PREFRONTAL LESIONS AND AVOIDANCE REFLEX DIFFERENTIATION IN DOGS

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Abstract. The effects of bilateral partial prefrontal lesions on go-no go differentiation with symmetrical or asymmetrical reinforcement trained by the avoidance procedure were investigated. Moderate impairment of these tasks was observed after large lateral or medial prefrontal lesions, while severe impairment after a deep incision of the fibers in the specific prefrontal region. It is suggested that the mechanism of avoidance differentiation is of a symmetrical type, which is directly related to the "motor act differentiation" but not to the drive–no drive differentiation.

INTRODUCTION

The role of the prefrontal region in fear-motivated behavior, studied in different animal species by various experimental procedures and ablations of different parts of the prefrontal area, has been discussed by a number of authors. Two controversial hypotheses have been put forward to explain the experimental data. The first one states that the impairment in avoidance behavior is a result of diminution of anxiety (14, 15, 18, 20). The second view, taking into account the disinhibitory effect after prefrontal lesions, has postulated temporal increase of a fear dirive (1).

Brutkowski's suggestion (4, 6) concerning the release of a drive excitation from the cortical inhibitory control after prefrontal ablations was based upon alimentary classical (3) and instrumental conditioning (2, 5, 6). This suggestion was extended to defensive conditioning when the disinhibitory effect was discovered in classical defensive differentiation after lobectomies in dogs (1). However, while the effects of prefrontal lesions with alimentary conditioning in dogs (3, 5, 6) were in agreement
with other data obtained in monkeys (7), rabbits (2) and cats (16, 19), inconsistent results were obtained when the defensive method was applied. Using classical defensive procedure in dogs and rabbits the disinhibitory syndrome was observed in some experiments (1, 11), whereas in the others was not (16, 17). On the other hand, in all experiments where the avoidance procedure was applied, inhibitory reflexes were not impaired after prefrontal ablations in cats (21-23) and dogs (12).

Regardless of the hypotheses of increased or decreased fear drive after prefrontal lesions, the question arises as to why prefrontal lesions have a different effect on retention of differentiation of instrumental conditioning when elaborated by alimentary or defensive procedures. Dissociation of impairment in symmetrical and asymmetrical alimentary differentiation after partial prefrontal lesions (8, 9) suggested that these two types of differentiation have two different mechanisms and that they are mediated by two different areas situated within the prefrontal region. Moreover, these experiments (9) showed also that there are many variations of the symmetrical type of differentiation, and within them only one is severely impaired after removal of orbital areas when the similar stimuli and responses are used. (The stimuli are regarded similar when they elicit a high rate of generalization.)

A detailed analysis of the experimental situation and comparison of the results obtained by alimentary (8-10) and avoidance (D. Kowalska et al., in preparation) procedures with symmetrical reinforcement has shown that there is no discrepancy between the effect of prefrontal lesions on retention of alimentary and avoidance differential reflexes, when dissimilar stimuli are used. But in the case of the asymmetrical procedure, removal of the medial prefrontal cortex impairs slightly the retention of asymmetrical alimentary differentiation (5, 6), whereas asymmetrical differentiation of avoidance reflexes is not impaired (12).

The aim of this paper is to verify the effects of partial prefrontal lesions on the retention of avoidance reflex differentiation symmetrically and asymmetrically reinforced in stimulus conditions which were most sensitive to specific cortical ablations when alimentary differential reflexes were used.

**MATERIAL AND METHODS**

**Subjects.** Sixteen naive mongrel dogs, about 2 years old, were used. Dogs were randomly assigned into experimental groups for purposes of different training and surgery.

**Experimental procedure.** The experiments were performed in a sound-proof chamber. CS\textsubscript{1} — a tone of 1,000 Hz and CS\textsubscript{2} — a tone of 700 Hz
were the conditioned stimuli. Both tones were delivered through one loudspeaker located in front of the animal. Onset and cessation of the tones were controlled by a mechanical switch, thus a click could be heard with the tone as was the case in previous experiments with alimentary conditioned reflexes. The unconditioned stimulus (US) was an alternating current from a transformer (50 Hz, 50–70 v, one pulse per second) applied to the left hindleg. Twenty trials a day were given. Intertrial intervals lasted 40 to 60 sec and were changed at random from trial to trial.

**Preliminary training.** While being presented with CS₁, the animal was required to make the instrumental response of placing his right foreleg on a bar. If the dog did not perform the response within the 5-sec presentation of CS₁, the US was delivered and lasted up to the moment when the response was elicited. Then the CS₁ and US were interrupted automatically. If the animal performed the instrumental response within the 5-sec presentation of the CS₁, it was stopped and US was not delivered.

The criterion of performance during preliminary training was 100% of correct avoidance responses made in one day (20 trials). Then the animals were divided into two groups, each consisting of eight dogs.

**Differential training.** CS₂ was introduced in this training. Ten trials with CS₁ and 10 with CS₂ were applied in random order. The required criterion was 95% of correct responses to both stimuli during 100 consecutive trials (5 days). One group of animals was trained in symmetrical differentiation, while in the second group asymmetrical differentiation was applied.

**Symmetrical differentiation.** The animals were required to perform the instrumental response within the 5 sec presentation of CS₁ and to withdraw this response within the 5 sec presentation of CS₂ to avoid US. If the animal did not perform the response to CS₁ or it responded to CS₂, US was given.

**Asymmetrical differentiation.** The animals were required to perform the instrumental response during the 5 sec presentation of CS₁ to avoid the US or to withdraw this response during the 5 sec presentation of CS₂. However, during operation of CS₂, US was never applied irregardless of whether the dog did or did not perform the trained movement.

When the animals reached the criterion, each of the two groups was divided into two subgroups for purposes of surgery. Each subgroup consisted of four dogs.

Bilateral ablation of the medial prefrontal cortex was made in four dogs (dogs 58, 59, 68 and 69) trained in symmetrical differentiation — symmetrical medial subgroup (SM), and in four dogs (dogs 62, 63, 105 and 106) trained in asymmetrical differentiation — asymmetrical medial
subgroup (AM). The lateral prefrontal cortex was bilaterally ablated in four dogs (dogs 60, 61, 67 and 108) trained in symmetrical differentiation — symmetrical lateral subgroup (SL), and in four dogs (dogs 64, 65, 107 and 109) trained in asymmetrical differentiation — asymmetrical lateral subgroup (AL). After allowing 7 days for recovery the testing was continued until the preoperative criterion was established.

Surgical and histological procedures. These were performed in the same way described previously (10). For a detailed analysis of the extent of cortical damage, a reconstruction was made in the following manner. Every 20th frontal section was projected onto the scheme of lateral or medial surface of the hemisphere depending on the place of the lesion. To eliminate the differences in shape of the dog brains and to facilitate comparison of these lesions, the results of the reconstructions were next transferred into standard drawings according to Kreiner's patterns (13).

RESULTS

Table I shows the performance of the task during preliminary training (elaboration of instrumental avoidance response to CS₁), preoperative acquisition and postoperative retention of symmetrical and asymmetrical differentiation. Criterion trials and errors are included in the scores. Considerable individual differences in the number of trials and errors performed by dogs to obtain criterion may be observed during preliminary training and also during acquisition of both types of differentiation. Differentiation training in these experiments was relatively long-lasting. However, there were no differences to be seen between the groups in relation to the acquisition of symmetrical and asymmetrical tasks. In all animals of both groups both types of errors were made: omission (errors to CS₁) and commission (errors to CS₂). The number of errors to CS₂, however, was greater. In postoperative retention there were no subgroup differences. All dogs except two (59 and 108) were moderately impaired. The number of errors in the last 100 preoperative trials (preoperative criterional period) and the number of errors in the first 100 postoperative trials were statistically analysed. The difference between these scores is highly significant (Wilcoxon matched-pairs, $T = p < 0.01$).

Figure 1 shows the relation between the number of trials to criterion in preliminary training, preoperative acquisition and postoperative retention in all applied types of task and lesion. It appears that the duration of preliminary training and the difficulty in elaborating differentiation had no effect on the postoperative number of trials to criterion in individual animals. Furthermore, there is also a lack of such correlation in the number of errors made by these animals (Fig. 2).
## Table I

Pre- and postoperative performance of symmetrical and asymmetrical differentiation in dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Dog</th>
<th>Preliminary training</th>
<th>Trials</th>
<th>Errors</th>
<th>Surgery</th>
<th>Retention</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CS₁</td>
<td>CS₂</td>
<td>Total</td>
<td>Trials</td>
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<td></td>
<td></td>
<td>68</td>
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<td>105</td>
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<td>890</td>
<td>5.7</td>
<td>170.8</td>
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Fig 1. The number of trials to criterion in preliminary training (white areas), preoperative acquisition (striped areas), and in postoperative retention (crossed areas).

Fig 2. The number of errors made in preoperative acquisition (striped areas) and in postoperative retention (crossed areas).
Nevertheless, it is unlikely that the severe impairment of three animals (dogs 61, 62 and 67) was accidental. It may have been related to the damage to a specific area of the prefrontal region.

Figures 3 and 4 show reconstructions of the lesions made on the medial surface of the hemisphere (subgroups SM and AM). Pregenual areas PG I, II and III were completely or almost completely bilaterally ablated in all dogs but one (dog 68) of these subgroups. In dog 68 only half of the area PG II was removed. Areas XM I and II were damaged bilaterally in all dogs. In five dogs (dogs 58, 59, 62, 63 and 105) these areas were almost completely bilaterally ablated; whereas in others there were partial lesions of this region. The medial part of the proreal gyri

![Figure 3. Reconstructions of the medial lesions in subgroup SM. The lined areas show shallow damage not involving all the cortical layers. Crossed areas, damage through the whole thickness of the cortex and involving to some extent the white matter lying just below the cortex. Black indicates deep damage of the fibers in the white matter.](image-url)
was completely removed in two dogs (dogs 59 and 63); while in others only partial damage of this area was observed. Other frontal areas situated on the medial surface of the hemispheres were also partially injured in several dogs. The largest lesion, partially involving genual and subgenual areas, was made in dog 59. The smallest lesion was found in dog 68. Purely cortical ablations were found in only two dogs (dogs 58 and 106). In other dogs there were small, deep, asymmetric damages to fibers located in the various parts of the prefrontal region. In one dog (dog 62) only there was symmetrical bilateral deep damage on the border of areas PR, PG I, II, III and XM, which transected the fibers so that the damage in fact involved not only the medial, but partially also lateral areas. Postoperative retention of the differentiation task was severely impaired only in this dog from both medial subgroups.
Figure 5 and 6 show the reconstructions of this lesion made on the lateral aspect of the hemisphere (subgroups SL and AL). The greatest damage of the lateral prefrontal areas occurred in dogs 60, 61 and 67. The smallest lesion was found in dog 108. In all dogs except one (dog 108) orbital areas ORB I' and ORB I" were completely removed bilaterally. Complete bilateral removal of ORB II was present in dogs 60, 61 and 65. In other dogs this area was damaged only partially. In all dogs the proreal gyri (PR) were extensively damaged; while PRL areas were only
partially removed. The polar region was also slightly damaged in all dogs. Moderate bilateral lesions of the paraorbital region (PORD") were made in dogs 61, 65 and 67 and PORD' was damaged unilaterally in dogs 61 and 67. In several dogs there were also slight damages of the white matter at various places under the lateral ablated areas, but only in two dogs (dogs 61 and 67) were these deep lesions. These were on the border of the PRL and ORB I' areas. Moreover, these lesions were bilaterally symmetrical. These were the only two dogs severely impaired.
DISCUSSION

Results of the present experiments have shown that postoperative retention in dogs trained by the defensive procedure in symmetrical and asymmetrical differentiation tasks is impaired. Comparison of these results with previous ones obtained in dogs trained by the alimentary procedure (8, 9) and by the defensive procedure (14, 15) showed some similarities, but also essential differences. Table II shows the relations between postoperative impairment and other factors, such as the types of differentiation task (symmetrical or asymmetrical), the type of reflexes (instrumental alimentary or defensive), the lesions (prefrontal medial or lateral), and the quality of the stimuli (similar — 1,000 Hz and 700 Hz tones, or dissimilar — 1,000 Hz tone and buzzer).

Postoperative impairment in avoidance reflex differentiation is related to the quality of the stimuli but not to the type of cortical lesion nor to the differentiation task. Symmetrical and asymmetrical defensive differentiation is moderately impaired after medial or lateral prefrontal cortical lesions when similar stimuli are used. And also, both types of defensive differentiation are not impaired after both types of partial cortical prefrontal lesions when dissimilar stimuli are used.

Postoperative impairment of alimentary differentiation is related to the type of differentiation, to the type of partial cortical prefrontal lesions, and to the quality of the stimuli. Lateral prefrontal lesions affect only symmetrical alimentary differentiation of two similar stimuli,
whereas such lesions have no effect on asymmetrical differentiation of similar as well as dissimilar stimuli. Medial prefrontal lesions have no effect on symmetrical alimentary differentiation of similar and dissimilar stimuli, but such lesions moderately impair asymmetrical alimentary differentiation of similar and dissimilar stimuli. The above comparison suggests that the lateral and medial regions of the prefrontal lobes play the same role in the retention of both types of differentiation of instrumental defensive conditioned reflexes and that the impairment after such lesions is only related to the quality of the stimuli.

However, the following results provide evidence against this suggestion. In the present experiment there were three dogs which were severely impaired. Histological verification of these dog brains showed a deep incision just under the presylvian fissure (dogs 67 and 61), and a deep cut of the fibers on the border of PG III and XM areas (dog 62). This fact shows that not only quality of the stimuli plays a role in postoperative impairment, but also the region in which the lesion was made. In a previous paper (10) it was suggested that such a deep lesion made either in a particular prefrontal region on the medial or on the lateral aspect of the hemisphere, causes similar damage of the fibers connecting orbital areas with other structures of the brain. It has been shown also in this paper that removal of exactly the same region is crucial for severe impairment of alimentary symmetrical differentiation of two similar stimuli.

Nevertheless in comparing alimentary and defensive instrumental reflexes, there are two unsolved questions. The first one is, why does section of the fibers in the prefrontal region impair severely both types of defensive differentiation (symmetrical in dogs 61 and 67 and asymmetrical in dog 62). The second question is, why does a large ablation of the lateral or medial prefrontal cortex impair only moderately both types of differentiation of defensive conditioned reflexes to two similar stimuli — whereas section of the fibers in the prefrontal region causes severe impairment of these differentiations.

To answer the first question let us consider an experimental situation during elaboration of avoidance reflex differentiation. During the preliminary training the conditioned stimulus $CS_1$ acquires the property of exciting the fear-drive center, and this excitation is stopped or inhibited by interruption of the stimulus sound. This is made by performance of the instrumental response. Thus, removal or suppressing of a fear is a reinforcement. Application of a second differential stimulus, similar to the first one elicits through generalization excitation of the fear-drive center. Animal performs the instrumental response but it does not remove the stimulus sound which prolonges and even increases the excitation level of this center. In such a case this response is repeated several times
up to the moment when the stimulus sound is interrupted. Thus the animal stops to respond to CS₂ by flexion movement because it is not adequate in new conditions. In the symmetrical procedure, performance of the instrumental response is punished by application of the unconditioned stimulus (electric shock to the paw) which additionally increases the excitation level of the fear drive center, which forces an animal to withdraw this response. In both cases, in symmetrical and asymmetrical procedures, the animal probably learns actively to withdraw the instrumental response to the differential stimulus. Thus, as a consequence of such training there is obtained only one type of differentiation—the symmetrical one. This suggestion is supported also by Zielinski's results (22) on cats in which prefrontal lesions impaired their ability to perform short-latency avoidance responses. Zielinski concluded that "...the short latency avoidance responses are based more on the direct CS–CR connections than on the indirect CS–Fear Drive–CR connections..." and that a prefrontal lesion in the cat "...affects the direct CS–CR connections...". A similar conclusion for alimentary instrumental differentiation was made in our previous papers (9, 10). If so, it may be supposed that the differentiation of instrumental defensive reflexes (avoidance) will be impaired after partial prefrontal lesions only in such conditions in which the symmetrical type of alimentary instrumental differentiation is impaired. Table III shows a comparison concerning alimentary symmetrical

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Medial</th>
<th>Lateral</th>
<th>White matter</th>
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</thead>
<tbody>
<tr>
<td>Stimulus</td>
<td>Dissimilar</td>
<td>Similar</td>
<td>Dissimilar</td>
</tr>
<tr>
<td>Alimentary</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(10)</td>
</tr>
<tr>
<td>Defensive</td>
<td>—</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td>avoidance</td>
<td>(iii)</td>
<td>(ii)</td>
<td>(iii)</td>
</tr>
</tbody>
</table>

(iv), lobectomy (D. Kowalska, personal communication). Other designations the same as in Table II.

and instrumental defensive procedure, when in defensive procedure groups with symmetrical and asymmetrical reinforcement are taking jointly. It may be seen that neither alimentary symmetrical nor defensive differentiation are impaired after lateral or medial prefrontal lesions if dissimilar stimuli were used. If similar stimuli were applied, alimentary symmetrical and defensive differentiations were severely impaired after small but deep cuts of the fibers in the region situated between the posterior part of the orbital areas on the lateral aspect of the hemisphere and the anterior part of the XM areas on the medial aspect of the hemi-
sphere. Alimentary symmetrical differentiation, however, is also severely impaired after cortical removal of the orbital areas; while defensive differentiation is only moderately impaired after both lateral as medial prefrontal cortical lesions. This last evidence suggest, that the crucial cortical area for defensive differentiation was not completely removed in the present experiments, and/or the deep incision of the white matter in the given region cut not only the fibers connecting the orbital areas with other brain structures as was postulated previously (10) but also fibers from other cortical prefrontal areas which may be important for defensive differentiation.

Another question is related to the omission errors specific for postoperative impairment of symmetrical alimentary differentiation. These errors were not observed during preoperative differential training in alimentary reflexes while they were observed in the postoperative retention (9). Contrary to those results, the present experiments show that the animals did not perform, or performed only a very small number of omission errors postoperatively, while such errors were made before operation. This finding suggests that either this type of error is not specific for symmetrical differentiation or the defensive instrumental differentiation has a mixed character. This problem and others discussed before require new experiments.

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