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The effects of simultaneous and serial lesions of the olfactory bulbs on muricide, irritability, and open-field activity in Long-Evans female rats

B. MICHAEL THORNE and ODIE L. BRACY
Mississippi State University, Mississippi State, Mississippi 39762

Muricide, handling, and activity were measured in either simultaneously or serially bulbectomized Long-Evans female rats. No evidence for sparing of function after two-stage damage was found on the behavioral variables investigated. All bulbectomized groups showed increases in muricide and activity when compared with controls, but no differences were noted between the control group and brain-damaged groups in the handling test.

Beginning with the pioneering work of Adametz (1959) and Ades and Raab (1946), a great deal of attention has been focused on sparing of function after serial brain lesions. In general, researchers have found that brain damage produced in stages results in greater sparing of function than the same amount of damage produced in one operation (for reviews, see Finger, 1978; Thorne, 1974), although a number of exceptions have been noted (e.g., Kircher, Braun, Meyer, & Meyer, 1970; LeVere, 1969; Thorne, Latham, & Thompson, 1973).

Despite the large number of brain regions that have been studied with serial lesions, relatively little attention has been devoted to the olfactory system (Macrides, Firl, Schneider, Barthe, & Stein, 1976; Rowe & Smith, 1973; Winans & Powers, 1974). Macrides et al. cut the lateral olfactory tracts of adult male hamsters in one or two stages, and no evidence was found for sparing of function of mating, marking, or nest building behaviors. Winans and Powers found that two-stage bulbectomy in adult male hamsters caused the abolition of sexual behavior in most animals. However, Rowe and Smith found no disruption of mating behavior following two-stage olfactory bulb (OB) removal with a 30-day interoperative interval in male mice.

In summary, two of three studies of serial damage to the olfactory system in rodents found no evidence for sparing of species-specific behaviors. In the study in which sparing was found, a relatively lengthy interoperative interval was used. Length of the interoperative interval is an important variable in serial-lesion effects (Finger, 1978).

Lesions of the olfactory system are of interest because of the marked behavioral changes often seen. For example, OB damage has been found to increase muricide in nonkiller rats (e.g., Karli, Vergnes, & Didiergeorges, 1969; Malick, 1970; Thorne, Aaron, & Latham, 1973, 1974), produce hyperirritability (e.g., Douglas, Isaacson, & Moss, 1969; Malick, 1970), and cause hyperactivity in the open-field test (e.g., Burge & Edwards, 1976; Klein & Brown, 1969; Sieck, 1972). There is evidence that hyperirritability is a function of the extent of damage to the olfactory system, and destruction of the olfactory bulbs alone often results in no change in irritability (e.g., Bandler & Chi, 1972; Phillips, 1970; Thorne et al., 1973, 1974).

The purpose of the present study was to assess the generality of sparing of function following serial OB damage. Muricidal behavior, irritability to handling, and activity in the open field were measured in rats receiving either one- or two-stage OB destruction. OB lesions were performed in a manner similar to that employed by authors reporting hyperirritability (e.g., Douglas et al., 1969). Specifically, the heads of the animals were elevated slightly during surgery to effect greater damage to the olfactory system. A 30-day interoperative interval was employed in the serially damaged group to maximize the potential for sparing.

METHOD

Subjects

The subjects were 48 Long-Evans female rats taken from the breeding stock maintained by the psychology department at Mississippi State University. They were housed in group cages until approximately 24 h before surgery, at which time they were placed in cages measuring 17.78 x 25.40 x 17.78 cm and housed singly until they were sacrificed. Ad-lib food and water conditions were in effect throughout the experiment. The subjects were kept on a 12 h on, 12 h off light-dark cycle.

The animals were randomly and equally assigned to four operative groups: Group OC, operated control subjects; Group OB-1, one-stage OB removal on Day 1 and control operation on Day 30; Group OB-30, control operation on Day 1 and one-stage OB lesion on Day 30; Group OB-1,30, unilateral and contralateral bulbectomy on Days 1 and 30. The average preoperative weight was approximately 321 g, and there were no

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significant differences between the groups on this variable. Because of death or illness, six animals were discarded, leaving 42 rats distributed among the groups as follows: Group OC, N = 12; Group OB-1, N = 10; Group OB-30, N = 9; Group OB-1,30, N = 11.

Apparatus

An open-field box measuring 76.2 x 76.2 x 25.4 cm was used to measure activity. The box was painted flat black, and the floor was divided into 25 equal squares with white lines. Wire mesh covered the top.

Surgery

The subjects were anesthetized with an injection of chloral hydrate solution and were given an injection of Duricilin (.2 cc) intramuscularly. Their heads were shaved and placed in the headholder from a Baltimore stereotaxic instrument. The toothbar was elevated 5 mm for each of the operated groups. After reflecting the scalp, the bone overlying the olfactory bulbs was removed. All animals in Groups OB-1 and OB-30 had their olfactory bulbs removed by subpial aspiration either on Day 1 or on Day 30. Animals in Group OB-1,30 received unilateral bulbectomy on Day 1 and corresponding contralateral damage on Day 30. Group OC rats received sham operations on Days 1 and 30, as did Group OB-1 and OB-30 rats on Days 30 and 1, respectively. In sham operations the bone overlying the OB was not removed, and the bulbs were not damaged.

After each operation, the wound was closed by autoclips, and the animal was returned to its cage for recovery. All subjects were given 14 days to recover after Day 30.

Procedure

On Day 44, a 20-min 24-h muricide test was given, in which an albino mouse was placed into each animal's cage. Latencies to kill were recorded during the 20-min observation period. After 24 h, any live mice or the remains of dead mice were removed from each cage, and a latency of 24 h was given to any animal killing after the 20-min observation period.

On Day 45, handling and activity tests began and continued for 4 days. All subjects were randomly ordered each test day to ensure that an ordering effect was not present. A clipboard was placed over each cage as it was removed from the rack, and the home cage of the animal was carried to the table on which the handling test was conducted. The handling test consisted of rating the animals on a scale used by Thorne et al. (1973). The scale consisted of five measures, each of which was rated on a 4-point scale (0-3); the ratings for the components were summed to give a daily total. Reliability checks were performed with the use of an independent observer, and the correlation was $r_S = .92$ ($p < .01$).

Histology

At the conclusion of testing, all bulbectomized animals were deeply anesthetized with chloral hydrate, the tops of the skulls were removed, and the heads were fixed in a 10% Formalin solution for at least 48 h prior to removal of the brains. The lesions were reconstructed on line drawings of the dorsal surface of a rat brain, and an estimate was made of the percentage of OB damage rostral to the frontal area (see Thorne et al., 1973, for details of the procedure).

In addition to reconstruction of damage rostral to the frontal area, any damage to the frontal area and/or to the olfactory system ventral to the frontal area was noted. Within each group, the animals were ranked on the total amount of brain damage sustained, and this ranking was used in assessing correlations between damage and the behavioral measures observed.

RESULTS

Histology

The average percentage of damage computed for each group rostral to the frontal area was approximately: OB-1, 94%; OB-30, 89%; OB-1,30, 97%; A K-W one-way analysis of variance was performed on the amount of damage, and a significant result was found [$H(3) = 8.98$, $p < .02$]. Individual comparisons using the Mann-Whitney U test revealed a significant difference between Groups OB-1,30 and OB-30 ($U = 11.5$, $p < .01$).

There was bilateral removal of at least 75% of the olfactory bulbs anterior to the frontal area in each rat. In addition, some animals in each group sustained damage either to the frontal area or to brain structures ventral to the frontal area, or both. The number of rats receiving extra OB damage in each group was: Group OB-1, 7/10; Group OB-30, 4/9; Group OB-1,30, 10/11.

Muricide

Using the Fisher exact probability test, six pairwise comparisons were made, and none was significant. A comparison between the control group and the combined bulbectomized groups revealed a significant difference ($p = .035$). The total number of killers in each group was: Group OC, 1/12; Group OB-1, 4/10; Group OB-30, 4/9; Group OB-1,30, 4/11. Because of the limited number of killers, a comparison was not made of latencies to kill.

Handling

All groups were relatively docile over all days. A K-W test was performed on the overall handling scores, and the result was not significant.

Habituation to handling occurred for all groups except Group OB-1. Using the Wilcoxon matched-pairs signed-ranks test, comparisons were performed between Day 1 and Day 4 handling scores for each of the groups, and three of four pairwise comparisons were significant. Scores on Day 4 were significantly lower than scores on Day 1 for all groups except Group OB-1 ($p < .01$). There was a significant correlation between the size of the lesion and the handling scores for Group OB-1,30 animals ($r_S = .77$, $p < .01$).

Horizontal Activity

Figure 1 shows that the OB groups consistently exhibited more horizontal movement than Group OC. A K-W test performed on the total 4-day scores revealed a significant difference between groups [$H(3) = 20$, $p < .001$]. The M-W test showed that all brain-damaged groups were significantly more active than the operated controls ($p < .001$) but did not differ from each other.

A K-W one-way ANOVA on the daily scores revealed a significant difference between the groups on each of the 4 days ($p < .01$). The M-W test showed that on Days 1, 2, and 4, all experimental groups differed from the control group and did not differ from each other. On Day 3 the M-W test revealed that five of six pairwise comparisons were significant, and the only one that was not significant was between Groups OC and OB-1. There was a significant correlation between the size of the lesion and the total number of squares traversed for Group OB-30 animals ($r_S = .77$, $p < .02$).

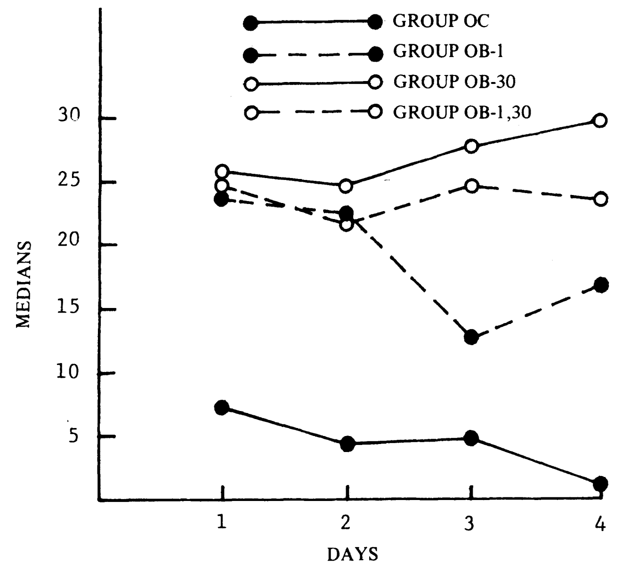

Figure 1. Median group horizontal activity plotted over days.

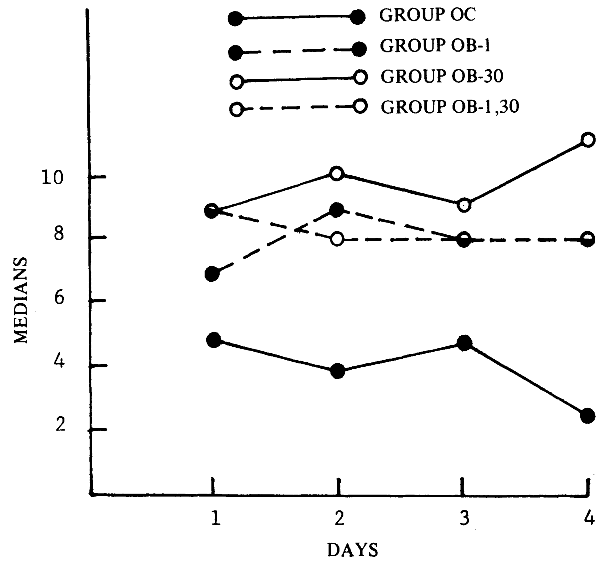

Figure 2. Median group vertical activity (rearing) plotted over days.

The Friedman two-way ANOVA comparing daily scores for groups was significant [$\chi_r^2(3) = 8.4, p < .05$]. For Group OB-1, a Wilcoxon matched-pairs signed-ranks test showed that four of six pairwise comparisons were significant. Scores on Days 3 and 4 were significantly lower than scores on Days 1 and 2 ($p < .025$).

Vertical Activity

Figure 2 shows that Group OC animals reared less than the brain-damaged animals, who were quite similar on this variable. A K-W test on the 4-day totals revealed significant differences among the treatment groups [$H(3) = 15.31, p < .01$]. Pairwise comparisons with the M-W test revealed that all brain-damaged groups reared more than the operated controls ($p < .01$) but did not differ from each other.

A K-W one-way ANOVA on each of the 4 test days showed significant differences on the last 3 days ($p < .01$). For all days on which a significant difference was found, the M-W test revealed that the brain-damaged groups differed significantly from Group OC.

DISCUSSION

The main purpose of this study was to investigate the generality of sparing of function after serial lesions of the olfactory bulbs in rats. The majority of previous studies reviewed did not find evidence for sparing of unlearned behaviors after serial OB damage in rodents (Macrides et al., 1976; Winans & Powers, 1974). The results of this study agree with the conclusion that unlearned behaviors are not spared after two-stage destruction of the olfactory bulbs. While previous studies focused on mating and/or maternal behaviors in hamsters and mice, we found no evidence of sparing of behaviors associated with emotionality and activity in the Long-Evans female rat.

In a study in which sparing of mating behavior was seen in male mice subjected to serial removal of the OB, a lengthy (30-day) interoperative interval was used (Rowe & Smith, 1973). Since length of the interoperative interval is an important variable (Finger, 1978), we employed a 30-day interoperative interval in our experiment in order to create optimal conditions for observing sparing. Despite the lengthy interval, no sparing was seen.

One problem for the unequivocal interpretation of the results is that Group OB-30 sustained slightly less damage to the olfactory system than did the other two bulbectomized groups. However, the behavioral similarities between bulbectomized groups were striking and led us to the conclusion that the confounding of lesion size was irrelevant to the behavioral outcome obtained. In addition, Groups OB-1 and OB-1,30 had equivalent damage, and no sparing was seen in Group OB-1,30 relative to Group OB-1.

A significant increase in muricide was noted when all bulbectomized animals were combined and compared with the control group. This finding is in agreement with previous research (e.g., Karli et al., 1969; Malick, 1970; Thorne et al., 1973, 1974), but the effect was not striking as in previous reports since only 40% of the bulbectomized rats killed. By contrast, over 75% of rats of the same strain and sex with comparable damage killed in the study reported by Thorne et al. (1974). One possible explanation for the low incidence of killing in the present study is the lengthy postoperative recovery period we employed (14 days for two groups and 45 days for the third). In studies reporting a higher rate of killing, the length of postoperative recovery has generally been much shorter (e.g., 5 days in the Thorne et al., 1974, report).

Unlike some studies (e.g., Douglas et al., 1969; Malick, 1970), we found no evidence for irritability to handling after OB removal. Despite our efforts to produce greater damage to the OB system by elevating the toothbar, none of the experimental groups showed hyperirritability relative to controls. Lack of hyperirritability has been reported by a number of other investigators (Bandler & Chi, 1972; Phillips, 1970).

A significant correlation was found between the handling scores and the amount of damage sustained by rats in Group OB-1,30. However, all of the animals had relatively low scores, and the highest 4-day score for any animal in the group was 12 out of a maximum possible of 60. Evidence for habituation to handling was found for all groups except Group OB-1.

In agreement with previous research (Burge & Edwards, 1976; Klein & Brown, 1969; Sieck, 1972), all bulbectomized groups displayed significantly more horizontal activity than the control group. Group OB-1 was more similar to Group OC than it was to the other two experimental groups, possibly because of its lengthy recovery period.

In agreement with previous studies (Baumbach & Sieck, 1977; Sieck, Turner, Gordon, & Struble, 1973), all bulbectomized groups reared significantly more than the operated control group. No significant differences between the bulbectomized groups were found.

In summary, we found no evidence for sparing of function in serially bulbectomized rats relative to one-stage lesioned controls. In general, the changes in behavior after OB damage in the present study agree with previous research. That is, an increase was seen in muricide and in horizontal and vertical activity in the open field, while no change in handling characteristics was noted.

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