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SUPPRESSION AND DISINHIBITION OF INSTRUMENTAL ALIMENTARY REACTIONS AFTER SUCCESSIVE LESIONS OF THE DORSOMEDIAL AND LATERAL AMYGDALA IN DOGS

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Abstract. Damage of the dorsomedial amygdala produced impairment of instrumental performance to CS+ with no changes to CS−. Subsequent lesion of the lateral amygdala resulted in restoration of instrumental performance to CS+ and transient disinhibition to CS−. These results support the hypothesis that the dorsomedial amygdala is involved in facilitation, while the lateral amygdala in inhibition of alimentary reactions.

INTRODUCTION

In previous papers (2, 5, 7) it was shown that retention of positive instrumental reflexes established to an acoustic stimulus were severely impaired after dorsomedial amygdala lesions. Subsequent lesions of the lateral amygdala produced an improvement of the impaired instrumental performance (10). It was suggested that through lateral amygdaloid damage the source of excess inhibition are removed, and therefore the previously suppressed instrumental reactions reappear, liberated from this inhibition. In view of these results it was assumed that the impairment of inhibitory mechanisms produced by the lateral amygdala lesion will be revealed by the disinhibition of responses to inhibitory stimuli. The aim of the present paper was to test this assumption.
Methods

The experiment was performed on six naive, mongrel male dogs.

Training: The training of instrumental responses to a tone of 1,000 cycle/s (CS+) was accomplished by a method of passive movements. The response consisted of putting the right foreleg on the food tray, which was reinforced by small morsels of bread moistened by broth and meat (US). When the instrumental reaction was established to a criterion of at least 90% of correct performance in the last 10 experimental sessions, differentiation training started. A tone of 500 cycle/s was introduced as CS−, which was not reinforced by food. Experimental sessions consisted of 10 reinforced (CSI+) and 10 nonreinforced (CSI−) trials interspersed randomly. The experiments were performed 6 days a week. When the dogs reached the criterion of 90% of correct responding by inhibition of reacting to the CS−, they were subjected to the first operation. After the operation, from 10th postoperative day, the daily experimental sessions were resumed. Five experimental sessions, i.e., 50 positive and 50 non-reinforced trials, were performed as a retention test in each dog. Beginning from 6th session retraining began, and was conducted by the same method as initial training. In total, 10 experimental sessions were applied in all dogs during the period between the two operations, with the exception of one dog that had 20 experimental sessions. After the second operation, the same procedure of retention test and retraining was applied.

Measurements of food intake. Food intake was measured during the 10-day period before the first operation, the subsequent 10 days, 10 days before the second operation, 10 days afterwards, and once again in a later period about 4–8 wk postoperatively. During these periods the dogs were fed ad lib. twice each day. In between meals no food was available. The ad lib. feeding was not available during the periods of training and retention tests when the dogs were fed only once daily at 2 p.m. with standard portions of food, consisting of about 1.5 kg of cereal soup with meat.

Surgery. Under Nembutal anesthesia and aseptic standard conditions, the electrolytic lesions of the dorsomedial amygdala (DMA) were made by DC, anodal 2–3 mA through stereotaxically directed electrodes insulated by enamel except for 1 mm on the tip. After 3–6 wk, the second operation was performed in which the lateral part of the amygdala (LA) was damaged in a similar way. The behavioral and surgical procedures have been described in more detail previously (4, 6, 8).

Histology. All lesions were verified by subsequent histological examination. After the experiment was completed the anesthetized dogs were perfused with Ringer's solution, followed by 10% buffered formalin. Then
the brains were stored in formalin until embedded in paraffin later, and sectioned frontally at 40 µm. Every 10th section was stained by Klüver and Nissl method alternatively.

RESULTS

The effects of the first operation (DMA)

In three dogs complete aphagia and adipsia were observed during the 6–12 days postoperatively, which was followed by subsequent hypophagia. In three others, only transient hypophagia was observed after the operation (Fig. 1). In addition, all dogs were apathetic, indifferent and showed complete symptoms of the dorsomedial amygdala syndrome, described in previous papers (4, 5, 7–9). The instrumental reactions were severely impaired or even completely abolished (Fig. 2). This impairment was highly significant statistically for both the whole group and each individual dog.

Fig. 1. The effect of DMA and subsequent LA operation on food intake. Blocks represent mean from 10 days ad lib. feeding. Mean data from six dogs.

![Food intake graph](image)

Fig. 2. The effect of DMA and subsequent LA operation on the instrumental performance. Blocks represent mean of the number of instrumental responses from 10 experimental sessions for the whole group. White blocks, responses to CSi⁺; black blocks, responses to CSi⁻. Mean data from six dogs.

![Instrumental responses graph](image)
(\(P \leq 0.001\) Mann–Whitney U test). Retraining of the instrumental responses was unsuccessful in five dogs. Instrumental performance reappeared in one dog during the course of retraining, so that in order to observe the further progress, the experimental sessions were continued for a

Fig. 3. The illustration of the effect of DMA and LA damage on the course of performance in individual dogs. Note the poor performance after first (DMA) operation in all dogs, and the restoration after subsequent (LA) operation, with the disinhibition of the response to CS\(^-\), in particular in dog Miętus.
longer period than in the remaining dogs. However, the responses consistently fluctuated and showed a tendency to extinguish rather than to improve (Fig. 3 below, Dog Troć). In the remaining dogs the instrumental reactions were not reestablished until after the second operation. As a rule, the instrumental response to the CS− did not appear.

The effects of second operation (LA)

All the dogs showed an increase in food intake after the second operation (Fig. 1). They became lively again, friendly and interested in surroundings. This effect of the subsequent LA operation upon dorsomedial amygdala syndrome was reported in detail previously (8–10).

The instrumental reactions were restored in all dogs (Fig. 2), and this restoration in three dogs occurred before retraining started, while in two others the instrumental response was noted in the first retraining trials. In all but one of the dogs, the course of restoration was slower than initial training. The increase of performance after the LA operation, when compared with the performance before it, was highly significant for the whole group (P, < 0.001, Mann–Whitney) and significant for each individual dog except one. In all dogs a further progressive increase of performance was observed, and they all reached 100% performance.

In two dogs disinhibition of instrumental reactions to the CS− was obvious, while in two others disinhibition was slight. This disinhibition was found during the first postoperative period of 1–15 experimental sessions only (Fig. 3 above, dog Miętus). The intertrial movements were also disinhibited during the first postoperative period in four dogs

<table>
<thead>
<tr>
<th>Dogs</th>
<th>After DMA</th>
<th>After LA</th>
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<tr>
<td>Tuńczyk</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miętus</td>
<td>10</td>
<td>119</td>
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<tr>
<td>Halibut</td>
<td>15</td>
<td>39</td>
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<td>Sansonet</td>
<td>18</td>
<td>73</td>
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<tr>
<td>Kondor</td>
<td>2</td>
<td>68</td>
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<tr>
<td>Troć</td>
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(Table I). Later the differentiation was restored, i.e., the dogs did not react to CS− with the instrumental response (Fig. 2). The number of intertrial reactions also decreased in time.
Anatomical verification

The results of the verification of the localization of lesions showed that in most dogs the first operation (DMA) involved the central and medial nucleus invading adjacent parts of basal nucleus, entopeduncular nucleus and internal capsule. The tractus opticus was damaged in three dogs bilaterally and in three others unilaterally. Two dogs also had slight

Fig. 4. The reconstruction of lesions based on histological slides. Stippled circles, first (DMA) operation; crossed areas, second (LA) operation.
olfactory tract or nucleus damage. The second operation (LA) involved the nucleus lateralis and adjacent part of nucleus basalis in all dogs. The temporal lobe and piriform cortex as well as the ventral hippocampus were also slightly damaged in some dogs. Figure 4 shows the reconstructions of lesions based on frontal sections of histological slides.

DISCUSSION

The present results furnished further support for our hypothesis put forward in the previous papers (4, 5, 8, 10) that the dorsomedial amygdala does not consist in a locus of neurons patterning the alimentary instrumental reflexes or pathways for CS–R connections, but exerts an excitatory influence upon the other parts of alimentary system. Therefore, damage to the DMA produced dominance of inhibitory influences. This source of inhibition derives mostly from the lateral amygdalar nucleus, which was shown previously as having an inhibitory role (1, 3, 6, 11). Subsequent damage of this area, removing the excess of inhibitory influences produced improvement of previously depressed instrumental reactions due to release from inhibition as well as restoration of other aspects of alimentary behavior.

The criticism may be made that the restoration of instrumental reactions might occur independently of the LA damage by the mechanisms of compensation, therefore the effect of LA operation may be more apparent than real. In this experiment we purposely performed the second operation only 3–4 weeks after the first to examine the effect of LA lesions before spontaneous compensation might occur. From the previous work (10) we know that prolongation of retraining after the first DMA operation, although may result in temporal enhancement of performance, in most dogs it does not result in a stable effect. The prolongation of observations for 3–4 months, or even longer, as was previously (9, 10) attempted, did not produce the total recovery, i.e., fluctuations of instrumental performance were observed in most of the DMA dogs continuously until the end of observations. In the single dog that had extended retraining in the present experiment, the retrained reaction also had the tendency to decrease in time instead of improve (Fig. 3, Troč).

Another interesting aspect of the present data is the disinhibition of instrumental reactions to the inhibitory CS− in the first postoperative period after the LA operation. This disinhibition goes in parallel with the restoration of instrumental alimentary reaction to CS+, which support our previous suggestion about the inhibitory role of the lateral amygdala. After damage of the lateral amygdala the excess of inhibition was suddenly removed, which produced the “rebound” of excitation, reflected
in the disinhibition of instrumental reactions to the inhibitory stimuli (CS−) and an increase in number of intertrial movements. After a few days or weeks, the inhibitory–excitatory balance was spontaneously reestablished more or less on a normal level and the differentiation became perfect again.

Starting from Klüver and Bucy's classical work (12) some authors have suggested the role which the amygdala plays in discriminatory functions. The impairment in discriminatory tasks after amygdala lesions have been observed by various authors in monkeys and rats. However, in view of our recent results, such impairment may be based on other than purely sensory discriminatory mechanisms. The so-called discriminatory tasks may be impaired because of various reasons. One of these mechanisms might be the low level of either general arousal or alimentary motivation. The animal may not react to the proper stimuli by definite reactions either because of low arousal, or because it is not motivated to perform the reaction. The animal in a depressed state may be so indifferent and not pay attention to any stimuli so that it is not able to concentrate on complicated tasks. Moreover, the animals may be negativistic postoperatively, and actively or passively oppose, to perform the tasks. This has been found for dorsomedial amygdala and lateral hypothalamic dogs. All of these factors acting together in result of dorsomedial amygdala lesions, may produce various kind of errors in discriminatory tasks mostly positive ones.

On the other hand, damage to the lateral amygdala may produce disinhibition of reactions to negative stimuli and in consequence also produce errors in successive discriminatory tasks. After the lateral amygdala lesions, the errors to CS− in one of the most disinhibited dogs reached about 30% during the first 10 experimental sessions after the operation. In some sessions it even reached 80% of uncorrect responses. This may appear as an impairment of discrimination, but in fact it is due to the rebound of excitation caused by damage of inhibitory mechanisms. From behavioral observations we know that the dogs are at the same time generally aroused, lively and hyperphagic.

Our two step lesion experiment shows that the solution of “discriminatory” tasks is changed in parallel with the general arousal and alimentary motivation. First, the DMA operation produced “errors” to positive stimuli (CS+), subsequent LA surgery produced transient “errors” to negative stimuli (CS−). These changes in performance cannot be attributed to a lack in discrimination of tones as the dogs are able to discriminate the stimuli, judged by their behavior; but rather they are either not motivated to do it (DMA damage) or too lively and motivated to eat in
order to inhibit the inappropriate reaction (LA damage). When the inhibitory–excitatory balance is restored the “discrimination” becomes perfect again.

It remains to add that the dogs had bilateral or unilateral partial impairment of the optic tract. This produced changes in the visual field (in most cases the lateral part of visual field was limited). However, these changes were transient and did not affect the restoration of instrumental performance after the LA operation. Also the damage of the olfactory tract or/and nucleus did not have any influence on the restoration of reactions produced by LA damage. It might also be suggested that the visual or olfactory discriminatory disturbances might be due to such damage. However, although after the DMA operation the dog did not seem to recognise the experimenter, we have evidence that this is produced by changes in motivational and emotional status. The dogs after DMA operation are apathetic, indifferent and negativistic. After subsequent LA operations, the dogs are able to recognize the experimenters at once, without any special retraining. Again, they become friendly and discriminated them from unknown persons. They could also find the way to the experimental chamber easily and start to perform the proper reaction to positive CSi. Their dull behavior after DMA operation and before LA may suggest that they are not able to recognise persons, understand calls and identify other objects, but the reappearance of all of these abilities after LA surgery, together with increased arousal, shows that the changes in discriminatory mechanisms are secondary.

We should, however, consider that the decrease of arousal may in consequence produce a decrease of discrimination without primary impairment of purely discriminatory mechanisms. It is known that stimulation of the reticular formation produced better discrimination of visual stimuli (13), and also enhances acoustic discrimination. So, it is possible that discriminatory functions might be temporarily diminished by low arousal after DMA operation and improved with increases in general arousal after LA damage.

Most of the investigators of amygdala functions made rather extensive lesions involving the total amygdaloid complex in most cases. Such lesions may differently affect dorsomedial or lateral amygdaloid regions in particular cases, and therefore produce different combinations of errors to \((\text{CS}^+)\) and \((\text{CS}^-)\), due to either the prevailing of inhibition or facilitation.

A final problem concerns the changes in instrumental performance that were not ideally parallel to changes in food intake. In the three dogs which were only hypophagic, the instrumental reactions were impaired after the DMA operation as well as in the others. Also the improvement of the instrumental alimentary reactions was not simply derived from in-
creasing voraciousness. The degree of the increase of food intake (hyperphagia) and the restoration of instrumental reactions did not run in parallel. This may indicate the presence of two separate mechanisms, one for the regulation of food intake and another for motivation of instrumental performance. The latter mechanism is probably closely connected with general arousal. Miller et al. (16) showed in rats, that, although the rats after ventromedial hypothalamic lesions were hyperphagic if food was offered free, they were less motivated to press the bar for food reinforcement with either increased effort or food that was less palatable. Morgane (17) showed that in the rat's hypothalamus the metabolic and motivational alimentary mechanisms are separate. It is probable that hypothalamic control of food intake and instrumental alimentary performance depends on different mechanisms, although they are linked with each other. A similar conclusion might be drawn concerning the amygdala. Instrumental performance seems to be much more dependent on the general arousal of the dogs, reflected in motility and interest in surroundings than on alimentary excitation. This finding may suggest that various functions of the DMA amygdala are controlled by separate neurons, exerting specifically an influence on different aspects of alimentary behavior, i.e., general arousal indispensable for any life functions, hunger or alimentary drive, food intake, and instrumental alimentary reactions. Our recent work on the effect of DMA lesions on salivary reflexes (14, 15) also showed the dichotomy in amygdalar mechanisms. We observed that in particular dogs the decrease of salivation was not dependent on the decrease in food intake, although as a rule the impairment of salivation was the most pronounced in those dogs in which aphagia was the most prolonged. The fact that our lesions were not exactly the same in all dogs suggests that various alimentary mechanisms might be differently affected by different lesions and speaks for the need of further investigations using the method of small lesions more precisely limited to particular nuclei.

In the present experiments the disinhibition effect observed after the second (LA) operation was the most pronounced in dogs in which the ventral-posterior part of lateral amygdala was damaged which suggest that this part might be crucial for inhibitory mechanisms. On the other hand, the fact that in most dogs the adjacent part of the piriform lobe was damaged and/or the ventral hippocampus was touched unilaterally should be taken into account.

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