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Hippocampal, granule cell and CA_{3-4} lesions impair formation of a place learning-set in the rat and induce reflex epilepsy

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Rats were pretrained on a place learning-set task, in which a platform, submerged in a swimming pool filled with opaque water, was moved to a new location each day. Then they received either: (1) suction removal of the dorsal hippocampus, (2) intrahippocampal microinjections of colchicine to remove dentate gyrus granule cells, (3) kainic acid to remove CA_{3-4} cells of the hippocampus proper, (4) suction removal of parietal cortex overlying the hippocampus, or (5) no surgery. Performance was then evaluated for 48 days. All lesion groups were chronically impaired with respect to the control group, but the rats with parietal cortex lesions retained the ability to solve the task, whereas rats with hippocampal damage did not. Training frequently induced task-related behavioural seizures in the rats with granule cell or CA_{3-4} lesions. The results show that the hippocampus, including granule cell and CA_{3-4} cell populations, is essential for the rapid acquisition of place responses in the swimming pool task. The finding that training on the task induced reflex epilepsy in granule cell and CA_{3-4}-damaged rats, but not those with aspirative removals, suggests that residual portions of the hippocampus are activated by training and are involved in production of the epileptic attacks.

INTRODUCTION

A substantial literature has now developed from attempts to evaluate the role of the hippocampal formation in spatial navigation in rodents[20]. There is no concensus on the relative effects of different types of lesions within subsystems of the hippocampal formation or on the process through which lesions produce their impairments[8–12,18,22,24,25]. Studies using swimming pool spatial navigation tasks have shown that rats with hippocampal damage are impaired in forming place responses requiring that they locate a platform hidden just below the surface of opaque water in a swimming pool[16–18,24,25]. It has been suggested that since the platform is not visible, the rats must navigate using the relational properties of distal visual cues. If they are presented with a similar task, except that the platform is visible, they show little or no impairment. These results support the idea that the hippocampal formation is relatively selectively involved in place navigation, and is not essential for the utilization of other navigation strategies. Other studies, however, using dry-land tasks, have reported less severe effects of hippocampal damage or only minor differences between place and cue tasks or complete recovery with practice[8–12].

Inconsistencies in results may occur for 4 reasons: tests are different, training durations are

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different, presurgical experience is different, or brain injury is different. If it is claimed that a structure is essential for a given function, then the following conditions would seem to apply. If a test fails to produce a deficit or if recovery occurs with practice, then the test fails to demonstrate that the structure is essential for the function. Although the question of presurgical experience is more difficult to deal with, within a restricted set of circumstances the same argument still applies. Finally, if damage to an intregral part of the structure fails to produce a deficit, then that component is either not involved in the solution to the problem, or the test is not measuring the function of that structure. Thus it would seem that the most rigorous test of hippocampal function should be one that animals with hippocampal damage fail if damage is extensive, do poorly on if damage is partial, and on which complete recovery does not occur after pretraining and extended practice. The purpose of the present experiment was to make a definitive test of the role of the hippocampus in place navigation. Accordingly, a test was designed that most obviously met the criteria for place navigation and the experiments were structured so that animals were provided every opportunity to succeed; that is, they were pretrained, and they were given extensive postsurgical practice. Finally, different hippocampus subsystems were damaged selectively to compare the effects of their removal with the effects of global damage.

According to the O'Keefe and Nadel[20] definition, place responses should be acquired in one trial, should be retained for relatively long periods of time, should be given up after a single unrewarded trial, and should depend upon the flexible use of the relational properties of distal cues, no one of which is essential. A 'learning-set' task that evaluates just these abilities has been developed for the swimming pool and was used for the experiment*. The task requires that the rats acquire a new place response each day[27,28]. After a brief period of training, normal rats will swim, on the first trial of each day's test, first to the previous day's correct location, thus demonstrating their retention of the previous day's problem. They then locate the platform at its new position. On the next trial, given a few seconds later, they swim to the new location quite quickly and accurately, demonstrating their ability to rapidly give up one place response and adopt a new one. Once the task is mastered, it can be given repeatedly. Since the task requires 16 trials on each problem each day, the impairments of animals subjected to experimental treatments can be evaluated, both between and within days. Finally, since the subjects can be pretrained, retrograde as well as the anterograde effects of experimental treatments can be evaluated. The task has definite advantages over a two-trial 'working-memory' task that uses only a latency measure of performance but is designed for use in the swimming pool[17]. This is because an error measure can be used to provide a direct assessment of accuracy, on the assumption that only direct swims from start to goal represent true place navigation. Also, the repeated testing on each problem permits a more robust measure of performance deficits. For the study, 3 types of damage were produced. Aspirative lesions of the entire dorsal hippocampus were made in order to produce a maximal impairment. Elements of the trisynaptic pathway[26] were selectively removed using relatively specific neurotoxins; colchicine[7,25] was used to remove granule cells, and kainic acid[19,25] was used to remove CA_{3-4} cells. Parietal cortex aspiration[13] was performed to control for extrahippocampal damage produced by the hippocampal lesions.

MATERIALS AND METHODS

Subjects

Male Long–Evans hooded rats, aged 120 days (275–350 g) at the time testing commenced, served as subjects. They were housed in pairs in

* In the learning-set task as used here, only four platform positions were used; however, it has been demonstrated that, once acquired, the ability to rapidly solve place problems can be utilized for new problems, even those administered in a different pool in a different room[28].

hanging wire mesh cages, with constant access to Purina Lab Chow and water. Behavioural testing was conducted during the light phase of the animal colony's lighting cycle (12 : 12 h light/dark).

Surgery

Rats were randomly assigned to one of five groups. Two of the groups (n = 4 animals per group) received bilateral intrahippocampal injections while anesthetized with sodium pentobarbital (50 mg/kg, i.p.), one group received aspirative lesions of the dorsal hippocampus (n = 6), one received bilateral aspirative lesions of parietal cortex (as a control for tissue damage that might be produced by the other lesion procedures), (n = 4), and one group received anesthesia only (n = 5). Intrahippocampal injections were stereotaxically placed and made through a 30-gauge stainless steel needle connected to a 5-μl microsyringe (Hamilton) fixed to a motorized microdrive.

The drugs were dissolved in Kreb's solution and at each injection site the volume injected was 0.5 μl over a 4-min interval. Kainic acid (0.1 μg/site) was injected into one group[19,25,31]. Injections were made at 3 sites within each hippocampus (coordinates: (1) P 2.5, L 2.3, V 3.3; (2) P 4.5, L 4.0, V 4.0; (3) P 5.0, L 4.5, V 7.5), with the skull leveled between bregma and lambda. Colchicine (2 μg/site) was injected bilaterally in the second group at 3 sites within each hippocampus (coordinates: (1) P 2.5, L 1.0, V 39; (2) P 4.0, L 2.0, V 4.8; (3) P 5.5, L 4.4, V 5.7)[7,25]. The aspirative lesions were performed by making an aperture in the skull overlying parietal cortex, and then removing either parietal cortex alone or parietal cortex and the underlying hippocampal formation[13,30].

Procedure

Swimming pool

The rats were tested in a large circular swimming pool 146 cm in diameter and 46 cm high, which was painted white and filled to a height of 25 cm with approximately 18 °C water[27,28]. Approximately 1,500 ml of instant powdered skim milk was dissolved in the water to make it opaque. The platform was a clear Plexiglas stand submerged 14 mm below the surface of the water so that it was invisible to a viewer inside the pool[28].

Learning-set task

The learning-set task is similar to that described previously[27,28]. Four different platform locations were used and the platform was moved each day to one of these locations according to a designated sequence. Location 1 was in the center of the south-east quadrant of the pool, location 2 was in the center of the south-west quadrant of the pool, location 3 was in the center of the pool, and location 4 was about 8 cm away from the wall between the north-east and north-west quadrants of the pool (it was slightly closer to the wall of the pool than were locations 1 and 2). The platform positions were chosen to frustrate a number of non-place learning strategies that normal rats may adopt. A rat may attempt to locate the platform by swimming in a circular path around the pool: if this strategy is adopted a platform located at position 3, in the pool's center, will not be found. A rat may turn away from the wall and swim at a given angle: this strategy will not help it reach platform location 4, which is immediately adjacent to a start position and located slightly closer to the wall than positions 1 and 2, and which requires that the rat swim toward the center of the pool to locate it. A rat may concentrate swimming in a quadrant or half of the pool: the asymmetric locations of the platforms will limit the utility of this strategy. Four start locations were used: north, south, east and west. The rats are gently placed into the water facing and touching the wall of the pool at these starting points.

Testing was conducted on consecutive days. Each rat received 16 trials on each day. If on a particular trial a rat found the platform, it was permitted to remain there for 5 s. A trial was terminated after 120 s if a rat failed to find the platform. Trials were given in pairs. The second trial of each pair was given immediately after the 5 s stay on the platform, and the same starting location was used. At the end of the second of each pair of trials, the rat was returned to its home cage and approximately 5–8 min elapsed (during which the remaining rats were tested) before the

next pair of trials from a new starting location was given, etc. Trial pairs were given so that the rats started from each of the 4 locations on each of the first 4 pairs of trials and each of the second 4 pairs of trials. The sequence in which starting positions were used was random.

Pretraining

All of the rats were pretrained on the task. Pretraining consisted of administering 16 training trials each day for 14 days. By this time all rats had achieved asymptotic performance on the task.

Videorecording

Videorecords were made continuously for 24 h of each rat that displayed a behavioural seizure during testing in the water task. Each animal was placed in a circular clear Plexiglas cage 38 cm in diameter and 28 cm high. Sawdust covered the floor of the cage, and food pellets were scattered in the sawdust. A water bottle was attached to the side of the cage. Videorecords were made with a Sony variable speed videocassette system, with a recording speed of 24 h and a playback speed of 1 h. During playback, behaviours displayed by the rats could be subject to careful scrutiny. Each rat that displayed a behavioural seizure was video-recorded continuously for one week (except for the time that testing in the swim task occurred). Videorecording was done during the last two weeks of swim task testing.

Data analysis

The swim path was drawn on a map of the pool as the rat completed each trial and latency (time to a tenth of a second) was recorded beside the swim path. Two measures were used for analysis; latency and errors. For the error measure, an 18-cm wide path from the start point to the platform was designated as the correct route, so that if a rat deviated from this route at any point it received a maximum of one error on that trial. Group differences were determined by analysis of variance with follow-up Newman–Keuls tests.

RESULTS

Histological findings

Fig. 1. shows an example of the typical extent of the aspirative ablations of the hippocampal formation. In general, all of the hippocampus including fimbria and fornix was removed but the ventral hippocampus was spared. The extent and

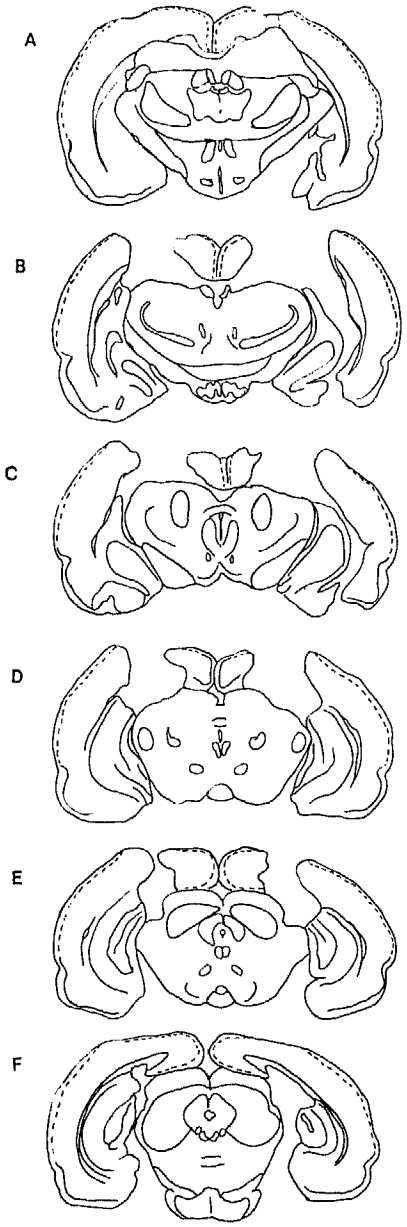

Fig. 1. Reconstruction of a representative hippocampal aspiration lesion.

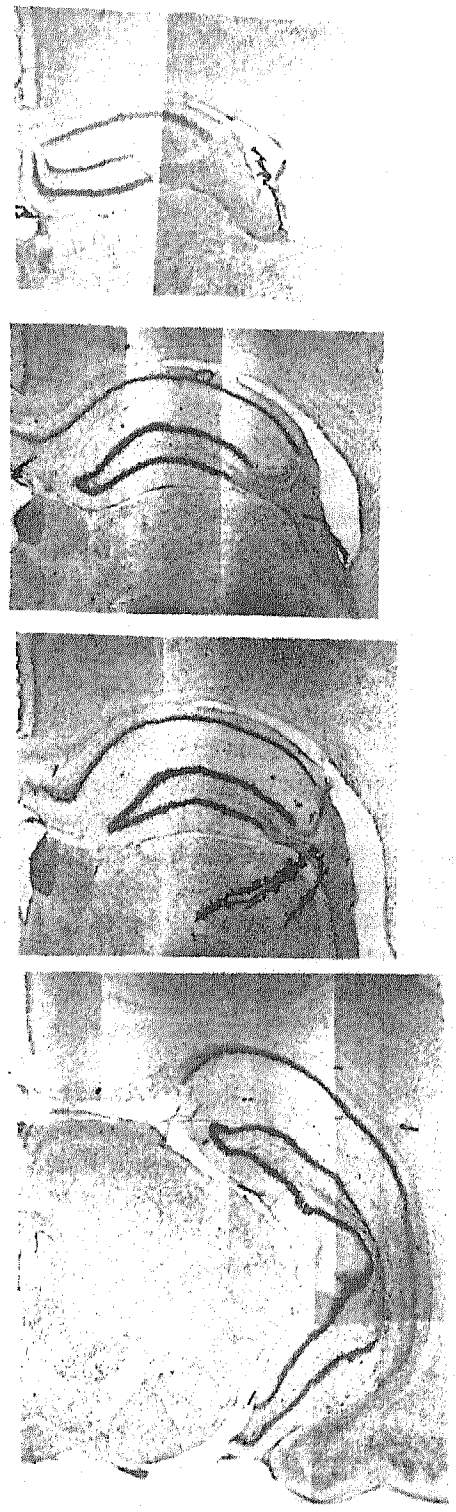

Fig. 2. Photomicrographs showing the extent of cell loss in CA_{3-4} produced by intrahippocampal microinjections of kainic acid (Cresyl violet).

location of the parietal ablations also involved a removal that was similar to that illustrated in Fig. 1, except the hippocampus was spared.

Fig. 2 shows the extent of kainic acid-induced hippocampal damage. As can be seen the CA_{3-4} fields were nearly completely removed from both the dorsal and ventral hippocampus. There was very minor and inconsistent cell loss in the medial portion of the CA_1 or the lateral portion of the CA_2 fields in two of the rats. The dentate gyrus was not affected apart from a reduction of the distance between the dorsal and ventral blades, presumably because of the loss of the intervening pyramidal cells of CA_{3-4}. A careful examination of the subicular regions showed no obvious cell loss or gliosis.

Fig. 3 shows the extent of the typical colchicine-induced damage to the granule cells of the dentate gyrus. The granule cells were largely removed and the cross-sectional area of the hippocampus was noticeably reduced. All of the rats had a few granule cells remaining at some coronal levels and one rat had a substantial number of granule cells remaining unilaterally in the most anterior portion of the hippocampus. Counts of numbers of granule cells at representative levels suggested that between 6 and 16% were spared in different rats. There was a small amount of loss to pyramidal cells of CA_1 and this was largely adjacent to the penetration of the injection cannula. There were two extrahippocampal areas that showed abnormalities in most animals. Directly above the hippocampus, along the injection needle tracts, minor damage to the corpus callosum was apparent and a small area of overlying parietal cortex was thinner than normal, probably reflecting diffusion of colchicine along the injection tract. Gliosis and darkly stained material was also present throughout the lateral posterior nucleus of the thalamus.

Task-induced behavioural seizures

Of the colchicine-lesioned rats, 3 had behavioural seizures or fits (n's = 11, 4, 1) at some time during testing, and of the kainic acid-lesioned rats, 3 had fits at some time during testing (n's = 11, 4, 1). The fits only began to occur after the first week of testing, and then happened

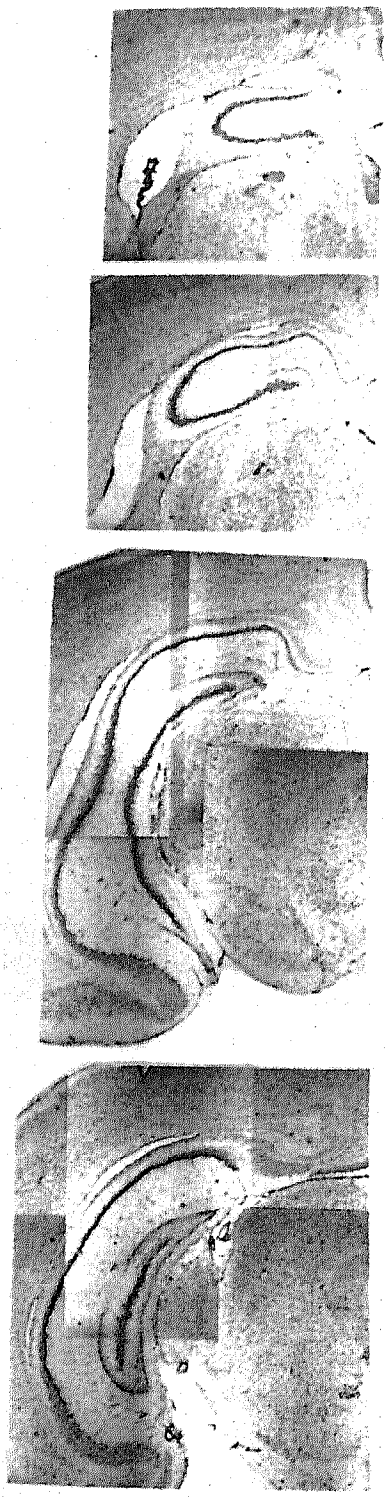

Fig. 3. Photomicrographs showing the extent of cell loss of granule cells in the dentate gyrus produced by intrahippocampal injections of colchicine (Cresyl violet).

periodically, but in none of the rats did they occur each day. On average, fits occurred on trial 6.3 (range: trial 4 to trial 16). Eight fits occurred on the first trial of the two trial pairs, whereas 24 of the fits occurred on the second of the two trial pairs. Furthermore, 31 of the 32 fits occurred when the rats were swimming, rather than when they were in the holding cages or on the platform. One occurred while a rat was in the holding cage. Inspection of the 7 days of video records showed that none of the 6 rats had a fit during the period when they were outside of the test situation.

The fits were recognized by pauses in swimming and by behavioural manifestations such as vibrissae twitching and body convulsions. When this occurred, the rats were removed from the water and replaced in their home cage and the swim trial was repeated 5 min later. The fits did result in abnormal performance for one trial after they occurred (Fig. 4). Student t-tests showed that there was a significant increase in latency between the trial preceding the fit and that following, $t_{31} = 3.43$, $P < 0.05$. But by the subsequent trial, latencies were again the same as they were on the trial preceding the fit. Since performance was adversely affected on the trial on which a fit occurred and on the trial following the fit, these trials were not included in subsequent analyses and supplementary trials were given as necessary.

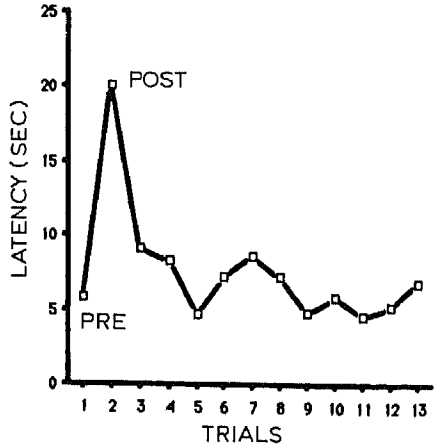

Fig. 4. Latency changes that followed behavioural fits. PRE: mean latency of rats on trials preceding the behavioural fit. POST: mean latencies on trials following the behavioural fit.

Learning-set acquisition

All of the rats reached asymptotic performance on the task by the time that the 14-day pretraining period was completed. Their asymptotic performance was identical to that displayed by the control group (see below) throughout the postoperative tests. Briefly, on the first trial of each day's test, the rats swam with about 70% accuracy to the site where the platform had been located on the previous day. On finding the platform absent they then searched the pool until they located it. Thus on the first trial their escape latencies were high, >10 s and the probability that they made an error with respect to the new problem was also high, >0.8. On the second trial, given a few seconds later, they swam directly to the new location and thus had low latencies, <5 s and the probability that they made an error was low, <0.3. Since the rats had almost achieved asymptotic performance on the second trial, improvement on the subsequent 14 trials was slight.

Postoperative performance

Following the surgical procedures, the performance of the rats that received brain damage was impaired. Their daily escape latencies were elevated, they made more errors, their improvement over successive trials on each day's problem was lessened, and with the exception of the animals with parietal cortex lesions, they never reacquired the learning-set response as defined by the performance of the control animals. Furthermore, when compared to preoperative acquisition, they displayed not only a complete retrograde loss of ability, but their performance was even worse than that obtained on acquisition. For example, on acquisition, normal rats have latencies reduced below 10 s by the second training day and error scores reduced below 5 errors by the sixth training day and they have mastered the task by about 7 days. Rats with hippocampal damage were initially worse than naive rats and showed little improvement throughout testing. These features of performance are described below. Since performance of the parietal ablated rats was quite different from the hippocampal groups, they are described separately.

Escape latency

Overall postoperative escape latencies are summarized in Fig. 5A. The control rats maintained a high level of performance throughout testing, escaping in a mean time of about 5 s, whereas the groups that had received hippocampal lesions were severely impaired even after 48 days of postoperative training, Group $F_{3,15} = 6.18$, $P < 0.006$. Nevertheless, the hippocampal lesion groups were more severely impaired in the early postoperative period and did show substantial improvement with training, Days $F_{47,705} = 8.85$, $P < 0.001$; Group by Days $F_{141,705} = 2.36$, $P < 0.001$. An interesting feature of the hippo-

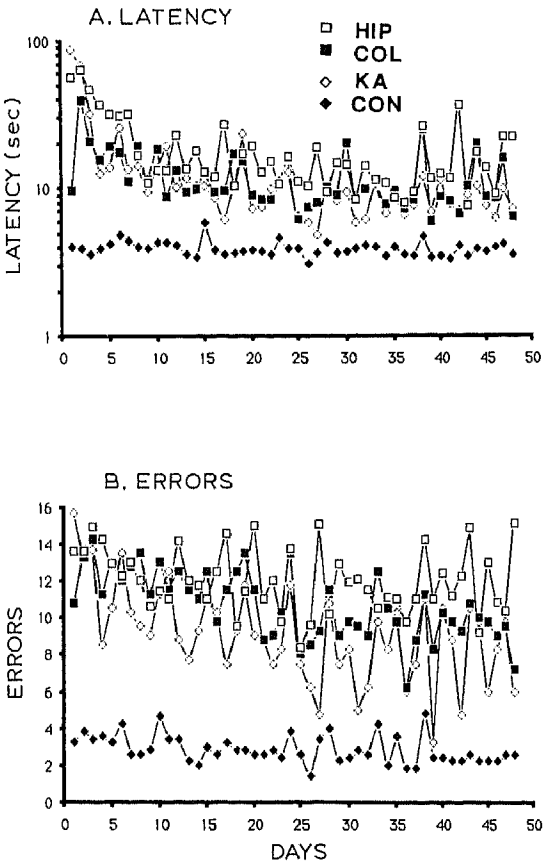

Fig. 5. A: mean escape latencies (plotted as a semi-log graph) for control and hippocampal-lesioned rats on each daily block of 16 trials through 48 days of postoperative testing. B: mean errors for control and experimental groups on each daily block of 16 trials through 48 days of postoperative testing. HIP, hippocampal aspiration; COL, colchicine injection; KA, kainic acid injection; CON, control.

TABLE I

Probability levels associated with group differences in overall postoperative latencies and errors between control rats and rats given kainic acid, colchicine or aspirative hippocampal lesions

Group	*Latency*			*Errors*		
	KA	*Col*	*Hip*	*KA*	*Col*	*Hip*
Control	0.008	0.003	0.003	0.003	0.001	0.001
Kainic acid (KA)	–	0.84	0.26	–	0.25	0.012
Colchicine (Col)	–	–	0.18	–	–	0.08
Hippocampal (Hip)	–	–	–	–	–	–

campal lesion groups' first postoperative days performance was that the colchicine group was only mildly impaired and became worse by day 2 postoperative, whereas the kainic acid rats were extremely impaired and improved rapidly over the next few days. These differences were probably due to the fact than granule cell degeneration induced by colchicine takes a few days to occur and kainic acid induces electrographic abnormalities that may persist for one to two days. Individual comparisons between groups are shown in Table I. The control group's performance was superior to that of the other groups, which did not differ from each other.

Errors

A summary of overall errors made by control and hippocampal lesion groups is given in Fig. 5B. The performance by the control group was excellent throughout the tests and seldom exceeded a mean of 3–4 errors on each of the 16 trial blocks. Comparatively, the other groups remained severely impaired over the 48 days of the tests, Group $F_{3,15} = 53.3$, $P < 0.001$. The hippocampal lesion groups, however, did show some improvement over the test period, Days $F_{47,705} = 4.72$, $P < 0.001$; Group by Days $F_{141,705} = 1.72$, $P < 0.001$. Follow-up comparisons (Table I) showed that the control group was significantly better than the other groups. Among the other groups, only the difference between the kainic acid group and the hippocampal group was significant.

Trial one to trial two changes

A feature of the performance of the rats that most obviously evaluates their ability to acquire a place response rapidly is the improvement shown between the first and second trials of daily training. Since rats in all groups did show some improvement in performance over testing, the performance on the first and second trials was summarized in 4-day blocks over the 48 days of training. The overall analyses gave significant Group, Trial and Block effects for both latency and errors (P's < 0.001). Follow-up analyses on individual groups showed that the first to second trial improvement shown by the control group was over 50% on both errors and latency on all trial blocks. Beginning by about the fifth trial block, the kainic acid group showed improvements between 35 and 45% on both errors and latency. The colchicine and hippocampal groups showed improvements of between 30 and 40% in latency on some trial blocks, beginning on about the fourth trial block, but at no time did they show improvements in error scores that exceeded 10%. When these latency and error results are taken together, they suggest that the control and kainic acid rats did show evidence of rapid place learning, although the kainic acid group was inferior to the control group. The colchicine and hippocampal aspiration groups, although able to reduce their latencies somewhat, remained inaccurate in their performance as was shown in their error scores.

The analysis of first to second trial improvement was repeated on individual animals to much

TABLE II

Probability levels associated with group differences in asymptotic latencies and errors between control rats and rats given kainic acid, colchicine or aspirative hippocampal lesions

Group	*Latency*			*Errors*		
	KA	*Col*	*Hip*	*KA*	*Col*	*Hip*
Control	0.001	0.001	0.001	0.001	0.001	0.001
Kainic acid (KA)	–	0.40	0.17	–	0.48	0.001
Colchicine (Col)	–	–	0.12	–	–	0.002
Hippocampal (Hip)	–	–	–	–	–	–

the same effect. All of the control animals showed consistent first to second trial task acquisition as judged by both errors and latency. All but one of the kainic acid group showed significant first to second trial improvements on both measures, whereas none of the colchicine or hippocampal aspiration groups showed consistent first to second trial improvements. Further details of group performances follow.

Asymptote latency

The trial by trial latencies for each of the groups on the last 8 days of training were compiled (days on which animals had behavioural fits were excluded from the analysis). The performances of the control group and the hippocampal lesion groups are illustrated in Fig. 6A. Analysis of variance showed that there were significant Group, $F_{3,14} = 3.68$, $P < 0.038$, Trials, $F_{15,45} = 4.65$, $P < 0.001$, and Group by Trials, $F_{15,45} = 4.65$, $P < 0.001$, effects. As shown by post hoc analyses, the control and kainic acid groups showed significant changes across trials and the colchicine and hippocampal groups did not. Furthermore, group comparisons showed that the control group performed significantly better than the hippocampal lesion groups, among which there were no significant differences (Table II). Analyses on first to second trial changes showed that the control group displayed a rapid reduction in latency between the first and second trial, so that it almost reached asymptotic performance by that trial. Changes in first to second trial performance were not sustained in the groups with hippocampal lesions. However,

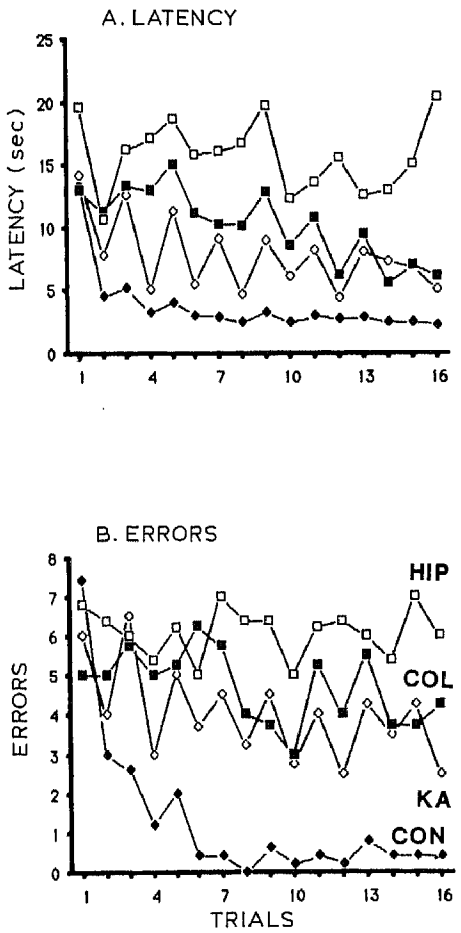

Fig. 6. A: mean escape latencies (plotted as a semi-log graph) across trials for the last 8 days of training. B: mean error scores across trials for the last 8 days of training. HIP, hippocampal aspiration; COL, colchicine injection; KA, kainic acid injection, CON, control.

the kainic acid group did display a consistent improvement in performance on the second of each of the pairs of trials.

Asymptote errors

Trial by trial errors for the control group and the hippocampal lesion groups were computed for the last 8 days of training (excluding days on which rats had behavioural seizures) and are illustrated in Fig. 6B. Analysis of variance gave significant effects of Groups, $F_{3,14} = 19.2$, $P < 0.001$, Trials, $F_{15,45} = 12.72$, $P < 0.001$, and Group by Trials, $F_{45,210} = 4.27$, $P < 0.001$. As shown by post hoc analyses, all groups except the hippocampal aspiration group showed significant changes in error scores across trials. Between-group comparisons also showed that the differences between all groups, excepting the colchicine and kainic acid groups, were significant (Table II). Analysis of first to second trial changes showed that only the control group showed a significant improvement between trials, but again the kainic acid group did show improvement between first and second trials of each trial pair.

Parietal cortex lesions

A summary of the effects of parietal lesions on acquisition latency and errors and asymptotic latency and errors is shown in Fig. 7. The rats with parietal lesions were impaired on acquisition latency, $F_{1,7} = 5.71$, $P = 0.048$, and on acquisition errors, $F_{1,7} = 21.5$, $P = 0.002$. They were also quite impaired for a few days following surgery, but within about 5 days they reached

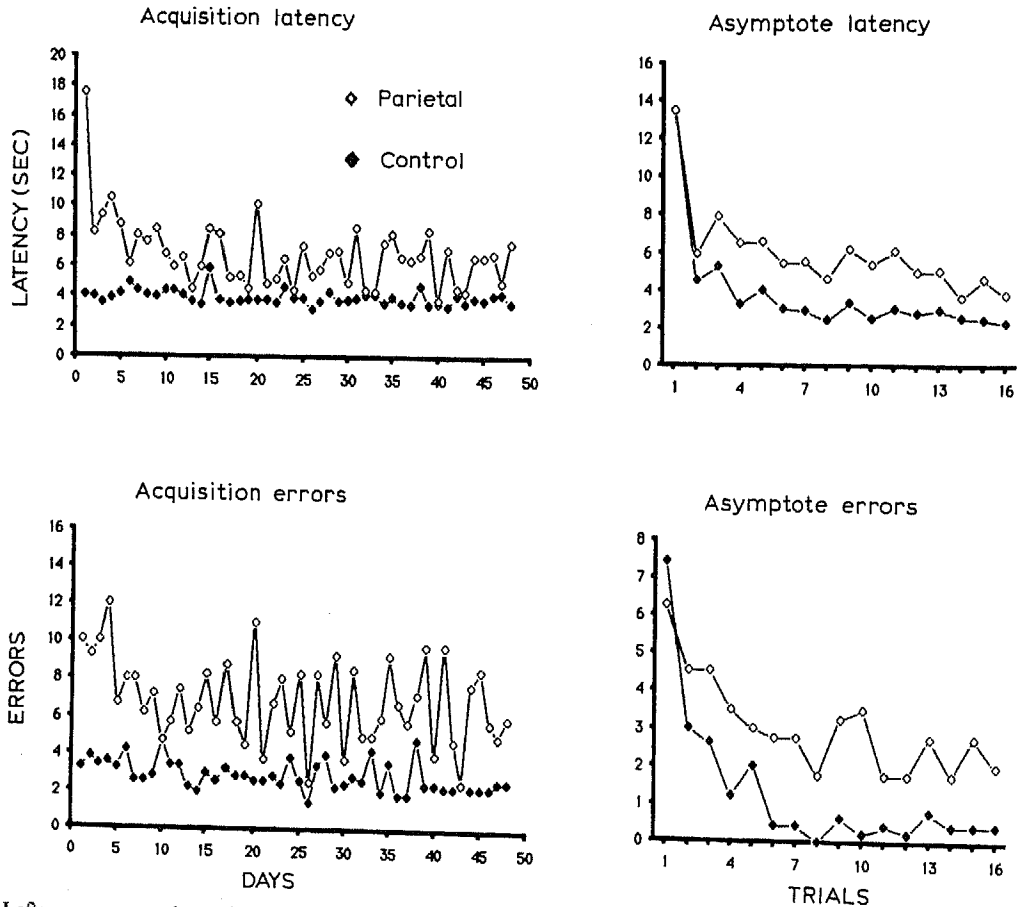

Fig. 7. Left: mean escape latencies and errors for control and parietal cortex-lesioned rats on each daily block of trials through 48 days of postoperative testing. Right: mean escape latencies and error scores across trials for the last 8 days of training for control and parietal-lesioned rats.

asymptotic postsurgical performance, Group by Trials interactions both being significant, $P < 0.001$.

Although the asymptotic performance on both latencies and errors of the parietal group was inferior to that of the control group (P's < 0.01), there were no group by trial interactions (Fig. 7). The first to second trial improvement on latency by both groups was significant (P's < 0.05) and the pattern of performance was similar, although the parietal group's latencies remained inferior to the control group. The first to second trial improvement in errors by the control group was significant, but that for the parietal group was not. Nevertheless, overall improvement did occur across trials although asymptotic performance did not reach control levels. In summary, the parietal group responded at asymptote in much the same way as the control rats, except with slightly elevated latency and error scores.

DISCUSSION

The study confirms previous work showing that normal rats readily form a 'place learning-set' in the swimming pool and perform it efficiently over long periods of testing[27,28]. In addition, 5 new observations were made. First, rats with complete dorsal hippocampal lesions were unable to perform the task even after they had been pretrained and they were never able to solve the new problems that were presented to them each day. Second, rats that had granule cells of the dentate gyrus removed by intrahippocampal microinjections of colchicine, performed almost as poorly as rats with aspirative hippocampal removals. Third, rats that had cells of CA_{3-4} removed by intrahippocampal microinjections of kainic acid perform the task slightly better than the two former hippocampal lesion groups and they displayed evidence of acquiring the new place responses, but they were significantly impaired compared to control rats. Fourth, the parietal lesions produced impairments, but the qualitative features of the rats' performance was similar to that of control rats. Fifth, rats that had received colchicine or kainic acid injections displayed behavioural seizures or fits only when required to perform the place tasks, suggesting that performance on the task itself may have been the trigger. Each of these observations contributes to the conclusion that the hippocampal formation is importantly involved in place navigation.

In the experiments the rats were pretrained on a 'learning-set' task, in which a platform, hidden beneath opaque water, was moved to a new location each day. Important features of this task suggest that it provides a robust evaluation of place navigation as it has been defined by O'Keefe and Nadel[20]; that is, acquisition and extinction should be rapid, retention should be relatively long-lasting and the response must be guided by distal cues. Control rats acquire each daily problem in about one trial and to do so they must give up the previous day's correct response as quickly. They also retain the previous day's correct response between tests, because on the first trial of a day's test, they reliably swim to that location before searching for the platform at a new location. Thus, they have high latency and error scores on the first trial because they swim first to the place where the platform was located on the previous day and they then search for it at its new location. On the second trial their latency and error scores are reduced almost to asymptote because they swim directly to the new location. Since the platform is hidden and the only available cues to guide the response are distal cues, the excellent performance of the control rats demonstrates their efficiency in generating new responses in relation to those cues. Additional virtues of the test are that it provides a sufficient number of daily trials to make a comparative evaluation of animals that are impaired and the tests can be repeated indefinitely.

The findings of the study, therefore, seem definitive. Despite pretraining and extensive practice, rats with hippocampal ablations, whether by suction or the neurotoxins colchicine and kainic acid, were severely impaired from the outset and remained so for the duration of the test. Their latencies were high, they made errors on virtually all trials and they failed to show the rapid one-trial acquisition on the daily test sessions that was characteristic of control rats. When it is considered that they were pretrained and that they

then received over 700 training trials postoperatively, their deficit does seem almost absolute. It also seems unlikely that further practice would have led to any significant improvement in performance. The results strongly imply that if the definition of place navigation includes the stipulation that acquisition and extinction be rapid, then that definition will identify a task for which the hippocampus is essential. In some respects this conclusion seems to favor the postulate that the hippocampus is necessary for working memory[8], which is defined as memory that is used only briefly for successful task performance. Unfortunately, the present results shed little light on just how the hippocampus is involved in place navigation. Views that the hippocampus holds spatial maps, is involved in inhibiting competing responses, is required for working memory, or is involved in calculating swim trajectories, could equally account for the present results. It is important to recognize, however, the rats with hippocampal damage were not completly devoid of all ability. They swam normally and their swim patterns permitted them to reach the platform on virtually all trials. For the most part, they made loops around the pool, and this was a strategy that, although not accurate, was effective. The swim patterns also seemed modifiable, for the animals did show latency, but not error, reductions between the first and second trials on the tests. The fact that these latency improvements did not carry over to subsequent trials suggests that they were not generated by a place navigation strategy but may rather involve modifications of some cue-based strategy. Furthermore, the neurotoxin-treated rats did display better performance than the rats with aspirations. For the colchicine-treated rats, this may have been due to residual cells. The treatments spared between 6 and 16% of the granule cells and a good relation between cell loss and degree of impairment was observed. Second, elsewhere it has been reported that residual functions can be sustained by small numbers of granule cells. Following neonatal focal X-irradiation or adult colchicine injections, as few as 6% of residual granule cells can still support hippocampal (RSA, rhythmical slow activity or theta rhythm).[25,29]

The kainic acid injections most severely impaired performance immediately following surgery, at which time electroencephalographic seizures may have contributed to the impairment. Kainic acid-treated animals gradually improved to the point that they were better than other hippocampal groups, but they remained severely impaired with respect to control rats. At asymptotic levels of performance, they did show improvement between the first and second trials of the successive trial pairs, but the improvements were not as great as those seen in the control rats and they seemed largely limited to individual trial pairs. It was almost as if the rats were restricted to learning the location of the platform from each of the start points de novo. This impairment bears some similarity to impairments displayed by rats with entorhinal lesions on a learning-set task that used olfactory cues[23]. The rats with entorhinal lesions were severely impaired if the retention intervals were increased beyond 2 min. This may mean, as was suggested, that hippocampal damage produces a rapid forgetting syndrome similar to that of humans with temporal lobe dysfunction, but this must be viewed cautiously. First, human temporal lobe patients do not show similar memory impairments on olfactory tasks as is classically found on other tasks[4]. Second, the impairment observed here with CA_{3-4} lesions was not the same as that observed with the aspirative ablations. Nevertheless, the result is sufficiently interesting to warrant more work to evaluate the time course of this apparent response decay.

Despite a number of lines of converging evidence that suggest that the hippocampus is involved in place navigation, the possibility that extra-hippocampal damage is responsible for the deficits cannot be categorically refuted[9–12]. However, in view of the close relation between granule cell damage and performance, the slow onset of the colchicine deficit, which parallels the slow action of the neurotoxin, and the absence of definitive cell loss in other structures, the hypothesis that it is the hippocampus that is responsible for the impairments is favoured. Furthermore, the performance of rats with CA_{3-4} lesions has now been evaluated in a number of studies. The finding that pretrained rats are initially impaired

postoperatively but subsequently show some recovery seems consistent in all reports, but descriptions of the extent of the initial deficits and degree of recovery do vary[8,10,24]. Likely the difference between the present study, in which full recovery was not obtained, and other studies, in which complete recovery was obtained[8,10], is due to differences in the demands made by the task. Thus, the view that task difference can account for performance differences between studies is favoured here and, accordingly, comparative studies using different tasks may be worthwhile.

The parietal cortex aspirations, performed as a control for the aspirative hippocampal lesions and for possible cortical damage following diffusion of the neurotoxins along the injection needles, produced a moderate impairment in both error and latency measures. Nevertheless, this group was significantly better in all facets of performance than the hippocampal lesion groups and their learning set acquisition at asymptote was similar to that of the control rats. These results confirm the results of many previous studies in which similar lesions have been made and they also confirm previous studies that evaluated spatial navigation in swimming pool tasks[13,14].

Hippocampal kainic acid injections have previously been reported to produce chronic electrographic and behavioural seizures[3,10,15]. After high doses of kainic acid, handling seems sufficient to produce fits but after doses as low as those used here, their occurrence seems much more restricted. Here we also report that hippocampal colchicine injections can result in chronic periodic fits. That fits in both groups occurred when the rats were performing in the learning-set task is interesting, for the effect is in some ways analogous to human reflex epilepsy, which is induced in some patients by reading, drawing, arithmetic, etc.[1,2,5,6]. If the fits are analogous to human reflex epilepsy, they provide further evidence for the hippocampal formation's essential involvement in place navigation. At least 3 lines of evidence suggested that the fits were task-related. First, they occurred only after the rats had been performing in the task for a number of trials and then an overwhelming number occurred on the second of the two trial pairs. Fits were not observed when the rats were sitting on the platform after trials or when they were in the holding cages between trials. This suggests that some demand features of the task may have provided the trigger for inducing the fits. Second, the rats were filmed continuously for one week and none were observed to have a fit. Thus the fits were not a regular manifestation of the rats' behaviour that was incidentally observed during swim tests. It is possible that the fits were induced by some other feature of the task, such as exercise from swimming, or from non-specific stress, or from a combination of these influences. Nevertheless, they are still interesting, for they demonstrate that rats with selective lesions of certain cell populations of the hippocampus can subsequently suffer epileptic attacks. These attacks may be related to extrahippocampal damage produced by the lesions, but it is more likely that they are due to hyperexcitability of remaining hippocampal cell populations, such as those of CA_1 or of the subiculum. The suggestion is made because the rats with hippocampal aspirations displayed no seizure activity in the same test conditions. Since animal models of epilepsy are relatively difficult to produce[21], some features of the present paradigm may be elaborated into a more comprehensive animal model. It is also noteworthy that the fits did result in task performance deficits for about 5 min; however, this is hardly surprising since it is known that hippocampal electrographic seizures also disrupt performance in spatial navigation tasks.

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