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Sibling Recognition in Spiny Mice (*Acomys cahirinus*)*

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Summary. Sibling recognition by spiny mice (*Acomys cahirinus*) was investigated by housing groups consisting of two pairs of littermates together and recording frequency of dyadic pairing. A total of 136 animals (68 pairs of siblings) were tested in three experiments. Sibling pairs were observed more often than pairings between nonsiblings; however, such preferences were no longer evident if the nonsiblings were exposed to one another prior to testing. Animals made anosmic through zinc sulfate treatment did not differ on their frequencies of sibling vs. nonsibling pairing and showed a higher incidence of group huddling (by all four animals) than did intact controls. Weanling *A. cahirinus* appear to be able to recognize (i.e., are attracted to) their littermate siblings through olfactory cues, which seems to be a modifiable attraction to odors to which the littermates were exposed rather than an irreversible imprinting-like process.

Introduction

The degrees of relatedness between individual conspecifics is a critical variable in recent theories seeking to explain the evolution of 'altruistic' behavior through kin selection and inclusive fitness (e.g., Hamilton, 1963, 1964; Maynard Smith, 1964). In instances where close as well as only distantly related (or unrelated) conspecifics overlap, some ability to discriminate between individuals as a function of their degree of relatedness to the individual in question would be a prerequisite for kin selection to control the evolution of altruism. Furthermore, even for such species as wolves (Mech, 1966), lions (Bertram, 1976) and many of the social hymenoptera (e.g., Michener, 1974; Wilson, 1971), for which conspecifics within a given area are probably closely related, recognition of close relatives would be necessary for mediating the rejection of individuals outside of the kin group. The most common example of such discrimination of kin

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is that of parent-offspring recognition which was documented in a wide array of species, primarily avian and mammalian. Although little work was reported concerning the recognition of relatives other than parents and offspring, several recent studies with rodents have shown that individual conspecifics can be recognized through chemical cues. For example, in mice (*Mus musculus*), laboratory rats, and gerbils (*Meriones unguiculatus*), individual animals were found to be able to discriminate between odors produced by different conspecifics (e.g., Bowers and Alexander, 1967; Carr et al., 1976; Dagg and Windsor, 1971; Mackintosh and Grant, 1966). Furthermore, Halpin (1976) recently reported that, while anosmic gerbils respond similarly to familiar as compared to unfamiliar conspecifics, control animals interact differently with strange vs. familiar animals.

The present study is concerned with littermate (sibling) recognition and the role of olfactory cues therein in the spiny mouse (*Acomys cahirinus*). Previous research with this species already revealed that olfactory cues are important in mediating mother-infant interactions (Porter and Ruttle, 1975; Porter and Doane, 1976) and that the specific properties of these chemical signals emanating from neonates and lactating females are dependent upon the diet of the latter (Porter and Doane, 1977; Doane and Porter, 1977).

Materials and Methods

The spiny mouse (*Acomys cahirinus*) is a murid rodent indigenous to the Near East. To date, behavioral research with *A. cahirinus* has focused primarily upon the uniquely precocial offspring (e.g., Dieterlen, 1962; Porter and Doane, 1976, 1977; Porter and Etscorn, 1974, 1975).

Subjects for the present series of experiments were randomly selected from the laboratory colony where breeding pairs are housed in individual cages along with their offspring (range of litter size = 1–6), with the latter being removed at approx. 1 month of age. Since the length of gestation is 38 days, weaning at 1 month insures that successive litters will not be present simultaneously in the home cage.

Throughout each of the three experiments reported below, groups consisting of four animals each were housed in individual 10-gallon terrariums (observation cages). A metal partition divided each terrarium into two 12×12 in. areas, with a 3×3 in. high opening centered at the bottom of the partition allowing free movement between the two sides. The floor of the observation cage was covered with bedding material (sugarcane waste) and food (Purina mouse chow) was available ad lib. Moisture was provided by pieces of fresh apple which were placed into the cage at the end of each day's observation session.

Specific procedural details for each of the experiments are described further in relevant sections under Results.

Results

Experiment 1: Sibling Preferences

The first experiment was conducted to determine whether littermate weanlings recognize and interact differently with one another as compared to unfamiliar conspecific agemates. Since numerous observations in our laboratory indicate that littermates spend a great deal of time huddled in contact with one another

while in their home cage, the specific question posed by experiment 1 was whether weanlings would maintain physical contact with siblings more often than with unfamiliar agemates.

On the day of weaning, two pairs of siblings (i.e., four animals born to two different females) were removed from their respective home cages and placed together into an observation cage. A total of nine groups (each consisting of two pairs of siblings) of weanlings were tested. Within each group, the sex composition of the two pairs of siblings was identical. Overall, three of the groups consisted of two pairs of male siblings; three were made up of two pairs of female siblings each; while the final three groups of paired siblings contained one male and one female in each of the two pairs. All animals were between 29–37 days of age when first removed from their home cages, marked for purposes of identification, and placed into the observation cage; with no more than two days age difference between the two pairs of siblings housed together in a given terrarium.

All four animals within each group remained together in their observation cage until the end of the fifth observation day (i.e., from Monday–Friday), at which time they were housed separately in individual isolation cages. Isolation lasted from Friday afternoon until Monday morning, when all four animals making up a group were again placed together into their terrarium for three more days of observation. Grouped weanlings were observed during six 5 min sessions per day on each of the 8 days (5 days prior to and 3 days following the isolation period) in the observation cage. The first observation session (on day 1) began 60 min after the animals had been placed into the observation cage to allow time for them to explore and become somewhat familiar with their new environment. At the beginning of each 5-min observation session, and at the end of each 60-s interval therein, the identities of any animals that were physically touching one another (touching of limbs, head or trunk; excluding touching of whiskers or tails) were recorded. Thus, for each 5-min observation session, there were a total of six observation points. The six 5-min observation sessions were distributed throughout the working day with at least 30 min between successive sessions.

In all nine cages of weanlings, sibling dyadic pairing (i.e., two siblings in physical contact with one another only) was observed more often than was nonsibling dyadic pairing. These same results were consistent over the two phases of the observation period-i.e., before as well as after the isolation period following the fifth day of observation. For statistical purposes, the Chi square test was used to compare the observed number of observation cages showing a greater frequency of sibling vs. nonsibling dyadic pairing against the expected frequencies based upon chance alone (χ^2 with Yate's correction $= 15.12$, d/f $= 1$, $P < 0.001$). Since nonsibling pairings would result from twice as many of the possible combinations of two animals (in each observation cage) than would sibling pairing, the expected number of observation cages showing a majority of nonsibling pairings would be six as compared to three for the expected number showing a majority of sibling pairings.

The mean number of observed sibling pairings per observation cage (summed across all eight observation days) was 281.2 as compared to 7.8 for the nonsibling

Table 1. Mean observed frequencies of sibling and nonsibling dyadic pairings for each of the three sex combinations of weanlings

	Sex combinations		
	Cages containing two pairs of *male* siblings ($n=3$)	Cages containing two pairs of *female* siblings ($n=3$)	Cages containing two pairs of *male/female* siblings ($n=3$)
Sibling pairings	243.3	297.3	303.0
Nonsibling pairings	6.3	7.7	9.3

pairings. Mean frequencies of sibling as compared to nonsibling dyadic pairings by sex combination of the weanlings are presented in Table 1.

Experiment 2: Modifiability of Sibling Preferences

Given that the littermate weanlings in the first experiment appeared to be able to recognize one another, a second experiment was designed to ascertain whether the preference for contact with siblings as opposed to unrelated agemates was unmodifiable, or whether such preferences would be reversed following a period of forced exposure to the latter.

On the day of weaning (Monday), littermate pairs of pups were assigned randomly to one of two conditions. In the experimental condition, two pairs of siblings (of comparable sex composition) were separated and housed in two cages, each containing two unrelated animals. For example, sibling pair A-1/A-2 and pair B-1/B-2 were housed in two cages containing A-1/B-1 and A-2/B-2 respectively. All paired animals remained in their appropriate cages until the fifth day post-weaning (Friday), at which time they were all placed into individual isolation cages. The following Monday (day 8 post-weaning), all four animals (two pairs of siblings) were placed together in a 10-gallon terrarium where they were observed for five consecutive days. Data collection began 1 h after the four animals were placed into their observation terrarium.

A total of nine cages of animals (four per cage) were tested in the experimental condition, with three replications of each of the three possible matched-pair sex combinations. An identical number of weanling littermate pairs assigned to the Control condition were treated comparably to the animals in the experimental condition except for being housed in littermate pairs (rather than nonsibling pairs) from weaning (Monday) until isolation (Friday). As for experiment 1, records were kept of frequency of dyadic pairings (i.e., two animals in physical contact with one another) and the identities of the paired animals. Further methodological details were identical to those of experiment 1.

The results of experiment 2 are summarized in Table 2 which contains mean frequencies (per cage) of total dyadic pairings, sibling pairings, and nonsibling pairings for the experimental vs. control conditions. Due to the death of a single animal in the experimental condition (in one of the cages containing two male/female pairs of littermates), data were collected on only eight cages of animals in that condition.

Table 2. Mean frequencies of dyadic pairing by animals housed with littermates (control condition) vs unfamiliar agemates (experimental condition) following weaning

	Condition			
	Experimental (housed with unfamiliar agemates for 5 days post-weaning) ($n=8$ cages)	Control (housed with littermates for 5 days post-weaning) ($n=9$ cages)	t	P
Sibling pairings	8.88	75.78	2.89 d/f=15	<0.02
Nonsibling pairings	7.25	12.67	0.88 d/f=15	>0.20
Total dyadic pairings (sibling+nonsibling)	16.13	88.33	2.82 d/f=15	<0.02

For statistical purposes, the units of analysis were the cages containing four animals each. As seen in Table 2, frequency of sibling pairing, as well as total dyadic pairing (sibling+nonsibling) was significantly greater (t-test for independent samples) in the control as contrasted with the experimental condition. The two conditions did not differ on mean frequency of nonsibling pairing.

A final series of analyses compared the observed number of cages showing a greater frequency of sibling vs. nonsibling dyadic pairings against the expected frequencies based upon chance alone. For the control condition, the number of cages (8/9) displaying a majority of sibling pairings was significantly greater than the number showing a majority of nonsibling pairings (1/9) (χ^2, with Yate's correction, $=9.88$, d/f$=1$, $P<0.01$). In the experimental condition, however, the number of cages showing sibling pairing vs. nonsibling pairing majorities did not differ statistically. Of the eight cages in the experimental condition, two had a majority of nonsibling pairings, two a majority of sibling pairings, one an equal number of sibling and nonsibling pairings, while in the remaining three cages, no instances of dyadic pairing were observed.

Experiment 3: The Role of Olfaction in Sibling Preferences

Based upon related research with several species of rodents (cf. Halpin, 1976; Bowers and Alexander, 1967), it was hypothesized that the most salient sensory modality for sibling recognition in *A. cahirinus* (as found in experiments 1 and 2) is olfaction. Accordingly, the final experiment assessed the role of olfactory cues in sibling recognition by comparing temporarily anosmic vs. intact control animals.

A total of 32 pairs of siblings were tested in experiment 3. On the day of weaning, each pair was randomly assigned to the anosmia (experimental) or control condition. Animals in the experimental condition were subjected to olfactory impairment through the administration of zinc sulfate into the nostrils, while control animals were treated comparably except that saline was

Table 3. Mean frequencies of dyadic pairing and huddling by zinc sulfate vs saline treated weanlings

	Treatment condition			
	Zinc sulfate ($n=8$ cages)	Saline ($n=8$ cages)	t d/f 14	P
Sibling pairings	8.9	108.5	2.91	<0.02
Nonsibling pairings	21.9	9.5	0.87	>0.20
Total dyadic pairings (sibling + nonsibling)	30.8	118.0	2.47	<0.05
Huddling (i.e., all four animals touching)	84.4	3.8	4.04	<0.01

substituted in place of zinc sulfate. Intranasal application of zinc sulfate was previously found to induce reversible anosmia in rats, mice, and hamsters (Alberts and Galef, 1971; Edwards and Burge, 1973; Devor and Murphy, 1973).

Upon removal from the homecage, each weanling was lightly anesthetized and approx., 0.01–0.02 ml of the appropriate substance (either 5% zinc sulfate solution or physiological saline) was injected into each nostril through a blunt 26 ga. needle inserted 1–2 mm into the nostril. As soon as the animals appeared to have recovered from the effects of the anesthesia, two pairs of littermates (i.e., born to two different females) in the same condition (zinc sulfate or saline treated) were placed together into a 10-gallon terrarium where their behavior was observed (beginning 1 h later) for five consecutive days. In each instance, the two pairs of siblings housed together were matched as closely as possible for age, with no more than 48-h age difference between them. Eight cages each containing four zinc sulfate treated animals (two pairs of siblings) were observed, while another eight observation cages each contained four saline treated animals. The sex composition of the two pairs of siblings in each cage was equated across the two treatment conditions. Aside from dyadic pairing, frequency of huddling by all four animals (i.e., all four animals grouped together and touching) was also recorded. Further details of the observation cage and procedure are otherwise identical to those of experiment 1.

Following the last observation session on day 5, the olfactory ability of the animals in each cage was tested by burying two pieces of fresh apple under the bedding material and recording the latency (with a maximum of 15 min) until one piece of apple was first uncovered by any animal in the cage.

The results of experiment 3 (Table 3) indicate that the saline treated control group of animals displayed significantly more dyadic pairing and sibling pairing than did the zinc sulfate treated (experimental) animals. Huddling together by all four animals in a cage was significantly more frequent amongst the zinc sulfate animals, however. The saline and zinc sulfate treated animals did not differ on mean frequencies of nonsibling pairing.

For the control animals, the number of cages showing a majority of sibling pairings ($n=7$) was significantly greater than those showing a majority of nonsibling pairings ($n=1$), ($\chi^2=8.24$; d/f 1; $P<0.01$). No significant difference

was found in the anosmia condition for the number of cages showing a majority of sibling ($n=3$) vs. a majority of nonsibling ($n=5$) pairings.

Finally, the results of the buried apple test following the last observation session (day 5), reveal that the zinc sulfate treated animals took significantly longer ($t=2.42$, d/f 14, $P<0.05$) to locate the apple (mean latency/cage=472.0 s) than did the saline treated control animals (mean latency/cage=166.4 s).

Discussion

That weanling *A. cahirinus* are able to discriminate between littermate siblings and strange agemates is evident from the results of experiment 1 (and of the control conditions in experiments 2 and 3) which reveal that sibling dyadic pairing is significantly more frequent than nonsibling pairing in a group housing situation. This preferential responsiveness to littermates is taken as empirical support for sibling recognition (or at least recognition of specific cues emanating therefrom) in this species. The results of experiment 2, however, suggest that such discriminative responding to siblings is not unmodifiable; it is no longer found when nonsiblings are housed together for five days prior to being grouped into two pairs of littermates. Furthermore, such exposure to nonsiblings in the absence of the subjects' own littermate leads to subsequent reductions in frequency of dyadic pairing per se, as well as frequency of sibling pairings. It would therefore appear that weanlings are no longer able to 'recognize' their littermates (or possibly odors associated with their littermates) following the absence of these siblings coupled with enforced exposure to a nonsibling agemate. Thus, for sibling recognition to have any impact on evolution in *A. cahirinus*, it may be necessary for animals to remain in proximity to one another or to interact frequently with each other, over an extended period of their lives. Whether or not this is the case for *A. cahirinus* in the wild (i.e., whether they live in physically close family units) is unknown since there has been very little research on this species in its natural habitat.

The critical role of olfactory cues in littermate recognition by *A. cahirinus* weanlings is seen in experiment 3; thereby reiterating the general importance of olfactory cues in mediating social interactions and individual recognition in rodents (cf. Alberts, 1976; Cheal, 1975). Animals made anosmic through zinc sulfate application appeared indiscriminate as to choice of partner for dyadic pairing, in contrast to the saline treated controls who preferred sibling over nonsibling pairings. Furthermore, the total frequency of dyadic pairings shown by the zinc sulfate treated animals was significantly lower than for the controls. This particular finding tends to corroborate a similar report by Alberts (1976) in which 5–20 day-old rat pups showed a reduction in bodily contact with an 'immobilized' target animal following zinc sulfate treatment.

While dyadic pairing was decreased in experiment 3 as a function of the zinc sulfate treatment, huddling involving all four animals was significantly increased in this condition as compared to the Controls. Since the anosmic animals apparently were unable to discriminate between individuals, they could not show consistent pair preferences, but rather chose to gather into a single

heterogenous grouping. Thus, in small groups, as tested in the present series of studies, dyadic pairings as opposed to larger groups involving several animals, may depend upon an ability to recognize individuals through chemical cues. That the zinc sulfate treatment actually resulted in olfactory deficits is supported by the results of the buried apple test (5 days after treatment) in which the saline treated control animals had faster latencies until finding the apple than did the zinc sulfate animals.

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