Changes in Amygdaloid Afterdischarges and Kindling Effect Following Olfactory Bulbectomy in the Rat

SHIGENORI WATANABE, HIROSHI NAKANISHI, SHIGENOBU SHIBATA AND SHOWA UEKI

Department of Pharmacology, Faculty of Pharmaceutical Sciences
Kyushu University, Fukuoka 812, Japan

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WATANABE, S., H. NAKANISHI, S. SHIBATA AND S. UEKI. Changes in amygdaloid afterdischarges and kindling effect following olfactory bulbectomy in the rat. PHYSIOL. BEHAV. 28(4) 687-692, 1982.—Changes in the thresholds for afterdischarges and the formation of kindling effect in the medial amygdala following olfactory bulbectomy were investigated in the rat with chronic electrode implants. The threshold for afterdischarges in the amygdala of the olfactory bulbectomized rat (OB rat) was significantly decreased on day 4 after olfactory bulbectomy, however, no significant difference was found between OB and sham operated rats on days 7, 14 and 21 since the threshold in the sham group was also decreased at these periods after the surgery. The formation of kindling effect was remarkably accelerated in the OB rats. In this case, the number of days required to reach the stage 1 (Racine’s classification) was significantly shortened. These results suggest that the activity of the medial amygdaloid nucleus is increased following olfactory bulbectomy.

Afterdischarges Kindling effect Amygdala Olfactory bulbectomy Muricide Rat

IT IS well known that afterdischarges are easily induced by electrical stimulation in the limbic system such as the amygdala [10]. Kindling effect was also found at this brain region: i.e. repeated electrical stimulation of constant strength at regular intervals causes a slight response at first which develops gradually to final generalized convulsions [4].

Several studies have been reported which examine the relationship between amygdaloid activity and aggression by measuring the threshold for afterdischarges and/or the kindling effect in the amygdala. Adamec [1] observed that the amygdaloid activity in the rat-killing cat was lower than that in the non-killer, while McIntyre [7] and Harry and Racine [5] suggested that the amygdaloid nucleus did not play a significant role in the manifestation of mouse-killing behavior (muricide) in the rat since the threshold for afterdischarges in the amygdala of spontaneous mouse-killers showed no significant difference from those of non-killer rats.

On the other hand, bilateral olfactory bulbectomy in rats has been shown to cause muricide [17]. The amygdaloid nucleus, particularly the medial nucleus, seems to have a facilitatory effect in the induction of muricide by olfactory bulbectomy because muricide in the olfactory bulbectomized rat (OB rat) is suppressed by destruction of the medial nucleus of the amygdala [14]. Further, a direct microinjection of noradrenaline (NA) or tricyclic antidepressants into the medial amygdala suppressed the muricide [18]. The mechanisms involved in functional changes in the amygdaloid nucleus induced by olfactory bulbectomy remain to be elucidated.

The present experiment was undertaken to investigate the changes in the activity of the amygdala following olfactory bulbectomy by examining the effect of bilateral olfactory bulbectomy on the threshold for afterdischarges in the medial amygdaloid nucleus and on the development of kindling effect.

METHOD

Animals

Male Wistar King A strain rats weighing 200–250 g at the beginning of the experiment, supplied by Kyushu University Institute of Laboratory Animals, were used. Animals were housed in groups in an air-conditioned room at 22±1°C with a 12 hr light-dark schedule (lights on at 7:00). Food and water were given ad lib during the experimental period. Rats were transferred to individual cages after olfactory bulbectomy or sham operation.

Surgery and Experimental Procedure

The animal's head was fixed in a stereotaxic instrument under pentobarbital-Na (40 mg/kg IP) anesthesia, and a bipolar stainless steel electrode (tip diameter 0.2 mm; uninsulated length 0.2 mm; polar distance 0.7 mm) was chronically implanted in the medial nucleus of the amygdala (A: 6.0, L: 3.5, H: −3.5) and the frontal cortex (A: 8.5, L: 1.0) on the same side according to König and Klippel’s brain atlas [6]. The experiment was commenced after a 10-day recovery period. The medial amygdala was stimulated several min after the
animals were transferred to an open-topped Plexiglas cage placed in a sound-proof shielded cage. The animals were allowed to adapt themselves to the new environment and became calm, prior to stimulation. Behavior of the animals was observed through a transparent plastic window on the sound-proof shielded cage. The electroencephalogram (EEG) and afterdischarges were biologically recorded on a polygraph. The threshold for afterdischarges in the amygdala was measured twice before olfactory bulbectomy or sham operation, and once on days 4, 7, 14 and 21 after surgery. Square pulses (0.5 msec, 50 Hz) were given for 5 sec at an interval of 30 min. For threshold measurement before the operation, a stimulus of 80 μA was given at first, and once afterdischarge was induced, a 60 μA stimulus was given. If afterdischarge was not induced by 60 μA stimulus, a medium strength, 70 μA stimulus was applied. A 100 μA stimulus was given if the first 80 μA stimuli failed to cause afterdischarge. When an afterdischarge was induced by stimulus, the third trial was made with a 90 μA stimulus. On the forth trial and thereafter, the stimulating current was increased or decreased by 3 μA until the minimum current eliciting amygdaloid afterdischarge of constant duration (about 70 sec) was obtained. For observation after surgery, a stimulus with a strength 3 μA lower than control threshold was given first. Thresholds were obtained in a similar way with preoperative measurement by increasing the stimulus by 30 μA if no afterdischarge appeared and by decreasing the stimulus by 20 μA if afterdischarge appeared. EEG recordings and muricide tests were carried out prior to threshold measurement at each time. Muricide was regarded as positive if the rat bit a mouse to death within 3 min after a mouse was introduced into the rat's home cage.

Electric stimulation with a constant strength (50 Hz, 0.5 msec, 60 μA) was applied to the medial amygdala for 5 sec once a day from day 4 after the olfactory bulbectomy or sham operation until a kindling effect was established. EEG recordings and muricide tests were carried out prior to amygdaloid stimulation. Rats in both OB and sham groups were housed individually after surgery.

Development of the kindling effect was measured and recorded under the following five stages according to Racine's method [12].

Stage 1: Mouth and facial movement, Stage 2: rhythmic head nodding; Stage 3: forelimb clonus; Stage 4: Rearing; Stage 5: Rearing and falling.

Data Analysis

Results of threshold experiment and kindling effect experiments were analyzed by Student's t-tests and Mann-Whitney U-tests, respectively. One-tailed Fisher's exact probability test was used for the analysis of the incidence of muricide.

Histology

After completion of the experiment, the animals were anesthetized with ether, and their brains were perfused with a 10% formalin solution through the carotid arteries. The brain was removed, fixed, and 50-60 μ frozen sections were prepared and stained with cresyl violet. The extent of olfactory bulbectomy and the site of the electrodes were verified histologically. If the electrodes were not located in the proper position or the extent of olfactory bulbectomy was not appropriate, the results from these rats were discarded from the data.

RESULTS

Changes in Threshold

The rats moved around the cage showing exploratory behavior for a while after they were transferred to the shielded cage from the home cage. After 5–10 min the animals adapted to the new environment and continued to sit in one corner of the cage. During this period, the amygdala was stimulated electrically. The threshold for amygdaloid afterdischarges before surgery was about 70 μA. Amygdaloid afterdischarges were consistently obtained by electrical stimulation with this current. The duration of afterdischarges was approximately 70 sec.

The amplitude of the EEG of the amygdala was decreased by olfactory bulbectomy, no remarkable change was found in the EEG of the cortex. Threshold for afterdischarges was significantly lowered to about 50 μA 4 days after olfactory bulbectomy relative to the sham group (p<0.05) (Fig. 1-a) (Table 1). Thresholds in the sham group, however, gradually decreased with repetition of stimulation and only slightly lower values in comparison with those of sham group were observed in the OB rats on day 7 (p<0.20). No significant difference was found between the OB and sham groups on days 14 and 21 (Fig. 1-a, Table 1).
### TABLE 1

**CHANGES IN THRESHOLD OF AMYGDALOID AFTERDISCHARGES FOLLOWING OLFACTORY BULBECTOMY IN THE RAT**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>4d</th>
<th>7d</th>
<th>14d</th>
<th>21d</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>7</td>
<td>69.9 ± 9.3</td>
<td>52.7 ± 9.2*</td>
<td>52.3 ± 8.9</td>
<td>46.2 ± 5.9</td>
</tr>
<tr>
<td>Sham</td>
<td>7</td>
<td>68.6 ± 8.8</td>
<td>65.9 ± 9.7</td>
<td>61.1 ± 9.8</td>
<td>50.5 ± 9.9</td>
</tr>
</tbody>
</table>

*p < 0.05 (Student’s t-test).

### Duration of the afterdischarge

Duration of the afterdischarge became longer when more measurements were repeated for both groups, together with increased amplitude of the discharges. Generalized convulsions were observed in both groups on days 14 and 21 after surgery. Spontaneous spike waves were also observed in the EEG of the amygdala and cortex during this period (Fig. 2-b, F, G). The incidence of muricide was 83% on the day 4 and reached 100% on day 21 after surgery (Fig. 1-b). No muricide was observed in the sham group throughout the experiment.

### Kindling Effect

A 60 μA stimulus was applied to the medial nucleus of the amygdala once a day from day 4 after the surgery to form a kindling effect. Slight afterdischarges in the amygdala were observed (duration approximately 10 sec) from the first stimulation with this strength in both the OB and sham groups. Seizure discharges were also observed in the frontal cortex (Fig. 2-a). While the afterdischarges were low in both

![FIG. 2](image-url)  
**FIG. 2.** Development of afterdischarges and changes in EEG on successive daily stimulation in the OB rat. Classification of each stage was made according to Racine’s method (see text for details). (a) Afterdischarges. A: stage 0 (the 1st stimulation); B: stage 1; C: stage 2; D: stage 3; E: stage 4; F: stage 5. AM: amygdala; FC: frontal cortex. Vertical bar 200 μV, horizontal bar, 10 sec. (b) EEG. A: intact; B: stage 0 (the 1st stimulation on the 4th day after olfactory bulbectomy); C: stage 1, D: stage 2, E: stage 3; F: stage 4; G: stage 5. AM: amygdala; FC: frontal cortex. Vertical bar, 50 μV, horizontal bar, 1 sec.
TABLE 2

CHANGES IN AMYGDALOID KINDLING EFFECT FOLLOWING OLFACTORY BULBECTOMY IN THE RAT

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>7</td>
<td>1.7 ± 0.5*</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.4</td>
<td>0.7 ± 0.7</td>
<td>2.1 ± 1.5</td>
<td>8.3 ± 1.3t</td>
</tr>
<tr>
<td>Sham</td>
<td>6</td>
<td>4.7 ± 1.4</td>
<td>2.8 ± 0.9</td>
<td>1.8 ± 1.1</td>
<td>0.7 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>11.3 ± 1.5</td>
</tr>
</tbody>
</table>

*p < 0.001.
†p < 0.005.
‡p < 0.05 (Mann-Whitney U test).

Development of the kindling effect, measured by Racine's method, is shown in Table 2. The number of days required for the establishment of kindling was significantly shortened by olfactory bulbectomy to 8.3 ± 1.3 days for the OB group and 11.3 ± 1.5 days for the sham groups (p < 0.005). More specifically, the number of days required to reach stage 1 was most remarkably reduced by olfactory bulbectomy; 1.7 ± 0.5 days were required in the OB group compared with 4.7 ± 1.4 days in the sham group (p < 0.001). Some cases in the OB group reached stage 1 at the first stimulation. The second significant reduction was shown in the number of days from stage 1 to stage 2 (p < 0.05). No significant difference was shown in the number of days required to reach stage 3, 4 and 5 between the OB and sham groups (Table 2).

The duration of discharges in the OB group was longer than that in the sham group from the beginning of stimulation to day 7, particularly at the beginning (Fig. 3-a). However, no difference in the duration of discharges was observed between the two groups, as they were compared at each stage (Fig. 3-b).

The incidence of muricide was 71.4% on the day when stimulation was commenced (day 4 after olfactory bulbectomy), and 100% on the second day. Only one of seven rats in the sham group manifested muricide on day 4 after the initiation of stimulation.

DISCUSSION

The threshold for amygdaloid afterdischarges in rats was significantly decreased to 75% of control on day 4 after olfactory bulbectomy. While the stimulus threshold in OB rats on day 7 was about the same as that on day 4, differences between the OB and sham groups became insignificant as the threshold in the sham group decreased. No significant difference was observed between the two groups on days 14 and 21. The reduction in the threshold to stimuli in the sham group following repeated stimulation may be due to the kindling effect [11, 16]. The duration of afterdischarges became longer...
and behavioral convulsions also took place frequently along with the reduction of threshold. However, the marked reduction in the threshold at 4 days of the OB rat, is obviously caused by the olfactory bulbectomy itself.

It has been shown that the amygdaloid afterdischarges are suppressed by benzodiazepines and that the stimulus threshold is increased by these drugs [3]. On the other hand, pentetrazol, a central nervous stimulant, decreases the threshold for amygdaloid afterdischarges [3]. These facts seem to suggest that the rise and fall in the threshold for afterdischarge reflect the increase and decrease in the excitability of these sites. Therefore, the significant reduction at the initial phase in the threshold for afterdischarges of the amygdaloid nucleus following olfactory bulbectomy suggests that the excitability of the medial amygdaloid nucleus is increased by olfactory bulbectomy.

The kindling effect in the amygdaloid nucleus was remarkably accelerated by olfactory bulbectomy. Detailed examination of the results in each stage according to the classification by Racine revealed that the number of days (stimulation) required to reach stage 1 was most significantly reduced and the reduction in the number of days between stage 1 to 2 was also significant. No significant difference from the sham group was found in the days required to reach subsequent stages. The duration of amygdaloid afterdischarges was remarkably increased on day 2 after the commencement of stimulation in the OB group, and reached about 85 sec on day 4 without showing further prolongation thereafter. While in the sham group, the duration of afterdischarges gradually became longer reaching about 80 sec on day 8. These results showed that only the initial phase of kindling effect was significantly accelerated by olfactory bulbectomy. Signs of stage 1 are mouth and face movement. Particularly oral behavior is easily induced by electrical stimulation of the amygdaloid nucleus [2]. Therefore, it is conceivable that quick attainment of stage 1 by the OB group suggests that the medial amygdaloid nucleus became readily excited by electrical stimulation after olfactory bulbectomy. As previously stated, significant reduction in the stimulation threshold for the amygdaloid afterdischarges was also observed in OB rats. Therefore, the electrical stimulation of the same intensity can be relatively more effective in OB rats than in sham rats and this may account for the facilitation of kindling formation in OB rats.

A high incidence of muricide was observed in the OB group reaching its peak at 4–5 days after olfactory bulbectomy. It has been shown that the amygdaloid afterdischarges are suppressed by benzodiazepines and that the stimulus threshold is increased by these drugs [3]. On the other hand, pentetrazol, a central nervous stimulant, decreases the threshold for amygdaloid afterdischarges [3]. These facts seem to suggest that the rise and fall in the threshold for afterdischarge reflect the increase and decrease in the excitability of these sites. Therefore, the significant reduction at the initial phase in the threshold for afterdischarges of the amygdaloid nucleus following olfactory bulbectomy suggests that the excitability of the medial amygdaloid nucleus is increased by olfactory bulbectomy.

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