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Sequential behavior after modified prefrontal lesions in the rat

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Ablation of the "orbital" prefrontal area, which includes the dorsal bank of the rostral third of the rhinal sulcus and the ventral surface of the frontal pole, strongly impaired performance of a new task that required sequential manipulation of two different and spatially distant manipulanda. Performance of the same task was only mildly, but significantly, affected by dorsomedial prefrontal lesions. The two groups did not significantly differ from sham-operated controls in the rate of barpressing for continuous reinforcement, in extinction of two operant responses, or in spontaneous alternation. Unexpectedly, the present variant of sequential behavior was more affected by the "orbital" prefrontal lesion than by the lesions of the cortex, which, in the rat, combines the medial prefrontal cortex and anterior cingulate areas and has been considered to be involved in the sequencing of behavioral chains.

According to neuroanatomy, the anteromedial cortex in the rat is a telescoped counterpart of the primate frontal eye field, dorsolateral prefrontal area, and the medial frontal cortex, including the anterior cingulate area (Divac, Kosmal, Björklund, & Lindvall, 1978; Krettek & Price, 1977; Leonard, 1969). The primate orbital cortex not only corresponds to the dorsal bank of the rostral third of the rhinal sulcus (Leonard, 1969), but also includes the ventral surface of the frontal pole, anterior to the fusion with the anterior olfactory nucleus (Divac et al., 1978; Krettek & Price, 1977).

Ablations of the anteromedial frontal area in rats result in breakdown of the normal sequence in behavioral chains (Michal, 1973; Slotnick, 1967; Thomas, Hostetter, & Barker, 1968). For example, operated mothers are capable of carrying their pups, but they show impaired retrieval of the litter back to the nest (Slotnick, 1967). Since impairments in complex behaviors, such as maternal or sexual ones, could be induced by a variety of dysfunctions, some of which have been attributed to damage of the prefrontal cortex (e.g., Brutkowski, 1965; Mishkin, 1964; Nauta, 1971; Pribram & Tubbs, 1967), we decided to test further the notion of involvement of the anteromedial cortex in the sequencing of behavior. We did so by determining what effects the ablation of the two subdivisions of the frontal cortex had on a simple task that required the sequential operation of two manipulanda. If the anteromedial area indeed regulates sequencing or temporal ordering of behavior, its lesion should impair performance of the present task. Two further tasks were employed: extinction of two learned operant responses (presumably sensitive to orbital lesions; Butter, 1969; Butter, Mishkin, & Rosvold, 1963; Kolb, 1974; Kolb, Nonneman, & Singh, 1974), and spontaneous alternation (found sensitive to medial ablations in the rat; Divac, Wikmark, & Gade, 1975).

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METHOD

Apparatus

Sequential behavior and extinction were studied in operant conditioning units—the "Student Research Model" of Ralph Gerbrands Company. This operant chamber contains a lever mounted on one wall, a rod that protrudes into the chamber from its ceiling, a signal lamp near the lever, and a reinforcement magazine into which 45-mg pellets are delivered accompanied by an audible click. Relevant responses in this chamber can be made either by pressing the bar or by displacing the rod from the vertical. The chamber was enclosed in a sound-shielded box, in which a fan produced masking noise and secured an exchange of air and a lamp provided constant illumination. A video camera was attached to the ceiling of the box and connected to a monitor outside the box. Solid state equipment controlled the experiment and recorded the barpresses.

Spontaneous alternation was studied in an open, light gray, one-unit T-maze with 22-cm-high walls and 12-cm-wide corridors. The stem was divided by a transparent guillotine door into a 20.5-cm-long startbox and a 24.5-cm-long runway. The length of each arm was 44 cm. At the end of each arm, a 4.3-cm-high metal barrier blocked the last 5.5 cm of the arm (these barriers conceal food cups in food-reinforced experiments). The maze was placed in the middle of a well-lit room in which other rats were present during testing.

Subjects

We used 15 male Wistar albino rats with a history of barpressing on a CRF-schedule in test chambers similar to the present one. They weighed approximately 300 g at the beginning of the experiment. The rats lived in single cages in a permanently lit room with water always available. They were fed commercial rat chow once daily after training and were maintained at approximately 90% of their ad-lib body weights during the study of sequential and extinction behavior. During the spontaneous alternation experiment, they had ad-lib access to food in their home cages. The rats were divided randomly into three equal groups: sham, and two groups with ablations of either the dorsal medial or orbital isocortex (see Figure 1A).

Procedures

Sequential task. Since the animals had had prior experience with test chambers of this type, habituation and magazine training were unnecessary. On Day 1, the rats were briefly reshaped, then given 15 min of continuous reinforcement (CRF) training, during which the signal lamp was permanently lit. The same training was given on Day 2. On Day 3, the animals began training on the sequential task in which a barpress would be reinforced only if it had been preceded by a rod displacement; only the first barpress following a rod response would be reinforced. The first barpress of the session was reinforced regardless of whether or not it had been preceded by a rod displacement. When the session began, the signal lamp was lit; it remained lit until the first barpress. Each rod response lit the signal lamp, and each reinforced barpress turned the lamp off. Thus, the light signaled that the next barpress would be reinforced. For each session, an efficiency ratio was arrived at by calculating the number of reinforcements as a percentage of the number of barpresses. A 60-min session was given on Days 3 and 4 and a 20-min session was given on Day 5. From Day 6 to the day of criterion performance, the animals had two daily 20-min sessions separated by 6 h. This training continued until the animal reached an efficiency ratio of 90% or better in each of three consecutive sessions. (The sessions on Days 3 and 4 did not count as criterion sessions, even when the efficiency ratio was at the required level.)

When a rat reached criterion, it was given a 10-day pause, followed by a retention test consisting of a 20-min session on each of 3 consecutive days. After the last session of the retention test, the animal received ad-lib food and was operated on the day following.

Following surgery, the animal was allowed a 10-day recovery period, at the end of which it was brought again to 90% of its normal weight. During the postoperative test and relearning period, the animal was tested for 20 min each day. The first three sessions were regarded as a postoperative retention test. Training continued until the animal reached the preoperative criterion.

Extinction of rod contact. After reaching criterion on the sequential task, an animal waited 1-5 days and then received one retraining session on the same task. Following retraining, the rat was given a 15-min session on each of 2 consecutive days. The signal lamp was now permanently lit, and each barpress provided a pellet regardless of the rod displacements. With the aid of the video camera and monitor, the experimenter registered the number of rod contacts (displacement of the rod off the vertical was not required). The number of rod contacts as a percentage of the number of barpresses was calculated.

Extinction of continuous reinforcement (CRF). During each of the following 3 days, 10 min of CRF barpressing was followed by a 5-min extinction period in which barpresses produce neither a pellet nor a click from the pellet dispenser. The signal lamp was kept lit throughout the session. The number of barpresses made during the extinction period was calculated as a percentage of the number of responses made in the CRF period.

Spontaneous alternation. Following the CRF extinction experiment, an animal received ad-lib food for 11 days. On the 12th day, its alternation behavior was tested. Each test consisted of two runs. The rat was placed in the startbox and immediately released. After it entered one of the arms, the rat was kept there for 60 sec, with the aid of a transparent door. The rat was then transferred to a cage for 15 sec, returned to the startbox of the maze, and immediately released again. After entering one of the arms, the rat was kept there for 60 sec, as before. Three such sessions were given. There was always a pause of 3 days between the sessions.

During all behavioral procedures, the experimenter was kept ignorant about which group an individual rat belonged to.

Surgery

The animals were anesthetized with Equithesin. All ablations were made by subpial suction with help of an operating microscope. The orbital lesion was made with the lateral approach, following retraction of the eye and a slit in dura above the rhinal sulcus. For further details, see Divac, Gade, and Wikmark (1975).

Histology

After the spontaneous alternation experiment, the rats were deeply anesthetized and transcardially perfused with saline followed by a 10% Formalin solution. Following perfusion, the brains were removed and allowed to sink in a 10% Formalin solution containing 20% sucrose. The brains were then rapidly frozen in isopentane cooled to −50°C and cut in a cryostat. The Nissl-stained sections were examined and the lesions were reconstructed with the aid of a microfiche reader.

RESULTS

The lesions are illustrated in Figures 1B and 1C.

The medial lesions invaded the cortex, which probably corresponds to the monkey frontal eye field, lateral prefrontal cortex, and anterior cingulate area. The cingulum, corpus callosum, and tenia tecta were not damaged.

The orbital lesions removed rostrally not only the suprarhinal, but also the ventral cortex of the frontal pole to the medial surface of the hemisphere. More caudally, the suprarhinal area alone was lesioned

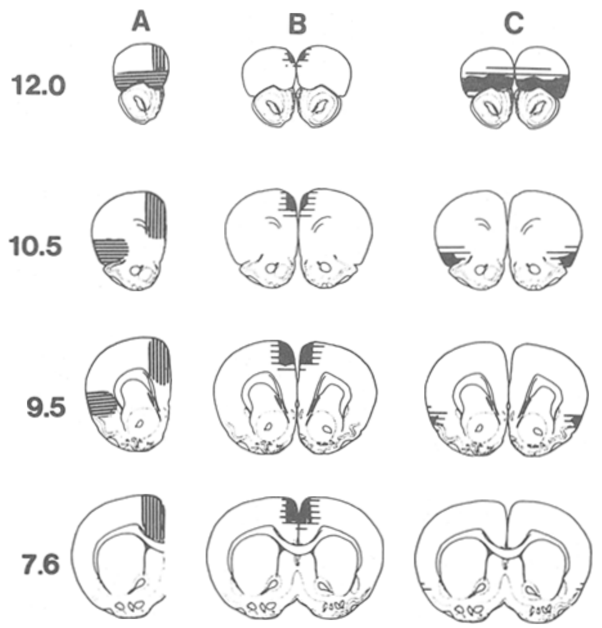

Figure 1. (A) Intended lesions. (B) Lesions of the medial cortex. (C) Lesions of the orbital cortex. Black = the tissue removed in all animals. Horizontal stripes = the tissue removed in at least one rat.

Table 1
Individual Percentages of Sequential/Total Barpresses During Pre- and Postoperative Retention Tests

Preoperative Days			Postoperative Days			
1	2	3	1	2	3	D*
			Control			
92.1	97.3	94.5	90.5	94.7	94.3	4.0
90.8	97.6	97.1	97.4	95.2	91.3	−0.3
93.2	97.3	98.3	93.6	92.4	98.8	4.7
97.7	98.2	99.0	95.2	100.0	100.0	3.8
88.8	97.1	93.2	91.2	89.4	87.7	2.8
			Dorsomedial			
93.3	94.9	95.9	75.8	91.2	88.4	20.1
91.4	98.6	98.1	91.7	96.4	96.9	6.4
90.5	96.8	99.4	91.9	83.3	90.2	7.5
95.0	99.4	100.0	84.9	87.3	79.2	15.1
91.0	97.8	98.2	78.3	96.3	92.0	19.9
			Orbital			
86.1	96.3	94.0	66.0	71.8	76.8	28.0
96.5	99.4	98.9	47.6	65.8	75.0	51.3
96.8	97.4	99.2	74.6	86.5	93.5	24.6
86.6	94.5	92.9	46.9	73.4	77.0	46.0
98.9	96.5	99.0	78.2	97.5	100.0	20.8

**Difference between the scores on the 3rd preoperative and 1st postoperative day.*

without involvement of either the claustrum or the dorsolateral cortex. Figure 1A indicates the extent of intended lesions based on previous anatomical results (Divac et al., 1978).

Both groups that received lesions were transiently impaired in retention of the sequential task. The orbital lesions produced a stronger and a longer lasting impairment: median days to criterion postoperatively were 0 for the control group, 3 for the "dorsomedial" group, and 5 for the "orbital" group. Table 1 shows the group differences, which were highly significant for both the preoperative-postoperative difference and the postoperative scores on the first day (Kruskal-Wallis test, $p < .01$; Siegel, 1956). The group differences after the first day are not statistically significant because of the small size of the groups and the rapid improvement shown by one of animals with orbital lesions. Profoundly affected by the lesions are three animals from the "orbital" group (Table 1). No correlation was found between the size of either lesion and degree of impairment in the sequential task.

Other measures of performance in the present experiment showed no significant group differences and no suggestive trends: Rod contacts were counted in extinction after the completion of the postoperative criterion in the sequential task and calculated as percentages of the number of barpresses during the same session. The percentages varied considerably in each group. The ranges around medians on the 2 test days were 1.1-27.8-115.4 and 0.0-1.9-26.3 for the control group, 5.1-21.4-42.0 and 0.0-2.1-12.8 for the "dorsomedial" group, and 5.3-9.9-67.6 and 1.1-2.4-5.6 for the orbital group. Extinction of barpressing after CRF revealed no significant differences between the groups (Table 2). In spontaneous alternation, one rat in the control group and two in each of the operated groups made one perseverative response in three trials.

Table 2
Individual Postoperative Performance During Extinction (EXT) of Continuous Reinforcement (CRF)

Day 1			Day 2			Day 3		
No. of Responses		Percentage	No. of Responses		Percentage	No. of Responses		Percentage
10-min CRF	5-min EXT	EXT/CRF Ratio	10-min CRF	5-min EXT	EXT/CRF Ratio	10-min CRF	5-min EXT	EXT/CRF Ratio
				Control				
74	39	52.7	82	11	13.4	84	7	8.3
79	18	22.8	85	11	12.9	84	13	15.5
80	12	15.0	85	8	9.4	83	22	26.5
50	27	54.0	60	17	28.3	62	17	27.4
80	44	55.0	78	20	25.6	79	14	17.7
				Dorsomedial				
37	20	54.1	52	12	23.1	48	17	35.4
64	19	29.7	71	27	38.0	72	14	19.4
43	50	116.3	43	20	46.5	44	11	25.0
66	25	37.9	67	19	28.4	66	11	16.7
69	28	40.6	67	17	25.4	71	10	14.1
				Orbital				
60	29	48.3	55	22	40.0	64	9	14.1
19	41	215.8	60	33	55.0	61	18	29.5
50	19	38.0	54	15	27.8	53	6	11.3
50	18	36.0	52	12	23.1	52	12	23.1
74	16	21.6	74	9	12.2	70	9	12.9

DISCUSSION

The main result of this experiment is the separation of groups with the modified orbital and medial prefrontal ablations in performance of the present sequential task. It seems that the impairment in this task cannot be attributed to "disinhibition" of either responses or drive (Brutkowski, 1965), since no overresponding was noted during the extinction test. The effects of mediofrontal ablations on other sequential behaviors (Michal, 1973; Slotnick, 1967; Thomas et al., 1968) can perhaps be attributed mainly to the destruction of the medioventral part of this area.

Absence of the effects of the ablations on the rate of continuously reinforced barpressing and on extinction appears to be contrary to previous findings (Brutkowski, 1965; Butter, 1969; Butter et al., 1963; Kolb, 1974; Kolb et al., 1974). It should be noted, however, that the suprarhinal lesions that impaired extinction in the rats included a substantial portion of the lateral wall of the hemisphere, thus extending beyond the projection field of the mediodorsal nucleus. Furthermore, there are many differences between the behavioral procedures in the cited investigations and the present study.

In a previous experiment (Divac, Wikmark, & Gade, 1975), rats with medial ablations seemed to respond randomly in the second run of spontaneous alternation. This finding could not be replicated in the present study. Whether this apparent discrepancy is due to differences in lesion site or in test conditions is not known. Spontaneous alternation, like the spatial reversal task, seems to be sensitive to prefrontal lesions only under special conditions, whereas delayed alternation has a higher sensitivity to lesion of the cortical target of the lateral part of the thalamic mediodorsal nucleus (Wikmark, Divac, & Weiss, 1973).

The present results with the sequential task cannot be plausibly interpreted before we know more about details of behavior during task performance and about other consequences of the modified prefrontal ablations.

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