INFLUENCE OF THE VARIETIES OF DIFFERENTIATION TRAINING AND PREFRONTAL LESIONS ON RETENTION AND REVERSAL LEARNING OF AVOIDANCE RESPONDING IN DOGS

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Key words: defensive reflexes, prefrontal lesions, differentiation, reversal, dogs

Abstract. The postoperative retention and reversal of go, no-go avoidance reflex differentiation with symmetrical and asymmetrical reinforcement were studied in dogs with prefrontal lesions and unoperated controls. The general pattern of the influence of such experimental variables as type of differentiation task and quality and arrangement of conditioned stimuli was similar to that observed preoperatively. However, prefrontal surgery changed the interactions between these experimental variables. The modifying role of the medial and the lateral parts of prefrontal cortex on the retention of differentiation and on reversal learning is discussed.

INTRODUCTION

In a previous study it has been shown that the quality of conditioned stimuli and their relative saliency exerted a strong influence on the rapidity of differentiation learning and on the number of commission errors when the asymmetrical procedure of reinforcement was used, whereas these effects were strongly attenuated when the symmetrical pro-
procedure was used (21). The early stages of differentiation learning in subjects trained with the symmetrical procedure were characterized by responses executed with shorter latencies, a larger percentage of errors on negative trials and numerous extra- and inter-trial responses than in subjects trained with the asymmetrical procedure. However, the retention test showed a greater stability of acquired differential responding under the symmetrical than under the asymmetrical procedure of reinforcement. The dogs trained with asymmetrical reinforcement required more trials to criterion after the control pause and committed more errors on negative trials, especially in the case of white noise intensity differentiation. Among the dogs trained with symmetrical reinforcement, only small differences between experimental groups were observed. The data also indicated a direct relation between the saliency of negative conditioned stimuli (CSi−), and the strength of the secondary punishing effect elicited by continuing the CS− after the barpress responses.

The present report describes the continuation of the experimental stages of the previous study (21). Specifically, we studied the interactions of lesions in the lateral or medial parts of prefrontal cortex with the type of differentiation task and the quality and arrangement of conditioned stimuli used in differentiation and reversal learning of avoidance reflexes. Two types of go, no-go differentiation (asymmetrically and symmetrically reinforced) are distinguished by the occurrence or non-occurrence of the unconditioned stimulus during negative trials. On asymmetrical differentiation, the painful unconditioned stimulus appeared only on positive trials, and there was no response — US contingency during CS−. In symmetrical differentiation, as described by Gross and Weiskrantz (13, 14) for alimentary reflexes, the food reward was given not only after instrumental response to the positive conditioned stimulus (CS+) but also after inhibition of such response to the CS−. During the symmetrical differentiation of defensive reflexes the subject should make the conditioned instrumental response to the CS+ to avoid the unconditioned painful stimulation, and should not make this response to the CS− to avoid the same US.

Also the influence of the quality and the arrangement between conditioned stimuli on the course of differentiation learning has been noted. There are many experiments indicating that the discrimination between CS+ and CS− is more difficult when physically similar CSi have been used (15, 21, 24–26) or when the arrangement of conditioned stimuli caused a stronger relative saliency of the CS− than the CS+ (16, 18, 21, 24, 27, 28, 30–32, 34, 36).

The data concerning retention of differentiated defensive responses
after prefrontal lesions in dogs are not clear. In contrast to the differentiated alimentary motivated responses (5, 6, 8, 9, 11, 17), in defensive reflexes double dissociation of the effects of partial prefrontal lesions on the type of differentiation task was not observed. It has been shown by Dąbrowska (10) that independently of the reinforcement contingency, the go, no-go differentiation of two similar conditioned stimuli (two tones: 1,000 Hz and 700 Hz) was moderately impaired after prefrontal, lateral or medial lesions, and severely impaired after damage of the white matter in the depth of the presylvian sulcus. Similar cortical prefrontal surgery exerted no effects on retention of go, no-go tone vs click differentiation with asymmetrical (18) or symmetrical (19) reinforcement. However, reversal learning was impaired after lateral lesions (18). Similar results for reversal learning after prefrontal lobectomy in cats has been obtained (35). Reversal learning data indicated that the relative saliency of the positive and negative conditioned stimuli was a crucial factor for effects of the prefrontal lesions to appear.

The aim of the present study was to obtain data concerning the influence of four experimental variables: (i) the type of differentiation task, (ii) the acoustical CS quality, (iii) the arrangement of stimuli pairs, and (iv) the kind of prefrontal lesion, on the retention and reversal of go, no-go differentiation of avoidance responding.

**METHODS**

Forty-nine male naive mongrel dogs with weight ranging from 5 to 19 kg were used in the four experiments. A factorial design was employed with four independent variables (Table I).

Training was conducted in an acoustically shielded chamber. The subjects from Experiment I were trained in a cage measuring 55 × 40 cm, and containing the bar, located 10 cm above the grid-floor in the middle of the oblong wall opposite to the entrance door of the cage. The sources of the CSi, located near the center of the cage ceiling, were a loudspeaker, through which a 1,000 Hz 65 dB (re: 20 μN/m²) tone was presented, and an electromagnetic relay, producing a rhythmic 5/s click. Alternating current of 50 Hz and 25 V to 100 V from a transformer was used as the US.

The dogs from Experiments II, III and IV were trained on a platform measuring 68 × 177 cm. Their limbs were secured in a harness. The sources of CSi, located facing the dog, were a loudspeaker for presenting
a 1,000 Hz 65 dB tone, or white noise of 50- or 70-dB intensity, and an electromagnetic relay which produced a 5/s click. A response pedal was located next to the subject within easy reach of the dog's right paw. A current of 50 V to 150 V from a condenser, one pulse per second, given through electrodes placed on the left hind leg, was used as the US. At the beginning of training the optimal voltage for each dog responding was chosen and remained constant throughout experimental sessions.

In the previous paper (21) data concerning acquisition of the avoidance response, go, no-go differentiation and retention after the control rest period were presented. The complete data for Experiment I and the results obtained for pre- and post-surgical differentiation of Experiment II were also published earlier (18, 19). This report comprises all the data obtained from the postsurgical retention of the differentiation and reversal learning in Experiments I-IV.

During differentiation and reversal stages, each daily session consisted of 10 positive and 10 negative trials. The order of positive and negative trials was changed each day according to a Gellerman series (12).

In both procedures of reinforcement (asymmetrical and symmetrical) on positive trials an avoidance response performed during the 5 s CS-US
interval automatically terminated the CS+ action and precluded US application; in the absence of a response, nociceptive painful stimulus was added to the CS+ after 5 s, and both stimuli continued until the dog terminated them by the escape response. A negative trial consisted of a 5 s CS− presentation independent of the instrumental barpressing response. No shock was given on negative trials in the asymmetrical procedure of reinforcement, whereas in the symmetrical procedure of reinforcement, each barpress response made on negative trials was punished by a brief shock.

The differentiation criterion was 90% of correct responses on positive and negative trials respectively in ten consecutive sessions.

Reversal training started the day after the subject reached postoperative differentiation criterion, and was continued for 50 experimental sessions. At this stage the signalled meaning of the conditioned stimuli was reversed. The new arrangement between conditioned stimuli is shown in Table II.

<table>
<thead>
<tr>
<th>Stimulus quality</th>
<th>Stimulus arrangements groups</th>
<th>Differentiation</th>
<th>Reversal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups 1</td>
<td>CS+</td>
<td>CS−</td>
</tr>
<tr>
<td>Click vs. tone</td>
<td>Click</td>
<td>Tone</td>
<td>Click</td>
</tr>
<tr>
<td></td>
<td>Groups 2</td>
<td>Tone</td>
<td>Click</td>
</tr>
<tr>
<td>White noise</td>
<td>Groups 1</td>
<td>70 dB</td>
<td>50 dB</td>
</tr>
<tr>
<td>intensities</td>
<td>Groups 2</td>
<td>50 dB</td>
<td>70 dB</td>
</tr>
</tbody>
</table>

During reversal training the reinforcement procedure was, as a rule, the same as on previous stages. However, five operated dogs, trained originally in the go, no-go differentiation symmetrically reinforced (two from group 1L, and ones from 2L, 1M and 2M groups respectively) were tested during the reversal training under asymmetrical procedure.

Following reversal training the subjects with prefrontal cortex damages were sacrificed using an overdose of Nembutal. After perfusion the brains were removed and subjected to histological analyzes by Klüver's techniques. Detailed examination of the extend of the cortical damage was made, and the results of reconstructions were transferred to Kreiner's patterns (23) to eliminate differences in shapes of the dogs brains, and to facilitate comparison of the lesions.

The main indices of behavior were: (i) the number of avoidance responses to CS+ (or CS'+), (ii) the number of disinhibited negative trials,
(iii) the number of extra responses to CS− (or CS'−) and intertrial responses (E + ITR), and (iv) the latencies of the avoidance response (measured to the nearest 0.2 s.). The E + ITR index might be considered as a good measurement for the general emotionality of dog. The dogs' behavior was observed through a one-way window from the room adjacent to the training chamber. The results were tested mainly by two or three-way factorial analyses of variance (for this reason the experimental groups were joined and the factors influencing the dogs' behavior were exposed).

RESULTS

Postoperative retention of go, no-go differentiation

The data obtained at this stage of the experiment show that after prefrontal cortex lesions, or the rest period for the normal dogs, no distinct differences in the level of performance between the two types of differentiation were observed. The factor which influenced the number and percent of trials with errors to postoperative criterion was the stimulus quality. A 2-way ANOVA of the effects of quality of stimuli and surgical treatment showed that dogs trained to discriminate two white noise intensities have a greater number and percent of trials with errors to both conditioned stimuli jointly, than dogs discriminating tone and click (for the number of trials with errors: $F_{1;43} = 4.65, P < 0.05$, and for percent of trials with errors: $F_{1;43} = 6.28 P < 0.025$). Similar stimulus quality effect was observed during the early differentiation learning (21).

During postoperative training stimulus quality factor affected the number and percentage of errors to the CS− alone. The number of errors to the CS− was greater for dogs trained with white noise intensities than discriminating tone vs click as it was shown by a 3-way ANOVA done without the stimulus arrangement factor ($F_{1;37} = 4.93, P < 0.05$). The percentage of errors to the CS− was also greater in the same groups as indicated by a 2-way ANOVA including quality of stimuli and surgery factors ($F_{1;43} = 6.99, P < 0.025$).

A 3-way ANOVA, combined the stimulus arrangement factor, showed that the joint action of the type of differentiation and the quality of CSi affected the number of sessions to criterion ($F_{1;37} = 7.72, P < 0.01$), the number of errors to CS+ ($F_{1;37} = 7.72, P < 0.01$), the number of errors to CS− ($F_{1;37} = 8.99, P < 0.005$), and the number of extra- and intertrial responses (E + ITR index) during the first five experimental
sessions after the operation \( (F_{1;37} = 4.18, P < 0.05) \). When the easily discriminable stimuli were used (tone and click), the subjects trained under the symmetrical procedure committed more errors to the \( \text{CS}^- \), more extra- and intertrial responses, and reached the criterion after longer training than the dogs trained with asymmetrical reinforcement. However, for the groups discriminating white noise intensities the symmetrical procedure was more effective; dogs reached the criterion faster, committed fewer errors to the \( \text{CS}^- \) and made less \( E + \) ITRs, than subjects trained with the asymmetrical procedure.

Another experimental factor, stimulus arrangement, also affected the \( E + \) ITR index. Subjects trained with \( \text{CS}^- \) more salient than \( \text{CS}^+ \) (groups 2) made more extra- and intertrial responses than those trained in groups 1, as it was shown by a 3-way ANOVA collapsing across quality of conditioned stimuli \( (F_{1;37} = 7.10, P < 0.025) \).

No significant difference in any indices were observed among the groups with lesions in the lateral (L) or medial (M) prefrontal cortex, or intact animals (N). However, the data showed that damage of prefrontal cortex may modify the influence of other experimental factors. As illustrated in Fig. 1, normal dogs, reached the differentiation criterion

![Fig. 1. Number of trials to retention criterion, and trials with errors to both \( \text{CSI} \) after the rest time for normal dogs. Open bars denote asymmetrical training and slashed bars represent symmetrical training. Closed bars indicate the trials with errors to \( \text{CS}^+ \) and crossed bars indicate the trials with errors to \( \text{CS}^- \). Groups 1: \( \text{CS}^+ \) more salient than \( \text{CS}^- \), groups 2: \( \text{CS}^+ \) less salient than \( \text{CS}^- \).]
after the rest time very quickly with the exception of two dogs making the differentiation of the click CS$^+$ and the tone CS$^-$. Three of the six medial prefrontal dogs (Fig. 2) also required prolonged postoperative training in order to differentiate tone and click CSI under symmetrical reinforcement. But the most trials to criterion and most frequent er-

Fig. 2. Number of trials to retention criterion, and trials with errors to both CSI for the dogs after the medial prefrontal lesions. Denotions as in Fig. 1.

Fig. 3. Number of trials to retention criterion, and trials with errors to both CSI for the dogs after the lateral prefrontal lesions. Denotions as in Fig. 1.
rors to both conditioned stimuli occurred in the medial subjects required to differentiate between 50 dB CS+ vs, 70 dB CS- white noise, asymmetrically reinforced. In the analogues experimental conditions only one lateral animal required more trials to criterion and made more errors to both CSI (Fig. 3) in comparison to the remaining lateral subjects which reached the postoperative criterion relatively quickly. An ANOVA pooling across the stimulus arrangement factor, showed that the interaction between kind of prefrontal surgery and quality of conditioned stimuli was significant for number of errors to the CS+. Normal dogs made more errors to tone and click CSI+ than to white noise CSI+, whereas the operated groups made more errors to the CSI+ when they differentiated intensities of white noise ($F_{2,37} = 3.33, P < 0.05$).

Fig. 4. Interaction between prefrontal surgery and the type of differentiation task for the mean number of E+ ITR index, during the first five experimental sessions after the prefrontal surgery or the rest time. N, normal, M, medial prefrontal lesion, L, lateral prefrontal lesion.

At the postoperative stage of training surgical treatment and the type of differentiation task interacted in determining the mean frequencies of extra- and intertrial responses. As can be seen in Fig. 4, normal dogs made more extra- and intertrial responses during symmetrically reinforced differentiation than in the asymmetrical procedure. In comparison with normal dogs the medial prefrontal animals made even more E+ ITRs in the symmetrical test. In contrast, the lateral damaged dogs showed a higher level of the E+ ITRs in the asymmetrically than in the symmetrically reinforced differentiation.

After surgery or the control rest period there were no systematic changes in the latency the instrumental responses among the normal, medial and lateral groups of dogs. As in the previous stages of the
experiment this measure was influenced by the type of differentiation task. Dogs trained with the symmetrical procedure of reinforcement made more short latency responses than dogs trained with asymmetric procedure, (3-way ANOVA collapsed across the stimulus arrangement factor; $F_{1;37} = 8.73, P < 0.01$).

Significant changes in general behavior were not observed during this stage of the experiment, but during the first session after the surgery an enhancement of the excitation level in four medial prefrontal and one lateral prefrontal dogs appeared (all subjects were trained with symmetrical reinforcement). Transient symptoms of aggressive behavior, barking and growling in two medial dogs trained with asymmetrical reinforcement were also observed.

**Reversal training**

As noted in the methods section, during reversal training the signalling values of the CSI were changed. The originally positive conditioned stimuli were changed to negative conditioned stimuli (CS$^+$→ CS$^-$) and vice versa. This means that, opposite to the original differentiation stages, groups 1 made a more difficult reversal (CS$^+$ less salient than CS$^-$) in comparison to groups 2 (CS$^+$ more salient than CS$^-$).

**Reversal with continuation of the type of differentiation task.** Among 44 subjects, in which the type of differentiation task during reversal training was not changed, only 12 dogs reached the criterion before of 50th experimental session: 3 dogs from Experimental I (groups: 1L, 2M, and 2N), 7 dogs from Experiment II (two from groups 1L and 2L and one from groups 1M, 2M, and 2N), and 2 dogs from Experiment IV (groups 2L and 2M). No one dog from Experiment III reached the criterion. The mean levels of performance for normal, medial and lateral dogs to both conditioned stimuli in five experimental sessions before the reversal (Block 0) and in ten consecutive blocks of reversal training, 5 sessions in each Block, are presented in Fig. 5 - 7. As can be seen in the figures, acquisition of the avoidance response to the new positive conditioned stimulus, (CS$^+$), was very fast, but extinction of the instrumental responses to the previously positive and now negative stimulus (CS$^-$) was very difficult. Among the normal dogs (Fig. 5) no differences in performance level to CS$^+$ were observed, except for the dogs differentiating 70dB CS$^+$ vs 50dB CS$^-$ white noise intensities with asymmetrical reinforcement (Exp. III, 2N). Bigger differences appeared in the level of performance to the CS$^-$. The influence of the stimulus arrangement is to produce better extinction in groups 2N than
Fig. 5. Changes of performance of normal dogs in consecutive blocks of sessions, before (Block 0) and during reversal learning (Blocks 1-10). Responses to positive CS are represented by black triangles and circles, responses to negative CS are denoted by open triangles and circles.

in groups 1N. It is worth mentioning that all normal subjects that reached the criterion by the 50th reversal sessions were trained in groups 2N.

Differences among the medial damaged subjects (Fig. 6) were determined by the quality of conditioned stimuli. Better extinction occurred to tone and click than to white noise intensities as the CSi

\[
\text{CSI}^{-1}
\]
Fig. 6. Changes of performance of dogs after medial prefrontal lesions in consecutive blocks of sessions, before (Block 0) and during reversal learning (Blocks 1-10). Denotations as in Fig. 5.

there was no influence of stimulus arrangement on the response level to either CSi, under a given stimulus quality or reinforcement contingency.

The effects among the lateral damaged dogs were somewhat different (Fig. 7). In contrast to the normal dogs trained under the asymmetrically reinforced procedure (Exp. I and III), the lateral subjects from
these experiments extinguished their responses to CS' faster in groups 1L than in groups 2L. Moreover, the response curves for the symmetrically reinforced procedure (Exp. II and IV) demonstrate for lateral dogs faster acquisition of response to the new signalization of conditioned stimuli than the medial and normal dogs showed.
The outcome of reversal learning was variable, and mastery of the reversal task was not specific to surgical treatment. To provide a more sensitive index of discriminative performance, Mann-Whitney U tests of the difference in response latencies on CS" and on CS' trials were calculated for each reversal training sessions for all subjects. Significant $P < 0.05$ differences between the two distributions of latencies might appear in two cases: (i) when the latencies on CS" trials were longer than CS', which means that subject did not detect the reversal of signalling values of the CSI, and (ii) when the latencies on CS' trials were shorter to CS', which means than the reversal of signalling values was detected.

A 3-way ANOVA (quality $\times$ arrangement of stimuli $\times$ prefrontal lesion) of the mean number of reversal sessions in which the latencies to CS" were significantly longer than to CS' indicated that the effects of quality and arrangement of conditioned stimuli were significant. The dogs had more difficulties with detecting of a new signalling values of CSI when they made reversal of two intensities of white noise in comparison with the tone and click reversal training ($F_{1,32} = 14.31, P < 0.001$). This index was also higher in groups 1, than in groups 2 ($F_{1,32} = 9.97, P < 0.005$). Interaction of these two variables with the surgery treatment was also significant (Fig. 8). In the case of the white noise intensity conditioned stimuli reversal, the groups were affected by the stimulus arrangement factor. Subjects trained in group 1 conditions had an elevated level of this measure in comparison to groups

![Fig. 8. Interaction among the prefrontal surgery, the quality and the arrangement of stimuli for the mean number of reversal sessions in which subjects responded according to the old signalling meaning of conditioned stimuli. N, normal, M, medial prefrontal lesions, L, lateral prefrontal lesion.](image)
2. Moreover, the dogs from group 1M had a highest number of reversal sessions in which they responded according to the old signalling meaning of the CSi. The responding of dogs during reversal of click and tone conditioned stimuli depended on the surgical treatment and also on the arrangement of stimuli. A big difference between the normal groups appeared. Group 1N had the largest, and on the contrary, group 2N had the smallest value of this index. The differences for the operated groups were not so big, the difference between the lateral operated subjects was opposite to that of the normal and medial dogs.

The mean number of reversal sessions with the latencies on CS' trials significantly shorter than to CS'− depended only on the quality of the CSi. Values of this index were higher when the dogs made the reversal of tone and click CSi than during reversal of white noise intensities ($F_{1;32} = 10.03, P < 0.005$). The influence of the stimulus quality factor on the mean number of correct response to either CS'− or CS'− was also significant ($F_{1;32} = 5.53, P < 0.025; F_{1;32} = 4.10, P < 0.05$, respectively) and indicated better performance of dogs during the reversal of tone and click stimuli than during reversal of white noise stimuli.

However, 3-way, ANOVA (differentiation task × quality × arrangement of stimuli) showed that during the last block of sessions (Block 10), mean number of errors on CS'− trials depended not only on the quality of conditioned stimuli ($F_{1;36} = 4.18, P < 0.05$) but also on the differentiation task ($F_{1;36} = 6.88, P < 0.05$) and indicated better response inhibition during negative trials punished by shock than during negative trials in the asymmetrically reinforced procedure.

In the course of reversal learning group differences have also been observed in general emotionality represented by the $E + IT_{R}$ index. This measure for normal groups (Fig. 9) depended on the relative saliency of the conditioned stimuli, and groups 1N had an elevated level of $E + IT_{R}$ index in comparison with 2N groups. During reversal learning with asymmetrical reinforcement (Exp. I and III) the quality of stimuli did not influence the magnitude of between-group differences. In contrast, for the symmetrically reinforced groups the difference between groups 1N and 2N depended on the quality of stimuli. Note especially that the magnitude and the between-group difference of the level of $E + IT_{Rs}$ were highest during tone CS'− and click CS'− symmetrical reversal learning (group 1, Exp. II). Normal subjects trained in the reversal of white noise intensities under the symmetrical procedure (Exp. IV) had a very low level of $E + IT_{R}$ measure. Moreover, the difference between groups 1N and 2N was very small. The relations among the medial groups of dogs (Fig. 10), were similar to those observed in corresponding groups of normal dogs.
Fig. 9. Rate of extra- and intertrial responses in consecutive blocks of sessions for the normal dogs. The thin lines refer to dogs trained in groups 1 and the thick lines refer to dogs trained in groups 2.

Fig. 10. Rate of extra- and intertrial responses in consecutive blocks of sessions for the medial dogs. Denotation as Fig. 9.
The relations between the groups of dogs with lateral prefrontal ablation (Fig. 11) were different to those observed in normal and medial groups. No difference between the level of $E^+ ITRs$ of 1L and 2L groups in asymmetrical reversal learning (Exp. I and III) was observed. During tone and click reversal training with symmetrical reinforcement (Exp. II) the level of this index in group 1L was much lower than the level for 1N and 1M groups from this experiment. The dogs making reversal of white noise intensities (Exp. IV) had a very few $E^+ ITRs$, similarly to the remaining subjects trained in this experiment.

A 3-way ANOVA (quality $\times$ arrangement of stimuli $\times$ prefrontal operation) showed that the influence of the stimulus arrangement on the $E^+ ITR$ index was significant ($F_{1;32} = 10.36, P < 0.005$), however, the interaction of this variable with the prefrontal surgery was not significant.

Speed of responding depended on different factors at the beginning and the end of reversal learning. Analysis of the effects of kind of differentiation task and arrangement of stimuli on median latency of instrumental responses on CS$^+$ trials showed a significant effect of quality of stimuli at the beginning (Block 1) of reversal learning ($F_{1;36} = 8.84$,
During tone and click reversal learning the latencies of responses to CS' were shorter than in white noise intensity reversal learning. However, at the end of training (Block 10) the crucial factor affecting the latency of instrumental responses was the kind of differentiation task \((F_{1,38} = 4.20, P < 0.05)\), and the median latencies of responses were shorter for the subjects trained under symmetrical than under asymmetrical procedure of reinforcement.

Response latencies to CS', before the reversal learning (Block 0) were compared to the same measure at the beginning of reversal (Block 1) and augmentation of responding during the first second of CS' action was found \((P < 0.001, \text{one-way binominal test, 29})\). It might be supposed that the enhancement of instrumental responses executed with

### Table III

<table>
<thead>
<tr>
<th>Experimental factors</th>
<th>Variations of independent variables</th>
<th>(r_s)</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiation task</td>
<td>asymmetrical</td>
<td>0.696</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>symmetrical</td>
<td>0.422</td>
<td>(P &lt; 0.05)</td>
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<td>Quality of stimuli</td>
<td>tone vs. click</td>
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<td>(P &lt; 0.01)</td>
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<td></td>
<td>white noise</td>
<td>0.316</td>
<td>NS</td>
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<tr>
<td>Stimulus arrangement</td>
<td>groups 1</td>
<td>0.624</td>
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</tr>
<tr>
<td></td>
<td>groups 2</td>
<td>0.488</td>
<td>(P &lt; 0.05)</td>
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<td>Surgery</td>
<td>lateral</td>
<td>0.771</td>
<td>(P &lt; 0.01)</td>
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<tr>
<td></td>
<td>medial</td>
<td>0.303</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>normal</td>
<td>0.615</td>
<td>(P &lt; 0.05)</td>
</tr>
</tbody>
</table>

* Spearman rank order correlation coefficient, one-way test (29).

The short latencies was related to the increase in number of E+ITR index in Block 1. The data shown in Table III indicate that most of the variables a significant positive correlation between the increase in short latency instrumental responses frequency and the increase of the E+ITR values.

**Reversal with the alteration of the type of differentiation task.** As mentioned in the methods section, for five dogs (four from Exp. II, groups: 1L, 2L, 1M, 2M and one from Exp. IV, group 1L) in the course of reversal learning not only were the signalling values of conditioned stimuli changed, but also the kind of differentiation task was changed so that after forming a symmetrical differentiation they received re-
versal training with the asymmetrical procedure. Interestingly, none of
the tested dogs reached the reversal criterion. Similarly to the subjects
described earlier, difficulties in the reversal training were not due to
a retarded acquisition of avoidance response to CS$^+$ but rather to re-
tarded inhibition of responses to CS$^-$. Dog D-26/70 had a smallest
(460) number of avoidance responses to the tone CS$^+$ and a highest
(33) number of inhibited responses to the click CS$^-$, whereas the
other dogs tended to respond to every CS presentation. Comparisons of
response latency distributions (Table IV), reveal that at the beginning

<table>
<thead>
<tr>
<th>CS$^+$</th>
<th>Number of dog</th>
<th>Group</th>
<th>Comparison of cumulative distribution of response latencies</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Difference</td>
</tr>
<tr>
<td>Tone</td>
<td>D-26/70</td>
<td>1L</td>
<td>$S_0 &lt; S_1$</td>
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<tr>
<td></td>
<td>D-24/70</td>
<td>1M</td>
<td>$S_0 &lt; S_1$</td>
</tr>
<tr>
<td>Click</td>
<td>D-27/70</td>
<td>2L</td>
<td>$S_0 &lt; S_1$</td>
</tr>
<tr>
<td></td>
<td>D-25/70</td>
<td>2M</td>
<td>$S_0 &lt; S_1$</td>
</tr>
<tr>
<td></td>
<td>D-11/71</td>
<td>1L</td>
<td>$S_0 &lt; S_1$</td>
</tr>
</tbody>
</table>

* $P < 0.05$;  *** $P < 0.001$ Smirnov two-tailed test (7).

of reversal learning (Block 1) latencies of responses to CS$^-$ were sto-
chastically shorter than CS$^+$ for the preceding Block 0. For this mea-
sure the performance of these subjects with a change of the different-
iation task to the asymmetrical procedure did not differ from the per-
formance of corresponding groups of dogs making the reversal with the
unchanged symmetrical procedure of reinforcement. The biggest dif-
fferences involved the E + 1TR index. As seen in Fig. 12 the mean
frequency of extra- and intertrial responses per min. was very high for
the medial prefrontal dogs, especially, when the reversal learning was
more difficult (group 1M). The differences between the 1M and 2M
groups were similar to those observed between other medial dogs from
Experiment II (see also Fig. 10). In contrast, after lateral cortex da-
mage the differences between the 1L and 2L groups were in the op-
posite direction, and, in the case of the reversal learning with CS$^+$
Fig. 12. Rate of extra- and intertrial response in consecutive blocks of sessions before (Block 0) and during reversal learning (Blocks 1-10) for the dogs with altered differentiation task.

more salient than CS' (group 2L), the level of E+ ITRs was higher than in the situation when the CS' was less salient than CS'− (group 1L) independent of the quality of conditioned stimuli.

The alteration of the differentiation task and the signalling values of CSi during the reversal learning caused a great enhancement of the excitation level, as measured by the frequency of E+ ITRs. However, this did not change the response relations among the groups of operated dogs in comparison to the operated subjects described in the previous part of this paper.

General behavior and emotional changes. The change in the signalling values of conditioned stimuli during reversal learning caused anxiety enhancement of all subjects. The dogs displayed various emotional reactions such as trembling, vocalization (barking, whining) and restless-
ness. During reversal learning with asymmetrical reinforcement the absence of response termination of the CS'- which had previously sig-
gnalled the likelihood of shock delivery, was the most stressogenic factor. Moreover, the addition of shock application with the CS'+, which before the reversal had never been associated with painful stim-
ulation, was also very stressogenic. The most quiet were the subjects trained with white noise intensity conditioned stimuli (Exp. III). Among them only small and transient increase of anxiety was observed.

The behavior of dogs which made the reversal under the sym-
metrical procedure of reinforcement was characterized by strong anxiety enhancement. At the beginning of reversal learning the dogs restrained from instrumental responding to the new positive stimulus (CS') which resulted in shock application after 5 s. On the other hand, instrument-
al barpress responses during negative trials not only did not interrupt the CS' (CS before the reversal) but also caused shock delivery. This contingency increased the frequency of barpress responses on negative trials at the beginning of reversal training. As a result of this a drastic increase of extitation was observed. Dogs tried to escape from the harness, they bit the pedal and harness, and vocalized. These symptoms were similar to those observed during the early stages of differentiation training and were maintained for a very long time.

**Histology**

The histological verification of the prefrontal lesions made in dogs from Experiment I and Experiment II were presented earlier (18, 19). Here, only the verification of damages made in prefrontal cortex of subjects from Experiment III and IV are presented.

As seen in Figure 13 and 14, the prefrontal cortex lesions on both sides of hemispheres were large, as intended. On the medial aspects of the hemispheres (Fig. 13) the pregenual area (PG I, PG II and PG III, according to Kreiner, 23) were removed completely, or almost completely (D-3/71). The medial parts of the proreal gyrus (PR) were bilaterally removed in all subjects. The polar area (POL) was bilaterally damaged in all subjects with the exception of one dog (D-3/71) which has only a partial lesion of this area. As intended, partial removal of the anterior part of the medial precruciate cortex (XM I and XM II areas) were made in all subjects. There were also small incisions in the anterior part of genual gyrus (G). The subgenualinis area (SG) was removed completely or almost completely in two dogs from Experiment III (D-2/71 and D-13/71). Incisions of the pregenual fibers bilaterally occurred in two dogs (D-13/71 and D-21/70) and unilaterally in three
Fig. 13. Reconstructions of the medial prefrontal lesions in dogs from Experiment III and Experiment IV.
Fig. 14. Reconstruction of the lateral prefrontal lesions in dogs from Experiment III and Experiment IV.
dogs from Exp. III. The largest cortical area was ablated in dog D-2/72 and the smallest area was damaged in dog D-3/71.

At the lateral side of the hemisphere (Fig. 14) the lateral parts of proreal gyrus (PR, PRL I and PRL II) and the larger parts of orbital cortex (ORB I' and ORB") were completely ablated in all subjects with the exception of one dog from Experiment IV (D-11/71) which had only partial ablation of area ORB I". Posterior parts of orbital cortex (ORB II and ORB III) were bilaterally removed in one subject (D-5/72) and unilaterally in one dog (D-20/70) both from Exp. IV. In the depth of the presylvian sulcus the anterior parts of PORD' and PORD" areas were bilaterally ablated in three dogs from Experiment III, and one dog from Experiment IV (D-5/72). This dog also had complete ablation of the polar area (POL), and a partial unilateral lesion of the subproreal gyrus (SPR I area). Generally, there were small differences between damaged areas among the lateral subjects.

It is worth mentioning that the lesions of the prefrontal cortex of dogs trained in Experiments III and IV did not differ from the analogous damages made in prefrontal areas of subjects from Experiments I and II.

**DISCUSSION**

*Factors influencing retention of differentiation.* According to the design of the study, the retention of the original differentiation was tested twice: (i) after 10 days rest period given to all subjects (21) and (ii) after the prefrontal surgery or the control rest period for the normal dogs.

Data presented in this study indicate that effects of three main independent variables such as type of differentiation task, quality, and arrangement of stimuli were similar during the two retention tests. However, after the second rest period the influence of type of differentiation task was less significant. This was the result of consolidation of go, no-go differentiation which appeared most clearly among the normal dogs trained with white noise intensities.

The normal subjects trained in tone/click differentiation showed better performance after the second rest period only when the tone was used as the CS+ and click as the CS− (group 2N). In the opposite stimulus arrangement (group 1N) the criterion was reached more slowly and more errors appeared on the negative trials. This phenomenon was also observed after the first rest period and it seems to be an effect of the reflexogenic strength of the click conditioned stimulus. Click,
as a very salient stimulus, caused in the case when the barpress response did not terminate the CS− a strong secondary negative reinforcement. As a result, better response inhibition to the click CS− than to the tone CS− was observed. This mechanism was described earlier in the Kowalska and Zieliński study (21). Moreover, the differences between the 1N and 2N groups might be explained also by generalization of the response to click as a very reflexogenic stimulus. Although during original differentiation training, generalization between the click and the tone conditioned stimuli was very small (particularly in the asymmetrically reinforced procedure), during the latter stages, when differentiation was well established, the response conditioned to click, a very salient stimulus, might be generalized to tone, a less salient stimulus. As a result of this in groups 2N in which click was the CS−, the inhibitory influence of this stimulus was seen even on positive trials, and the escape response to the tone CS+ appeared. In contrast, in groups 1N less errors to the click CS+ were observed than to the tone CS−, causing the disinhibition syndrome, particularly in the symmetrically reinforced procedure, in which the generalization processes were more intensified, as was suggested in the preceding paper (21).

Aside from the variables used in these experiments the level of consolidation of the conditioned reflexes seems to be a very important factor. In the preceding study (21) it was shown that after a control pause of 10 days the go, no-go differentiation was disturbed only in dogs trained with the asymmetrical procedure of reinforcement. This fact might provide an explanation for the divergence between our results and data obtained by Dąbrowska (10) on the retention of defensive reflexes after prefrontal cortex lesions. She obtained impairment of the differentiation performance after the partial prefrontal lesions, but the subjects from her experiment were not submitted the preoperative retention period and were damaged immediately after they reached the criterion of differentiation learning.

Factors influencing reversal learning. The course of reversal training revealed similar effects of the main experimental factors like those observed in original differentiation learning. During reversal the transfer of avoidance responding to the new positive stimulus was very rapid, while inhibition of responding to the previously positive conditioned stimulus was very retarded. Similar to the beginning of differentiation learning at the very beginning of reversal learning a strong generalization between responses elicited by the new positive and negative CSi was observed. In contrast to the beginning of the original differentiation learning this generalization appeared in both asymmetrically
and symmetrically reinforced procedures. So, the most important factor determining the speed of reversal learning was the quality of conditioned stimuli. However, at the end of reversal training, when the subjects can detect the new signalling meaning of the conditioned stimuli, dogs trained under the symmetrical procedure made fewer errors on negative trials and made avoidance responses with shorter latencies than dogs trained in the asymmetrical procedure. A similar phenomenon at the end of the original differentiation learning was also observed.

Reversal was also affected by the arrangement between conditioned stimuli. The data showed that the reversal was more difficult for groups 1 (CS' less salient than CS') than for groups 2. This conclusion was supported by the influence of the stimulus arrangement factor on the magnitude of extra- and intertrial responses, a measure of the subjects' level of excitation as evoked by the experimental situation (18, 20, 21, 35). Generally, a higher level of E + ITR index in groups 1 than in groups 2 was observed during the reversal learning.

**Medial lesions effects.** The results indicate that the behavior of dogs is modified by prefrontal ablations. After medial lesions, dogs required during retention test a similar number of sessions to reach the differentiation criterion and made a similar number of errors to the CS-, as did normal dogs. This was true when the easily discriminated conditioned stimuli were used (tone and click) or when the CS+ was more salient than CS-. But, when the test conditions were more difficult e.g., the dogs differentiated intensities of white noise, and the CS- was more salient than the CS+, the medial subjects required more sessions to reach the criterion, and made more errors not only to the CS- but also to the CS+ than did normal dogs. Moreover, the medial prefrontal dogs had a highest level of extra- and intertrial responses in symmetrically reinforced differentiation in comparison to other animals.

During reversal training the medial dogs were marked by lack of the stimulus arrangements and the type of differentiation factors effects on the level of performance on CS' trials. As a result, both in symmetrical and asymmetrical procedures medial dogs discriminate the tone CS' vs click CS' stimuli better than did the normal dogs. But, when they did the reversal of signalling values of white noise intensities, such differences between medial and normal dogs were not observed. However, the magnitudes of the E + ITR indices were for groups 1M higher than for groups 2M similarly, as for the normal dogs trained with the same arrangement of the stimuli. Thus, it may be concluded that for medial dogs, stimulus arrangement factor had no effect on the rapidity of CS' extinction, but as in normal dogs it affected
the general level of emotionality expressed by the \( E + I TR \) magnitude. Moreover, medial dogs exhibited the highest level of the \( E + I TR \) measure also during reversal learning with asymmetrical reinforcement after symmetrical differentiation training. These results suggest that dogs subjected to the medial prefrontal lesions are more sensitive to the arousing factors of the experimental situation than are normal and laterally damaged dogs. This suggestion is supported by the data obtained in experiments testing the tone frequency generalization of alimentary reflexes (1, 2) and defensive reflexes (2, 22, 33). The dogs with medial prefrontal lesions were the most sensitive to the reinforcement density. The generalization tests in these studies were carried out either in blocks of test trials among partially reinforced trials in the daily session, or as a mass generalization during complete extinction sessions. When generalization was done in blocks of trials, the medial dogs had the broadest generalization gradients, whereas during the mass generalization test, under complete extinction, the medially damaged subjects showed the strongest extinction effect. It was postulated before that the drive disinhibition syndrome resulting in enhancement of responding is a specific effect of the medial prefrontal lesions in dogs (3, 4, 8, 9, 17). However, the results obtained in this study and also in the generalization tests indicate that after these lesions either the enhancement or the inhibition of acquired behavior could be obtained depending on the experimental paradigm.

**Lateral lesions effects.** The results provide evidence that the postoperative retention was not disturbed after lateral prefrontal lesions. However, the postsurgical comparison of the \( E + I TRs \) levels revealed that, opposite to normal dogs, after lateral damages there were a smaller number of these responses in symmetrically than in asymmetrically reinforced procedures. Similarly, some dogs with lateral prefrontal lesions required more trials to reach postoperative criterion of differentiation with asymmetrical than with symmetrical reinforcement.

During reversal learning the lateral prefrontal lesions changed the direction of differences between the groups of dogs trained with the two opposite CS arrangements, relating to normal dogs. The reversal learning with the \( CS' + \) tone and the \( CS' - \) click was mastered better by laterally lesioned than normal dogs, whereas the reversal with \( CS' + \) click and \( CS' - \) tone was mastered with greater difficulty by laterally damaged than by normal dogs. Similar relations between lateral and normal dogs trained with two intensities of the white noise were observed. The differences between the 1L and 2L groups in the numbers of \( E + I TRs \) emitted during reversal training were also markedly re-
duced in comparison to the relative groups of normal and medially damaged dogs.

All of these data indicate that the discrimination ability was not disturbed after lateral prefrontal lesions. The results of the generalization tests done under alimentary and defensive reinforcement as well, after lesion of the lateral prefrontal cortex (1, 2, 22, 33), confirmed this suggestion.

On the ground of the results obtained after the partial (lateral or medial) prefrontal lesions in dogs, it may be concluded that in differentiation of defensive reflexes, prefrontal cortex damage did not change drastically but only modified the dogs' behavior. The strength and the direction of these modifications are related to the variables used in experimental situation.

This investigation was supported by Project 10. 4.01.4 of the Polish Academy of Sciences.

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Accepted 10 December 1985