Social responsiveness and Zn-induced anosmia in rats: A replication

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Virgin male Long-Evans rats receiving multiple intranasal applications of ZnSO₄ solution showed significantly less social investigation of novel females than did controls, when tested 17 days posttreatment. Experimental and control groups all sustained a transient loss of weight following treatment, with Zn-treated males requiring a longer time to regain body weight than did controls. Verification of anosmia in experimental animals using an independent appetitive test of olfactory ability, in conjunction with the body weight data, suggest that the observed reduction in social-investigatory behavior may be attributed to depressed olfactory acuity and not to physical debilitation produced by the toxic effects of Zn absorption.

In the rat, peripheral anosmia abolishes intermale territorial aggression and appears to be intimately related to a decline in social investigation of either sex by Zn-treated males (Flannelly & Thor, 1976). Decreased social investigation between male gerbils has been reported following bulbectomy or ZnSO₄ treatment (Christenson, Wallen, Brown, & Glickman, 1973).

Recently, Thor & Flannelly (in press-b) found that naive male rats given multiple intranasal applications of ZnSO₄ solution exhibit a deficit in social investigation of castrate females for 20 days posttreatment (duration of testing). These findings suggest that the prolonged decrement in normal social responsiveness may be due to a sustained olfactory blockade. Alternatively, social behaviors may have decreased because of a general lethargy induced by the toxic effects of Zn (Sieck & Baumbach, 1974). Subsequent work in this laboratory indicates that anosmia of several months duration may be produced by a 3-day sequence of multiple intranasal applications of ZnSO₄ solution. The transient effects of Zn toxicity, on the other hand, appear to last only a few weeks, as indicated by a precipitous loss and gradual recovery of body weight following treatment (Thor & Flannelly, in press-a).

The present study was a replication of the previously observed reduction in social-investigatory behavior following ZnSO₄ treatment. The time course of possible Zn-induced toxic effects was observed prior to behavioral testing by monitoring body weight, and verification of anosmia was examined by a food preference test.

METHOD

Subjects

Subjects were 63 male Long-Evans rats, approximately 90 days of age, acquired from Perfection Breeders, Inc., Douglassville, Pennsylvania. Each was housed in a 17.5 x 24.5 x 17.5 cm wire mesh cage, and a 12:12 reversed (dark 6:00 p.m. to 6:00 a.m.) light-dark cycle was maintained throughout the experiment. Food and water were freely available except as specified prior to the odor discrimination task.

Procedure

Animals were randomly assigned to three treatment groups: zinc sulfate ($n = 33$), isotonic saline ($n = 15$), and air ($n = 15$). Under light chloroform anesthesia, .2 ml of 7.65% ZnSO₄ solution, isotonic saline solution, or air was slowly injected into the nasal cavity through a polypropylene tube inserted 2 cm into either naris. The animal was held in a head-down position during both the application procedure and the recovery from anesthesia in order to allow excess solution to drain from the nasal cavity (Thor, Carty, & Flannelly, 1976). Three applications were administered each day at 2-h intervals on Days 1-3.

Body weights were recorded immediately before the first treatment each day, 24 h after the final treatment, and at 4-day intervals thereafter until Day 20.

On Days 21-23 each animal was removed from its home cage and placed into a clean pan cage (41 x 51 x 22 cm) for a 5-min test of social responsiveness toward a novel female (Flannelly & Thor, 1976; Thor & Flannelly, in press-b). After allowing the animal 5 min to adapt to the cage, an ovariectomized female was placed with the male and the frequency of male social-investigatory, sexual, and aggressive postures were recorded (Grant & Mackintosh, 1963). Social-investigatory behaviors included: approach, follow, anogenital sniff, investigate, crawl over, crawl under, and groom. Duration of anogenital sniffing was also recorded since this measure had previously been found to be most affected by Zn treatment.

Following 23-h food deprivation, on Days 25 and 26, each animal was placed for 30 min into a pan cage containing five small pieces of sharp cheddar cheese (to establish cheese as a familiar food). The next day, under 23-h food deprivation, each animal was placed in a similar cage for a 5-min cheese preference test. Two metallic containers, one containing cheese and the other empty, perforated with numerous puncture holes, were situated on opposite sides of the test cage. Duration of sniffing (tip of nose within 1 cm of the container) each container was recorded.

RESULTS

Table 1 presents the measures of social investigation of females by experimental and control animals collapsed across the three test trials. No differences between the two control groups were found and their data were combined. Aggressive posturing and copulatory attempts were minimal and did not differ significantly between treatments. During the posttreatment

Table 1
Mean Frequencies and Standard Errors of
Social-Investigatory Behavior

Measure	Group			
	Experimental		Control	
	Mean	SE	Mean	SE
Anogenital Sniff	3.1	1.2	11.3	2.2*
Approach-Follow	29.6	3.0	43.4	3.0*
Investigate	29.6	3.0	45.8	2.9*
Crawl Over/Under	1.6	.4	3.6	.6*
Groom	1.6	.4	1.5	.4

* $p < .01$

interval, four Zn-treated animals and two air-treated controls died from undetermined causes.

There was a significant between-treatment difference in frequency of anogenital sniff [$F(1,55) = 10.48$, $p < .01$], approach-follow [$F(1,55) = 13.20$, $p < .01$], investigate [$F(1,55) = 14.58$, $p < .01$], and crawl over/under [$F(1,55) = 7.50$, $p < .01$]. Significant Trials by Treatment interactions were found in male approach-follow [$F(2,110) = 4.29$, $p < .05$] and investigation [$F(2,110) = 5.94$, $p < .01$] of females. The main effect of trials was significant for anogenital sniff [$F(2,110) = 3.81$, $p < .05$] and groom [$F(2,110) = 3.83$, $p < .01$]. The frequency of anogenital sniff and the number of animals displaying this posture increased over trials in both treatment groups. Zn-treated animals showed an increase in grooming with repeated testing which was not exhibited by controls. No other main effects or interactions were statistically significant.

By the end of testing, 75% (21/28) of controls but only 24% (7/29) of Zn-treated animals had exhibited anogenital posturing. Mean duration of anogenital sniffing per test, for animals which displayed the posture, was nearly identical for the two treatment groups, however: controls, $M = 16.8$ sec ($SD = 16.9$); experimentals, $M = 16.3$ sec ($SD = 14.5$). Across groups, the total frequency and duration of anogenital sniff were positively correlated [$r(56) = .93$, $p < .01$], and both measures were also positively correlated with overall frequency of the other social-investigatory postures [$r_s = .65-.73$, $p < .01$]. Correlations of measures within groups were also significant [$r_s = .61-.99$, $p < .01$].

Control animals showed a 68.2% ($SD = 15.0$) group mean preference for sniffing the cheese container, while Zn-treated animals displayed a mean cheese preference of 56.7% ($SD = 21.4$) [$t(55) = 2.35$, $p < .05$]. Groups did not differ in total time sniffing containers.

Figure 1 presents the mean body weight of Zn-treated and control groups. Saline and air control groups did not differ in body weight, so data from these groups were combined. Differences in body weight between experimental and control groups prior to treatment were not significant. Body weight data of animals which died before behavioral testing were excluded from all analy-

ses. Zn-treated and control groups lost appreciable body weight during the 3 treatment days. By Day 4 (24 h after the final treatment), experimental and control animals had lost a mean of 12.4% ($SD = 3.4$) and 13.3% ($SD = 2.3$), respectively, of their pretreatment body weight. By Day 8, however, control animals did not differ significantly from their pretreatment weight, while body weight of experimental animals remained depressed. Mean body weight of Zn-treated and control groups differed significantly on Day 8 [$t(55) = 2.70$, $p < .01$] and Day 12 [$t(55) = 2.07$, $p < .01$]; group mean differences were nonsignificant on Day 16 and Day 20.

DISCUSSION

In concordance with previous findings, the results of the present study indicate that anosmia reduces the frequency of specific social-investigatory behaviors characteristic of the rat and other rodent species (Christenson et al., 1973; Flannelly & Thor, 1976; Thor & Flannelly, in press-b). This alteration in responsiveness apparently reflects the degree to which conspecific recognition is dependent upon olfaction. The high correlation of anogenital sniff duration and frequency with the combined frequency of other social-investigatory behaviors suggests that this particular measure may have utility as a representative measure of social recognizance. Once initially observed in a few of the Zn-treated animals, the duration of anogenital sniffing did not differ from that of control animals which displayed the posture.

The results of cheese preference testing verify that experimental subjects suffered impaired olfactory ability. While body weight data demonstrate that the treatment regimen has a transient debilitating effect on both experimental and control groups, the extended depression of body weight in the experimental animals would seem to be associated with the toxic effects of Zn absorption (Sieck & Baumbach, 1974). Since experimental and control groups did not differ appreciably in body weight at the time of testing (17 days posttreatment), it seems unlikely that the social deficit of Zn-treated animals can be attributed to treatment factors other than anosmia.

Weight loss of the control group during the treatment phase (Figure 1, Days 1-4) may be attributed to repeated exposures to chloroform. Comparable weight losses of saline-treated controls

Figure 1. Mean ($\pm$ SE) body weight of experimental and control animals. Treatment was administered on Days 1-3.

have not been observed in previous investigations when anesthesia was accomplished with ether (Thor & Flannelly, in press-a). Although chloroform offers more rapid induction and recovery, the weight data suggest that ether is the preferable anesthetic.

Finally, multiple intranasal applications of ZnSO_4 solution appear to confer anosmia of unusually long duration. By allowing sufficient posttreatment time prior to behavioral testing, the confounding effects of Zn poisoning and anosmia may be avoided. Furthermore, chronic peripheral anosmia affords an attractive alternative to bulbectomy and other radical surgical procedures.

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