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Function of Prefrontal Cortex in Timing Behavior of Monkeys

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Three groups of monkeys—with prefrontal lobectomy, cingulectomy, and normal controls—were trained on the DRL operant conditioning schedule. Lever presses by the subjects resulted in food reward, provided no response was made during a predetermined delay period. Subjects were first trained on a 10-sec delay to the criterion of 50% rewarded responses in three consecutive sessions. The delay was then lengthened by steps of 5 sec to a maximum of 70 sec. All subjects met criterion on delays as long as 25 sec. On longer delay settings subjects in each group failed to meet criterion. During the course of training, subjects reduced their response rates and responded with unimodal interresponse time distributions during criterion sessions, with mean interresponse times near the delay settings. The findings that prefrontally ablated and cingulectomized monkeys were unimpaired in reducing their response rates and in developing timing responses are contrary to the hypotheses that frontal lobes are essential to the inhibition of responses, recent memory, or temporal patterning of behavior.

Introduction

Experimental investigations of the behavioral role of the prefrontal lobes have indicated a persistent impairment by lobectomized animals on tasks of delayed response (2, 3, 4, 6) and delayed alternation (1, 5). On the basis of these experimental findings several hypotheses have been proposed, namely, that prefrontal cortex is implicated in temporal patterning of behavior (2), recent memory (2, 3), or inhibitory processes (4). In arriving at these interpretations particular consideration has been given to the experimental requirements that the subject adapt his responses to a temporal delay. The significance of prefrontal lobe functions in timing behavior as such, however, has not been clearly established.

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With the development of operant schedules of reinforcement it is now possible to investigate timing behavior in animals more precisely. The operant schedule that has been found most suitable for this kind of investigation is the differential reinforcement at low rates (DRL) schedule (11), where the subject is rewarded for pressing a lever, provided it has refrained from responding for a predetermined delay period. Analyses of response patterns for animals trained on this schedule clearly express timing behavior, with measures of interresponse times as functions of the delay period (9). In the present experiment two aspects of timing behavior were examined: the ability of monkeys to delay lever presses for relatively short periods on delay settings of 10 to 25 sec; and the longest delay on which each monkey can give timing responses. Prefrontally ablated monkeys were trained together with normal and cingulectomized control groups. The hypothesis was examined that prefrontally ablated monkeys are impaired, compared to the other groups, in developing adequate patterns of timing behavior.

Method and Procedure

Seventeen immature rhesus monkeys were used. Before the start of the experiment six monkeys[2] (group F) had bilateral ablations of dorsolateral prefrontal cortex and four operate control monkeys (group C) were subjected to bilateral cingulectomy. Seven unoperated subjects (group N) served as normal controls (one monkey in this group died during the course of the experiment).

Each subject was tested in a portable cage ($16 \times 13 \times 22$ inches) that was placed in a sound-absorbing converted icebox. The front of the cage faced a white lucite panel from which a lever protruded 1 inch into the cage. A food cup was beneath the lever. A dim overhead light provided constant illumination, and a blower provided air circulation and a masking noise.

Control and recording panels were located in an adjacent room. The subjects were trained on the DRL schedule of reinforcement. For this schedule the first lever press in each session was rewarded with a 48-mg dextrose pellet, and subsequent presses were rewarded only if they occurred after a predetermined delay period. If the monkey pressed during the delay, the timer reset so the subject had to wait again until the delay terminated before it could receive a reward. Concomitant with each

[2] The terms "frontal lobe" or "frontal cortex" refer to dorsolateral prefrontal cortex, anterior to arcuate sulcus.

reward a white light behind the lucite panel was turned on for 2 sec. Responses and rewards were recorded on counters, a cumulative recorder, and an Esterline Operation Recorder.

After the monkey had learned to jump into the testing cage, it was adapted to the apparatus and the lever pressing procedure by 30-min daily sessions, with the delay first set at 2 sec, then at 5 sec, and finally at 7.5 sec until at least 100 rewarded responses were made in one session on each of these delays. Individual subjects required a maximum of eight sessions on this procedure.

Each subject was given 40-min daily sessions, generally six sessions per week. The delay was first set at 10 sec and training continued until the criterion was attained of at least 50% rewarded responses on each of three consecutive sessions, or during the first, second, and fourth of four consecutive sessions. On the following day the delay was increased to 15 sec and training continued until criterion was again attained. This procedure of step-wise increments in delay by 5 sec was continued to the maximum delay setting of 70 sec, or until a subject failed to reach criterion. If a subject did not attain the criterion on a given delay setting after twenty-five testing sessions, that delay was increased by 5 sec and testing was continued for a maximum of fifteen sessions. Monkeys which attained criterion on this lengthened delay were continued in the experiment. Monkeys which failed again were discontinued from this phase of the experiment.

Results

Anatomy. The intended limits for prefrontal ablations were from frontal pole to anterior bank of arcuate sulcus and from midline to orbital surface, including the banks and depth of principal sulcus. As represented by Fig. 1, cortex within these limits was successfully ablated, except for sparing of the tip of one frontal pole in subject 507.

The surgical procedure and landmarks for cingulate ablations were similar to those previously described (8), except that resection of cingulate cortex continued posteriorly, approximately to the level of the splenium of corpus callosum. Reconstructions of these ablations (Fig. 2) show nearly complete destructions of cingulate cortex, with occasional damage to adjacent cortical structures and to corpus callosum.

Responses for delay settings: 10 to 25 sec. The criterion of 50% rewarded responses in three sessions was met by all subjects for delay

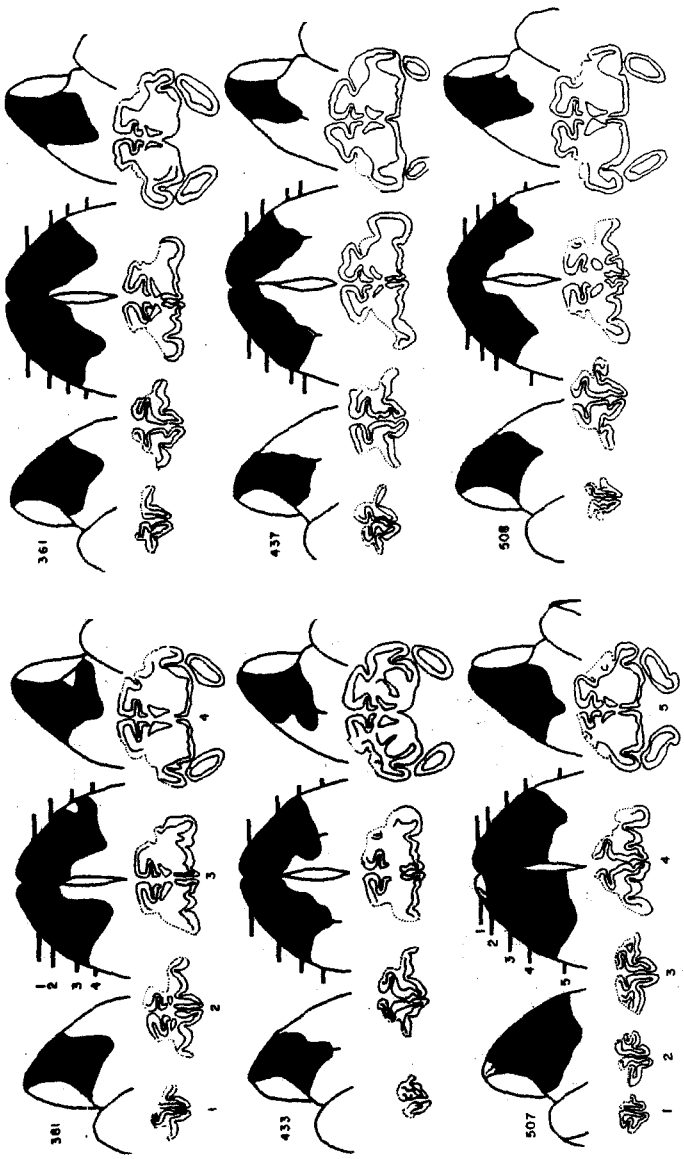

FIG. 1. Reconstructions of dorsolateral prefrontal ablations. Ablated areas are indicated in black. For each brain the cross sections correspond to the levels indicated for 381, except for those of 507.

settings up to and including 25 sec. For the longer delay settings, however, some subjects in each group failed to meet the criterion. The present section will present the results for all subjects on delays up to 25 sec.

Each of the groups of monkeys required about the same number of training sessions for this phase of testing. Group medians for all training

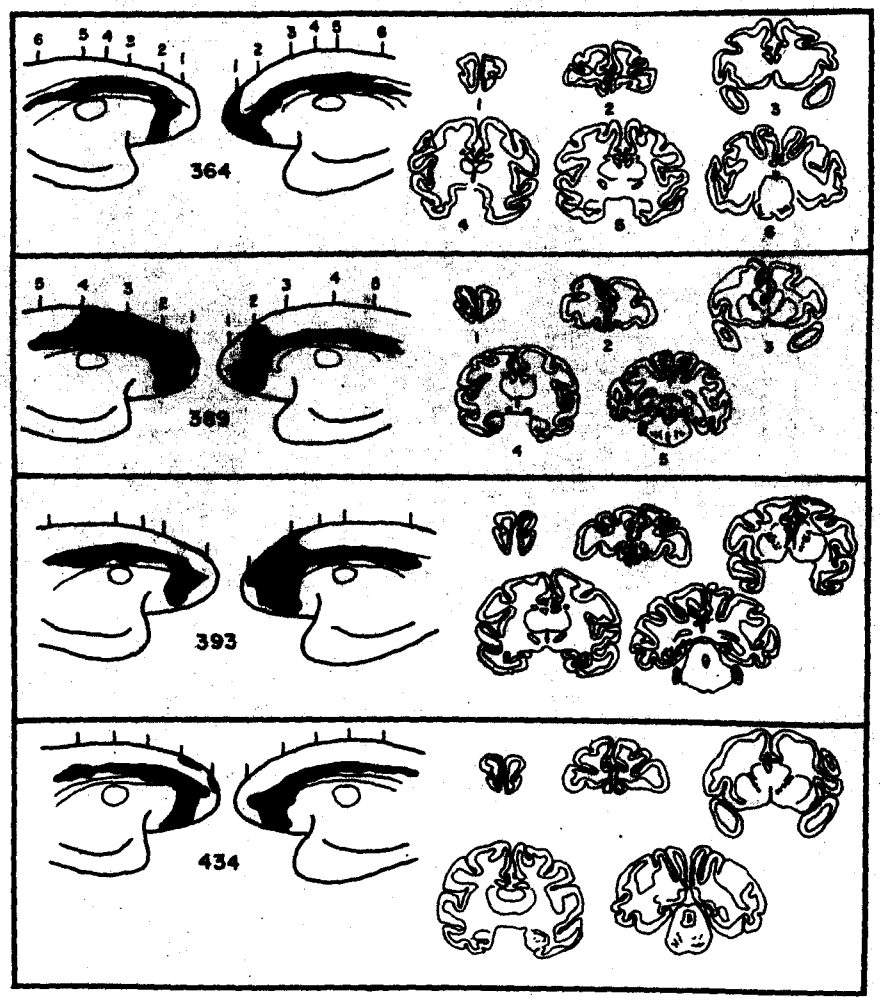

FIG. 2. Reconstructions of cingulate ablations. Ablated areas are indicated in black. For each brain the cross sections correspond to the levels indicated for 389.

sessions, including those on 25-sec delay, were: 46 for group N, 45 for group F, and 53 for group C.

The groups of subjects differed markedly in their response rates (mean number of responses per minute) during the initial sessions on the 10-sec delay setting, as seen in Fig. 3. The high response rate of the prefrontally ablated monkeys (10.3 presses per min) is an expression of general hyper-

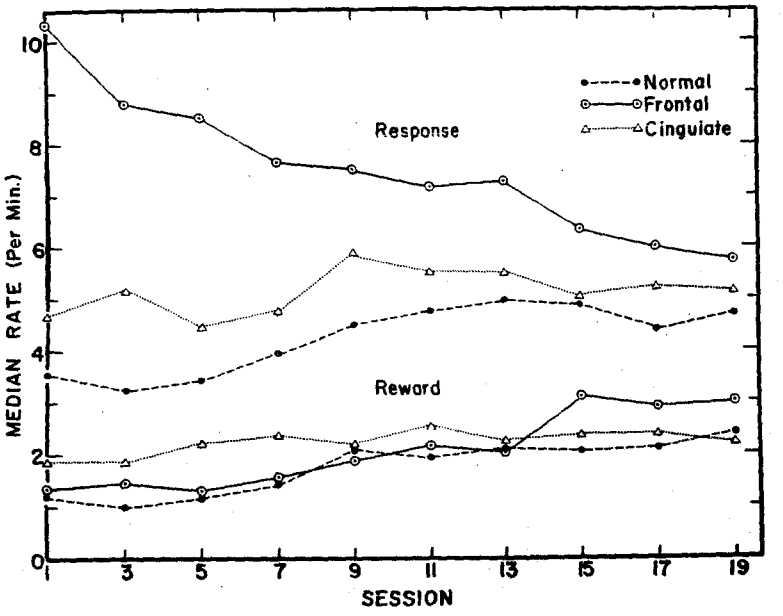

FIG. 3. Group medians of response rates (upper curves) and reward rates (lower curves) for three groups of subjects on alternate days under delay setting of 10 sec.

activity, which has been reported in other investigations. During the course of training (median of 19 days) on the 10-sec delay these subjects systematically reduced their response rates, so that they attained criterion within the same number of training sessions as required by the normal (median of 20 days) and cingulectomized groups. The normal subjects modified their distributions of interresponse times during the course of training. During the initial sessions rewarded responses were frequently followed by series of rapid lever presses, and then the subjects waited for periods much longer than 20 sec, before again responding. During the

criterion sessions normal subjects responded after interresponse intervals which were distributed around the 10-sec delay setting.

The reward rates during testing on the 10-sec delay (Fig. 3, lower curves) show only slight differences among the three groups, except for somewhat higher initial rates by group C. The reward rates for each of the groups increased during training from rates on the first day of 1.2, 1.3, and 1.85 rewards per minute, respectively, for groups N, F, and C, to maxima during the criterion sessions of 2.6, 3.0, and 2.85, respectively, for each of these groups.

Timing behavior was evaluated by analyses of interresponse times (IRT); i.e., the intervals between successive lever presses. These data were obtained from the Esterline Operation Recorder traces. Most of the subjects obtained clearly unimodal IRT distributions, with mean values near the delay settings, during the 10-sec criterion sessions and during subsequent testing. For the criterion sessions on the 25-sec delay the means and standard deviations of IRT distributions were computed for every subject. One subject in each of groups N and F obtained unusually high mean IRT and standard deviations on this delay. The normal monkey (384) met the criterion only because it responded at exceptionally low rates, and its IRT distributions for all delay settings were highly positively skewed. There is no indication that this subject ever developed patterns of timing responses. The prefrontal subject (508) also responded with highly skewed distributions during criterion tests on the 25-sec delay. On the shorter delays, however, its IRT distributions were only slightly skewed, and the standard deviations were within normal limits.

Group means of interresponse times and standard deviations were computed for all subjects, except the two mentioned above. These statistics (Table 1 and Fig. 4) express no appreciable differences among the groups in timing responses. The results presented thus far indicate that under the present experimental procedures, all monkeys, with the exception of one normal and possibly one prefrontal, were able to develop adequate patterns of timing behavior. In no respect were the prefrontally ablated monkeys deficient, compared to the normal or cingulectomized subjects.

Delay settings of 30 to 70 sec. During the course of testing with gradually increasing delay settings, monkeys in all groups failed to meet the criterion. Thus, only four of seven normal monkeys met criterion on the 35-sec delay and two of six subjects (one monkey died) on the 70-sec delay. Of the six prefrontal subjects, four met criterion on the 60-sec

delay and three on 70-sec delay. Only two of the cingulectomized monkeys met criterion on the 30-sec delay and none did so beyond the 55-sec delay. Group medians for maximum delays passed were: 50 sec for group N, 65 sec for group F, and 35 sec for group C.

TABLE 1

MEAN INTERRESPONSE TIMES AND STANDARD DEVIATIONS IN SECONDS

Delay (sec)	Normal group			Prefrontal group			Cingulate group		
	N[a]	Mean	SD	N	Mean	SD	N	Mean	SD
25	6	27.1	11.1	5	25.9	9.5	4	26.3	8.8
30	6	32.8	12.2	4	33.1	10.5	3	32.1	9.7
50	3	54.2	22.5	4	51.7	20.6			
60	2	65.9	15.1	4	64.4	22.1			

[a] Number of subjects.

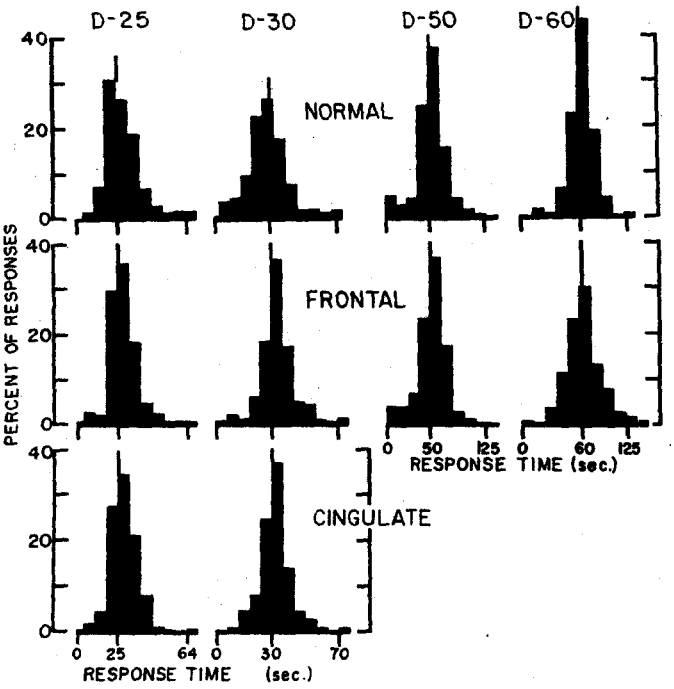

FIG. 4. Interresponse time distributions for three groups of monkeys on DRL schedule. Only subjects which met criterion on the delay settings are included (see Table 1). Units of interresponse times are 6.4 sec for 25- and 30-sec delays, and 12.8 sec for 50- and 60-sec delays.

Among the monkeys which met criterion on the longer delay settings, the prefrontal subjects appeared to require somewhat less training than did the other groups. Figure 5 presents curves of cumulative number of training sessions for subgroups of the three normal and four prefrontally ablated monkeys that met criterion on the 55-sec delay. For delays longer than 30 sec the prefrontal subgroup attained each criterion consistently more rapidly than did the normal monkeys. For the five delay settings of 35 to 55 sec the prefrontal subjects required from twenty to forty-six

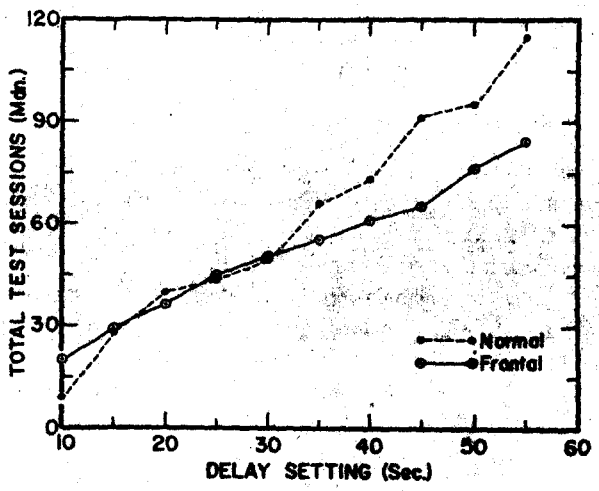

FIG. 5. Cumulative number of training sessions on delay settings of 10 to 55 sec for subgroups of monkeys (four prefrontal, three normal), which met criterion on these delays.

sessions compared to thirty-eight to eighty-five sessions for the normal subjects. Although there was overlap in these scores between the groups, the rapid learning by the ablated subjects is impressive, especially the performance of two prefrontal monkeys which responded at criterion on several successive delays during the first three sessions on each setting.

Interresponse times were computed for those subjects which met criterion on delay settings of 30, 50, and 60 sec. Mean interresponse times and standard deviations for the subgroups are shown in Table 1 and Fig. 4. These results clearly express distributions of timing responses for each of the subgroups, with mean IRT slightly above the delay settings.

The significance of shifts in means of the IRT distributions for criterion

sessions between successive delay settings was evaluated by *t*-ratios for the difference between means. For the shift from 25- to 30-sec delay the *t*-ratios were significant beyond 1% probability for the four normal, five prefrontal, and two cingulate subjects which had met criterion on the 30-sec delay. All of the ablated subjects which had not met criterion on this delay obtained significant *t*-ratios ($p < 0.01$) for differences between means on shorter delays. For the remaining two normal monkeys, however, which obtained wider IRT distributions than did the others, *t*-ratios were significant ($p < 0.01$) only for shifts over a 10-sec range (25 to 35 sec). For each of the two normal and four prefrontal monkeys which met criterion on the 60-sec delay, the shift in mean IRT from the 50- to 60-sec delay was statistically significant (*t*-ratio at $p < 0.01$).

Discussion

Under the procedure of gradual delay increments on the DRL schedule, as used in the present experiment, the subject was required to reduce its response rate to a very low final level, and to refrain from responding for a period approximately equal to that of the delay setting. We found that on this schedule the animals generated unimodal interresponse time distributions. The findings that all prefrontally ablated subjects met these requirements without impairment, and that some ablated monkeys met criterion on the 70-sec delay setting, appear contrary to the hypotheses that frontal lobes are essential to the inhibition of responses (4), recent memory (2, 3), or temporal patterning of behavior (2).

The distributions of interresponse times (Fig. 4) with the method of gradual delay increments are similar to those reported by other investigators (e.g., 9), except that we did not observe the high proportions of very short IRT (less than 2 sec), which in Sidman's report comprise 15% to 45% of the total number of responses. In that experiment rats were placed on the 20-sec DRL schedule after only brief preliminary training and for testing sessions of 2-hour duration. Thus, it appears that the incidence of short IRT is dependent upon the experimental procedure employed. Gradual increments in delay and training to a high criterion level on each delay setting result in very low proportions of short IRT. Further evidence in support of this finding has been obtained in a subsequent experiment (unpublished) where delay increments of 60 sec resulted in high incidence of short IRT, with bimodal IRT distributions.

On the basis of the results of the present experiment the behavioral deficits found in prefrontally ablated monkeys on tasks of delayed

response and delayed alternation cannot be adequately explained in terms of unitary processes, such as short-term memories or response inhibitions. The behavior of brain-damaged monkeys seems affected not only by the experimental task, but is also influenced by the experimental procedure employed and by the subjects' prior testing experiences. Several of these variables have been investigated in other experiments with prefrontally ablated monkeys (7) and by means of electrical stimulation of intact frontal cortex (10).

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