

NEOSTRIATAL LESIONS IMPAIRED RATS' DELAYED ALTERNATION PERFORMANCE IN A T-MAZE BUT NOT IN A TWO-KEY OPERANT CHAMBER

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Abstract. Rats with lesions in the neostriatal region that belongs to the prefrontal system were trained in two versions of delayed alternation. They performed as proficiently as intact animals in a two-key operant chamber. The same operated rats took many more trials to reach criterion when subsequently compared with the same control group in a T-maze. This finding demonstrates that variants of delayed alternation are not equivalent for animals with lesions in the prefrontal system. Observations suggested that delayed alternation in the operant chamber may be mastered by positional mediation.

INTRODUCTION

Rats with electrolytic (19) or kainic acid (8) lesions in the region of the neostriatum that belongs to the prefrontal system (6, 16) are severely impaired in delayed alternation in a T-maze. On the other hand, synaptic blockers of cholinergic or dopaminergic transmission implanted in the same neostriatal region in rats induced only a marginal impairment in rats alternating (without imposed fixed delay) between two bars in an operant chamber (A. Gade and I. Divac, unpublished results). One possible explanation of this discrepancy is that the two variants of this task are not equivalent: in a T-maze each run starts from a box into which the animal is placed by hand, and the delay (the time between "acquisition" of the essential information on the previous trial and the moment of the operant response) is spent outside the T-maze. Therefore, the possibility for positional mediation is practically nil. In an operant chamber with two keys and a centrally-placed feeder, the

subject remains in the chamber during the delay, and positional mediation is possible. Some species, notably dogs, after prefrontal lesions perform successfully delayed alternation by means of positional mediation (10, 15). In the present experiment we compared directly the performance of the same group of rats in two variants of delayed alternation: in a T-maze and a two-key operant chamber. The rats had lesions of the neostriatal region associated with the prefrontal cortex (4, 6).

METHODS

Subjects. Seven adult, male, experimentally naive, Wistar albino rats participated in the experiment. They lived in individual cages in a colony room with a 12 h on-off light cycle, on at 6.00 a. m. The animals were kept at 80% of their ad lib. body weight.

Surgery and histology

Four rats (L1–L4) received an electrolytic lesion in the anteromedial neostriatum [19] 40 days before the start of training. Three rats (N1–N3) remained unoperated throughout the experiment. After training the operated animals were perfused in deep anesthesia, their brains were cut in a cryostat and the lesions reconstructed from Nissl-stained sections in a microfiche reader.

Apparatus

An operant chamber (29×26×24 cm) was placed in a sound-attenuating cubicle. On one wall the box had a food tray and three transparent nose keys; the central key was not used. The peripheral keys, 1.8 cm in diameter and 9 cm center to center, were placed 16.5 cm above the floor grid. A press of at least 0.1 N (= 10 g) was recorded as the key response. Each key could be lit from behind by a 3 W bulb. Each key was surrounded by ring-shaped (diameters 1.8 and 5.0 cm) metal probes connected to a proximity detector adjusted to detect the presence of the rat within a distance of about 2 cm from the nose key. The food tray was placed centrally, 1 cm above the floor and behind a 5×6 cm flap. A pellet dispenser delivered into the tray a 45 mg pellet accompanied by an audible click and a 0.5 s illumination of the tray. A video camera in the cubicle was connected to a monitor in a neighbouring room. Solid state equipment was used for programming and recording of responses.

The T-maze was described in detail previously [12]. In short, it was an open, light gray, one-unit T-maze; the stem was divided by a transparent guillotine door into a startbox and a runway. At the end of each arm a metal barrier concealed a food cup. The maze was placed in a well lit room in which other rats were present during testing.

Behavioral procedure

General procedure. Following surgery the rats were left to recover for 40 days (the last part of this period consisted of a deprivation period in which their body weights were gradually reduced to 80% of the ad libitum body weight). They were

first trained in the operant chamber. After this training the rats were given *ad libitum* food and left for another period of 40 days without training (in the last part of this period they were again gradually reduced to 80% of their *ad lib.* body weight). Then the T-maze experiment started.

Operant chamber. Two rats with lesions (L1, L2) underwent extensive shaping in order to develop mediating behavior (description below).

The remaining rats (two operated: L3, L4; and three intact: N1, N2, and N3) received response-independent food pellets on a variable-time 2 min schedule for two sessions as magazine training. Then for one session, the left or right key would start blinking (0.2 s period). The experimenter delivered a food pellet and turned off the light when the rat approached the blinking key and soon (within 20 trials) rats pressed the lit key. After ten trials of responding to the blinking key, the procedure changed to the proper delayed alternation (DA) task. The rats remained in the chamber and the remainder of this session was treated as the first session with the DA task.

Sessions began with the onset of blinking light on both side keys. A response to either key produced pellet delivery and turned off the key lights. After a fixed delay the blinking lights on both keys were turned on again and activation of the key on the opposite side to the key chosen on the previous trial delivered a pellet and turned off the key lights. Activation of the same key as on the previous trial turned off both key lights and did not deliver a pellet. In the latter case the same trial reoccurred until activation of the key on the other side. This response delivered a pellet. Thus each trial had the same stimulus conditions, with pellet delivery contingent on activation of the key opposite the key chosen on the immediately preceding trial. The delay was 5 s until the rats alternated on at least 70% of the trials. The delay was then increased gradually to 10 s as the performance improved. There were 100 trials per session during the 16 sessions run with the terminal 10 s delay.

The two lesioned rats (L1 and L2), which received shaping of mediating behavior, first had magazine training for two sessions and then shaping of responding to a lit key for one session, as the other rats. To shape appropriate pre-trial mediating behavior, the stimulus onset was made contingent on the rats being on the side of the chamber where the stimulus would turn on. After the stimulus onset, pellet delivery was contingent on the activation of the lit key. The stimuli alternated strictly among left and right, with a minimal interstimulus interval of 6 s. Initially, the experimenter turned on the stimulus when the rat's head pointed toward the side of the chamber where the stimulus would come on. Gradually, the requirement was increased so that eventually the entire body of the rat should be on that side before the stimulus turned on. The procedure continued for five sessions.

After this phase, for two sessions both keys were turned on simultaneously when the rat was on the "correct" side of the chamber. In addition, the key on the "incorrect" side was a steady white light rather than a blinking light. At this stage

a correction procedure was introduced, so that activation of the same key as on the previous trial turned off both key lights and did not deliver a pellet. In such cases the same trial recurred until the activation of the "correct" key.

During the last approximation to the DA task, which lasted for two additional sessions, the stimulus on the "incorrect" side was the same as the stimulus on the "correct" side. The procedure was now almost the same as the proper DA task, except that the experimenter turned on the key lights only when the rat was on the "correct" side of the chamber. The minimal delay between trials was gradually increased to 7 s for rat L1 and to 8 s for rat L2 during these two sessions.

Rats L1 and L2 were then transferred to the proper DA task with no intervention on the part of the experimenter during sessions. The delay was gradually increased to 10 s for rat L1 and to 15 s for rat L2.

T-maze. In the T-maze, all seven rats were given identical treatments. The procedures of this training have been described in detail previously (13). Rats were habituated to the maze (with free access to food in both arms) 15 min daily for 2 days. On day 3 the rats were shaped by being repeatedly placed in the start box and released. If the rats did not enter one of the arms within 5 s after their release, they were removed from the maze and given a 15 s delay before the next trial. The shaping continued until the animal in 20 consecutive trials entered either arm within 5 s after its release. After shaping the rats were given 21 trials each day. The first trial was always reinforced; for trials 2 to 21 the rats were reinforced only when they entered the arm not visited on the preceding trial. As reinforcement, the rats were allowed to eat mashed rat chow for 8 s. The delay between the end of one trial and the start of the next was 8 s after reinforcement and 16 s after non-reinforcement. The training was terminated when the rat reached a proficiency of at least 90% correct choices in 5 consecutive days (calculated on the pooled results from all 5 days) and no more than 3 errors on any of these 5 days.

RESULTS

Anatomy. The lesions, illustrated in Fig. 1, destroyed the anteromedial part of the neostriatum, and were in their position and extent similar to the lesions made in earlier experiments (8, 14, 19), in which they strongly impaired delayed alternation and related behaviors.

Behavior

Operant chamber. Both operated and control rats eventually alternated on almost all trials. Figure 2 shows the percent of trials with alternation for each rat and session. For lesioned rats L1 and L2 with explicit training of differentiated mediating behavior, the shift from experimenter-controlled to time-controlled trial onset did not markedly disrupt alternation. Before the shift the rats, by procedural

necessity, alternated on each trial (i. e., 100% alternation). The shift brought about a decrease to about 80% alternation which subsequently improved to highly accurate terminal levels. For lesioned rats L3 and L4 with no shaping of mediating behavior and no prior history other than magazine training and shaping of respon-

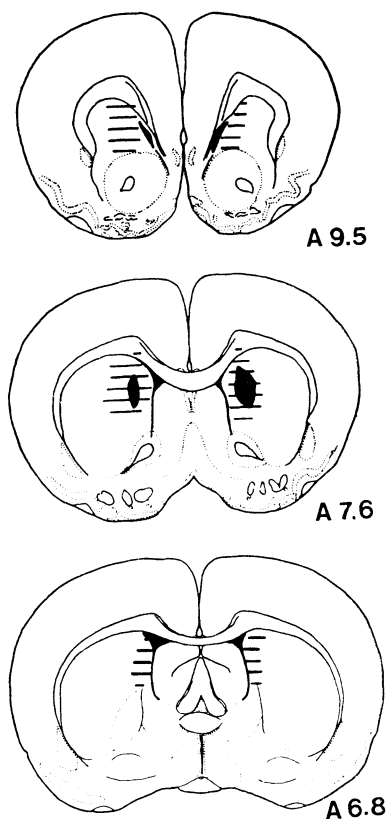


Fig. 1. Schematic illustration of the lesions. Black: the tissue destroyed in all animals. Horizontal stripes: the tissue destroyed in at least one rat.

ding to lit keys, alternation increased from about chance level to the same high level as for rats L1 and L2. For unlesioned rats N1, N2, and N3 with similar histories as rats L3 and L4, alternation also gradually increased to high terminal levels. The gradual and uneven increase of the delay across the rats prevented a quantitative comparison of acquisition across training conditions. However, the lesioned rats L3 and L4 certainly did no worse than unlesioned rats.

The lesioned rats L1 and L2 maintained the mediating behavior the experimenter had established during pretraining. The lesioned rats L3 and L4 as well as the unoperated rats N1, N2 and N3 all developed mediating behavior much resembling

that explicitly developed for the rats L1 and L2. Figure 3 illustrates in segments of event records of trials, nose key recording, proximity to the keys, and experimenter-recorded mediating behavior.

In all conditions, a few seconds after pellet retrieval the rat typically went to the side of the chamber opposite to where the last response was reinforced. The rat then

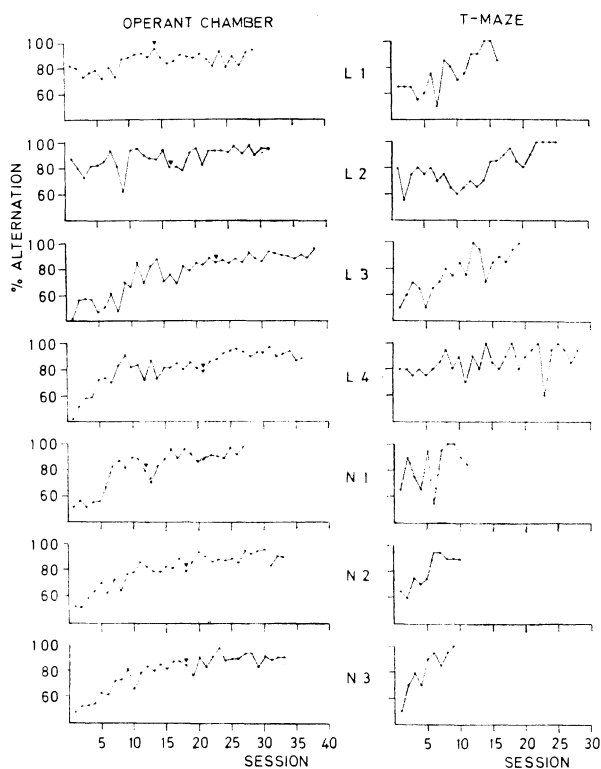


Fig. 2. Individual day-to-day performance of the experimental rats in the two variants of delayed alternation. The animals L1 and L2 alone were shaped to use positional mediation in the chamber. The groups differ only in the T-maze. The lengths of training in the two tasks cannot be compared because only in the operant chamber task the delay was gradually extended. Triangles indicate the first session with the terminal delay in the operant chamber.

remained on that side as evidenced by both the recorded proximity to the key and key responses on that side prior to trial onset. Then after trial onset, the rat pressed the key on that side. Deviations from this pattern invariably resulted in failures to emit the "correct" nose-key response. Two deviating patterns dominated. With one pattern, the rat would go to the "correct" side after pellet retrieval, but would then shift side, orient to the key on the other "incorrect" side, and press that key after trial onset (A's in Fig. 3). With the other pattern, the rat would go to the

“incorrect” side immediately after pellet retrieval, remain on that side, approach the key, and after trial onset press the key on that, “incorrect” side (B’s in Fig. 3). Occasionally, rats went to the side just reinforced and then shifted side later in the trial (C’s in Fig. 3) or alternated between sides late in the trial (D in Fig. 3).

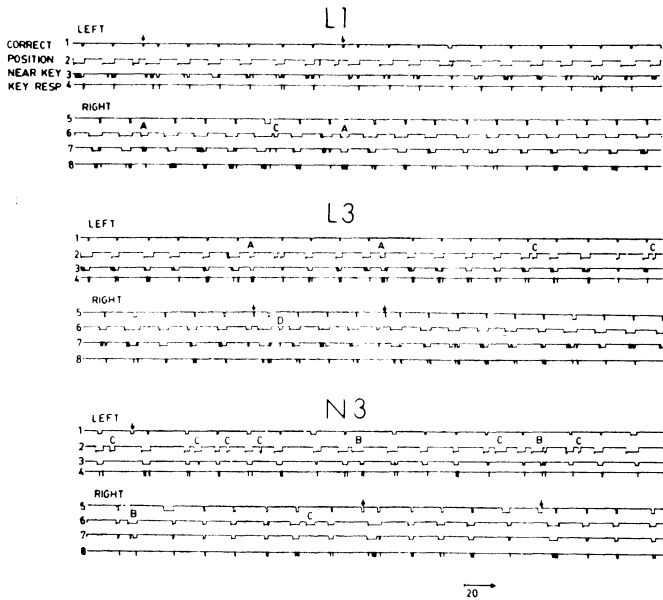


Fig. 3. Segments of event records for one rat for each condition. Pens deflect downwards for activations. Lines 1 and 5 show the “correct” side for the next response. Lines 2 and 6 show experimenter recorded position of the rat during the delay; the head and at least half of the body should be over the chamber midline before the experimenter recorded position to a side. Lines 3 and 7 show automatically recorded proximity to the keys. Lines 4 and 8 show nose-key activation. Trials with failures to emit a “correct” response are indicated by arrows. Data appear for the rats L1 (lesioned and shaped to mediate), L3 (lesioned, no shaping of mediating behavior) and N3 (unlesioned, no shaping of mediating behavior).

The performance can be described as a three-stage pattern: the first stage is alternation in position within the chamber after pellet retrieval, the second stage is maintenance of the position through the delay, and the third stage is responding to the nearest key after light onset. Thus, failures to emit a “correct” alternation response to the key *after* trial onset resulted from disruptions of positional mediation *prior* to trial onset.

T-maze. As shown in Figure 2 all rats eventually reached criterion in the T-maze version of the delayed alternation task. However, the lesioned rats took a considerably longer time to reach criterion. The number of sessions required for the rats to reach criterion in the T-maze were: L1 : 16, L2 : 25, L3 : 19, L4 : 28, N1 : 11,

N2 : 10 and N3 : 9. The total number of errors made by the individual animal during the entire training period were: L1 : 78, L2 : 101, L3 : 87, L4 : 78, N1 : 37, N2 : 39 and N3 : 33. In both measures, the absence of overlap between the groups indicates significant group differences.

DISCUSSION

Neither the small groups nor the differential training of the lesioned rats invalidates the present basic observation: formally the same task may or may not detect brain damage, depending on the circumstances of its presentation. In our experiment the groups were not significantly different in one but were so in the other apparatus (Mann-Whitney $n_1 = 3$, $n_2 = 4$, $U = 0$, $P = 0.028$, one-tailed test because only impairment of the lesioned animals could be expected (17)). It is unlikely that the lesioned rats developed the delayed alternation impairment during the prolonged postoperative period, because such an impairment was observed soon after similar lesions in earlier experiments (8, 14, 19).

The present procedures established that rats with a neostriatal lesion within the prefrontal system are unimpaired in delayed alternation in an operant chamber with nose keys. Explicit training of differentiated mediating behavior turned out to be unnecessary for the development of alternation. All rats developed mediating behavior of a very similar topography.

The two variants of delayed alternation used in the present experiment are not equivalent. The task in the 2-key operant chamber did not detect the prefrontal system damage, which was clearly demonstrated by subsequent testing in the T-maze. Earlier investigators, who claimed to have shown impaired delayed alternation in an operant chamber following lesions in the prefrontal system (2, 9) used tasks that differed from the "classical" delayed alternation in which an uninterrupted sequence of identical trials is presented. In one of these experiments rats were reinforced for alternating between two bars; each error was punished by a time out in darkness. A response following an error served only to initiate a new alternation sequence (2). In the other experiment, the same investigators required rats to press sequentially first the left and then the right bar for one reinforcement (9). In comparison with these methods our procedure which formally followed the classical delayed alternation paradigm is not difficult to master for rats with damage of the prefrontal system. Different outcomes of formally similar tests when given in manually and automatically driven apparatuses have been described earlier (1, 11). Results obtained with monkeys (1) are very similar to the present ones. Absence of the screen and gradual increase of the delay in the automatic apparatus were considered responsible for different outcome in two variants of delayed alternation.

The results point to the possibility that our operated animals showed no impair-

ment, as compared with the normal controls, in the operant chamber, because they could rely on positional mediation, thereby decreasing or eliminating the effects of imposed delay. In other words, individuals with prefrontal lesions from some species, under some conditions, perform successfully in the tasks with imposed delay by assuming a body position right after the directional cue (external or internal) becomes available. This position is maintained until the release signal. In this way a delay, regardless of its objective length, is in practice reduced to zero. This interpretation is in agreement with the use of mediating strategies by all present rats, irrespective of the shaping procedures. Similarly, Lawicka (10) demonstrated that dogs with prefrontal ablations recover proficient performance of delayed alternation by positional mediation. Finally, monkeys with prefrontal ablations under so-called zero second delay condition are unimpaired. As soon as the delay is lengthened, a significant number of errors appears (18). It should be pointed out, however, that prefrontally lesioned cats as well as rats can perform efficiently delayed alternation even when the positional mediation is made impossible (e. g. (3) and present data in the T-maze).

An unexpected outcome of the present experiment was the ultimate mastering of delayed alternation in the T-maze. In previous experiments (19) rats with similar lesions showed no relearning. The unexpected ability of the present operated rats to master delayed alternation in a T-maze may be attributed to an incomplete lesion (in spite of apparent similarity with the earlier, more effective lesions), positive transfer of training in the operant chamber, or an unusually long period of postoperative recovery.

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