Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5885–5887, September 1984
Neurobiology

Hippocampal denervation causes rapid forgetting of olfactory information in rats

(amnesia/neurology/temporal lobe syndrome)

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Communicated by Richard F. Thompson, June 4, 1984

ABSTRACT **Rats were trained on a succession of two-odor discriminations for a water reward in a modified radial maze. A different odor pair was used each day. After three or four pairs, rats would learn to choose the correct odor in only 3–5 trials. Animals were then subjected to electrolytic lesions in the lateral entorhinal cortex, which is innervated by the lateral olfactory tract, or in the dorsal entorhinal cortex, which is not a target of the olfactory system. Lesions of the first type did not interfere with performance, provided a short interval (30 sec to 2 min) was used between trials. However, the rats were severely impaired when trials were separated by 3–10 min. Dorsal lesions had no effect on olfactory discrimination irrespective of length of delay. In additional experiments, the rats were trained for 10 trials with short inter-trial intervals and then tested 1 hr later with the significance of the cues reversed. Animals with dorsal lesions continued to respond to the formerly correct odor while those with lateral entorhinal damage immediately reversed their response choices. These results provide evidence that lesions to the hippocampal system produce a rapid forgetting syndrome in rats comparable with that reported for humans with temporal lobe damage or dysfunction.**

Damage to the temporal lobes and hippocampal formation in humans is associated with a profound anterograde amnesia (1–3). Recent studies have shown that this effect is due to the rapid forgetting of some but not all types of recently acquired material (2, 4, 5). This has led to the suggestion that the brain-damaged systems are involved in the memory system for specific information (or "data") (6, 7). In rats, lesions to the hippocampal formation or its connections severely impair the animal's performance in various spatial mazes, a result that has been interpreted as showing that hippocampus is a "spatial map" (8) or that it participates in a "working memory" system (9). The relationship of these deficits in rats to the human amnestic syndrome is uncertain and has been the subject of considerable controversy (7, 10). This presents a serious obstacle to the development of generalized hypotheses about the role played by hippocampus in memory storage.

Olfactory learning offers two potential advantages for comparing memory deficits after production of hippocampal lesions in rats with those found in "higher" mammals. First, the olfactory bulbs in rats form extensive monosynaptic connections with the lateral entorhinal cortex (11), a primary source of afferents for the hippocampus (12). This anatomy is comparable and probably homologous with that found in other mammals, including primates (13); this similarity may not be true for the connections between other sensory modalities and the entorhinal cortex. Second, recent studies indicate that the learning of successive olfactory (but not visual or auditory) discrimination problems by rats closely resembles the acquisition of learning sets for various sensory cues by primates (14, 15). Thus, the memory system used by the rat in olfactory learning may be comparable with that routinely employed by larger-brained mammals.

These points led us to test the effects of lesions to the entorhinal cortex on an olfactory memory problem in rats. The results indicate that such lesions do in fact produce memory deficits that qualitatively resemble the rapid forgetting found in humans with temporal lobe damage.

MATERIALS AND METHODS

Adult male Sprague–Dawley and Long Evans rats (270–320 g) were used in all experiments. The animals were trained using a water reward in a radial maze (consisting of a round center place from which eight symmetrical alleys extend in a radial fashion) with the same arm always serving as a starting position. Two distinct odors were ejected by air pressure from tubes in different arms of the maze (i.e., one odor per arm). The location of a given odor was randomized across five arms in different trials; the remaining arms were blocked on each trial. One (correct) odor led to a water dish placed at the end of the arm, the other, to an empty dish. When the rat selected the incorrect odor, a flashing light was turned on for 15 sec when it reached the end of the arm. After a trial, the animal was returned to its home cage for 30 sec to 10 min and then a second trial was initiated. Twenty trials were run per day. After the animal reached a criterion of 80% correct on the second 10 trials of the first discrimination (usually in 3–5 days), a second pair of odors was introduced. After three or four such pairs, the animals would learn to choose the correct odor in three to five trials. Having performed at least six discriminations of 20 trials, they were then subjected to one of two bilateral electrolytic lesions. The first was directed at the ventral, lateral entorhinal cortex, the region innervated by primary and secondary projections from the olfactory bulb (11). A second group (control) received lesions at a more dorsal and medial level.

Two types of histological analyses were carried out at the conclusion of testing. The lesion-induced damage was graphed from serial horizontal sections onto a two-dimensional reconstruction of the entire dorso-ventral extent of the retrohippocampal region from the hippocampal-subicular border to the rhinal fissure. In addition, sections through hippocampus were stained for acetylcholinesterase. A dense band of this esterase is known to appear in those dendritic zones denervated by lesions to the entorhinal cortex (16); this provides a convenient means for assessing the extent of denervation produced in hippocampus by damage to the retrohippocampal region.

Behavioral testing was resumed 2 days after surgery. Each day, a new pair of odors was used with inter-trial intervals of different lengths used on different days.

RESULTS

The results are shown in Fig. 1. The animals with control lesions behaved in a fashion that was indistinguishable from

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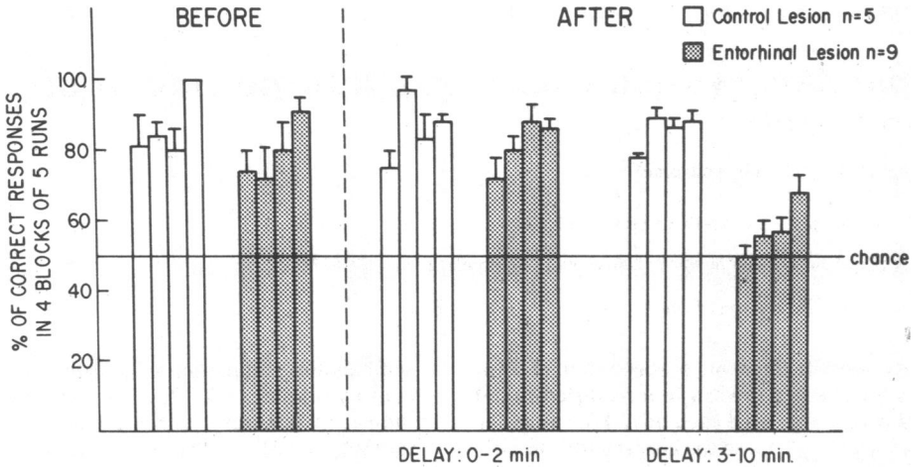

FIG. 1. Mean number (percentage) of correct olfactory discrimination choices over 20 trials (in four successive blocks of five trials) by a group of five rats with control lesions versus nine rats with entorhinal lesions. The means (±SEM) of the last two discrimination problems before the lesion for each group are shown. After-the-lesion performance for each group is shown according to short delays between trials (0–2 min) across three discrimination problems or according to long delays (3–10 min) across five discrimination problems.

preoperative performance. Moreover, the number of errors was about equal whether short or long delays were interposed between trials on a given day. The entorhinal lesion group acquired the correct response nearly as quickly as controls on test days on which short delays were used but were severely impaired (compared with controls, $P < 0.001$ by U test) when the trials were separated by 3 or more min. The animals were nearly randqm in their choices for the early trials and showed only slight improvement over the test episodes. No attempt was made to determine whether the rats could learn the correct response with additional blocks of 10 long-delay trials because this would have involved very long testing sessions.

This pattern of results suggests that the rats acquired information about the correctness of the cues but were unable to retain it for more than a few minutes. To further explore this possibility, we measured retention of two well-practiced discriminations. Control rats and the entorhinal lesion rats were first trained on a given odor pair for 10 trials using minimal delays between trials. Over the last five trials, both groups showed excellent and nearly identical performance (Fig. 2). The animals were placed in their home cages for 1 hr and then returned to the testing apparatus for a second set of 10 trials, again with brief delays between trials, but with the significance of the odors reversed. As expected, normal rats have some difficulty with the first few reversal trials; this was found for the control animals in the present experiments (Fig. 2). The entorhinal lesion animals, however, exhibited no preference for the previously correct odor and immediately switched their response to the previously incorrect (now correct) odor. On the first reversal trial, 100% of the controls, but only 44% of the entorhinal lesion rats, chose the formerly correct odor. From this we conclude that entorhinal lesion rats rapidly forget even well-learned information. What appears to be a similar effect after large lesions of hippocampus and amygdala has been reported in a recent abstract (19).

Histological reconstruction indicated that the ventro-lateral lesions included the lateral entorhinal cortex or the perforant path or both; the deafferentation expected from this pattern of damage was confirmed by histochemical evidence of a dense band of acetylcholinesterase in the entorhinal projection areas within the hippocampus. The control lesions invaded the retrohippocampal region to various degrees but did not typically produce damage to the lateral entorhinal area or perforant path; increased acetylcholinesterase staining was found unilaterally in two of the five control rats.

DISCUSSION

These data suggest that the hippocampal formation plays a critical role in olfactory memory. The rats rapidly acquired an olfactory discrimination in which the delay between trials was very short, indicating that the lesions did not interfere with prior learning about the task nor with the sensory, motor, and motivational components needed to perform it. The impairment found with longer delays between trials seems to be due to a deficit in the memory system for specific olfactory information. The reversal experiments also point to this conclusion. Lateral entorhinal lesion rats that had clearly mastered an olfactory discrimination had no difficulty in reversing their choices 1 hr later; thus, and in marked contrast to control animals, the first training episode had no effect on the second.

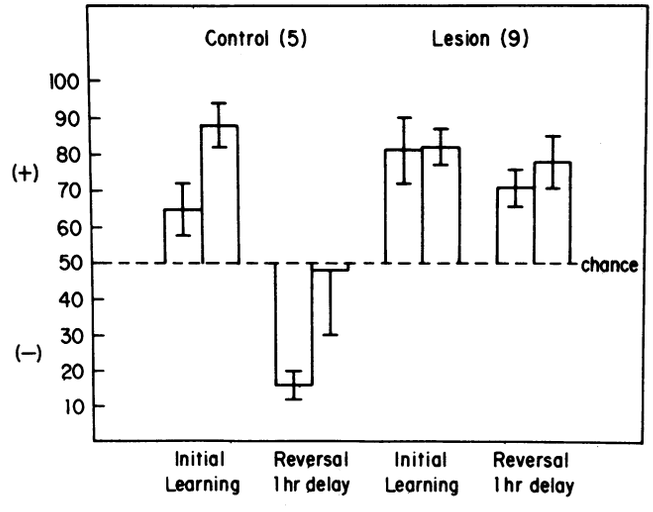

FIG. 2. Mean number (percentage) of correct responses in an olfactory discrimination reversal problem by a group of five rats with control lesions versus nine rats with entorhinal lesions. The data used for each bar are the means for each group (in successive blocks of five trials) across two reversal problems with a 1-hr delay interposed between the first 10 choices and the second 10 choices.

The present results are not readily explained by the widely discussed hypothesis that the hippocampus serves as a spatial map (8), because the odors were used as randomly positioned intramaze cues. Thus, on a trial-by-trial basis, spatial information was irrelevant to performance of the task. It has also been suggested that hippocampus serves as a working memory system for the rat (9). However, the learning paradigm used in our study does not match a working memory task because the odor was correct for 10 or 20 successive trials. (Working memory involves storage and utilization of different cue information across a series of responses.)

There is little evidence in the literature for rapid forgetting of nonolfactory information after hippocampal lesions in rats. Thompson (17) found that ablation of hippocampus caused a disruption of Y-maze reversals when rats were tested 60 min but not 5 min after training. Interpretation of this result is complicated by evidence that hippocampal rats learn mazes using different cues than do normals (8) and indeed the hippocampal rats in Thompson's experiments were substantially impaired in the acquisition as well as the retention of the task.

Why then is rapid forgetting in rats with hippocampal lesions so apparent for olfactory cues but not for other types of stimuli? One possibility is that tasks of the type used in the present experiments involve a memory system somewhat different from that typically tested with non-olfactory stimuli. The rats in the present experiments were slow to acquire the first discrimination, but solved the third and fourth problems in only three or four trials. Slotnick and co-workers (14, 15) who observed a similar effect, argued that rats acquire primate-like learning sets (i.e., information about the learning task itself as opposed to memory of specific items used in the task) during the course of successive olfactory discriminations. Using the same testing paradigms, they also found that rats did not acquire learning sets when auditory or visual cues were used instead of smells. Possibly then the rapid forgetting effect after damage to the hippocampal system is detected only in those tasks involving the acquisition of new specific information against a background of a previously learned organizational framework. In light of Slotnick's work, we suggest that successive olfactory problems are particularly well suited for revealing this contribution of hippocampus to memory.

In several important respects, the above-described deficit resembles that found in humans with hippocampal damage. These patients rapidly forget specific data (e.g., names and faces), even when these have been well rehearsed (1–4); as shown in Fig. 2, entorhinal lesions produce a similar effect in rats. Hippocampal damage in humans does not impair the recall of older memories excepting those for a relatively brief period immediately before the occurrence of the damage (7). Denervation of the hippocampus in the present study did not interfere with the animals' ability to retrieve information acquired before surgery; for example, the rats clearly remembered the maze and the location of the water dishes and were able to use the learning set when the delays between the trials were short. (The question of whether any retrograde amnesia existed for events occurring immediately before surgery was not addressed by our experiments.) Finally, recent work has shown that the anterograde amnesia caused by hippocampal damage in humans is not complete and that these patients are able to master certain types of problems (e.g., puzzles) that require a considerable amount of practice by normal subjects. It has been suggested that the learning, storage, and recall of rules or procedures is not included in the temporal lobe amnesia syndrome (6, 7). Rats with extensive hippocampal damage are known to learn a variety of problems (8). It is especially noteworthy that large lesions of the pyriform cortex and olfactory tract, which should eliminate any olfactory input to the entorhinal cortex, do not block the acquisition of learning sets over the course of successive olfactory discriminations involving very short intertrial delays (15). If, as can be expected from this result, rats with entorhinal lesions are able with practice to acquire strategies or rules, then the pattern of olfactory memory deficits produced by these lesions would be in excellent agreement with that found for all types of material in humans: i.e., rapid forgetting of specific information combined with normal learning of rules. It should be noted that two groups have shown that lesions of the dorso-medial nucleus of thalamus or its projection targets in frontal cortex severely impair acquisition of strategies or learning sets for olfactory problems in rats (15, 18) and this appears to be the case in our paradigm as well (unpublished data).

To summarize, we propose that damage to the hippocampal system produces effects in rat olfactory learning that are similar to those reported for a variety of non-olfactory memory tasks in humans. Therefore, paradigms of the type used in the present experiments may be valuable in analyzing the cellular processes through which the hippocampus participates in memory.

This work was supported by research grants from the National Science Foundation (BNS 76-17370) and the National Institute of Mental Health (MH 19793-12). U.S. holds a fellowship from the Swiss National Science Foundation.

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