

The Development of Food Search Behavior by Rats: The Effects of Hippocampal Damage and Haloperidol

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Food deprived rats were required to locate four pellets of food located in 4 of 16 holes in an enclosed arena. Three groups of animals were studied in 11 testing sessions: rats with bilateral hippocampal damage; rats with bilateral neocortical damage; and an unoperated group. Half of each group received haloperidol and half received saline injection 20 min before Sessions 4 through 10. No injections were given on the first three sessions or on the final, 11th session. Animals with hippocampal lesions visited more nonfood holes than control animals and did not develop consistent sequences of food-hole visits. The administration of haloperidol reduced the number of consistent sequences of food seeking behaviors by intact animals without significantly affecting the efficiency of performance as measured by the number of nonfood holes visited. Haloperidol reduced the number of visits to nonfood holes of animals with hippocampal lesions.

Animals with extensive bilateral hippocampal damage perform poorly on behavioral tasks in which information about spatial location is important (Douglas and Isaacson, 1966; Olton and Isaacson, 1968; O'Keefe and Dostrovsky, 1971; O'Keefe, *et al.*, 1975; O'Keefe, 1976). The animals misuse the information about the environment and repeat the selection of particular locations (Olton, 1977) and they also tend to have a preference for spatial hypotheses in problem solving (Isaacson and Kimble, 1972). Using birds, Oades (1976) has shown that limbic lesions disrupt normal searching strategies in the environment.

This paper reports the results of an investigation of the exploration and food searching of intact rats and rats with neocortical and hippocampal damage. There was no single correct method to solve the task. Each rat was allowed to develop its own method of finding food pellets located in 4

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out of 16 holes in an enclosed arena. Such a design allows for an analysis of the patterns of search and exploration used by the animals that is not severely limited by reward or response contingencies.

As an extension of this research, we undertook to evaluate the effects of the neuroleptic agent haloperidol on both lesioned and intact animals. This was based on observations that this drug and others that reduce dopaminergic effectiveness reduce the exaggerated number of responses made in DRL-20 tasks and the number of task irrelevant responses made in other operant tasks by animals with hippocampal lesions (Schneiderman and Isaacson, 1976; Fish, 1976). The question we wished to address was whether haloperidol would reduce the extraneous responses made by the lesioned animals in the rather free environment of the open field as well as in an operant test situation.

METHOD

Subjects

The subjects were 42 male Long Evans hooded rats from Charles River Farms. They weighed between 250 and 350 g at the time of surgery. There were three experimental groups: 16 animals with bilateral hippocampal (plus neocortical) lesions; 14 with bilateral neocortical lesions; and 12 unoperated controls. With the exception of the first three testing sessions and the last testing session (11th), all animals were tested after haloperidol or saline injections, i.e., Sessions 4 through 10. Of the animals with hippocampal damage, nine received haloperidol (Group H_D) and seven received saline injection (H_S); with neocortical damage, seven received haloperidol (N_D), and seven received saline injection (N_S); six of the intact animals received haloperidol (C_D), and six received saline (C_S).

Operative Procedure

All rats, including unoperated controls, were anesthetized with 50 mg/kg sodium pentobarbital anesthesia. All surgical procedures were performed in one stage using clean surgical techniques. Lesions were made by aspiration using the methods described by Isaacson and Woodruff (1975). The animals received 100,000 units of Bicillin im immediately after operation. At the end of the experiment the rats were perfused with saline followed by 10% formalin solution. The brains were then frozen and 20- μ m sections were cut and stained with thionin. These procedures resulted in bilateral lesions which involve 60 to 90% of the hippocampus as well as in removal of part of the overlying posterior neocortical surface. The lesions were comparable with those produced in studies originating in this laboratory (e.g., Woodruff and Isaacson, 1972). After surgery or anesthetization, the animals were allowed to recover for 2 to 3 weeks. When the rats had regained their preoperative weights they were placed

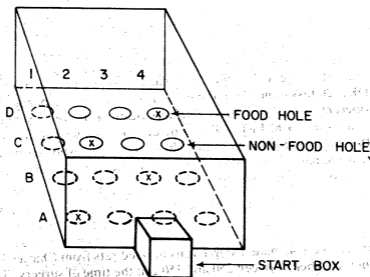


FIG. 1. Schematic drawing of testing apparatus. Numbers and letters designate rows and columns as described in text. The X symbol in the four holes indicates the placement of food for most subjects.

on a food deprivation schedule which maintained them at 80% of their preoperative body weight. They had free access to water at all times outside of the test arena.

Apparatus

The food-search tests were conducted in an arena measuring $70 \times 70 \times 50$ cm high. In the wooden floor were 16 holes, 3.5 cm in diameter, below which hung cups 2 cm deep. The adjacent holes were set 10 cm apart and peripheral holes were set 13 cm from the wall. Entrance was from a start box midway along one wall (Fig. 1). The arena was dimly illuminated by a 40-W lamp covered with red plastic film, 150 cm above the center of the arena. The rest of the room was dark.

Training

Animals were first exposed to the apparatus for 30 min on each of 5 consecutive days. For the first 2 of these 5 days no food was in any of the holes. For the remaining 3 days, one Noyes food pellet (35 mg) was placed at the bottom of every hole. All rats rapidly learned to visit every hole and to obtain the pellets. Two days later, formal testing began.

During testing, food pellets were located in holes A_1 , B_3 , C_2 , and D_4 , as shown in Fig. 1 for most rats. However to control for possible left or right preferences with respect to external cues, eight rats were presented with a mirror-image array of food pellet placements (i.e., A_4 , B_2 , C_3 , D_1). What-

ever pattern of food holes was selected for an animal, it remained consistent for that animal throughout training. Performance for the two patterns of reward placements was similar and results from both were combined. To keep food and odor cues to a minimum the floor and all food cups were cleaned after every trial.

A trial commenced with the raising of the door of the start box. The animal entered the arena almost immediately. A trial ended when the rat found and ate the fourth food pellet. All food pellets were eaten at the place where they were located. A visit to a hole was scored when the nose of the rat turned to the edge of a hole, moved over, or was placed into it. At the end of a trial, the rat was replaced in the start box and food pellets were placed in appropriate holes of the arena before the next trial.

Training sessions of 10 consecutive trials were given each morning and each afternoon for 5 consecutive days. The intertrial interval was approximately 20 sec. The intersession interval was never less than 4 hr. Thus, each rat received over 10 training sessions in a 5-day period.

Three days after the 10th session an 11th testing session was conducted. No injections were given before testing on this day.

Drug Administration

All injections given on Sessions 4 through 10 were given subcutaneously 20 min prior to testing. Volumes of physiological saline and haloperidol were given such that an animal received 7 ml of fluid per kilogram of body weight. All animals given haloperidol first received a 0.4 mg/kg dose. However, many animals particularly control animals, were inactive after this dose. They would move slowly, defecate, and sometimes become immobile for up to 2 hr. When this syndrome appeared, the dose of haloperidol was decreased for the next testing session. Doses were adjusted on an individual basis so as to achieve a dose that would allow normal locomotor levels. There were no significant differences between groups in the dose levels that allowed reasonable locomotor behavior. For example, on the final drug testing session, the mean dose given animals with hippocampal damage was 0.3 mg/kg, the mean dose given those with neocortical damage was 0.25 mg/kg, and the mean dose of the intact group was 0.275 mg/kg. However, even with decreased doses, marked behavioral changes were sometimes observed, especially lethargy and immobility. On sessions where this appeared, the immobile rat was removed from the arena and replaced at 15-min intervals until the rat began to move around the arena spontaneously.

All between group statistical comparisons were made by Mann-Whitney *U* tests, and probabilities presented are for two-tailed tests of independent samples.

RESULTS

Pattern of Hole Visits

No group exhibited a tendency to visit one particular hole first throughout all testing sessions, but during the course of a particular 10-trial session each rat tended to visit first one hole more often than any other. By the third session, rats with hippocampal damage revisited their preferred hole less than intact rats ($0.02 < P < 0.05$). This difference was maintained through the 11th session ($P < 0.05$). From the fourth session onward rats with hippocampal damage revisited their preferred first hole less than rats with neocortical damage alone ($0.02 < P < 0.002$).

The number of times the "first selected hole" was changed from one session to the next was higher for rats with hippocampal damage than either those with neocortical damage or intact animals ($P < 0.002$) from the third session onward.

The number of trials on which intact animals or those with neocortical damage visited their "first selected hole" was decreased by injection of haloperidol (see Fig. 2). This effect occurred for drug-injected animals with neocortical damage relative to their saline-injected counterparts from the seventh session onward ($P < 0.014$) and for drug-injected intact animals relative to those that received saline injections from the ninth session onward ($P < 0.042$). There was no significant drug effect for animals with hippocampal damage.

Haloperidol produced more frequent changes of the preferred first hole in Sessions 4 through 10 in animals with neocortical damage than similar animals given saline. No such effect was found in the other groups.

The largest number of trials in a session when a particular sequence of food-hole visits occurred (e.g., CBA, ABC, etc.) was termed a "preferred sequence." The data were considered separately for preferred sequences that were found on three or more consecutive trials within a session and those that did *not* occur on consecutive trials in a session. Results are reported for nonconsecutive sequences, but they are entirely consistent with the data from consecutive sequences.

Intact animals did not develop preferred sequences on more than 50% of trials until the later sessions (see Fig. 3). Animals with hippocampal damage repeated their preferred sequence *less* than intact animals ($P < 0.05$) and animals with neocortical damage ($0.002 < P < 0.02$) on Sessions 10 and 11. Animals with hippocampal damage also showed many more changes of preferred sequences from Sessions 3 to 11 than either of the other experimental groups ($P < 0.002$). Haloperidol treated intact animals and those with neocortical damage showed fewer preferred sequences than their saline-injected counterparts ($P < 0.042$ and $P < 0.008$, respectively). Animals with neocortical damage changed their preferred se-

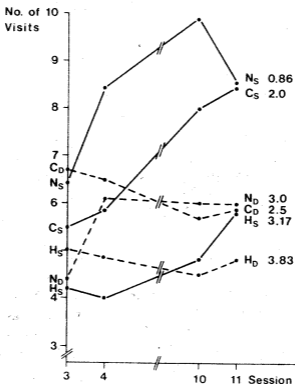


FIG. 2. The mean number of visits to a preferred "first hole" exhibited by the six groups of subjects on Sessions 3, 4, 10, and 11.

quence of hole visits more often after haloperidol than after saline injection ($P < 0.01$).

Efficiency

Animals with hippocampal damage visited many more nonfood holes than intact animals or those with neocortical damage ($P < 0.002$). There was no significant difference between the number of errors made by the latter two groups (Fig. 4).

Both intact animals and those with neocortical damage avoided nonfood holes more than animals with hippocampal damage ($P < 0.0002$). The percentage decreases of the mean number of visits to nonfood holes from Session 4 to Session 10 were smallest for animals with hippocampal damage (Group H_D, 38%; Groups H_S, 36%). Greater decreases were for the intact and neocortically lesioned animals receiving saline before testing (Group N_S, 68%; Group C_S, 75%) but those receiving the drug improved almost as much (Group C_D, 51%; Group N_D, 60%).

A significant increase in the number of visits to nonfood holes occurred on the last, nondrug Session 11 ($P < 0.016$) for animals with hippocampal

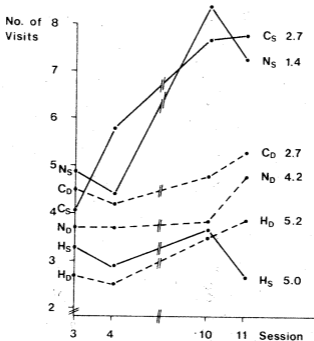


FIG. 3. The mean number of preferred hole-selection sequences (as defined in text) exhibited by the six groups on Sessions 3, 4, 10, and 11.

lesions receiving haloperidol. No influence of the drug on the number of visits to nonfood holes after the drug was found in the other groups on Session 11.

Data from Sessions 3 and 4 and from 10 and 11, as shown in Fig. 3, indicate changes of the patterns of behavior between the two groups of animals with hippocampal damage.

Between Sessions 3 and 4 the proportion of central (holes C_1 , B_2) to peripheral (holes A_1 , D_1) food holes visited increased for drug-injected animals with hippocampal damage with respect to their saline-injected counterparts. ($P < 0.016$). The number of nonfood holes visited (especially in the center) increased but showed no significant change ($P < 0.095$).

DISCUSSION

In this task rats were required to locate four pellets of food placed in 4 of 16 holes in an arena. The design allowed each rat to acquire an individual sequence of visits to these holes. Intact rats learned not to visit empty holes and acquired a preferred sequence of visits to food holes. From this it may be inferred that each rat acquired a strategy used in finding the holes containing food. Animals with hippocampal lesions vis-

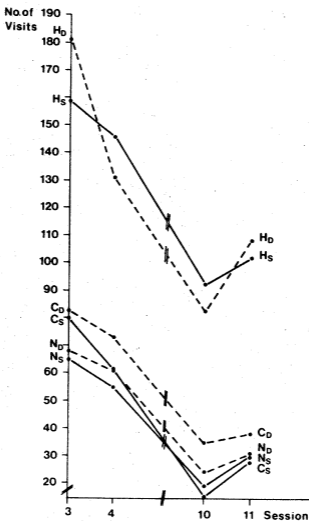


FIG. 4. The mean number of visits to nonfood holes made by the six groups on Sessions 3, 4, 10, and 11.

ited more nonfood holes and did not develop consistent sequences of food seeking.

Injection of haloperidol retarded the development of food hole sequences by intact animals without significantly affecting the number of nonfood holes visited, however animals with hippocampal damage made fewer visits to nonfood holes after haloperidol.

Animals with neocortical damage behaved like intact animals in most respects except that when tested after haloperidol they showed more changes in preference for the first hole to be visited and in their preferred hole-seeking sequence between sessions. This suggests that the neocorti-

cally damaged animals may be more sensitive to haloperidol than intact animals. A similar result was found in the experiments by Fish (1976) done in this laboratory in which animals with neocortical damage were disrupted in the performance of an operant visual discrimination task at lower doses of the drug than intact animals or those with hippocampal damage. In her dissertation, Fish found that haloperidol reduced the excessive number of responses made by animals while performing on the DRL-20 task and during "time out" portions of the operant visual discrimination task described in Woodruff and Isaacson (1972). She also found that animals with hippocampal lesions were *more* sensitive as measured by the effect of *d*-amphetamine given while the animals were performing a FR-6 task. Her results played an important basis for the present study.

The large number of visits to nonfood holes by animals with hippocampal damage may be related to locomotor hyperactivity. These animals were more active than those in other groups, and the proportion of nonfood hole visits decreased less for them than for the other groups over the course of testing. This deficit would also be consistent with the many descriptions of animals with hippocampal damage being impaired in withholding responses to a formerly positive stimulus or location (see Douglas, 1972; Isaacson, 1974, for review).

The reason why animals with hippocampal damage do not develop a preferred sequence of food-hole visits is open to several interpretations. They may have the impaired ability to use spatial maps of the environment suggested by Olton (1977), but they also may be less likely to change directions of movements rapidly in open areas. In fact, Kimble (1963) found that animals with hippocampal lesions performed poorly in simple mazes because there were relatively long open areas in which "runs" could be made. Animals with hippocampal lesions performed well in more complicated mazes where motor tendencies were interrupted by frequently confronted walls. Another interpretation could be based on a hyperresponsiveness to the frustration induced by the variable reinforcement resulting from the preponderance of empty holes. Visiting many empty holes may be analogous to the characteristic bursts of unrewarded operant responses observed when intermittent operant schedules are introduced to rats with hippocampal damage (Isaacson, 1974).

All rats made equivalently large proportions of visits to nonfood holes between finding their third and fourth food pellets (range, 45 to 62%). No differences were found among the groups in visits to nonfood holes in this period. The preponderance of errors made after finding the third food pellet may reflect a decrease in food motivation and the correlated release of exploratory activity. If this is so, neither brain damage nor haloperidol influenced these variables.

The improvement of the performance of animals with hippocampal

damage following haloperidol was a consequence of an increased number of visits to holes in the center of the arena. The animals exhibited less of a tendency to move along the walls surrounding the arena. On Day 11, when the animals did not receive the drug, the animals did not revert to following the walls, but there was an increase of visits to centrally located nonfood holes. Improvements in efficiency found after haloperidol, beginning on Session 4, could be explained as a result of a decreased tendency to stay near the walls on the outside of the apparatus and of the relationship between food and nonfood holes. When an animal is in the middle of the apparatus, two of the four center holes contained food. This is not true for most locations on the periphery. Therefore, if haloperidol reduced thigmotactic tendencies, it would also enhance the likelihood of finding the centrally located food pellets.

Caution must be exerted in interpreting the results of haloperidol administration since the doses given were adjusted on an individual animal basis. The fact that locomotor behavior was impaired by the 0.4 mg/kg is undoubtedly due to the fact that the animals were being tested twice each day and an additive effect of the drug would be expected. The twice-a-day injection regime also resulted in a carryover of the drug effects to the next day, since there were no differences between morning and afternoon testing sessions. In general, however, most animals were tested with a total of about 0.4 to 0.5 mg/kg being given daily, a dose that when given in a single administration does not impair locomotor activity (e.g., Fish, 1976). Therefore, it is likely that the disabling effects of the drug were a consequence of the injection and training program, as well as the brain lesions, to which the animals were subjected. It should also be stressed that the dose of the drug was adjusted on an individual basis to one that allowed essentially normal rates of locomotion. Haloperidol in these doses disrupted the development of consistent sequences of visits to food holes by control animals. The development of habits of visiting food holes in a particular sequence develops during Sessions 4 to 10 in the saline-injected control animals. This might indicate that the development of acquired, specific, motor acts depends on the activity of dopaminergic systems that were blocked, at least in part, by the drug. Dopaminergic activity might also be associated with the performance of species-typical acts, such as thigmotaxis, and haloperidol could improve performance by producing greater flexibility in behavior. Schirring (mentioned in Randrup and Munkvad, 1974) found that rats tested after 5 mg/kg *d*-amphetamine took increasingly restricted routes in an open field. This would suggest the view that enhanced dopaminergic activity is correlated with inflexibility of behavioral reactions, a view also advanced by Lyon and Robbins (1975). The fact that hippocampal destruction leads to reduced flexibility of behaviors and exaggerated responsiveness also suggests that one effect of

such damage may be secondary alterations in forebrain dopaminergic systems.

REFERENCES

- Douglas, R. J. (1972). Pavlovian conditioning and the brain. *In* R. A. Boakes and M. S. Halliday (Eds.), "Inhibition and Learning," pp. 529-553. London: Academic Press.
- Douglas, R. J., and Isaacson, R. L. (1964). Hippocampal lesions and activity. *Psychonomic Science* **1**, 187-188.
- Fish, B. S. (1976). "Catecholamine Modulation of Behavior following Bilateral Hippocampal Damage." Ph.D. dissertation, University of Florida.
- Isaacson, R. L., and Kimble, D. P. (1972). Lesions of the limbic system: Their effects upon hypotheses and frustration. *Behav. Biol.* **7**, 767-793.
- Isaacson, R. L., and Woodruff, M. L. (1975). Spontaneous alternation and passive avoidance behavior in rats after hippocampal lesions. *In* B. L. Hart (Ed.), "Experimental Psychobiology," pp. 102-109. San Francisco: Freeman.
- Kimble, D. P. (1963). The effect of bilateral hippocampal lesions in rats. *J. Comp. Physiol. Psychol.* **56**, 273-283.
- Lyon, M., and Robbins, T. (1975). The action of central nervous system stimulant drugs: a general theory concerning amphetamine effects. *Curr. Develop. Psychopharmacol.* **2**, 81-162.
- Oades, R. D. (1976). Persistence of the pattern of feeding in chicks with hyperstriatal lesions. *Brain Behav. Evol.* **13**, 69-90.
- O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Exp. Neurol.* **51**, 78-109.
- O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* **34**, 171-175.
- O'Keefe, J., Nadel, L., Keightley, S., and Kill, D. (1975). Fornix lesions selectively abolish place learning in the rat. *Exp. Neurol.* **48**, 152-166.
- Olton, D. S. (1977). Spatial memory. *Sci. Amer.* **236**, 82-96.
- Olton, D. S., and Isaacson, R. L. (1968). The importance of spatial location in active avoidance tasks. *J. Comp. Physiol. Psychol.* **65**, 535-539.
- Randrup, A. and Munkvad, I. (1974). Pharmacology and physiology of stereotyped behavior. *J. Psychiat. Res.* **11**, 1-10.
- Schneiderman, B., and Isaacson, R. L. Pharmacologic changes in performance of normal and brain damage rats. *Behav. Biol.* **17**, 197-211.
- Woodruff, M. L., and Isaacson, R. L. Discrimination learning in animals with lesions of the hippocampus. *Behav. Biol.* **7**, 484-501.