

EFFECTS OF OLFACTORY BULBECTOMY AND PERIPHERAL DEAFFERENTATION ON REACTIONS TO CROWDING IN GERBILS (*MERIONES UNGUICULATUS*)¹

ELAINE M. HULL,² KAY L. HAMILTON, DOUGLAS B. ENGWALL, AND LINDA ROSSELLI

State University of New York at Buffalo

Male weanling gerbils sustained either olfactory bulbectomies, surgical ablation of olfactory receptors, or sham operations, and lived in one of several density conditions for 2 mo. Differential density conditions significantly affected only sham animals. All anosmic animals showed deficiencies in social-interaction tests. Only bulbectomized crowded animals showed extreme aggression in the home cage. Olfaction is shown to be an important factor mediating deleterious effects of crowding. However, since the presence of the bulbs in receptor-ablated animals was sufficient to control home-cage aggression, that displayed by bulbectomized crowded animals cannot be attributed to mere anosmia but rather to loss of a nonolfactory, limbic function of the bulbs.

It has recently been shown (Hull, Langan, & Rosselli, 1973) that high levels of population density diminish gerbils' territorial marking, aggression, and social-interaction scores. Furthermore, crowded males had lighter body, ventral gland, and testis weights than did low density gerbils, and heavier adrenal glands. These findings are in general agreement with results of many, though not all, population studies with other animals. The above pattern of results has been interpreted as resulting from the psychological stress of crowding (Christian, 1963; Thiessen, 1964), and appears in crowded animals whether or not they have fought or been wounded more than low density animals (Christian, 1959). Furthermore, it has been shown that subordinate animals show the greatest degree of crowding-induced stress (Barnett, 1958; Davis & Christian, 1957).

Among rodents, olfaction is probably the

primary sense by which animals identify one another, both individually and as members of a colony (Bowers & Alexander, 1967). Thus, if animals are deprived of their olfactory sense, they may not interact to form appropriate dominance/subordination relationships, and their reactions to crowding may be different from normal animals. The traditional means of depriving animals of their olfactory sense has been to ablate their olfactory bulbs. However, there has been recent evidence that bulbectomy results in more than a simple loss of olfaction. Wenzel, Albritton, Salzman, and Oberjat (1969) found that anosmic pigeons performed more poorly on a visual discrimination task as a result of their altered reactivity to nonolfactory stimuli. Karli (1956) and Bernstein and Moyer (1970) found that not only did olfactory bulbectomy facilitate mouse killing in previously nonkilling rats, but that the kills made by these animals appeared to be far more emotional than kills made by naturally predatory rats. Spector and Hull (1972) found that bulbectomy, although not peripheral deafferentation, facilitated the emotional form of mouse killing by rats. This differentiation between effects of peripherally and centrally induced anosmia ruled out the mere loss of smell as the primary factor leading to the emotionality or irritability

¹ We wish to thank Jill Joyce and Richard Hull for assistance in data acquisition and processing, and Dorothy Budzynski for histological assistance. This research was partially supported by State University of New York Research Foundation Fellowship and Grant-in-Aid 0689-03-050-71-0 to the first author and a National Institutes of Health Institutional Funds Grant.

² Requests for reprints should be sent to Elaine M. Hull, Department of Psychology, State University of New York, 4230 Ridge Lea Road, Amherst, New York 14226

often observed after bulbectomy (Bernstein & Moyer, 1970; Douglas, Isaacson, & Moss, 1969; Karli, 1956).

Gerbils were selected as subjects for this experiment partly because their territorial marking has been extensively studied (Baran & Glickman, 1970; Thiessen, Lindzey, & Nyby, 1970; Whitsett & Thiessen, 1972) and is easily observable. In contrast, mice and rats apparently mark territory by means of pheromones released with urine or feces, making it difficult for an observer to determine when animals are marking (Gleason & Reynierse, 1969). An additional reason for use of gerbils is that both marking and social interactions of gerbils have been shown to be affected by population density (Hull et al., 1973; Thiessen, Lindzey, Blum, & Wallace, 1970). Finally, gerbils have not been selectively bred for certain traits for many generations, as have most common laboratory animals, and therefore possess a relatively natural repertoire of social behaviors.

The present study was designed to determine the reactions of bulbectomized and peripherally deafferented gerbils to several levels of population density in order to differentiate olfactory and nonolfactory functions of the bulbs in reactions to crowding. Exploratory activity, ventral gland marking, and social interaction measures were examined, as well as several physiological indices.

METHOD

Subjects

The subjects were 90 male Mongolian gerbils (*Meriones unguiculatus*), obtained at weaning (4 wk. of age) from Tumblebrook Farms, Inc. They were housed in 10-gal. aquariums in groups of 10 before surgery. Thirty animals were randomly assigned to the bulbectomy group, 30 to the peripheral deafferent group, and 30 to the sham group. Surgery was performed between the ages of 40 and 65 days in balanced order. Twelve deaths and apparent failure to produce anosmia in 8 animals reduced the final number of animals to 70 (17 bulbectomized, 23 deafferented, and 30 sham). Data from only these 70 were statistically analyzed except for the smell tests, which included all surviving animals. Food, water, and a wooden block for gnawing were available ad lib throughout the experiment. The aquarium floors were covered

with wood shavings, and paper for shredding and nest building was supplied once a week. The walls of the aquariums housing isolated animals were opaque.

Apparatus

The open-field apparatus measured 1 m², was painted gray, and was lined off into 16 squares of equal size. Pegs made of plastic dowels 2 cm. high were inserted into holes at the corners of each square except along the boundary of the field. The sides of the field were 48.0 cm. high and were hinged at the middle.

A sniff board, similar to the one described by Thiessen, Lindzey, and Nyby (1970), was used to test olfactory sensitivity. It consisted of a board 16 × 6 cm., into which 2 3-cm.-diam. circular holes were drilled. Into the holes were inserted 2 pieces of tissue paper, one of which had been rubbed against the ventral gland of a mature, intact male gerbil; the other was clean. A piece of copper screen wire was inserted over each piece of tissue. The board could be suspended from a wall of the aquarium.

Surgical Procedure

Surgery was done under either 50 mg/kg of Diabutal or ether anesthesia, the ether being preceded by an injection of 1 cc of Koagamin to reduce bleeding. Bilateral olfactory bulbectomy was accomplished by aspiration in the usual manner. Peripheral deafferentation was also accomplished by aspiration. A 2 × 4 mm. section of frontal bone immediately rostral to the cribriform plate was removed by dental drill, thus exposing the posterior portions of both nasal cavities. A blunt 20-ga. needle, connected by tubing to a suction pump, was used to aspirate out mucous tissue from the walls of the cavities and to remove, insofar as possible, all turbinates. The aspirating needle was also passed systematically across the anterior ventral surface of the cribriform plate to remove any remaining axons passing through the plate to the bulbs.

In all operations hemostasis was obtained by means of Gelfoam, and sulfathiazole was sprinkled sparingly over the Gelfoam to minimize infection. After scalp incisions had been sutured, blood was aspirated from the trachea and nose. Sham operations consisted of drilling holes through the skull over the frontal lobes with no damage to underlying tissue. Animals were allowed to recover for 5 days in individual cages before being placed into experimental conditions.

Experimental Procedure

After recovery the animals were earmarked for individual identification and placed into 10-gal. aquariums in groups of 1, 2, or 10, all having the same type of operation. There were 10 animals in each operation-density condition.

After 60 days of differential housing, behavioral

testing was begun. Each animal was first run individually in the open-field apparatus for 10 min. Number of lines crossed with all 4 feet and number of ventral rubs on either pegs or floor were tallied. Only those responses which included perceptible lowering of the back were counted as marks. After each trial the apparatus was cleaned with a solution of Alconox in water.

Next, each animal was allowed to interact successively with 2 other gerbils with the same type of operation but from the other 2 density levels. The social-interaction tests were run for 10 min. in the open-field apparatus. Each time an animal initiated chasing, fighting or biting, sniffing and body contact, or ventral rubbing, the appropriate score was recorded. Chasing was defined as pursuit of one animal by the other in which the animal being pursued actively ran away. Cases in which one animal walked and the other followed were not counted as chases. In order to be classified as fighting or biting, a response had to involve apparent harm to either or both animals. Most common was a ventral-ventral clasp, in which the animals rolled on the floor and scratched or bit each other. The other common response was simple biting of the other animal without the ventral-ventral clasp. Postures which appeared to be aggressive, such as standing on the hind legs and boxing or arching the back and moving sideways towards the opponent were counted as contacts rather than fights because no harm was inflicted. Sniffing and contact was the broadest category of responses, including any nose or body contact with the other animal which did not appear to result in harm to that animal.

Animals were marked on the back of the head with a felt-tipped pen for identification. Two experimenters watched the animals and quietly called scores to a third person, who recorded and kept time. In all cases agreement was reached between the 2 experimenters before a score was recorded. All but a few trials were run blind, with the scorer selecting and marking the animals without the judges' knowledge of subject conditions. In the few exceptions to the blind procedure, only 2 experimenters were available to test animals, and 1 of the 2 selected and marked the animals. Again, the apparatus was cleaned after each trial.

In addition to the above behavioral measures, a dominance score was computed for each animal in each social interaction. Whenever an animal's score on any 1 of the 4 measures exceeded his opponent's by 3 or more points, he received 1 dominance point. Thus, each animal could receive a maximum of 4 dominance points per encounter; no dominance points were awarded to either animal if all scores were evenly matched.

After the social-interaction tests, each animal was again tested individually in the open field. Since decrements in marking from the first to the second open-field trial were not statistically significant, data from both trials were combined. At least 24 hr. separated each test of each animal. All

testing was done during the light half of a 12-hr. light/dark cycle, with room lights on and air circulation system providing a constant background noise.

Olfactory sensitivity was tested by means of a cotton swab soaked with Hai Karate cologne placed in front of each animal's nose while he was alone in his home cage (Thiessen, Lindzey, & Nyby, 1970). A naive observer judged whether the animal could smell it on the basis of whether he avoided the swab, ignored it, or chewed on it. Each animal was also tested for olfactory sensitivity with the sniff board (Thiessen, Lindzey, & Nyby, 1970) while he was alone in his home cage. The board was left in the cage for 2 min., and time spent sniffing or chewing at the screen in front of each well was recorded.

At the termination of behavioral testing, animals were sacrificed by means of chloroform inhalation, and body weight was recorded to the nearest gram. Ventral glands, adrenal glands, testes, and seminal vesicles were removed and weighed to the nearest milligram. Left adrenal glands from 4 animals from each of the 9 operation-density conditions were fixed in Formalin, embedded in paraffin, sectioned, and stained with Oil Red O. Right adrenals from the same animals were fixed in Formalin, frozen, sectioned, and stained with H and E. Brain and olfactory passages of bulbectomized and peripherally deafferented animals were exposed for verification of the operation.

RESULTS

The most striking result was the extreme aggressiveness in the home cage of crowded bulbectomized animals. Fighting broke out as soon as these animals were grouped following their postsurgery recovery period. Within the first 2 wk. of the experiment, these animals reduced their numbers from 10 to 4 as a result of frequent, bloody fighting. Although no wound by itself seemed to be serious enough to have caused death, the combination of bleeding from many superficial wounds and the stress of the almost constant fighting may well have led to stress-induced death (Christian, 1963; Selye, 1956). Two of the paired bulbectomized animals also died within the first 14 days of pairing, one of which had several visible wounds. Throughout the duration of the experiment, all 4 remaining bulbectomized crowded animals exhibited some open wounds and considerable scar tissue, especially on their tails. One animal was judged to have relatively few wounds, another to be severely wounded and usually alone; the

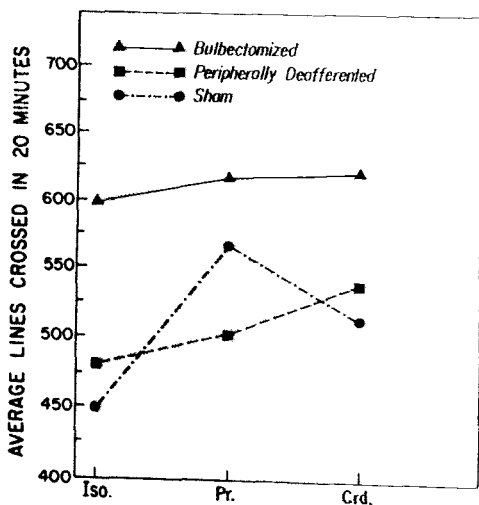


FIGURE 1. Open-field activity scores.

other 2 showed intermediate degrees of wounding.

As seen in Figure 1, bulbectomized animals were also more active in the open-field apparatus than were the other 2 groups ($F = 5.79$, $df = 2/61$, $p < .01$), crossing an average of 612 lines in 2 10-min. trials, compared with 506 and 508 lines crossed by peripherally deafferented and sham animals, respectively. In the social-interaction tests, bulbectomized animals were only slightly more aggressive than the other 2 groups, the differences in fighting scores being nonsignificant. Sham animals engaged in far more sniffing and body contact than did either group of anosmic animals ($F = 22.99$, $df = 2/61$, $p < .0001$). Bulbectomized and peripherally deafferented animals had average scores of 23 and 25, respectively, while sham animals had an average of 62 such encounters (see Figure 2).

Both open-field and social-interaction marking decreased with increasing density ($F = 5.47$, $df = 2/61$, $p < .01$; $F = 7.44$, $df = 2/61$, $p < .01$) (see Figures 3 and 4). Declines in fighting and sniff and contact scores with increasing density approached significance ($F = 2.80$ and 2.83 , $p < .07$ for each), again with sham animals showing greatest declines. In order to test density effects on sham animals separately from the other 2 groups, one-way analyses of vari-

ance were performed for each of the 3 groups. There were no density-related differences on any measure for the bulbectomized animals; peripherally deafferented animals showed a slight increase in body weight and a slight decrease in relative testis weight with increasing density ($F = 4.72$, $df = 2/22$, $p < .05$; $F = 4.99$, $df = 2/22$, $p < .05$). However, sham animals showed density-related differences at the .01 level on 5 measures (open-field activity: $F = 6.41$; open-field marking: $F = 7.02$; social-interaction marking: $F = 6.47$; body weight: $F = 11.44$; relative ventral gland weight: $F = 7.22$). Three one-way analyses of variance were computed to determine the effects of density on the dominance measure for each type of surgical operation. There was no significant difference in dominance scores for peripherally deafferented animals ($M = 1.1$, .6, and .9 for isolated, paired, and crowded animals, respectively). Isolated bulbectomized animals produced somewhat higher dominance scores than paired or crowded bulbectomized animals ($M = 1.3$, .7, and .7, respectively; $F = 4.52$, $df = 2/31$, $p < .05$); while sham ani-

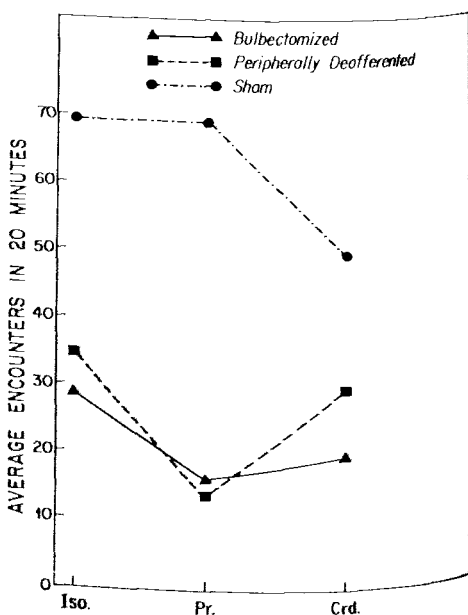


FIGURE 2. Sniff and contact scores for social-interaction tests.

mals showed a highly significant decrease in dominance scores with increasing density ($M = 2.0, 1.2, \text{ and } .2$; $F = 14.85, df = 2/57, p < .001$).

There was a significant increase in body weight as density increased ($F = 10.07, df = 2/61, p < .001$) (see Table 1 for all physiological measures). Relative ventral gland weights decreased with increasing density ($F = 5.79, df = 2/61, p < .01$). Type of operation significantly affected both relative and absolute adrenal gland weights ($F = 6.16, df = 2/61, p < .01$; $F = 8.41, df = 2/61, p < .001$, respectively), with bulbectomized animals having the heaviest adrenals and peripherally deafferented animals the lightest. There were no significant differences for testis or seminal vesicle weights.

Pearson correlation coefficients computed among all behavioral and physiological measures indicated relatively consistent relationships among behavioral variables. Open-field marking correlated with social-interaction marking ($r = .31, df = 68, p < .01$), sniff and contact scores correlated with chasing ($r = .35, p < .01$), and sniff and contact scores correlated with social-interaction marking ($r = .31, p < .01$). Fighting was significantly correlated with relative seminal vesicle weight ($r = .50, p$

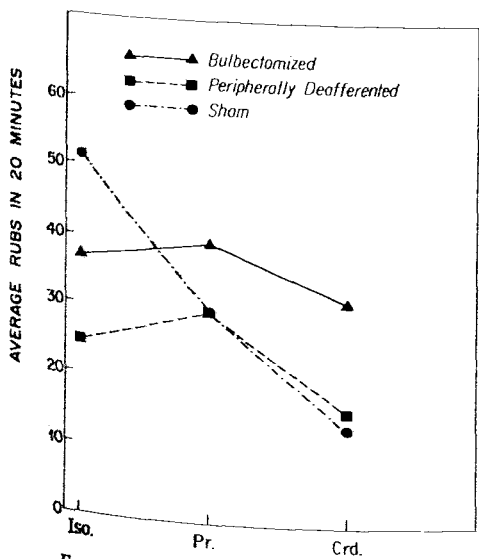


FIGURE 3. Open-field marking scores.

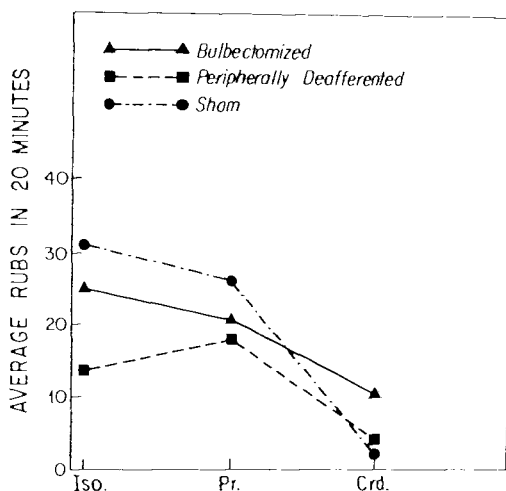


FIGURE 4. Social-interaction marking scores.

$< .01$). As expected, body weights correlated with most organ weights at the .01 level (body weight and absolute testis weight: $r = .69$; body weight and absolute ventral gland weight: $r = .65$; body weight and absolute seminal vesicle weight: $r = .50$; body weight and relative testis weight: $r = -.56$; body weight and relative adrenal weight: $r = -.46$). Testis weight correlated both with ventral gland weight ($r = .52, p < .01$) and seminal vesicle weight ($r = .59, p < .01$), and seminal vesicle weight correlated with ventral gland weight ($r = .44, p < .01$).

Histological examination of adrenal glands showed little consistency among measures for all anosmic animals. Sham-crowded animals had fewer lipid droplets and larger cells in zona fasciculata compared to sham paired and isolated animals. Both of these measures have been regarded as indicators of stress reaction of adrenal glands (Barnett, 1958). However, it should be noted that adrenal glands of sham crowded animals did not significantly increase in weight.

There was a clear difference between sham and anosmic animals on the Hai Karate smell test. Twenty-five of the 30 sham animals clearly avoided the swab, 1 chewed it, and 4 ignored it. Only one bulbectomized and one peripherally deafferented animal avoided the swab; the others either chewed

However, bulbectomy does more than simply render the gerbil anosmic. Bulbectomized animals in the present experiment became much more aggressive in their home cage, though only slightly more aggressive in the social-interaction tests. The relative lack of aggression in the social-interaction tests may have been related to the bulbectomized animals' inability to locate or interact with the other animal in the large, strange enclosure. Alternatively, since the field was large and the animals were not forced into close proximity, conditions may not have fostered irritability. Previous experiments utilizing bulbectomized rats (Bernstein & Moyer, 1970; Douglas et al., 1969; Spector & Hull, 1972) have suggested that bulbectomy leads to greater irritability in rats, though not necessarily to greater intermale aggressiveness. We suggest that irritability or hyperreactivity was a factor in producing the rampant home-cage fighting in our crowded bulbectomized gerbils. Experiments are presently underway to determine whether similar aggression is displayed among bulbectomized female gerbils and among castrated males. If so, such aggression may with some confidence be classified as irritable rather than territorial or intermale.

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(Received January 11, 1973)