breeding biology has not been studied.

Sternal glands in adult male Virginia opossums (*D. virginiana*) consist of a region of hypertrophied sebaceous and apocrine sudoriferous glands on the chest over the sternum (Holmes, 1987; Holmes Meisner, 1986). These glands produce a yellow-orange, musky secretion. Females lack specialized sternal glands, and produce no visible exudate. The staining of the sternal region of males is most striking in late November and December in Ohio, prior to the

onset of breeding. The amount of sternal exudate produced, as well as activity and histological development of skin gland units, varies considerably among males of similar age, size, and condition (Holmes, 1987). Although a study of testosterone levels in male opossums in Florida did not show reliable seasonal variation (Ryser, 1990), the peak for scent gland secretion in Ohio opossums corresponds approximately to the onset of increased sperm production reported by Winegarner (1982) in Florida.

Virginia opossums are nocturnal, non-gregarious, and seasonally polyestrous. *Didelphis* species were thought to be promiscuous (Gardner, 1982; Hunsaker, 1977), but recent data suggest a polygynous mating system (Ryser, 1990; Sunquist et al., 1987). Sternal secretions in *D. virginiana* are thought to function in sexual recognition or advertisement (Gardner, 1982; Pippitt, 1976; Ryser, 1990; Streilein, 1983), but this hypothesis has never been investigated experimentally.

The objectives of this study were first to

determine whether female opossums in breeding condition can discriminate the gender of conspecifics using sternal odors alone, and whether estrous status influences responses of females to odors of males and females presented together. In addition, I examined responses of females to sternal odors of conspecifics presented in same-sex combinations to determine whether there was evidence of individual recognition using these odors alone.

METHODS

Fourteen 1- to 2-year-old adult opossums were used, including six female test subjects, four male odor donors, and four female odor donors. All animals had been captive since before reaching sexual maturity or for at least 6 months before testing. All animals had prior social (including sexual) experience, but subjects and donors had been separated for at least 3 months. In no case was a subject known to be related to a donor, although one subject and two of the male donors had been trapped at the same site. Subjects were maintained individually in adjacent indoor dog kennels. All female donors and two of the male donors were individually housed in stainless-steel primate cages (1 by 0.75 by 1 m); the remaining two male donors were housed together in a large indoor dog run. Subjects and odor donors were housed at opposite ends of the building to minimize olfactory contact between them. Subjects were accustomed to the presence of humans, but had not been handled except as required for routine care. Diet consisted of dry high-protein cat food supplemented weekly with raw liver, eggs, and fruit, and water was supplied ad lib. Animals were exposed to natural light and temperature variation and to indirect fluorescent lights during testing.

Testing was conducted May–July, in the second one-half of the 1985 breeding season, during the opossums' most active nocturnal period (2000–2400 h EST). Sternal odors were collected by rubbing the chest of each donor animal 10 strokes with clean, dry paper tissue, then rubbing the tissue 10 times on a 30-cm-diameter plastic disk. Odor collection from male donors always resulted in visible staining of the paper tissue and transfer of the characteristic musky odor to the disk, whereas any secretion transferred from females was not readily detectable. The amount

of sternal exudate collected from males varied markedly among donors, and the collection procedure was intended to preserve this variability. Disks were steam cleaned prior to initial use, and they were washed after each test with hot water and an alcohol-based cleanser, then dried with clean paper towels. I processed all odor samples myself, and washed my hands after handling each sample. Immediately following odor collection, a pair of these artificially scent marked disks was placed ca. 1 m apart in the female subject's home cage so that she could investigate either disk freely. Odors were presented in the combinations male-female, male-male, or female-female. Every subject was used in 12 trials using 12 different male-female odor combinations (72 male-female trials total), and six trials each involving different male-male and female-female comparisons (36 male-male and 36 female-female trials total). Male-male and female-female trials were administered at random between male-female trials, for a total of 144 tests (24/subject) in the entire study. The position of the disks in the home cage was alternated from one trial to the next, randomizing the position within a complete set of trials for each subject. The order in which subjects and donors were used also was randomized. To minimize habituation of subjects to stimulus odors and depletion of sternal exudate from donors, no subject or donor was used in more than two tests per night, and testing was conducted no more than 4 nights/week. The amount of exudate collected from male donors did not vary visibly between first and second trials on a given night. All male donors usually were used every test night, minimizing the variability in time between odor collections among them.

Each trial consisted of a 5-min test period which began when the animal first approached within 5 cm of a disk. I scored the number of 5-s intervals during the test period in which the subject directed investigative behavior toward a disk, which included sniffing, licking, and biting or attempting to dislodge or overturn the disk. I used the same protocol to obtain separate scores for face rubbing, anogenital dragging, or dancing near a disk. Face rubbing and anogenital dragging are putative forms of scent marking in this species. Anogenital dragging is displayed routinely by opossums after urination and defecation (Holmes, 1987, 1990; McManus, 1970; Reynolds, 1952), and may serve to deposit proctodeal

and anal sac gland secretions (Brown, 1972; Müller-Schwarze, 1983; Schaffer, 1940), as well as secretions of the urogenital tract (Holmes, 1987, 1990). Dancing is an agonistic display that involves dragging the tail and shuffling the hind feet (McManus, 1967; Reynolds, 1952); this behavior is associated with dominance in female opossums (Holmes, 1987, 1990).

Estrous cycles of all subjects and the four female odor donors were monitored for 2 months before and during the study by microscopic examination of cells collected every other day by lavage of the urogenital tract (Fleming and Harder, 1981*a*, 1981*b*; Jurgelski and Porter, 1974). To eliminate a possible source of observer bias, smears taken during the test period were not examined until the experiment was over. All subjects exhibited estrous cyclicity before and during testing, and the experiment was long enough (ca. 10 weeks) to include at least one full cycle (ca. 28 days) for each female.

RESULTS

Subjects approached a disk in the first 30 s in 86% of the trials. In male-female trials, five of six females directed significantly higher levels of investigative behavior toward the disk marked with the sternal odor of a male than toward odors of females. Median scores for investigative behavior (median number of 5-s intervals during the test period in which investigative behavior occurred) directed toward male and female odors, respectively, for the six subjects were: 19.0 and 12.5; 32.5 and 17.0; 9.0 and 6.0; 31.0 and 13.0; 9.0 and 2.5; 20.5 and 16.5. Results of Wilcoxon paired-samples tests (two-tailed—Zar, 1984) for the six subjects were: $T_- = 3$, $n = 12$, $P < 0.005$; $T_- = 2$, $n = 12$, $P < 0.005$; $T_- = 9.5$, $n = 12$, $P < 0.05$; $T_- = 0$, $n = 11$, $P < 0.001$; $T_- = 4.5$, $n = 10$, $P < 0.02$; $T_- = 24$, $n = 12$, $P > 0.05$. Subjects failed to approach either disk at all during the 5-min test period in three of 76 trials; these were excluded from the analysis. In no case did a subject show more interest in odors from females than males, hence females clearly were able to discriminate the gender of conspecifics using sternal odors. Because sternal odors of females were never presented in combination with an odor

blank, however, results of male-female trials cannot be taken as evidence that female donors lacked a sternal odor entirely.

When the available data from male-female tests were sorted into two subsets by estrous status (proestrus or estrus compared to metestrus), samples were too small to test for differences in responses to male and female odors for every subject separately. Data from male-female tests pooled for all subjects and subsetted by estrous status showed significantly more investigation of male odors overall in both subsets with no indication of an influence of estrous status. For subjects in proestrus or estrus, median scores for investigative behavior directed toward male and female odors respectively were 20.0 and 13.0 (Wilcoxon paired-samples test, two-tailed, $d.f. = 38$, $P < 0.001$). For subjects in metestrus, medians for male and female odors, respectively, were 20.0 and 8.0 (Wilcoxon paired-samples test, two-tailed, $d.f. = 25$, $P < 0.001$). In the absence of a nonparametric alternative to repeated measures analysis, these results should be interpreted with caution, but female opossums apparently need not be in proestrus or estrus to discriminate between male and female sternal odors. Female odor donors also exhibited estrous cyclicity, but no effect of donor estrus or proestrus on responses of subjects was suggested.

Data from male-male and female-female trials, designed to test for individual recognition, were analyzed separately from male-female trials. Five of six females showed a trend toward higher levels of investigation scores (averaged for the two disks) in male-male than female-female tests, but Mann-Whitney U tests performed separately for each subject revealed no significant differences (two-tailed, $P > 0.05$). Subjects failed to approach either disk at all in one of 36 male-male trials and seven of 36 female-female trials; these were excluded from the analysis. Medians of scores for investigative behavior averaged for the two disks for male-male and female-female tests, respectively, for the six subjects were as follows: 21.50 and 11.26; 20.75 and 4.50; 8.50

and 16.00; 23.75 and 18.0; 10.50 and 3.00; 24.50 and 15.50. To test for overall differences in responses to odors of individual males in male-male trials, the observed distribution of subjects' responses to combinations of odors from two different male donors was compared to a randomized distribution of responses generated by a Monte Carlo simulation, based on the null hypothesis of a 0.50 probability of one odor receiving more investigative behavior than another. Scores for investigative behavior directed toward individual male odors in all possible combinations of male-male pairs did not deviate significantly from random (test statistic = 0.063; 95% critical value = 0.14; $n = 35$; $P = 0.35$), hence, females showed no preference for or aversion to the odor of any individual male. I saw no obvious relationship between the amount of stain on the sternal region and other subjective indicators of fitness in male donors (e.g., weight or physical condition), nor were males generally housed in such a way that a dominance relationship could be established among them. Although a significant preference for one odor over another establishes the ability of an animal to discriminate between them, failure of opossums to demonstrate a discrimination is not sufficient evidence that this ability is lacking.

Females responded to sternal odors of individual female donors in a significantly nonrandom fashion in female-female trials (test statistic = 0.224; 95% critical value = 0.18; $n = 29$; $P = 0.017$), clearly demonstrating an ability to discriminate among odors of female conspecifics. A lack of specialized skin glands in the sternal region of females evidently does not preclude the production of an individual olfactory signature that may be socially relevant; it is possible that females can make a similar discrimination using odors from other body regions.

Face rubbing, anogenital dragging, and dancing in general were performed at slightly higher levels near disks marked with male than female odors, but had average scores below one 5-s interval per trial. Although these behaviors occurred too infrequently

for inferential analysis, the results are consistent with the possibility that detection of male odors may stimulate females to deposit chemosignals containing information about reproductive status, and may facilitate contact with potential mates during behavioral estrus.

DISCUSSION

Results of radiotracking studies of *D. virginiana* and its congener, *D. marsupialis*, indicate that individuals travel widely within a flexible system of overlapping home ranges, and ranges of males often include those of several adult females (Fitch and Shirer, 1970; Gillette, 1980; Ryser, 1990; Sunquist et al., 1987; Telford et al., 1979). Although home ranges of males may overlap, female opossums are more philopatric than males (Ryser, 1990; Wright, 1989) and are likely to encounter females with which they have had prior social experience. Recent data suggest intense competition, including fighting, among *Didelphis* males for estrous females (Ryser, 1990; Sunquist et al., 1987), and adult males commonly sustain severe bite wounds on the neck, shoulders, and head during the breeding season (Austad and Sunquist, 1986). Virginia opossums of both sexes generally chest-rub infrequently compared to some other didelphids, including *Monodelphis domestica* (Fadem and Cole, 1985; Holmes, 1987; Hunsaker and Shupe, 1977), but they may deposit sternal odors indirectly in conjunction with the vigorous face and saliva rubbing typical of breeding adults. I have seen captive males chest rub females occasionally during courtship, but the possible value of sternal chemosignals in competition for females is unclear. Females are only receptive for one mating per behavioral estrus, and the role of female choice in opossum mating systems also is unknown. Captive females occasionally chest rub objects or substrate during fights with other females, but I observed this only rarely.

The results of this study suggest that sternal odors function in opossum social com-

munication, as do skin gland secretions of other nocturnal, nongregarious mammals (Clark, 1985; Leyhausen, 1965; Waser and Jones, 1983). The capacity for opossums to maintain social contact among conspecifics via chemical communication networks indicates that sociality in this species is better developed than previously recognized.

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