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Studies on Retrograde and Anterograde Amnesia of Olfactory Memory after Denervation of the Hippocampus by Entorhinal Cortex Lesions

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The effect of hippocampal denervation on olfactory memory in rats was tested after interrupting the lateral olfactory tract projections at the level of the entorhinal cortex. When lesioned animals were trained to learn new odors, they showed no evidence of retention 3 h after acquisition. These results confirm earlier data on rapid forgetting in rats after hippocampal deafferentation and are in parallel to the anterograde amnesia typically found in humans with hippocampal damage. On the other hand, preoperatively learned information was minimally impaired after hippocampal deafferentation even if it was acquired within less than 1 h before the lesion. This finding differs from reports on humans as well as monkeys with hippocampal damage where memories formed during a critical time span of months or even years before the lesion are found to be impaired. This may suggest that the consolidation process in humans and rodents has different time scales or that the roles of the human and the rat hippocampal structure in memory formation are somewhat different. © 1986 Academic Press, Inc.

A comparison of the rat olfactory system with other sensory modalities reveals a striking difference that sets olfaction apart: The primary projections from the olfactory bulb terminate monosynaptically in cortical structures; that is, there are no thalamic or nuclear relays between sensory input and cortical target area (e.g., Kosel, Van Hoesen, & West, 1981). Beyond the olfactory cortex (which includes piriform and entorhinal cortices) the olfactory projections lead via one synapse to the hippocampus and the dorsomedial thalamic nucleus (DMN). Dysfunction of these sites has been shown to cause learning deficits in olfactory discrimination tasks (Eichenbaum, Shedlack, & Eichenbaum, 1980; Slotnick & Katz, 1974; Staubli, Ivy, & Lynch, 1984). The data suggest that neither the DMN nor the hippocampus is involved in the sensation and discrimination of smells, but that they both participate in higher order olfactory functions and are used to process information about particular odors. Specifically,

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as the studies indicate, a primary encoding deficit appears to be responsible for the anterograde amnesia observed after DMN lesion (Staubli, Schottler, & Nejat-Bina, 1986b), whereas in rats with hippocampal dysfunction, new information is encoded normally but then forgotten rapidly (Staubli, Ivy, & Lynch, 1984).

Moreover, the hippocampus and the DMN are two structures that are particularly relevant to human memory (e.g., Milner, 1972; Squire 1982), and it is well established that damage to both these brain sites causes anterograde amnesia in humans. These memory disorders have prompted a considerable research effort aimed at finding animal models to characterize the functional role of these critical brain regions (Kesner & DiMattia, 1986). The rapid forgetting phenomenon found in rats for olfactory memory constitutes such a parallelism to the anterograde amnesia of humans with temporal lobe damage.

A further interesting aspect of olfactory memory is that rats show interproblem learning when presented with a series of olfactory discrimination problems; that is, the time to learn a particular discrimination is progressively reduced with increasing number of solved problems (Slotnick & Katz, 1974). This “learning to learn,” also called “learning set” formation, is characteristic for “higher mammals” such as primates and humans (Harlow, 1949), but is reported to be poorly developed in rodents outside the olfactory modality (Slotnick & Katz, 1974).

Clinical reports suggest that the human temporal lobe syndrome includes retrograde amnesia that is limited to events preceding the injury within a certain time span (1–3 years); if the memory tested is sufficiently old, retention is found to be intact. This observation has been interpreted as evidence that the hippocampal structure plays a crucial role in memory consolidation for some limited time after learning: “If the function of the medial temporal region is interrupted before its role in the development of memory is completed, memory can be irretrievably lost” (Squire, 1982). The gradient and nature of retrograde amnesia are not fully understood, in contrast to anterograde amnesia which is complete and remains permanently.

Whether the hippocampus of nonhuman primates is similarly involved in the consolidation of new data is not clear. A recent study has provided some evidence for retrograde amnesia in monkeys: After bilateral combined lesions of the hippocampus and amygdala, they showed no retention of a set of visual discrimination problems acquired 32, 16, 8, 4, and 2 weeks prior to surgery (Salmon, Zola-Morgan, & Squire, 1985).

Even less is known about retrograde amnesia in rodents with hippocampal dysfunction. Preliminary studies on spatial learning did not provide evidence for retrograde amnesia in rats with bilateral complete transections of fimbria and hippocampus (U. Staubli, unpublished observation). In that study eight rats were first familiarized with the procedure of an eight-
arm radial maze during several days and were then trained to enter one designated arm to retrieve a reward. Ten trials were given, and 24 h later the animals were tested for their memory of the specific arm. They all remembered perfectly and chose the correct arm in the first trial. An hour later, the animals were trained to enter a different arm within 10 trials. Testing was resumed 24 h later, followed by training to a new arm. In addition, a bilateral complete transsection of fimbria and hippocampus was performed in five rats immediately following the 10 training trials. Ten days later, the animals showed perfect retention and chose the correct arm in that they entered the last learned arm, as did three control rats that had received a sham lesion.

As noted above, hippocampal denervation in rats causes anterograde amnesia for olfactory information. Since olfactory learning in rodents bears a number of similarities to primate memory formation (Slotnick & Katz, 1974; Staubli, Fraser, Faraday, & Lynch, 1986a) and is extensive, accurate, and persistent, studies using a successive odor discrimination paradigm would seem to be suitable for the detection of retrograde amnesia. Existence of a temporally limited retrograde amnesia in rats, in addition to their dense anterograde amnesia, would support the hypothesis that rodent olfactory memory is an appropriate model for higher mammalian (i.e., human) memory.

METHODS

Apparatus. The experiments used a radial maze consisting of six arms in which the rat was required to direct itself to the odor source by first locating it at a central point and then moving toward it to find a reward. The arms (90 cm long) were separated from each other by 25-cm high walls with one arm designated as the permanent starting arm. During a given trial, two of the five randomly selected arms contained a tube (tygon tubing; i.d., 3/16 in.) that extended from the periphery of the maze toward the center platform. The entrances to the remaining three choice arms were blocked for that trial.

Odors. Odors were presented by forcing a clean airstream at a fixed pressure through 1-liter flasks containing different, commercially available odors (imitation flavors and extracts, McCormick, Inc.) diluted in a fixed amount of water. The outlet of each flask was connected to a tube (described above) that carried the odorized air. The tubes ended halfway down the arms where they ejected the odors in direction of the center of the maze. To ensure rapid clearing of the odors from the maze, an exhaust fan was mounted above the center platform to pull the odors down the arms and out of the testing area. Concentrates were diluted individually such that they were perceived by humans as being of similar intensity. It has been observed that strength of the cues is not a critical variable in tasks involving different odors (H. Eichenbaum, personal
We found that rats are capable of learning to discriminate two identical smells that differ in intensity (1:2/3), but that the animals need two to three times more trials than when learning two different odors (unpublished data). Together, these findings suggest that rats prefer to use a difference in quality over a difference in concentration as a means to discriminate between two odors.

Procedure. The choice of the arms containing the odor tubes was randomly determined for each trial, thus making use of spatial cues irrelevant. The correct odor led to a hidden well containing five drops of water at the very end of the arm, while following the wrong smell ended at a hidden but empty well. A flashing light signaled an incorrect response which the rats were not allowed to correct. After completing a trial, the rat was removed to its homecage for the length of the intertrial interval, which varied between 3 and 10 min. We found performance to be independent of the length of intertrial interval, at least within the time range of 15 min.

Subjects. The subjects of this study were 29 adult male Sprague-Dawley rats that were 60 days old at the beginning of their training. They were housed individually and were trained and tested during the dark phase of their light-dark cycle.

Training. The animals were kept on a deprivation schedule that allowed access to water for 15 min daily. Before the start of training the animals were familiarized with the apparatus by receiving 15-min exploring sessions for 3 consecutive days. During this phase the animals found a few drops of water each time they arrived at the end of any of the six arms of the maze. Training on a series of olfactory discrimination problems started on Day 4. Twenty trials were given per day. Learning was considered as demonstration of at least 80% correct runs during the second 10 trials. Rats typically acquired the first problem in 3 to 5 days. Subsequent problems using different pairs of odors were solved successively faster, and starting with the fourth or fifth problem, performance was usually 80% correct during the first 10 trials.

Surgery. Before surgery the rats were anesthetized with intraperitoneal injections of sodium pentobarbital (Nembutal, 50 mg/kg). The bilateral electrolytic lesions of the entorhinal cortex were placed under stereotaxic guidance (AP: 0.8, L: 3.0, DV: −6.0, −4.0, −2.0; L: 4.0, DV: −7.0, −5.0, −3.0; L: 5.0, DV −7.0, −5.0, −3.0; all in reference to lambda, at an angle of 10° from the vertical midline and with the tooth-bar set at −5.0). Current of 1 mA was passed for 45 s at each of the nine placements in both hemispheres through an insulated stainless steel electrode that was cut at the tip. Control animals were subjected to the same surgical procedure; that is, the electrode was lowered to the same locations but no current was delivered.

Histology. In the experiments described in the following, some of the
rats had received bilateral electrolytic lesions of the entorhinal cortex. These animals were deeply anesthetized upon completion of training and were perfused transcardially with physiological saline followed by 10% formaldehyde solution (in 0.1 M phosphate buffer). The brains were removed and stored in the formaldehyde solution for several days. The day before sectioning they were put in 20% saccharose solution (in 0.1M phosphate buffer) overnight. The brains were then frozen and sectioned coronally at 45 μm in anterior–posterior direction starting at the fornix. Horizontal sections at 45 μm were taken from the last third of the hippocampus and the adjacent entorhinal cortex. Every third horizontal section was stained with cresyl-violet, and every third coronal section was stained for acetylcholinesterase. A dense band of this esterase is known to appear in those dendritic zones denervated by lesions to the entorhinal cortex (Lynch et al. 1972). This provides a convenient means for verifying the extent of denervation produced in hippocampus by damage to the retrohippocampal region. The sections of all brains were examined microscopically. Analysis of the horizontal sections revealed that all lesions included the lateral entorhinal cortex, the projection site of the lateral olfactory tract axons. In the majority of cases (80%) the whole entorhinal cortex was found to be lesioned together with the caudal end of the hippocampus. A representative lesion is schematically illustrated in Fig. 4. Histochemical evidence of a dense band of acetylcholinesterase in the entorhinal projection area confirmed the massive deafferentation expected from this pattern of damage.

**EXPERIMENT 1**

A group of 16 rats was familiarized with the maze during 3 days as described above. No exposure to odors was given before start of the actual experiment. Following this, the animals were trained on a single olfactory discrimination problem for 4 consecutive days (one session per day with 20 trials). Immediately after learning on Day 4, 9 rats received bilateral electrolytic lesions of the entorhinal cortex. The remaining 7 animals were sham lesioned. After recovery from the lesion 10 days later, all animals were tested again on the original discrimination except that the significance of the two odors was now reversed (reversal). Only 10 trials were given on the reversal problem. The intervals between trials were kept minimal (45 s) since we know from a previous study (Staubli, Ivy, & Lynch, 1984) that rats with entorhinal lesions are able to acquire information nearly as fast as control animals, provided the delays between individual trials are short. When the trials are separated by 3 or more minutes, entorhinal lesioned rats are severely impaired and only show slight improvement over a 20-trial session.

The advantage of reversing the significance of the original problem is to enable detection of the particular memory which otherwise is likely
to be masked by a ceiling effect due to rapid acquisition (once learning set performance has been established novel problems are learned within a few trials). Intact rats typically respond in a reversal situation by choosing the previously correct (but now incorrect) odor, at least during the first few trials, and this is reflected in a below chance performance.

Results

Figure 1 shows the first 10 trials (two blocks of 5 trials) of the last training session and of the testing session following the lesion. The prelesion

![Graph](image-url)
scores of lesioned and control animals indicate rapid acquisition. In the reversal situation control animals as expected showed below random performance during the first and even during the second block of 5 trials. Obviously, they had no difficulty in remembering the two odors with their previous significance, even after the 10-day interval.

The animals that received bilateral entorhinal lesions also showed below random performance and thus almost normal memory retention throughout the two blocks of five trials. That the lesioned animals made slightly less errors than control animals in the reversal might point to some memory impairment, or to attentional or motivational disturbances affecting the overall behavior. However, the differences between lesioned and control animals were small and statistically not significant according to the $t$ test (trials 1–5: $t = 1.38, p > .1$; 6–10: $t = 1.29, p > .2$).

These results confirm the conclusions reached in earlier studies (Staubli et al., 1984) that the hippocampus is not the site of memory storage. They further suggest that the hippocampus in the rat is either not involved in the consolidation process as it appears to be in primates, or that this consolidation process is shorter than the time span between training and lesion. Since the animals in this experiment were given several days of training to a single odor, it is possible that learning accelerated or reinforced the consolidation process. We repeated this experiment using a single odor discrimination and shortened the time between acquisition and the lesion to less than one hour.

**EXPERIMENT 2**

Thirteen rats were trained during 9 days on a series of five different olfactory discriminations. Demonstration of learning set formation occurred after solving four problems. They were then presented with two novel odors for 20 trials. Following learning bilateral electrolytic lesions of the entorhinal cortex were performed in seven rats, and a sham lesion in the remaining six animals. After recovery from the lesion 10 days later, all animals were tested on the reversal of the original problem with short delays (< 1 min) between trials.

**Results**

The results obtained confirmed the findings of the first experiment: Memories of odors learned immediately before the lesion were intact in both lesioned and control animals, as is illustrated in Fig. 2. Thus, if the hippocampus should indeed play a role in consolidation of rat olfactory memory, the critical period of consolidation would have to be less than 1 h. As in Experiment 1, lesioned animals committed slightly less errors than control rats, at least in the first trial block, which could be taken as evidence for some weak retrograde effect caused by the lesion. The difference, however, was statistically not significant ($t$ test, trials 1–5: $t = 1.04, p > .2$; trials 6–10: $t = 1.19, p > .2$). Compared to the reversal
FIG. 2. Effect of hippocampal deafferentation on olfactory memory (after limited training). A group of 13 trained rats was given 20 trials on an olfactory discrimination problem. Immediately afterwards 7 animals received bilateral entorhinal lesions; the remaining 6 animals received sham lesions. Ten days later, the animals were tested on the reversal of the original problem. Ten trials spaced 1 min apart were given. Performance was calculated as mean percentage of correct choices in consecutive blocks of 5 trials and is shown in reference to the random performance level of 50%. During acquisition of the original problem, trial 1 of the first block was not taken into account to express the mean, since its outcome was random.

In the previous experiment, the scores of both groups were somewhat higher and the control animals started to acquire the correct response in the second five trials (above chance performance). It appears that the efficiency of learning a reversal is inversely related to the amount of training received on the original problem, at least in control animals.
EXPERIMENT 3

We have previously reported that entorhinal lesions cause rapid forgetting of olfactory information (Staubli, Ivy, & Lynch, 1984). Histological analysis in that study revealed that the lesions which caused olfactory anterograde amnesia typically involved damage to the lateral entorhinal area (which is the site that receives extensive monosynaptic connections from the olfactory bulbs) or to the perforant path or to both. Control lesions to the medial entorhinal cortex did not cause anterograde amnesia. In the two studies outlined above the lesions were aimed at the entire caudal entorhinal area and accordingly they were expected to cause anterograde amnesia. Thus, in order to experimentally verify the locus of lesion of a part of the animals used in the preceding experiments, and, second, in an attempt to confirm our previous finding on rapid forgetting, we did the following study: Both the lesioned and control animals of Experiment 2 were given 20 trials on each of four novel olfactory discriminations. With delays of 45 s between trials the lesioned animals showed excellent, nearly identical performance as the control rats over the last two trial blocks. Following learning on the fourth day, the animals were placed in their homecage for 3 h and then returned to the olfactory maze for another 10 trials separated by 45 s, but with the significance of the odors reversed. A separate group of 20 trained rats (11 entorhinal-lesioned and 9 sham control animals) that had previously been used in a different set of experiments were tested in the same manner for rapid forgetting of newly acquired olfactory information.

Results

The data obtained from 18 lesioned rats and 15 control rats were pooled and are illustrated in Fig. 3. As usual, control animals predominantly chose the wrong odor in the first few trials of the test phase. Entorhinal-lesioned rats, however, exhibited no preference for the previous correct odor (trials 1–5, lesion vs control: $t = 7.1$, $p < .001$). This outcome confirms our previous finding that entorhinal lesioned rats rapidly forget well learned information (Staubli et al., 1984). Also important in this context, the data imply that the lesions performed in Experiment 2 did successfully separate the hippocampus from its primary olfactory afferents. In the second five trials control animals switched their response to the previously incorrect, but now correct, odor and greatly improved their performance. The lesioned animals were somewhat slower in learning the problem and did not improve as rapidly during trials 6–10 on retest as did the controls (trials 6–10: $t = 3.09$, $p < .01$); this may indicate that some loss in memory occurred in the minute between trials when the animals were removed and reintroduced to the maze.
Fig. 3. Rapid forgetting after hippocampal deafferentation. Rats with bilateral entorhinal lesions (n = 18) that disconnected the hippocampus from its lateral olfactory tract inputs and a group of intact rats (n = 15) were trained on an olfactory discrimination. Twenty trials with a 45-s intertrial interval were given. Three hours later, the same two odors were presented again but with their significance reversed. Ten trials with 45-s intertrial intervals were given. Performance was calculated as mean percentage of correct choices in consecutive blocks of five trials and is illustrated in reference to the random performance level of 50%. Only the last two blocks of the initial discrimination are shown.

DISCUSSION

We analyzed the role of the hippocampus in the formation and maintenance of olfactory memory. This was done by determining existence of retrograde and anterograde amnesia after interrupting the lateral olfactory tract projections to the hippocampus at the level of the entorhinal cortex.

The data obtained confirmed our earlier findings that hippocampal deafferentation causes anterograde amnesia due to rapid forgetting of newly acquired information. In this respect the deficit in rodents parallels the human temporal lobe syndrome. It also suggests that the hippocampus is needed to form memories about odors. Interestingly, hippocampal deafferentation did not have a significant impact on previously stored memories. Experimental work on temporal lobe patients has similarly indicated that anterograde and retrograde amnesia differ in their severity. Retrograde amnesia has been observed to affect information acquired as much as 1 year before the lesion. Although no formal rigorous testing has been reported yet (Squire, 1982), evidence for this has come from several case studies including a recent report on retrograde amnesia in
a human with a circumscribed lesion to a subfield of the hippocampus (Zola-Morgan, Squire, & Amarel, 1985). This has led to the hypothesis that the temporal lobe including the hippocampal structure is essential for a considerable time after learning to consolidate memory.

In marked contrast, only a minor impairment of preoperatively acquired

Fig. 4. This graph schematically illustrates on consecutive horizontal sections the extent of a representative lesion of the entorhinal cortex.
information was found in the rat, even if the interval between acquisition and lesion was less than 1 h. There may be several explanations for this apparent lack of a clear retrograde amnesia in rodents. First, the time scale for the consolidation process in humans and rodents is vastly different and is complete in rats within an hour, but continues to act upon human memory for months or even years. Second, the rules for consolidation in olfaction differ from those for other modalities. The sparse information available about retrograde amnesia in humans does not appear to include data about memories for odors. Third, and perhaps most likely, the type of memory sampled in our experiments may not be of the same type, or stored in the same fashion, as that in humans after brain injury. This point can be discussed as part of the question of how any representation of the learned event that is stored in the hippocampus is used in retests involving the learned material. As is well known, hippocampal synapses are changeable and can remain in a potentiated state for very long periods; Bliss and Lomo (1973) described long-term potentiation (LTP) lasting for weeks and we have found LTP in rat field CA1 that persists unchanged for at least weeks (U. Staubli and G. Lynch, manuscript in preparation) following brief periods of stimulation. If comparable effects occur during behavior, the question then arises as to what they contribute to behavior. The task used in the present experiments required a simple recognition memory and clearly did not require the hippocampus once the memory had been stored (episode). More complex memories about the learning event, such as the context (episode) within which it occurred, might be more dependent upon hippocampal traces. One might also imagine that modified output from the hippocampus serves to facilitate the retrieval (as opposed to simple recognition) of information from other parts of the brain until such time as that information becomes associated with other items in storage. Tests of these ideas (e.g., does the rat remember the particular cage in which it learned a particular odor after lesions) are yet to be conducted.

To answer these questions, further studies on amnesia in rodent olfactory and nonolfactory modalities as well as more specific and quantitative information on retrograde amnesia in primates are needed.

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