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Territorial behavior of laboratory rats under conditions of peripheral anosmia

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Two experiments investigated the influence of peripheral anosmia with zinc sulfate solution on aggressive and nonaggressive behaviors of rats in the colony intrusion paradigm. Experiment I was a replication of a previous observation that anosmic intruders are *not* attacked by resident males. No significant differences were found in aggressive response to normal or anosmic intruders. Experiment II tested colony residents under normal and anosmic conditions for aggressive and nonaggressive response to intruders. The results were in agreement with previous reports that anosmia abolishes differential responding between males and females as well as all aggressive acts normally directed against unfamiliar males.

Unfamiliar male conspecifics are often attacked and killed when entering the established territory of a colony of wild (Alberts & Galef, 1973; Barnett, 1958) or domesticated rats (Blanchard, Fukunaga, Blanchard, & Kelly, 1975; Luciano & Lore, 1975; Thor & Flannelly, in press, a; Luciano, Notes 1, 2). Peripheral anosmia induced by intranasal application of a zinc sulfate solution (Alberts & Galef, 1971; Thor, Carty, & Flannelly, 1976), however, virtually eliminates resident aggression against intruders (Alberts & Galef, 1973; Luciano, 1975, Notes 1, 2). Treatment of only the intruders also eliminates aggression by intact colony residents (Luciano, 1975, Notes 1, 2). Although Alberts and Galef (1973) reported no diminution of social-investigatory behavior by anosmic residents toward intruders, Luciano (1975) found that residents treated with zinc sulfate demonstrated significantly less social-investigatory behavior when confronted with foreign males. Thus, the elimination of aggression associated with peripheral anosmia may conceivably be attributed to a general reduction in all social interactions and not necessarily to a sensory deprivation specific to the motivation of aggression. Sieck and Baumbach (1974) have indicated that zinc sulfate treatment may be toxic and that some behavioral effects may be more properly attributed to systemic poisoning.

The present experiments were conducted to further examine the question of decrement in social interaction by peripheral anosmia with zinc sulfate, and specifically the contention by Luciano (1975) that (a) anosmic male intruders are not attacked by intact colony males, and (b) that social investigation of intruders by anosmic colony males is significantly curtailed.

EXPERIMENT I

Luciano (1975, Notes 1, 2) found that zinc sulfate treatment of intruders 24 h prior to their presentation to a colony prevented the initiation of attack by residents. Aggressive posturing of residents was also reduced and social investigation by residents appreciably decreased in comparison to resident social investigatory behaviors toward intact intruders. Weight loss of anosmic intruders was comparable to untreated controls, although no wounds were sustained by anosmic animals. The present experiment was essentially a replication of this particular aspect of Luciano's general investigation of sensory control of intraspecific aggression in the laboratory rat.

Method

Subjects

A total of 24 male and 16 female rats of the Long-Evans strain were used. Sixteen of the males, 70-75 days old and 280-380 g, were designated as intruders. Females had been ovariectomized at 40-45 days of age, and were comparable in size to the males. All animals were socially reared in small like-sex groups since weaning. The remaining 8 males, 400-500 g, previously observed to display aggression toward unfamiliar males, were selected from a larger group of agemates (140-160 days old) for use as residents. Each resident male was housed individually in a clear polypropylene pan cage (41 x 51 x 22 cm) for 2 weeks before testing.

Procedure

Aggression tests. Residents were given two aggression tests with a 1-day rest interval between tests. Each resident was tested with both saline-treated and zinc sulfate-treated intruders. Half of the residents received a saline-treated intruder and half received a zinc sulfate-treated intruder on the first test. Type of intruder was reversed on the second test.

Test sessions were each 24 h in duration, beginning with the presentation of an intruder into the home cage of a resident. Intruders were weighed before and after testing. The following aggressive measures were recorded during the first 15 min of each test: frequency of aggression posturing by residents (Grant & Mackintosh, 1963), frequency of attack-fight sequences (Barfield, Busch, & Wallen, 1972), latency to aggressive posturing, latency to first attack, and frequency of chases. After testing, weight loss of intruders and number of body wounds were determined. Intruders were killed by ether inhalation, body hair removed with electric clippers, and the skin carefully examined. The method described by Luciano and Lore (1975) was used to score wounds.

Anosmia treatment. Treatment of intruders was administered 24 h before exposure to residents. Following light ether anesthesia,

each intruder received a 0.2-ml volume of 7.65% $ZnSO_4$ solution (isotonic) or 0.9% sterile saline solution applied by syringe (0.1 ml into each nostril) through an inserted polypropylene tube attached by a plastic tubing adapter to a 1-ml syringe. The tube was inserted 2 cm into the nasal cavity. Upon completion of the intranasal injection, the animal was held head downward, with tip of nose against an absorbent towel. An estimated volume of 0.1 ml was drained from the nares in this manner during recovery from the ether. Each animal was then returned to its home cage. See Thor et al. (1976) for a more detailed description of the application procedure.

Anosmia tests. Approximately 30 min before initiation of aggression tests, intruders were removed from their home cages and placed individually into clean cages (46 x 24 x 18 cm). Following a 5-min adaptation interval, an ovariectomized female was placed with each intruder and the freqency of anogenital sniffs and mounts by intruders were recorded during a 5-min observation. Preliminary testing indicated that the frequency of social investigation of ovariectomized females by intact males was comparable to that exhibited toward nonreceptive intact females. Castrates were therefore used as stimulus animals since they provided standard stimulus properties without requiring a determination of estral state before testing.

Results

The results of intruder anosmia tests supported the presumption that zinc sulfate-treated animals were anosmic. Social investigation of females by zinc sulfate-treated animals was minimal and none exhibited anogenital sniffing or mounting. Mean frequency of anogenital sniffs and mounts by saline-treated intruders were 12.9 and 5.8, respectively. Zinc sulfate-treated animals also displayed considerably less exploration of the test cage preceding introduction of the female.

Resident aggression scores toward both types of intruder are presented in Table 1. All aggression scores were subjected to analyses of variance with two within (intruder treatments) and two between (order of intruder presentation) measures. No significant main effects were found in resident aggressive responses toward intruders of either type. Order of intruder presentation and Order by Treatment interactions were also nonsignificant.

Table 1
Means and Standard Deviations* of Resident Aggression Toward Intact and Anosmic Intruders

Measure	Type of Intruder	
	Intact	Anosmic
Latency to aggressive posturing (seconds)	99.8 (84.2)	226.0 (322.7)
Frequency of aggressive posturing	21.5 (15.4)	18.8 (13.2)
Latency to first attack (seconds)	316.1 (374.3)	463.5 (396.9)
Frequency of attacks	2.3 (2.4)	2.5 (2.1)
Frequency of chases	2.0 (3.0)	1.3 (1.9)
Wound score	17.4 (23.8)	32.6 (46.3)
Weight Loss**	6.5 (7.4)	6.7 (4.9)

**Standard Deviations are reported in parentheses.*
***Weight loss is reported in percent body weight.*

Discussion

Luciano (1975, Notes 1, 2) reported that zinc sulfate treatment of intruders eliminated attack by colony residents. The present data are contrary to these findings, since colony residents failed to display differential responding toward intact and anosmic intruders. In the present study, zinc sulfate-treated intruders showed a marked reduction in social interaction with females. Luciano (1975) similarly reported that social investigation of residents by anosmic intruders was completely curtailed. The observation, noted by Alberts and Galef (1973), that immobility appreciably decreases the aggression-eliciting capability of a target animal, may offer one explanation for the discrepant results. Since Luciano's residents were not selected for aggressiveness, intruder immobility may have been sufficient to block vigorous attack; less intense aggressive acts were noted against a few intruders. Thus, resident tolerance of zinc sulfate-treated intruders in the Luciano (1975) study may have been a consequence of decreased intruder activity and not necessarily of anosmia.

EXPERIMENT II

Luciano (1975) reported that anosmic residents spent significantly less time than intact residents in social investigation of intruders. Previous research in this laboratory suggests that social-investigatory behavior of residents is inversely related to aggression, with initial attack upon a strange male often preceded by minimal social investigation (Thor & Flannelly, in press, a, b). On the other hand, although resident males rarely attack ovariectomized females, they display a full complement of social-investigatory behaviors toward them. In order to assess the detailed consequence of peripheral anosmia upon social investigation and aggression, zinc sulfate-treated residents were tested with intact male and ovariectomized female intruders.

Method

Subjects

Four males displaying the highest levels of aggression in Experiment I were used as residents. Sixteen male and 16 females of the same strain were used as intruders. All were socially reared (like-sex groups of four or five animals) and comparable in age and size to animals used in Experiment I. Females were ovariectomized at 40-45 days of age.

Procedure

Tests were conducted in the home cage (41 x 51 x 22 cm) of each resident. In addition to the aggression measures used in Experiment I, the frequency of resident social-investigatory behaviors (Grant & Mackintosh, 1963) toward intruders were also recorded.

On the first day of testing, each resident was given two tests. Half of the residents received a male and half a female intruder on the first test. Type of intruder was reversed on the second

test. Fifteen-minute test sessions were separated by 15-min intervals. Following the completion of both tests, each resident was treated with zinc sulfate solution. Treatment of residents was the same as in Experiment I for intruders; under light ether anesthesia, 0.1 ml of 7.65% $ZnSO_4$ solution was applied to each side of the intranasal epithelium by intubation. A second series of tests was administered 24 h after zinc sulfate treatment. Following a 3-week period, to permit full recovery of all residents from zinc sulfate treatment, the sequence of testing and treatment was repeated. Order of intruder presentation was counterbalanced throughout. Thus, each resident was tested eight times in all, twice with males and twice with females under each of the two conditions (normal and anosmic).

Results

Tables 2 and 3 present the social-investigatory and aggressive behavior of saline- and zinc sulfate-treated residents toward intact male and ovariectomized female intruders. Scores on the first and second series of tests were combined and an analysis of variance performed on the data, with resident treatment and type of intruder as within-subject measures. Resident frequency of approach-follow, $F(1,9) = 10.2$, $p < .05$, investigate, $F(1,9) = 19.7$, $p < .01$, and anogenital sniff, $F(1,9) = 8.62$, $p < .05$, were significantly greater toward castrated female than intact male intruders. Zinc sulfate treatment significantly reduced resident social investigation: approach-follow, $F(1,9) = 30.7$, $p < .01$; investigate, $F(1,9) = 74.9$, $p < .01$, and anogenital sniff, $F(1,9) = 21.9$, $p < .01$. Type of Intruder by Resident Treatment interactions were significant for approach-follow, $F(1,9) = 7.91$, $p < .05$, and investigate, $F(1,9) = 20.6$, $p < .01$.

Resident aggression against ovariectomized intruders was limited to low-intensity postural displays. The intense aggression shown toward male intruders was virtually eliminated by zinc sulfate treatment (see Table 3). Resident mounting of male intruders was enhanced by anosmatization.

Table 2
Mean Frequencies and Standard Deviations* of Social-Investigatory Behaviors of Resident Males Toward Male and Female Intruders

Social Behavior	Intruder**	Resident Treatment: Saline	Resident Treatment: Zinc Sulfate
Approach-Follow	IM	10.0 (3.9)	3.8 (3.7)
	CF	23.8 (11.4)	4.6 (3.5)
Investigate	IM	9.0 (4.4)	3.8 (3.9)
	CF	20.0 (6.3)	3.6 (2.3)
Anogenital Sniff	IM	3.9 (1.7)	.9 (1.5)
	CF	15.8 (10.3)	1.4 (2.3)
Mount	IM	1.9 (3.4)	5.4 (9.0)
	CF	10.0 (6.0)	2.5 (6.3)
Other***	IM	2.4 (1.6)	2.5 (3.5)
	CF	8.0 (4.8)	3.3 (2.6)

**Standard deviations are reported in parentheses.*
***IM (intact male); CF (castrate female)*
****attend, stretched attention, nose, crawl over, crawl under, and social groom*

Table 3
Mean Frequencies and Standard Deviations* of Aggressive Behavior of Male Residents Toward Male and Female Intruders

Aggressive Behavior	Intruder**	Resident Treatment: Saline	Resident Treatment: Zinc Sulfate
Latency to aggressive Posturing (seconds)	IM	125.3 (150.6)	811.3 (186.5)
	CF	782.5 (220.8)	847.5 (148.5)
Frequency of aggressive Posturing	IM	23.4 (12.5)	1.1 (2.2)
	CF	1.8 (3.4)	.3 (.7)
Latency to first attack (seconds)	IM	196.9 (299.2)	900.0
	CF	900.0	900.0
Frequency of attacks	IM	3.3 (2.6)	0.0
	CF	0.0	0.0
Frequency of chases	IM	1.5 (1.7)	0.0
	CF	0.0	0.0
Wound score	IM	4.3 (4.2)	0.0
	CF	0.0	0.0

**Standard deviations are reported in parentheses.*
***IM (intact male); CF (castrate female)*

Discussion

The present results are in agreement with Luciano's (1975) findings that zinc sulfate treatment of residents causes a decline a social-investigatory and aggressive behaviors. The data further indicate that anosmia abolishes differential responding toward males and females, as reported by Alberts and Galef (1973), who noted that anosmic males displayed considerable copulatory behavior toward anestrous females and intact males. They hypothesized that in the rat, homosexual mounting by males is normally inhibited by male olfactory cues which also serve to elicit aggression. Anosmia eliminated recognition of the aggression-eliciting properties of unfamiliar males and released copulatory behavior. Unpublished observations by the authors (Thor & Flannelly, in press, b), in conjunction with the present findings, support the view that the presence or absence of male olfactory cues is the determining factor in the elicitation of aggressive and copulatory behaviors, respectively. Mount attempts often appear in sequences of aggressive behavior, however, suggesting that copulatory behavior may not be actively suppressed by male pheromones, but that its probability of occurrence is reduced by competing aggressive tendencies.

Although the reduction in aggression following treatment may be due in part to the elimination of specific olfactory cues indicating the sexual identity of males, in the present experiment the specificity of olfactory control of aggression cannot be separated from the gross decline in social investigation. This general decrement in social interaction could represent a physical debilitation of residents by zinc

poisoning and/or a general decrease in arousal following the loss of a vital sensory avenue.

GENERAL DISCUSSION

The present results confirm the findings of Alberts and Galef (1973) and Luciano (1975, Notes 1, 2) that loss of olfactory capacities dramatically decreases social aggression in the rat. However, the data further indicate that application of a gross sensory manipulation, such as peripheral anosmia, does not permit any conclusion as to the specificity of this effect apart from the observed overall behavioral decline. Use of other peripheral treatments such as the intranasal application of local anesthetics (Latané, Joy, Meltzer, & Lubell, 1972; Doty & Anisko, 1973) may allow an assessment of the relative contribution of poisoning and anosmia to this behavioral decrement (Sieck & Baumbach, 1974).

In other rodent species, hormonal manipulations and superficial alterations of olfactory cue properties of target animals have revealed the subtle control of urinary pheromones upon social (Dixon & Mackintosh, 1975) and agonistic behavior (Mugford & Nowell, 1971; Payne, 1974a, b). Recent work in this laboratory indicates that aggressive-eliciting and aggression-inhibiting properties of males and females are not exclusively dependent upon current gonadal functioning (Thor & Flannelly, in press, b). As found in mice (Dixon & Mackintosh, 1975), females appear to be protected from male aggression by their lack of male odorant. Apparently, as suggested by Alberts and Galef (1973), olfactory recognition of a conspecific as male elicits aggression, while the absence of male olfactory characteristics elicits copulation. In the rat, gonad influence upon male characteristics necessary for sexual discrimination by conspecifics seem to be principally developmental (Thor & Flannelly, in press, a, b).

Luciano (1975, Note 1) has emphasized that the reaction of a potential target toward an aggressor is a major component affecting the aggressor's behavior. He hypothesized that anosmic intruders were not attacked because, failing to detect the resident's odor, they did not respond in an aggression-eliciting manner. Alberts and Galef's (1973) findings and those of Luciano (1975) suggest that decreased movement of victims following attack may be one factor reducing the probability of continued aggression. While the present study failed to find decreased aggression toward anosmic intruders, manipulations of intruder responding preceding and following attack should elucidate the nature of the interaction between aggressor and victim.

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