HYPEREMOTIONALITY INDUCED BY LESIONS IN THE OLFACTORY SYSTEM OF THE RAT

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Abstract—In order to clarify the neural mechanisms underlying the hyperemotionality induced by bilateral olfactory bulb ablations in rats, changes in emotional behavior were measured after various parts of the secondary olfactory structures had been lesioned and olfactory bulb ablations had been carried out. When the anterior olfactory nuclei, lateral olfactory tracts and prepiriform cortices were bilaterally lesioned simultaneously with olfactory bulb ablations, many rats died from lack of food intake, while on the contrary, the rats with bilateral lesions in the olfactory tubercle, anterior olfactory nuclei and olfactory bulb developed marked hyperemotionality immediately after the lesioning. Hyperemotionality of the latter rats included not only hyperreactivity similar to that observed in the septal rat but also a muricide of 90% in incidence, which is similar to rats with olfactory bulb ablations. From these results, it can be concluded that changes in the activity of the olfactory tubercle were the most important for the development of hyperemotionality following bilateral ablations of the olfactory bulb in the rat.

Since Watson (1) reported that bilateral olfactory bulb ablations caused hyperirritability in the rat, a number of investigators (2–6) have studied this hyperemotionality, including mouse-killing behavior (muricide) and a comparison has been made with that induced by lesioning in the other brain structures.

The present authors (7) have also compared the emotional behavior of the rat with bilateral olfactory bulb ablations (O.B. rat) with that of the rat with septal lesions (septal rat), and clarified that hyperemotionality of the O.B. rat was characterized by gradual development after olfactory bulb removal and offensive aggression in nature, whereas that of the septal rat developed immediately after septal lesioning and was defensive in nature. However, the neural mechanisms underlying such aggressiveness of the O.B. rat are still unknown.

The neuroanatomical studies on the neuronal connection in the olfactory system of the rat have revealed that the afferent fibers originating from the olfactory bulbs connected with the secondary olfactory structures such as the anterior olfactory nuclei, olfactory tubercles, amygdaloid complex and prepiriform cortices (8–11).

The authors (7) speculated that hyperemotionality of the O.B. rat could be related to changes in activities of these secondary olfactory structures following removal of the olfactory bulbs, especially due to degeneration of the primary olfactory afferent fibers. The present investigation was therefore undertaken to clarify changes in emotional be-

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behavior of the rat following lesioning in the secondary olfactory structures, and an attempt was made to elucidate the neural mechanisms of hyperemotionality in the O.B. rat.

MATERIALS AND METHODS

Subject: One hundred and three male Wistar-King A rats, weighing 200-250 g at the beginning of the experiments, were used. The animals were housed individually in wire-mesh cages of $18 \times 17 \times 17$ cm, and were given food and water ad libitum.

Surgery: The subjects were divided into 6 groups as shown in Table 1. The animals in all groups, anesthetized with sodium pentobarbital 40 mg/kg i.p., were placed

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Extent of brain lesions</th>
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<tbody>
<tr>
<td>I</td>
<td>17</td>
<td>OB</td>
</tr>
<tr>
<td>II</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>14</td>
<td>+</td>
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<tr>
<td>IV</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>13</td>
<td>+</td>
</tr>
<tr>
<td>VI</td>
<td>28</td>
<td>-</td>
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- : Non-lesioned.
+ : Part of the region was bilaterally lesioned.
++ : Approximately half of the region was bilaterally lesioned.
+++ : Major parts of the region were bilaterally lesioned.

OB: olfactory bulb
AON: anterior olfactory nuclei
OT: olfactory tubercle
PC: prepiriform cortex
LOT: lateral olfactory tract
N: number of rats used.

Fig. 1. Ventral view of the extent of lesions in the olfactory system.
Black area in the lower diagram was ablated in each group.
on a stereotaxic instrument. After trepanation was performed in the skull and the olfactory bulbs were bilaterally exposed, various parts of the olfactory system were lesioned. In rats of group I, the olfactory bulbs were bilaterally removed by suctioning, and a part of the anterior olfactory nuclei was also ablated (Fig. 1 and 2).

In group II and III, the ablations were further enlarged to the extent of 4 and 6 mm posterior respectively to the rostrum of the frontal cortex, of approximately 3 mm lateral to the midline and 1 mm deep from the ventral surface of the brain, as illustrated in Figs. 1, 2 and 3. Thus, the lesions were extended to the anterior olfactory nuclei, olfactory tubercles, lateral olfactory tracts and prepiriform cortices in groups II and III, as shown

![Fig. 2. Lateral view of the extent of lesions in the olfactory system. Black area in the right diagram was ablated in each group.](image)

![Fig. 3. Coronal sections showing the site and extent of lesions in the olfactory system. Black area in the lower diagram was ablated in each group. LOT: lateral olfactory tract, OT: olfactory tubercle, PC: prepiriform cortex, SE: septal area.](image)
in Table 1, though the extent of lesioning in the latter 3 areas was larger in group III than in group II.

In group IV, lesioning was restricted to the medial parts within 2 mm lateral to the midline, as shown in Fig. 1 and 2; thus the olfactory bulbs, anterior olfactory nuclei and olfactory tubercles were bilaterally ablated (Table 1).

In group V, lesioning was extended to the lateral parts (Fig. 1, 2 and 3); i.e. the olfactory bulbs, anterior olfactory nuclei, lateral olfactory tracts and prepiriform cortices were lesioned (Table 1).

Group VI consisted of the sham operated rats which were subjected to the same surgical procedure as done in the other groups except for the brain lesioning.

All animals were given penicillin 150,000 units s.c. after surgery.

Extent of the brain lesions was verified histologically after termination of the experiments, by means of the frozen-tissue and hematoxine-eosine staining technique (Fig. 3). It was confirmed that lesions did not invade the septum and preoptic area in any animal.

Experimental procedures: Changes in emotional behavior of the rats following lesioning in various parts of the olfactory system were determined by measuring the hyperemotionality and the general activity, as described in a previous report (7).

Hyperemotionality was measured by scoring the following 5 responses to given stimuli, i.e. 1) attacking and/or killing response to a mouse, 2) attacking and/or biting response to a rod presented, 3) jumping and/or startle response to tapping on the back, 4) flight or escape response to pinching the tail with forceps and 5) squeak (vocalization) during handling or capture. These responses in each item were graded as follows: 0: no reaction; 1: slight, 2: moderate, 3: marked and 4: extreme response.

The general activity was measured during a 3 min period in a novel situation by using the Hall's open-field apparatus (7, 12). As parameters of the general activity, ambulation (the number of blocks traversed), rearing (upstanding position) and preening in an open-field apparatus were employed.

The tests were performed every day during the initial 5 day period after the brain lesioning, and were thereafter repeated every other day.

RESULTS

Changes in emotionality of the rats in groups I, II and III after brain lesioning are illustrated in Fig. 4, in terms of scores of the emotional responses to various stimuli.

Rats of the sham operated group VI, showed no changes in the emotional responses to various stimuli after the surgery, and none of them killed a mouse.

In group I (O.B. rat), the total emotionality score increased gradually during approximately 10 days after lesioning, thereafter the increased score was maintained at least for a month as far as observed. Hyperemotionality of group I was demonstrated predominantly in attack responses showing attack on a mouse or a rod presented in their cages, and 30% of them displayed mouse-killing behavior (muricide), while on the contrary, the jump, flight and squeak responses to respective stimuli were not significantly increased.
The rats of group III, however, developed aggressive behavior soon after the surgery, and 70% of them exhibited muricide. They attacked any body part of a mouse and killed the mouse differently from group I which invariably attacked the nape of the mouse. In addition to these attack responses, the jump and startle responses were also increased in these rats as has already been observed in the septal rat. Thus the total emotionality score of group III was higher than that of group I, and the high score was maintained at least for a month after the surgery. However, food taking was so impaired in the rats of group III that their body weight gradually decreased and 4 out of 14 rats died during 5 to 7 days after the surgery as indicated in Fig. 4.

The development of hyperemotionality in the rats of group II was essentially similar

**Fig. 4.** Changes in emotional responses to various stimuli following lesions in the olfactory bulbs and the other olfactory system of the rat.

- △ : Group I (O.B. rat)
- ● : Group II
- ○ : Group III
- × : Group VI (Sham rat)

†: Animals died of emaciation.

Ordinate shows scores of emotional responses and abscissa shows the day after brain lesioning.

Animals used were 9, 11, 14 and 10 rats for group I group II, group III and group VI, respectively.

**Fig. 5.** Changes in emotional responses to various stimuli following lesions in the olfactory system of the rat.

- ○ : Group IV
- ● : Group V
- × : Group VI (Sham rat)

†: Animals died of emaciation.

Ordinate and abscissa are the same as in Fig. 4.

Animals used were 11, 13 and 10 rats for group IV, group V and group VI, respectively.
to that in group III, however the emotionality score was lower and the incidence of muri-
cide was approximately 40% (Fig. 4). It was also observed that 2 out of 11 rats of group
II died of emaciation.

The rats of group IV exhibited marked hyperemotionality soon after lesioning (Fig.
5). This high emotionality score was maintained unchanged as far as observed for a month
after the surgery, although Fig. 5 illustrates only the results obtained during 15 days. The
incidence of muricide was approximately 90% in group IV. The rats attacked any
body part of a mouse, and crushed a rod violently, like those of group III. Half of the
muricidal rats in group IV stopped killing mice within 5 to 6 days after the surgery, while
the other half continued the muricide as well as the other hyperemotional responses
at least for a month as far as observed. Food taking was not impaired in any of the
rats of group IV, differently from those of group III. The hyperemotionality of group
IV was generally similar to that of group III except in food taking.

In the rats of group V, food taking was severely impaired and 7 out of 13 rats died
during 5 to 10 days after the surgery, therefore it was difficult to observe completely the
changes in their emotionality throughout the experiments. However, 4 out of 13 rats
gradually developed slight hyperemotionality, such as attack, jump and startle responses
to given stimuli. The incidence of muricide was 23% in group V.

Qualitative differences in hyperemotionality among the rats of group I, group IV and
the septal rats, at the peak development, are illustrated in Fig. 6. The results of the
septal rats were obtained in a previous experiment (7). It is obvious from Fig. 6 that
the rats of group IV exhibited not only attack responses to a mouse or a rod (offensive
aggression), as seen in group I, but also hyperreactivity, such as jump and flight responses,
similar to that seen in septal rats. Group I was different from group IV in that there was less hyperreactivity, and the septal rat was different from group IV in the absence of muricide.

In parallel with the observation of hyperemotionality, general activity was determined in an open-field situation after lesionings of the olfactory system. The changes in general activity of the O.B. rat and group IV are shown in Fig. 7. Both ambulation and rearing of the O.B. rat increased gradually and reached a maximum within about 10 days. On the other hand, ambulation of group IV was increased immediately after the surgery and was maintained at least for a month. An increase in rearing in group IV was relatively slow, but it was higher than that of the O.B. rat. Preening increased slightly after olfactory bulb ablations but it was not significantly different from that of the sham operated group (group VI).

**DISCUSSION**

It is well known from the studies of a number of investigators that the rhinencephalic or limbic structures are important as the neural substrate of emotional behavior. The neuronal connection between olfactory bulbs and other limbic structures is
schematically illustrated in Fig. 8. It has been neuroanatomically confirmed that the afferent fibers from the olfactory bulbs of rats are projected to the anterior olfactory nuclei, olfactory tubercles, prepiriform cortices and amygdaloid complex (8–11). Concerning the neural mechanisms underlying the aggressive behavior induced by bilateral ablations of the olfactory bulb in the rat, Douglas et al. (4) hypothesized that the changes in amygdaloid activity resulted from either disruption of the fiber connection between the olfactory bulbs and the amygdaloid complex or irritation due to degeneration of the olfactory afferents, would be important for the development of aggressiveness. In addition, they speculated that hyperemotionality induced by septal lesions might also result from either damages or inactivation of some parts of the olfactory structures. Douglas’s hypothesis would be acceptable as well from the fact that the period of 7–10 days needed for the development of aggressiveness after olfactory bulb ablations coincides well with the time required for degeneration of the nerve fibers.

The present authors have also speculated that degeneration of the olfactory afferents following olfactory bulb ablations may cause changes in activity of the secondary olfactory structures, which in turn results in the occurrence of aggressive behavior. The present investigation was done to determine what areas of the secondary olfactory system were the most important to induce aggressiveness in the O.B. rat.

At first, a larger extent of the olfactory structures was lesioned, like the rats of group II and group III. As shown in the experimental results, the rats of group III developed marked aggressive behavior soon after brain lesioning and 70% exhibited muricide as well, although food taking was impaired. In group III, the olfactory bulbs and anterior olfactory nuclei were totally ablated and the lateral olfactory tracts, olfactory tubercles and prepiriform cortices were half ablated. It is therefore suggested that activity changes in some of these secondary olfactory structures are directly concerned in the development of aggressiveness of the O.B. rat.

Next, the lesions of group III were divided into the medial (group IV) and lateral (group V) parts of the olfactory system. Then the symptoms exhibited by group III were also separated; i.e. group IV developed marked aggressiveness including muricide in a higher percentage immediately after the lesioning, while group V showed marked impairment of food taking and gradual development of aggressiveness in a lower percentage. The lesions in group IV included the olfactory tubercles in addition to the anterior olfactory nuclei and olfactory bulbs, while in group V the olfactory tubercles were not lesioned. These results strongly suggest that the olfactory tubercles play an important role in the development of aggressiveness of the O.B. rat.

Zyo (11) and White (8) reported that degeneration of the afferent fibers originating from the olfactory bulbs, was found in the olfactory tubercles after olfactory ablations. Spiegel (13) reported that lesioning of the olfactory tubercles induced rage reaction in the cat. It is therefore postulated that the afferent fibers in the olfactory tubercles originating from the olfactory bulbs and anterior olfactory nuclei degenerate after ablation of these structures, which in turn alters the activity of the olfactory tubercles connected
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with the septum and hypothalamus, and results in the development of aggressiveness. The period of 7 to 10 days prior to the development of aggressiveness in the O.B. rat may be enough for complete degeneration of these fibers. The findings in the present experiments suggest that the olfactory tubercles are the most important in occurrence of aggressiveness induced by olfactory bulb ablations.

On the other hand, Karli & Vergnes (14) reported that none of the rats with lesions in the olfactory tubercles killed a mouse, but lesions of either the olfactory bundles or the prepiriform cortices were able to induce muricide. King & Meyer (15) emphasized the importance of the amygdaloid complex in emotional alteration, on the basis of the fact that hyperemotionality induced by septal lesions was markedly reduced by amygdaloid lesions. Karli & Vergnes (16) and Horovitz et al. (17) also observed that muricide of long-term isolated rats was inhibited by small centromedial amygdaloid lesions. Ueki et al. (7) observed that hyperemotionality of the O.B. rat was also inhibited by amygdaloid lesions. Recently, it was found in our laboratory that the EEG activity of the amygdala was changed in voltage after the olfactory bulb ablations in rats with chronically implanted electrodes (18). Moreover, Boisacq & Callens (19) observed in their electrophysiological experiments that excitability of the prepiriform cortex increased after removal of the olfactory bulb, and Callen et al. (20) suggested that releasing from the inhibitory influences of the olfactory neurons accounted for this phenomenon. Therefore, it cannot be excluded that activity changes in the amygdaloid complex and prepiriform cortex are possibly related to the occurrence of aggressiveness in the O.B. rat, and the present investigation indicates the great importance of the olfactory tubercle.

In the observation of general activity, ambulation of group IV increased immediately after the surgery, differently from that of the O.B. rat. Ueki et al. (7) have reported that exploratory activity of the septal rat was lower than that of the sham operated rat. Thus, the rats of group IV were more similar to the O.B. rat than to the septal rat in the changes in general behavior.

As shown in Fig. 6, the rats of group IV with lesions of the olfactory bulbs and olfactory tubercles shared with the O.B. rat not only offensive aggression such as muricide and attacking a rod but also hyperreactivity such as jump, flight and squeak responses with the septal rat. This may be due to the fiber connections between the olfactory tubercles and the septal nuclei. These neural mechanisms, however, should be clarified in further investigations.

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