Use of tactile cues by *Notiosorex crawfordi* (Insectivora) in response to topographical features in the environment

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Abstract: *The present studies were conducted on the ability of males of the gray shrew, Notiosorex crawfordi to use tactile cues to detect and respond to changes in topographical features of their environment. The animals were videotaped, digitized, and analyzed through video recordings. All subjects showed habituation (decrease in distance traveled) to arena conditions, followed by dishabituation when positions of the blocks were changed. Intact (INT) and sham controls (SHC) shrews traveled significantly greater distances and showed higher exploration velocities as compared to animals with shaved vibrissae (SHV). Results provide empirical evidence that N. crawfordi can effectively use tactile cues to detect and respond to changes in its environment.*

Key words: *Notiosorex*, *Tactile cues, Topographical features.*

Introduction

The degree to which shrews rely on various sensory modalities to respond to their environment can vary in accordance with environmental conditions and mode of life. It is generally acknowledged that most species of soricids have poor vision (Churchfield, 1990) and rely on varying degrees on olfactory, auditory and/or tactile cues to monitor their surroundings to search for food, nest sites and mates (Baxter and Meester, 1982; Punzo and Gonzalez, 2003). For example, olfactory lobes of most shrews that forage on or beneath the ground are typically well developed (Hutterer, 1985). However, in water shrews such as *Neomys fodiens* which hunt primarily underwater, their brain structures are reduced (Churchfield, 1985). With respect to audition, many shrews send and receive acoustic signals to locate prey and conspecifics, as well as detecting predators (Churchfield, 1990).

Tactile cues seem to be of paramount importance to soricids. A distinctive feature of most shrews is the presence of an elongated snout provided with numerous short and long mystacial vibrissae, which protrude from the sides of the snout. All of these vibrissae are well supplied with sensory neural connections enabling them to receive tactile information from sensitive mechanoreceptors (Pernetta, 1977). In captivity, shrews can be readily observed to move about their cages, as they explore their environment and search for food, alternately tapping the substrate with the tip of the snout (tactile) and waving their snouts in the air (olfaction) (Punzo, 2003a).

The gray shrew, *Notiosorex crawfordi* (Coues) (Insectivora; Soricidae) occurs in northern and central Mexico, extending into Texas and westward to southern California (Carraway and Timm, 2000). It is found in a variety of microhabitats including semi-desert scrub associations, riparian woodlands, and mesic canyons (Armstrong and Jones, 1972; Whitaker, 1996). Although there are detailed studies on the ecology and behaviour of shrews from other genera (*Crocidura*, *Sorex, Suncus,* and *Neomy)* (Churchfield, 1990; Innes, 1994),

far less information is available for *N. crawfordi* (Simons and Van Pelt, 1999). Previous studies have included analyses of diet composition (Punzo, 2003b), habitat preferences (Hoffmeister and Goodpaster, 1962; Coulombe and Banta, 1964), selection and construction of nests (Punzo and Lopez, 2003), activity patterns (Fisher, 1941; Duncan and Corman, 1991), and certain aspects of reproduction (Hoffmeister and Goodpaster, 1962; Lindstadt and Jones, 1980).

Although empirical evidence is lacking, it has also been suggested that *N. crawfordi* relies primarily on auditory and tactile information in detecting and locating potential prey (Armstrong and Jones, 1972; Lindstadt and Jones, 1980; Hutterer, 1985). Prey of *N. crawfordi* consists of arthropods and small vertebrates (Punzo, 2003b). This shrew typically moves over the ground surface, beneath leaf litter or other forms of decaying vegetation and between rock crevices in search of prey (Punzo, 2003a). Because many prey items are found under conditions of darkness or dim light, we hypothesize that tactile cues may play an important role in locating prey. The purpose of this study was to assess the ability of *N. crawfordi* to use tactile cues to detect and respond to topographical features of its environment.

Materials and Methods

Subjects, housing and management: All animals used in this study were from a captive-bred population of *N. crawfordi* that originated from males and females originally collected in Brewster County, Texas, during March and April, 2001. Shrews were reared and maintained according to procedures of Punzo (2003a), and University of Tampa guidelines for animal care (AC-201). Briefly, shrews were housed, either individually or in mated pairs, in styrene rodent cages (35 x 25 x 20 cm; Model RG4W, Bush Herpetological Supply, Neodosha, Kansas), and placed in a room maintained at $22 \pm 1.0^\circ$ C and 60 to 65% relative humidity (RH), and 10L:14D photoperiod regime. Each cage was provided with: (1) a lick-tube water bottle; (2) an opaque nest box (11 x 11 x 7 cm) with one entrance containing

dry grass and cotton wadding; (3) a glass food dish; (4) a small wooden board (placed in one corner) for the deposition of fecal pellets (latrine station); and (5) a substrate consisting of sterilized sand mixed with small pebbles. Shrews had constant access to water and were fed on a 2 g diet mixture twice per day consisting of equal parts of chopped beef heart, Ralston mink chow (Ralston Purina, St. Louis, Missouri), ground crickets (*Acheta domesticus*), and ground adult tenebrionid beetles (*Tenebrio molitor*). The substrate and nest box materials in each cage were replaced every third day, and the cages were washed with soap and water and wiped with ethyl alcohol.

All the test adult male shrews were 35 days old, ranging from 3.8 to 4.1 g in body weight. These test subjects were housed individually as described above.

Experimental arena and recording of observations: Experiments were conducted in a rectangular opaque arena (90 x 50 x 12 cm) made of stainless steel. The floor of the arena was covered with sterilized sand to a depth of 3 cm. After each trial, substrate was replaced, and the arena was cleaned with soap and water. Behavioral experiments took place in another room maintained at $23^{\circ}C$ and 60 to 63% RH. All trials were videotaped using a Pansonic videocamera placed 1.0 m above the arena. Digitization and analysis of video recordings were accomplished by using a Videomex 5.0 tracking device (Model 0800-40-D39; Columbus Instruments, Columbus, Ohio) that contains a binary frame grabber type digitizer and a Motorola Model 68000 microprocessor. Maximum digitizing rate was 30 frames/sec. All arena walls were assigned position labels: the long wall at the top of the video image was designated as North (N), and proceeding in a clockwise direction, subsequent walls were designated as East (E), South (S) and West (W).

All subjects were tested individually for 30 min per day until the completion of a particular experiment. Trials were conducted between 0900 to 1000 hr. Subjects were removed from home cages in an opaque plastic circular dish (6 cm in diameter), and placed inside the arena against the center of the East wall (inverted plastic dish served as a restraining device). After 5 min of restraint, the dish was removed, and shrews were allowed to explore the interior of the arena. Video recordings were initiated immediately after release.

Experimental testing: Shrews were randomly assigned to one of the following groups: (1) intact (INT; no anesthesia; vibrissae intact; *n* = 8); (2) shaved (SHV; *n* = 4); and (3) sham-control (SHC; *n* = 4). SHV subjects were anesthetized by intraperitoneal injection of ketamine (75 mg/kg; Bristol Laboratories, Syracuse, New York). Vibrissae (including mystacial vibrissae) were then removed by shaving with a Schick Model 17L electric trimmer. SHC subjects were anesthetized, but vibrissae were left intact. SHV and SHC animals were allowed to recover fully from anesthesia (for 3 days), and were then subjected to behavioral testing within 48 hr following recovery. Preliminary observations had shown that it takes between 2.5-3.0 weeks for vibrissae to begin

regeneration. To ensure that visual cues were not used, eyes of all subjects were covered with a rectilinear piece of soft black cloth fastened by a plastic clip on each side of the jaw and held in place by a thin rubber band. Previous observations on other adult gray shrews indicated that they appear to adapt readily to these blindfolds, and began to exhibit normal movement, foraging and feeding patterns within 15 min after the blindfolds were applied.

All subjects were tested in the following sequence: (1) barren arena; (2) with a series of equally spaced wooden blocks (4 x 4 x 4 cm) placed in the middle of the arena, along the E/W axis; and (3) equally spaced blocks placed in the middle of the arena along the N/S axis. These blocks resulted in variation in arena topography in contrast to a barren arena.

Once INT subjects habituated to barren conditions, they were placed back into their home cages for 24 hr, while wooden blocks were added along the N/S axis as described above. Blocks made it more difficult for subjects to move directly across the center of the arena to the opposite side. This was also true for trials where blocks were oriented along the E/W axis. Presence of blocks also presented subjects with novel objects to explore (smell, see, touch). Each animal received one trial / day for 4 days.

The same 8 subjects were then tested with blocks in the E/W direction for 24 hr after the final trial with blocks in the N/S direction. Each subject was tested only once, thus serving as a single probe trial. Movement of blocks from N/S to E/W presented each subject with novel test topography. The same testing sequence was administered to SHV and SHC groups.

Data analysis: All methods used for statistical analyses followed those described by Sokal and Rohlf (1995). A Bartlett's test showed homogeneity of variance, and G-Tests showed that error variances were normally distributed. Digitized paths recorded various positions of subjects and provided data on distance covered by animals as well as velocity. Distances traveled during a 30 min trial were used to determine average movement velocities and were used as indices for measuring exploratory behavior. Data were analyzed using a repeated measures MANOVA and post-hoc '*t*' tests.

<div align="center">Results</div>

Intact subjects / barren arena: Blindfolded shrews moved within the arena (barren, and with blocks), alternately waving their snouts in the air and pushing them into the substrate. When their vibrissae or snouts came in contact with arena walls, they either moved for variable distances while maintaining contact with walls (thigmotaxis), or moved back towards the center, eventually making contact with other walls. When a block was encountered, shrews made contact with its sides using their vibrissae or tip of the snout. In some cases, they raised up on their hind legs with forelegs in contact with the block. After 3 days of trials, subjects were less thigmotactic, and distance traveled declined over the testing period (Fig. 1, B1-B4) indicating habituation of subjects to testing condition. Subjects moved at a velocity (exploration velocity) of 278.3
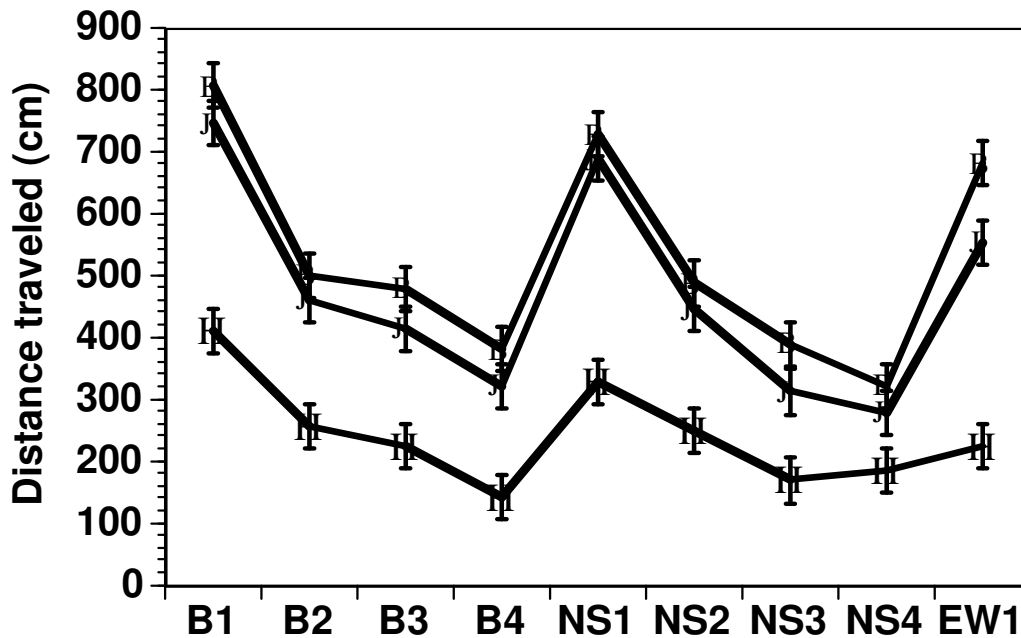
Fig. 1: Distances traveled (exploratory behavior) by males of *Notiosorex crawfordi* in a test arena. There were 3 groups of test subjects: Intact (squares), sham-controls (circles), and shaved vibrissae (triangles). B1 – B4: trials in a barren arena, for days 1 – 4; NS1 – NS4: wooden blocks placed along North-South axis of arena, for days 1 – 4; EW1: blocks placed along East-West axis, for day 1. Data expressed as means ± SE.

cm/min. INT shrews traveled a mean of 804.3 cm during trial 1, and 411.6 cm by trial 4. A significant reduction in locomotor activity occurred across trials (F = 60.46; df = 1,3; p < 0.01), and between trials 1 and 2 (t = 23.07; df = 2; p < 0.03).

Intact subject s- North/South and East/West block configurations: All subjects exhibited dishabituation to the first trial of a novel topographical situation. Upon reintroduction into the arena from their holding cages, subjects traveled a mean distance of 748.8 cm. There was significant increase in distance traveled during trial 4 under barren conditions (t = 19.45; df = 2; p < 0.02). Shrews also decreased locomotor activity during contact with blocks, typically nudging edges and sides of blocks with their snouts. As a result, exploration velocity decreased significantly, from 278.3 to 154.7 cm/sec in the barren arena (t = 18.99; df = 2; p < 0.01). All subjects habituated to the N/S configuration as shown by the decrease in distance traveled, from 748.8 cm in trial 1 to 378.4 cm in trial 4 (Fig. 1, NS1-NS4). With blocks in the E/W configuration, all subjects exhibited habituation to test conditions upon exposure to the new configuration (Fig. 1, Intact, EW1; t = 33.03; df = 2; p < 0.01). Exploratory behavior approached the same levels as observed in the beginning of the experiment. Exploration velocity (148.7 cm/sec) did not differ significantly from that exhibited by subjects under N/S configuration (p > 0.50).

Sham control subjects: These shrews exhibited a pattern of exploratory behaviors as shown by INT subjects (Fig. 1). Similar movement patterns and thigmotactic behavior were also observed under all the other test conditions. Animals exposed to barren as well as N/S and E/W configurations habituated

over the 4 trial training period. Exploration velocities under barren (259.2 cm/sec), N/S (163, 7 cm/sec), and E/W (158.5 cm/sec) were similar to those observed for INT subjects.

Shaved subjects: The exploratory behavior of these subjects was significantly less than that shown by INT and SHC animals (Fig. 1). However, these animals did exhibit habituation under barren and N/S conditions after 4 trials. Subjects moved at significantly slower velocities (45.3 cm/sec) as compared to exploration velocities of INT and SHC subjects (p = 0.01). There was also a significant reduction in distance traveled across all trials (F = 57.91; df = 1.3; p < 0.01). Shrews typically bumped into wooden blocks, either with the tip of their snout or side of the head, and usually became motionless for a variable period of time before moving. They also showed a tendency to remain at the position where they were placed at the beginning of a trial for a longer period of time (48.7 sec), as compared to INT (24.3 sec) or SHC (32.6 sec) subjects (t = 17.04; df = 2; p< 0.05). Additionally, when they made contact with an arena wall they showed a greater tendency to stop at that position, waving their snouts in the air, and appearing hesitant to continue walking.

Discussion

These results indicate that males of *N. crawfordi* use their vibrissae to detect and respond to tactile cues when exploring their environment. Furthermore, locomotor activity of blindfolded shrews decreased as a result of repeated 30 min exposures to a test arena in which no topographical changes occurred. Activity immediately increased when subjects were returned to an arena where novel topographical features were

introduced following a 24 hr period in their home cages. This suggests that a pattern of habituation followed by dishabituation is characteristic of these shrews when they experience topographical changes in their environment. This pattern cannot be explained merely as a response by shrews to being transferred from their home cages to the test arena because dishabituation occurred only after spatial changes were introduced.

Additionally, habituation to all configurations persisted over a 24 hr period without constant exposure to test conditions. This suggests that shrews exhibited latent learning linked to the recognition of particular topographical features of their environment. Latent learning, also referred to as exploratory learning, involves an animal using experience gained at one time to modify behavior at a later time. This is especially relevant when animals process spatial information. For example, rats and hamsters that have been allowed to explore a test arena (with an entrance and exit door on opposite sides) that contains specific topographical features associated with physical objects such as running wheels, tunnel tubes, platforms, etc., learn to navigate the maze to obtain a food reward at a later time much faster than rats introduced into the arena for the first time (Poucet *et al.,* 1986). Similar examples of latent learning in association with structural features of the environment have also been reported for maze learning tasks in rats (Davey, 1989).

Under natural conditions, the ability of a predator to detect and respond to topographical features of its environment and presence of potential prey organisms should contribute to its overall fitness (Macphail, 1982; Punzo and Farmer, 2004). In order to manifest such an ability, an animal should have the capacity to learn and remember specific physical features of microhabitats that are associated with: (1) foraging routes that lead to locations where prey is more abundant (Able, 1991); (2) escape routes leading to shelter sites (Punzo, 2002); (3) spatial patterns of physical objects such as shrubs or rocks that lie in close proximity to a nest or food source (landmark learning) (Biegler and Morris, 1996; Punzo and Farmer, 2003). Engaging in exploratory behavior allows an animal to initiate investigations of physical features of its microhabitat that will eventually lead to consolidation of experiential information within the central nervous system resulting in optimization of for aging activities (Benhamou and Poucet, 1996; Punzo and Chavez, 2003).

In order to realize these capacities, animals must rely on their sensory organs to detect various environmental cues for spatial information. This study presents empirical evidence that *N. crawfordi* can use tactile cues to detect and respond to changes in topographical features of its environment, and detect and locate prey, even in the absence of visual cues.

Acknowledgments

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