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Reversible inactivation of the medial septum differentially affects two forms of learning in rats

S.J.Y. Mizumori, G.M. Perez, M.C. Alvarado, C.A. Barnes and B.L. McNaughton

Department of Psychology, University of Colorado, Boulder, CO 80309 (U.S.A.)

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The contribution of the medial septum to different aspects of spatial information processing was assessed by examining the effects of reversible septal inactivation on radial maze performance of rats. In addition, the selectivity with which the medial septum affects learning was studied by testing the effects of septal inactivation on the acquisition of non-spatial information. Rats were first trained according to a spatial working memory procedure that included a 30-min delay between the first 4 (forced) choices and subsequent test (free) choices. The forced choices comprised the sample phase of the experiment while the free choices comprised the test phase. Saline or tetracaine (a local anesthetic) was injected into the medial septal area either before the sample phase, after the sample phase (i.e. at the beginning of the delay period), or just before the test phase. In contrast to the saline injections, tetracaine injected just before the sample or test phases produced a significant increase in errors at test. Tetracaine injection at the beginning of the delay period did not affect test choice accuracy. EEG records showed that septal inactivation drastically, yet temporarily, reduced the hippocampal Θ rhythm. Thus, when septal inactivation occurred either before the sample phase or at the beginning of the delay period, hippocampal Θ recovered by the time of the test phase. Septal inactivation also produced a significant retardation of learning on a non-spatial reference memory task, although clear improvement over trials did occur. Moreover, the results of subsequent saline injections suggest that at least some of the performance deficit was due to variables other than learning per se. Although such performance effects could at least in part account for the errors made when septal inactivation occurred before the test phase of the spatial task, it could not account for errors made when septal inactivation occurred before the sample phase. This result, together with the finding that septal inactivation drastically alters hippocampal single unit activity[27,28], is consistent with the hypothesis that normal septal activation (of perhaps the hippocampus) is required for acquisition of spatial information. Maintenance of spatial memory, on the other hand, may not rely on such activity.

INTRODUCTION

The hippocampus has for some time been implicated as a critical brain structure for normal spatial cognition[36,39,42]. Although the details of the specific contribution of this structure to spatial function remain uncertain (cf. ref. 4), the results of a number of electrophysiological and lesion experiments suggest that the hippocampus participates in the formation of complex sensory associations which in turn enables animals to learn to identify locations in space. It is likely that important spatially relevant sensory information arrives in hippocampus via the large afferent system from the entorhinal cortex, which in turn receives input from most neocortical regions[18,19,22]. Medial septal fibers comprise the second largest hippocampal afferent system[9,45]. Although the septal input is much smaller than the entorhinal input, septal terminals are strategically located such that septal neurons can potentially exert significant control over information processing in the hippocampus[11,12]. Indeed, past studies of medial septal influences on hippocampal-dependent behaviors indicate that, similar to the results obtained in hippocampal lesion experiments (e.g. refs. 1, 16, 32, 39), permanent medial septal or fimbria/fornix lesions produce spatial learning deficits in rat trained on a variety of different tasks[33,37,44]. Since the temporary removal of septal afferents also results in spatial performance deficits[28], permanent lesion-induced changes in hippocampal synaptic organization or retrograde degeneration do not appear to be necessary for the spatial impairments.

If the medial septum importantly modulates hippocampal function, it might be expected that forms of learning which are unaffected by hippocampal lesions would also be unaffected by septal lesions. The extent to which this expectation is correct is not yet clear. For example, although hippocampal lesions produce either no effect or only a short-lived impairment in non-spatial learning (e.g. refs. 17, 30, 32, 48), reports of either no effects or significant impairments can be found in experiments involving septal or fimbria/fornix lesions (e.g. refs. 33, 35, 37, 44). The apparent discrepancies are

Correspondence: S.J.Y. Mizumori, Department of Psychology. Present address: University of Utah, Salt Lake City, UT 84112, U.S.A.

difficult to evaluate since a variety of tasks and procedures were employed by the different investigators.

Medial septal influences on hippocampal function have also been assessed in terms of the physiological properties of hippocampus following septal stimulation or inactivation. For example, electrical stimulation of the septal region increases the excitability of dentate gyrus granule cells[3] by attenuating feed-forward inhibition of the interneuron population[5,6,10,26]. Also, reversible septal inactivation selectively affects hippocampal single units such that location-specific discharge[23,31,34] by hilar/CA3c cells become less spatially tuned while the location-specific discharge of CA1 cells is maintained during impaired performance on a spatial working memory task[28]. Thus, the spatial firing characteristics of different populations of hippocampal cells appear differentially regulated by the medial septum. Since both permanent and temporary septal deafferentation eliminates the movement-sensitive Θ-modulation of the hippocampal EEG (e.g. refs. 7, 14, 28, 49), it is also thought that the medial septum contributes to the rhythmic discharge pattern exhibited by many hippocampal cells.

Taken together, the behavioral and physiological results described above provide clear evidence that the medial septum exerts a dramatic influence over hippocampal function. However, there are issues that require further clarification. For example, it remains to be determined which aspect of spatial cognition is influenced by the septum. The present study examines this issue by assessing the effects of reversible septal inactivation on the acquisition, maintenance, and recall processes involved in performance on a spatial maze. Furthermore, since the extent to which the medial septum selectively participates in learning is presently not clear, the effects of septal inactivation on the ability of rats to learn new non-spatial information were also investigated.

MATERIALS AND METHODS

Subjects

Twelve experimentally naive, 9-month-old male Fischer 344 retired breeder rats (Charles River Laboratories, Wilmington, MA) were housed singly and provided food and water ad libitum for 1 month prior to behavioral training. Rats were maintained at about 80% of their free feeding body weight during behavioral testing. The colony was maintained at 25 °C on a 12-h light/dark cycle (lights on at 06.00 h). Behavior testing occurred between 07.00 and 13.00 h.

Apparatus

Rats were trained to perform a spatial memory task on an 8-arm radial maze[38] elevated 75.6 cm above the floor, with a central circular platform (19.5 cm diameter) and 8 alleys, or arms (58 × 5.5 cm), radiating outward. Small food wells, containing chocolate milk reward, were positioned at the distal ends of each arm. The arms were hinged such that the experimenter could control access to each arm by raising or lowering the proximal portion by a remote switch. Various objects such as a vacuum cleaner, ladder, tables, and chairs were located at different locations within the maze room which was illuminated by a single 40-W light bulb.

Following completion of testing on the spatial memory task, the same rats were trained on a non-spatial version of the radial maze task. Distinct cue inserts in each maze arm served as intramaze cues. These consisted of balsa wood covered with various grades of sandpaper, satin, wool, raised bars positioned horizontally or vertically on contact paper, toothpicks, hardware cloth, or small colored spheres. For training and testing on the non-spatial task, the maze was located in a different room from that used for the spatial task, and was surrounded by a black curtain. Illumination of the maze was by eight 35-W track lights located symmetrically overhead.

Surgical procedures

Rats were food and water deprived for 24 h before surgery. A dose of 30 mg/kg sodium pentobarbital (Nembutal 50 mg/ml) was used for initial anesthesia, followed by supplements of 0.05 ml as necessary. Small burr holes were drilled in the skull for stereotaxic placement of a 25-gauge stainless steel guide cannula[28] above the medial septum and bilateral EEG recording electrodes in dorsal hippocampus. The guide cannula contained a 33-gauge stainless steel stylet. The recording electrodes were constructed from 114-μm-diameter teflon-coated stainless-steel wires. Two strands of wire were twisted together, then cut blunt such that 1 wire extended about 700 μm beyond the other. The stereotaxic coordinates[40] for cannula and electrode placements were as follows: medial septum — AP 0.7 mm anterior to Bregma, L 0.0 mm (midline), DV–4.5 mm from the dural surface; dorsal hippocampus — AP 4.0 mm, L ± 2.4. mm, DV 3.0 mm. The final depths of the recording electrodes were determined electrophysiologically on the basis of hippocampal multiple unit activity.

In addition to the cannula and recording electrodes, a reference electrode was placed in corpus callosum (AP–0.5 mm, L 0.5 mm) and a ground lead was attached to a jewelers screw that was fastened to the skull. All leads were attached to amphenol pins that were inserted into a 9-hole connector[29]. Dental acrylic was applied to the base of the cannula, recording and reference electrodes, ground lead, and the 5 jewelers screws which anchored the assembly to the skull. Following surgery, 0.1 ml Bicillin was injected (i.m.) into each hindleg and antibacterial ointment (Furacin) was applied to prevent infection. Animals were allowed free access to food and water for 7 days after surgery. Food was then restricted and postsurgical training was begun.

Recording procedures

During postsurgical training trials, the animals were connected to a headstage comprised of 5 FET (unity gain) preamplifiers. The analog signals were amplified 1000 times, then filtered between 0.5 and 30 Hz (one-half amplitude cut-off). The signals were digitized in 2.56-s epochs at a sampling rate of 100 Hz. Following daily test sessions, the EEG data were subjected to fast Fourier transform analysis and then were converted to spectral power that was integrated between 6 and 9 Hz to provide a measure of the hippocampal Θ rhythm.

Hippocampal EEG was recorded while animals performed both spatial or non-spatial versions of the radial maze task. EEG was sampled while animals traversed arms during the sample and test phases of the spatial experiment (described below). In addition, the EEG was observed during the delay period to verify that tetracaine injections during this time reduced hippocampal Θ. The effects of saline and tetracaine on the relative power of Θ were quantified by calculating the fractional change between records collected during the sample and test phases. The specific calculation involved first taking the difference in Θ power during the two experimental phases, the dividing by the sum of these two values.

At least 5 EEG epochs were collected before the daily pretrial saline or tetracaine injection in the non-spatial task (see below). Since hippocampal Θ is most pronounced during movement[47], the

rats were encouraged to move about the central platform while pretrial EEG was collected. EEG was then recorded while animals traversed maze arms. Drug-induced changes in the EEG were evaluated by calculating the fractional change in relative power of Θ recorded during pretrial and trial epochs.

Drug injection procedures

The reversible inactivation procedure represents a modified version of that presented by Malpeli and Schiller[21] and Schiller et al.[41]. The specific injection protocol has been described previously[28]. The local anesthetic tetracaine (Sigma Chemical Co.) was dissolved in 0.9% NaCl to produce a 2% solution. Tetracaine, 0.5 μl, was air-pressure injected through a 33-gauge injection needle that was connected to a Picospritzer (General Valve Corporation). This particular dose of tetracaine produces physiological effects in hippocampus that last about 15 min[28].

Behavioral training procedures: spatial task

Before surgery, 12 rats were trained on the spatial version of the radial maze task. During the initial phase of training, all arms were presented simultaneously and the rats were required to enter each arm only once during a trial to obtain all of the food reward. Re-entries into previously visited arms were counted as errors. When rats entered all 8 arms at least once in 15 min, forced-choice training began. During this procedure, the experimenter first presented 4 arms sequentially (sample phase) and then presented all 8 arms simultaneously (test phase). The spatial location of the first 4 forced-choice arms differed from trial to trial and were selected randomly. The rats performed 1 trial daily until a criterion of 7 correct in the first 8 choices, and no more than two errors total, was achieved for 3 consecutive days. After reaching criterion, delays of progressively increasing lengths were imposed between choices 4 and 5, that is, between the sample and test phases of the session. The delay intervals were 1, 2, 5, 15 and 30 min. Each rat was required to perform at criterion for 3 days before advancing to the next delay interval length.

Following criterion performace with 30-min delays, the rats were given free access to food for 3 days, after which surgery was performed. After recovery from surgery, rats were retrained with 30-min delays. Following re-attainment of criterion performance, the rats first underwent a preliminary series of 4 injections (2 tetracaine and 2 saline injections) to adapt them to the injection procedure. All rats were then tested in *each* of the following 8 injection conditions: tetracaine or saline was injected either before the sample phase, immediately after the sample phase (i.e. at the beginning of the delay period), or immediately before the test phase. Tetracaine or saline was also injected immediately after the sample phase on probe days when the delay interval was reduced to a minimum ('0 min delay'). The latter condition was included to assess the effects of septal inactivation on performance that did not rely on information processed during a longer delay period. The order of testing was counterbalanced with the restriction that tetracaine or saline was not injected for more than 2 consecutive days. Each animal was assigned a different order of testing. Behavior testing was conducted blind with respect to the identity of the drug injected. Since each rat contributed data to all of the 8 injection conditions, a within-subjects analysis of variance test was used to assess the statistical reliability of the results.

Behavioral training procedure: non-spatial task

Within 7 days of completion of testing on the spatial task, 8 of the 12 animals began training on a non-spatial reference memory procedure. On their first exposure to the cued maze, the rats were allowed to traverse all 8 of the differently cued arms to obtain chocolate milk reward. Reward was replaced immediately following a visit to an arm. Each rat remained on the maze for about 15 min to ensure multiple exposures to each cue. On subsequent days, only 1 cue was associated with reward. This was considered the goal cue. Rats were assigned to experimental (tetracaine) or control (saline) groups. The animals were matched such that the correct goal cue was the same for each pair of control and experimental animals. Different correct cues were assigned to different pairs of rats.

On the first of 4 daily trials, only the goal cue was presented to the rat. After this sample trial, the rat was confined to the central platform for about 15–30 s. Then, the rat had to select the correct goal cue amongst the 2, 4 and 8 arms presented on trials 2, 3 and 4, respectively. The spatial location of the goal cue, as well as the spatial arrangement of cues relative to one another, varied randomly from trial to trial. Entries into arms that did not contain the goal cue were considered errors. When rats made on average no more than one error per day over a period of 5 days, the training procedure was modified to include one sample trial, followed by 4 trials in which all 8 cues were presented simultaneously. The rats performed according to this modified procedure until they made on average no more than one error per day over 7 consecutive days. The day after this criterion was met, performance was observed following either thorough cleansing of the goal cue or replacement of the goal cue with a new and clean one. This procedure assessed the contribution of goal cue-specific odors to performance.

Septal inactivation effects on non-spatial learning were then tested as animals were trained to select a new correct goal cue from a different set of distinct cue inserts. Before each daily training session, either saline (control) or tetracaine (experimental) was injected into the medial septum. Each training session consisted of one sample trial and 4 trials in which all 8 cues were presented. Training continued until an average of one error was committed per day over a 7-day period. On the next day, control animals received a tetracaine injection and experimental animals received a saline injection into the medial septum. This final probe for the control group served to establish whether septal inactivation affects retention of previously learned information in this task. The probe session for the experimental animals established the degree to which such learning was state-dependent. For those experimental animals that did not achieve criterion performance within 24 days, the probe session served to determine the extent of learning that occurred during the preceding septal inactivation trials.

Histological procedures

After the experiment, the rats were injected (i.p.) with 1 ml Nembutal, then perfused intracardially. The brains were removed, allowed to sink in 30% sucrose formalin, then embedded in gelatin. Frozen coronal sections, 40 μm thick, were stained with Cresyl violet for identification of the electrode and cannula placements. Any rat whose cannula was not directed toward the medial septum was excluded from behavioral and EEG analysis.

RESULTS

Electrode and cannula placement

The bilateral recording electrodes were located within dorsal hippocampus as shown in Fig. 1A, and the cannula tips above the medial septum are shown in Fig. 1B. Using a 1 in 5 series of Cresyl violet-stained sections, the percent damage of the medial septum and vertical limb of the diagonal band was estimated independently by two experimenters. Damage to the medial septum ranged from 1.9 to 32.6%. The mean (± S.E.M.) septal damage observed was 12.2 ± 2.8%. The diagonal band was damaged on average 10.7 ± 2.2%, with a range from 1.4 to 25.7%. Illustrations of septal regions suffering the least and greatest amount of damage are provided in Fig. 2. There were no systematic variations in behavior or EEG scores as a function of degree of damage to septal or diagonal band nuclei.

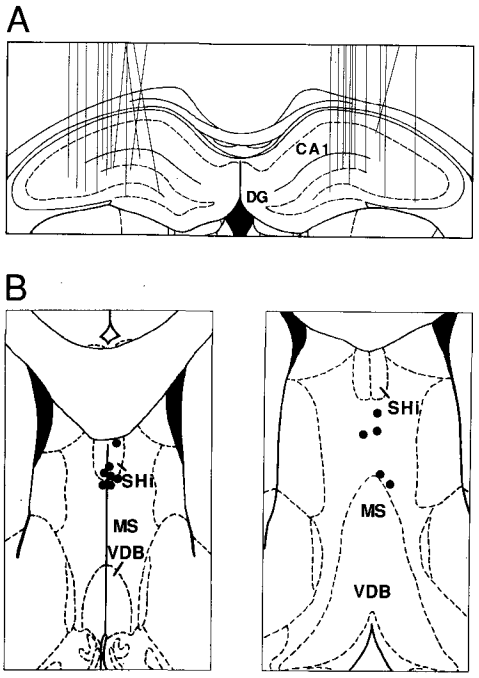

Fig. 1. A: schematic diagram of a coronal section of rat brain. The dashed lines represent the principal cell layers of hippocampus, and solid vertical lines show the location of the EEG recording electrodes within dorsal hippocampus. B: schematic representations of a coronal section through the medial septal region. Solid dots reveal the locations of the guide cannula tips. Since the injection needle was placed 1 mm beyond the cannula tip, it was estimated that the tip of the injection needles were situated either just dorsal to, or in the dorsal aspect of, the medial septum. A: DG, dentate gyrus; MS, medial septum; VDB, vertical limb of the diagonal band; SHi, septohippocampal nucleus. (Adapted from Paxinos and Watson[40].)

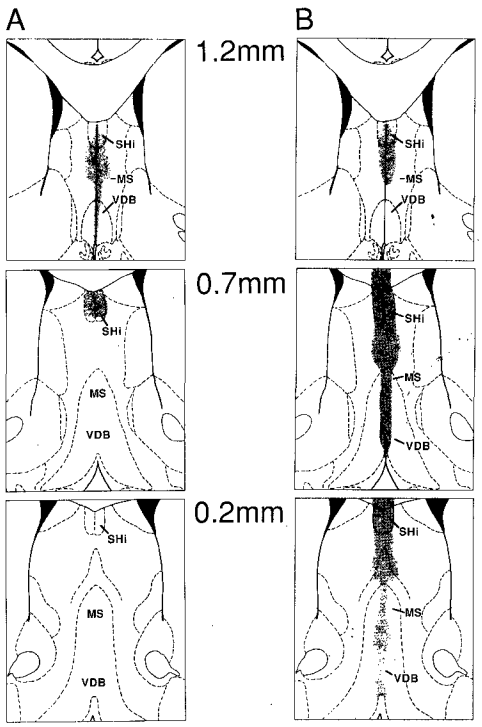

Fig. 2. Schematic illustrations of (A) the least and (B) the most tissue damage observed in the medial septal region, as determined by histological examination.

Spatial task

Animals required, on average, 32.0 ± 1.6 days to reach criterion performance before surgery. Following surgery, the animals quickly regained asymptotic performance within 12.8 ± 1.4 days. A summary of the mean number of errors made per rat during each of the injection conditions can be found in Fig. 3. A 2-way repeated measures analysis of variance (ANOVA) revealed a significant main effect of drug treatment $F_{1,22} = 32.4$, $P < 0.001$, as well as a significant interaction effect of drug and injection condition, $F_{3,166} = 4.26$, $P < 0.01$. Subsequent pairwise comparisons with the Scheffé test ($\alpha = 0.05$) confirmed that while saline injections did not affect choice accuracy regardless of the time of injection, tetracaine injection selectively impaired choice accuracy when injected before the sample phase or just before the test phase of 0- or 30-min delay trials.Tetracaine injection at the beginning of the 30-min delay had no effect on choice accuracy at test.

Additional analyses were conducted to determine the extent to which septal inactivation affected the pattern of arm selection at test. Consistent with reports of other investigators (e.g. ref. 48), the removal of septal afferents did not significantly affect the overall turn bias of animals as they excited one arm to select the next ($P > 0.10$). However, occasionally an animal did respond to the tetracaine injection by adopting a specific response strategy. For example, an animal might consistently turn clockwise upon exciting an arm, then enter the second arm

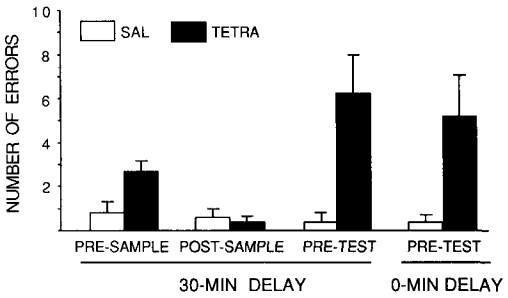

Fig. 3. Mean number of errors following medial septal injection of either saline (SAL) or tetracaine (Tetra) before the sample phase of 30-min delay trials (Pre-sample), immediately after the sample phase and at the beginning of the 30-min delay period (Post-sample), or just before the test phase (Pre-test) following either 30- or 0-min delays. Injections of saline did not affect choice accuracy at test. In contrast, pre-sample and pre-test injections of tetracaine produced a significant increase in errors after either 30- or 0-min delays. Pre-test injections resulted in more total errors than the pre-sample injections. The data provided in Fig. 4 suggest an explanation for this result. Post-sample tetracaine injections did not impair choice accuracy at test.

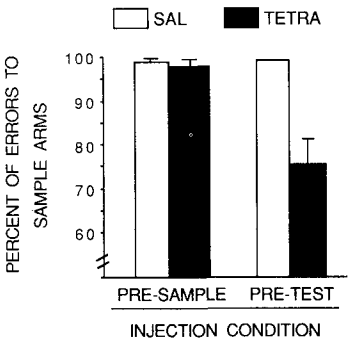

Fig. 4. Percent of test errors that involved re-entries to arms chosen during the sample phase of the experiment. When tetracaine was injected into the medial septum before the sample phase, animals made errors at test by re-entering arms chosen during the sample phase. When tetracaine was injected just before the test period, errors involved re-entries to arms selected during the sample phase *and* to new arms selected during the test phase. These findings, along with those of Fig. 3, support the hypothesis that septal inactivation impaired acquisition of new spatial information in the pre-sample condition.

over from the one just visited. Although this pattern was observed, it was not consistent between or within animals.

Whether or not errors made at test represented re-entries to arms presented during the sample phase or re-entries into new arms selected during the test phase was also examined. The data presented in Fig. 4 show that the type of errors made at test depended on whether or not tetracaine was injected before the sample phase or before the test phase, $F_{2,10} = 6.39$, $P < 0.01$. When septal inactivation occurred before the sample phase, test errors tended to occur to sample arms. When septal inactivation occurred just before the test phase after 0- or 30-min delays, errors were made to sample arms *and* to new arms selected at test. Similar numbers of different

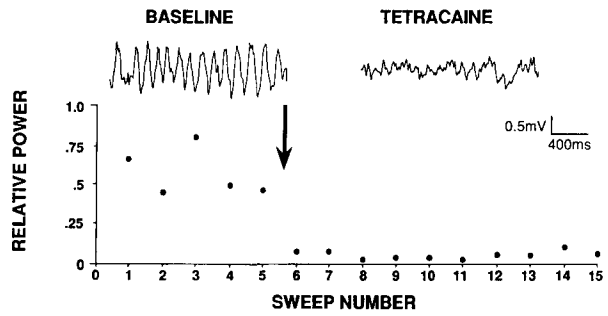

Fig. 5. Analog examples of hippocampal EEG collected before (Baseline) and after (Tetracaine) tetracaine injection into the medial septal area. The relative power (within a 7–9 Hz range) of the Θ rhythm is plotted as a function of successive sweeps collected as an animal moved radially inward on the maze during the spatial memory task. The arrow indicates that tetracaine was injected between sweeps 5 and 6, just prior to behavioral testing. After injection, the animal proceeded to make several errors.

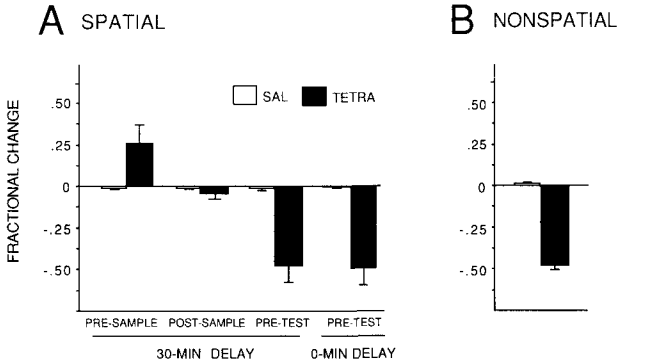

Fig. 6. Drug-induced changes in hippocampal Θ were quantified by calculating the fractional change before and after injection (see text for further explanation). A positive fractional change indicates that more Θ was observed during the test phase than during the sample phase. Conversely, a negative fractional change reflects less Θ during the test phase than during the sample phase. A: Pre-sample injections of tetracaine resulted in significantly more Θ during test choices than during sample choices in the spatial task. Post-sample injection of tetracaine did not significantly alter Θ at test. In contrast, pre-test tetracaine injections dramatically reduced Θ during test choices. B: during non-spatial training, hippocampal Θ was compared before the training trial, as the rat moved about the central platform of the maze, and while the rat performed on the maze. Drug injection occurred after the pre-trial sweeps were collected and before the animal performed the task. It can be seen that tetracaine, and not saline, significantly reduced hippocampal Θ when the rats performed this task.

arms were selected at test by animals injected with tetracaine before the sample (mean = 6.33) or test (6.25) phases, $F_{1,22} = 0.03$, n.s.

The hippocampal effects of septal inactivation were monitored by observation of the hippocampal EEG. Fig. 5 provides an example of the change observed in the analog EEG signal and relative power of hippocampal Θ following septal injection of tetracaine. The first 5 sweeps were collected during the sample phase while the rat traversed arms of the maze. Following the delay period, i.e. just prior to the test phase, this animal received a tetracaine injection. It can be seen that Θ was virtually absent during subsequent test performance.

Fig. 6 summarizes the average fractional change in Θ from sample to test phases as a function of injection condition. A 2-factor ANOVA revealed an overall main effect of the tetracaine injection, $F_{1,14} = 5.23$, $P < 0.05$. For trials in which 30-min delays were imposed, pre-sample tetracaine injections resulted in significantly more Θ during test responses than during sample responses (Scheffé test, $\alpha = 0.05$). This result indicates that Θ was reduced during the sample phase of the experiment, and that Θ had recovered by the test phase. No significant differences were observed in the amount of Θ present during sample and test choices when tetracaine was injected at the beginning of the delay period. Thus, Θ had recovered by the time of the test phase. When

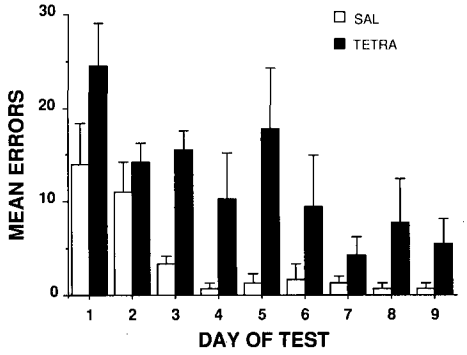

Fig. 7. Mean number of errors during the acquisition of non-spatial information while under the influence of daily injections of either tetracaine or saline. Saline-injected animals learned the task within a few days. The performance of tetracaine-injected animals, on the other hand, was highly variable and, as a group, required significantly more time to reach criterion than did controls.

tetracaine was injected before the test phase (after either 0- or 30-min delays), significantly less Θ was present during test responses than during sample responses.

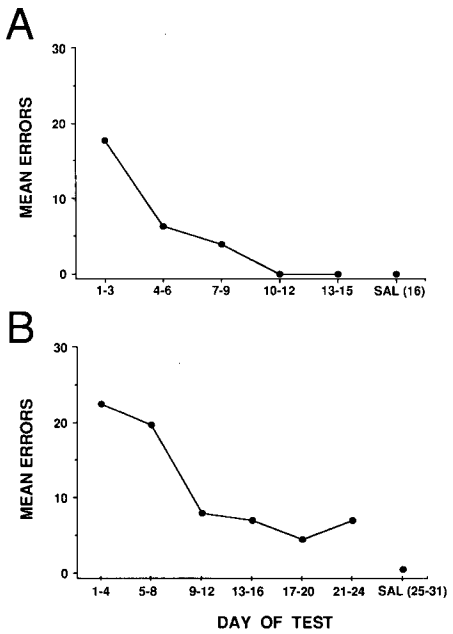

Fig. 8. Individual examples of the rate of acquisition of a non-spatial task for animals injected with tetracaine before each daily training session. A: The 'best' tetracaine animal learned the task within about 10 days and continued to perform with no errors for several days after. After this rat reached criterion performance, saline was injected to determine whether the improvement was state-dependent. Since there was no increase in errors, it was concluded that learning under the influence of tetracaine was not state-dependent. B: The 'worst' tetracaine animal initially showed improvement but continued to make errors even after 24 days of training. On days 25–31, saline was injected instead of tetracaine and the animal performed at criterion levels. These data provide evidence that this animal actually learned the non-spatial task under the influence of septal inactivation, but that accurate performace was influenced by other effects of the injection.

Non-spatial task

Following completion of the spatial tests, 8 animals began training on the non-spatial task. Of these, data from one animal were discarded due to technical difficulties that developed with the implant. The 7 remaining animals required 28.6 ± 4.0 days to achieve criterion performance before septal inactivation tests began. There was no significant difference in the rate of acquisition for experimental and control animals ($P > 0.10$). Choice accuracy was not affected by the olfactory probe trial ($P > 0.10$).

The effects of medial septal injection of either saline ($n = 3$) or tetracaine ($n = 4$) were assessed while animals learned to distinguish a new goal cue. The hippocampal EEG was monitored before, during, and after injection to verify that Θ was significantly reduced while tetracaine-treated animals performed the task, $F_{1,105} = 4.79$, $P < 0.001$ (see Fig. 6). Tetracaine-injected animals showed a significant decrease in Θ during training trials when compared to the pretrial period. In contrast, saline-treated animals showed an increase in Θ when pretrial EEG epochs were compared to EEG epochs collected during the training trials. The differential effects on the fractional change in Θ by saline- and drug-injected animals translates to about an 85% reduction in Θ during acquisition trials following septal inactivation.

The mean number of errors made during the first 9 days of non-spatial training was significantly greater for tetracaine-treated animals (2-factor ANOVA: $F_{1,8} = 6.80$, $P < 0.05$; see Fig. 7). Although the mean error rates were statistically different, examination of data from individual animals revealed some evidence of learning. For example, Fig. 8 illustrates the acquisition curves for the 'best' and 'worst' experimental animals. The upper acquisition curve shows that this animal learned the task within about 10 days. The lower curve shows that although the worst tetracaine animal showed improvement over the 24 days of training, it continued to average about 4–5 errors by the end of this period. Injections of tetracaine were replaced with saline injections for an additional 7 days. This animal performed at criterion during this 7-day period, averaging only 0.57 errors per day. No detrimental effects were observed during saline probe trials of other tetracaine-injected animals.

After saline-injected animals reached criterion performance, tetracaine was injected into the septal region to assess whether septal inactivation affected recall of previously learned information. Of the 3 animals tested, 1 made no errors on the tetracaine probe day, 1 made 10 errors, and 1 made 3 errors. The errors made were almost exclusively perserverative in nature, in that animals tended to repeat response sequences.

DISCUSSION

The contribution of the medial septum to different aspects of spatial cognition was the first of two issues addressed by the present study. When the medial septum was reversibly inactivated during only the sample phase of the experiment, errors made at test where with respect to choices made during the sample phase and not to new choices made at test. When septal inactivation was restricted to the delay period between sample and test phases of the experiment, test accuracy was similar to that of controls. These results show that normal function of the medial septum is important for the acquisition of trial unique spatial information, and that the effects of septal disruption cannot be attributed merely to performance variables. When septal inactivation began just prior to the test phase, errors consisted of re-entries to sample *and* new test arms.

The second issue addressed by the present study was the selectivity with which the medial septum contributes to learning. Septal inactivation substantially slowed the rate of improvement on a non-spatial reference memory task that required rats to remember the same information from trial to trial. However, it appears that the comparatively poor performance of some animals was at least in part a result of effects of the tetracaine treatment on performance variables. Some of the evidence for this conclusion is presented in Fig. 8. Three of the 4 animals tested reached criterion performance between 8 and 20 days. The fourth tetracaine animal (Fig. 8B) showed an initial improvement, but errors continued to be made for 24 days. When saline was substituted for tetracaine, the errors dropped abruptly to criterion and remained at this level for the next 7 days. Therefore, at least for this animal, medial septal inactivation may have affected other aspects of behavior such that the demonstration of this form of learning was not apparent.

If the inactivation of the medial septum produced effects on behaviors that interfered with an animal's ability to demonstrate new learning in the non-spatial reference memory task, then the possibility exists that a similar situation occurred while animals performed the spatial working memory task. However, the poor test scores of animals given injections of tetracaine before the sample period provides evidence that this was not always the case. If learning actually took place during the sample period, these animals should have performed as well as controls at test since the tetracaine effects had worn off. Instead, these animals performed poorly by making errors almost exclusively to arms selected during the period of septal inactivation, i.e. during the sample phase of the experiment. This finding suggests that septal inactivation impaired the acquisition process.

The contribution of septal inactivation-induced non-specific effects to poor choice accuracy following pre-test injections is less clear. Given the results of pre-sample injections, it is likely that any learning that normally takes place during the test phase was impaired. However, based on the results of the non-spatial test, non-specific effects of septal inactivation may also have influenced test behaviors. Therefore, it is not possible to resolve at this time the extent to which poor test performance following pre-test tetracaine injection resulted from altered recall processes. A similar difficulty would apply to the interpretation of earlier investigations (see Introduction) of the medial septum's role in learning, because those experiments involved permanent lesions.

The use of the reversible inactivation procedure to dissociate performance factors from learning processes illustrates an important advantage of the reversible inactivation technique for the study of functional connectivity. Unlike permanent lesions, this procedure effectively allows one to test animals after *removing* the effects of deafferentation, and then to determine (in the absence of performance effects) the extent of learning that took place when the target nucleus was non-functional. In this way, animals can demonstrate whether impaired performance during the period of deafferentation resulted from non-specific performance deficits or an impairment in learning per se.

A question that arises regarding any experiment involving the permanent or temporary inactivation of an afferent nucleus is whether or not the effects resulted from alterations in afferent cellular activity or the fibers of passage. Recent evidence from other laboratories supports the contention that inactivation of septal cells produced the results observed in this study. Givens et al.[13] recently reported that injection of either tetracaine or muscimol into the medial septum reduced hippocampal Θ and impaired spatial learning abilities. (Muscimol is a GABA-$_A$ agonist that does not affect fibers of passage.) Also, Brioni et al.[8] have reported that medial septal injection of muscimol impairs spatial learning in a pool of water.

It might be argued that the tetracaine injected into the medial septum impaired hippocampal-dependent behaviors because it diffused to hippocampus. The results of several control experiments argue against this possibility. For example, if tetracaine diffused to hippocampus, records of hippocampal unit activity should always show a reduction of activity following septal injection of tetracaine. Previous investigations have shown that simultaneously recorded hippocampal single units do not necessarily show the same response to the septal injection[27,28]. Some units exhibit increased activity, while others show reduced or no change in firing rate. Further

discussion of this issue can be found in Mizumori et al.[27,28].

Assuming that inactivation of septal cells contributed to the pattern of behavioral effects described in this study, it remains to be determined whether or not these findings resulted from the inactivation of septohippocampal afferent fibers, or from inactivation of septal afferents to other structures, possibly including those that convey information to and from the hippocampus. For example, it is known that the medial septum projects to layer II of entorhinal cortex, which in turn provides a majority of the afferent fibers to hippocampus[2,43]. It is possible that septal inactivation compromised information flow from the entorhinal cortex to hippocampus. One must also consider the possibility that septal afferents to the subicular complex[15,46] were importantly affected by septal inactivation, and this may have resulted in a functional isolation of the hippocampus from its cortical targets.

Although the present findings could be interpreted in terms of septal inactivation effects on either spatial abilities or working memory, the findings of others (such as those described above by Givens et al.[13] and Brioni et al.[8]) provide evidence for a role of the septohippocampal system in spatial learning. In this study, reversible inactivation of the medial septal region significantly reduced the hippocampal Θ rhythm (see Figs. 5 and 6; ref. 28) and choice accuracy (Fig. 3). Septal inactivation has also been observed to alter unit discharge in hilus/CA3c and fascia dentata[28]. Therefore, to the extent that the behavioral changes were spatial in nature, and a consequence of alterations in the septohippocampal afferent system, normal hippocampal unit activity and/or intact hippocampal Θ may be important for acquisition of recent spatial information. The finding that tetracaine injection had no effect on choice accuracy when injected at the beginning of the delay period is consistent with previous failures to disrupt retention of new spatial information over relatively short delay intervals (e.g. refs. 20, 25). It may be that septal inactivation, as well as the experimental manipulations of past studies, failed to affect storage, or maintenance, of spatial information because such processes do not rely on persistent patterns of unit discharge by hippocampal cells. Rather, the hippocampus may code recent spatial events in terms of a specific distribution of synaptic strengths (c.f. ref. 24). It is important to keep in mind that since the medial septum projects to a number of brain regions other than hippocampus[45], it is possible that these structures also contribute to behavioral alterations induced by temporary or permanent inactivation of the medial septum.

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REFERENCES

1 Aggleton, J.P., Hunt, P.R. and Rawlins, J.N.P., The effects of hippocampal lesions upon spatial and non-spatial tests of working memory, *Behav. Brain Res.*, 19 (1986) 133–146.
2 Alonso, A. and Köhler, C., A study of the reciprocal connections between the septum and the entorhinal area using anterograde and retrograde axonal transport methods in the rat brain, *J. Comp. Neurol.*, 225 (1984) 327–343.
3 Alvarez-Leefmans, F.J. and Gardner-Medwin, A.R., Influences of the septum on the hippocampal dentate area which are unaccompanied by field potentials, *J. Physiol.*, 249 (1975) 14P–16P.
4 Barnes, C.A., Spatial learning and memory processes: the search for the neurobiological mechanisms in the rat, *Trends Neurosci.*, 11 (1988) 163–169.
5 Bilkey, D.K. and Goddard, G.V., Medial septal facilitation of hippocampal granule cell activity is mediated by inhibition of inhibitory interneurons, *Brain Research*, 361 (1985) 99–106.
6 Bilkey, D.K. and Goddard, G.V., Septohippocampal and commissural pathways antagonistically control inhibitory interneurons in the dentate gyrus, *Brain Research*, 405 (1987) 320–325.
7 Bland, B.H. and Bland, S.K., Medial septal modulation of hippocampal theta cell discharges, *Brain Research*, 375 (1986) 102–116.
8 Brioni, J.D., Decker, M.W., Gamboa, L.P., Izquierdo, I. and McGaugh, J.L., Muscimol injections in the medial septum impair spatial learning, *Soc. Neurosci. Abstr.*, 15 (1989) 1173.
9 Chandler, J.P. and Crutcher, K.A., The septohippocampal projection in the rat: an electron microscopic horseradish peroxidase study, *Neuroscience*, 10 (1983) 685–696.
10 Fantie, B.D. and Goddard, G.V., Septal modulation of the population spike in the fascia dentata produced by perforant path stimulation in the rat, *Brain Research*, 252 (1982) 227–237.
11 Freund, T.F. and Antal, M., Postsynaptic targets of the septohippocampal GABAergic projection in the rat, *Soc. Neurosci. Abstr.*, 14 (1988) 245.
12 Frotscher, M. and Leranth, C., Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study, *J. Comp. Neurol.*, 239 (1985) 237–246.
13 Givens, B.S. and Olton, D.S., Modulation of medial septal area output alters working memory, *Soc. Neurosci. Abstr.*, 15 (1989) 80.
14 Green, J.D. and Arduini, A., Hippocampal electrical activity in arousal, *J. Neurophysiol.*, 17 (1954) 533–557.
15 Hjorth-Simonsen, A., Some intrinsic connections of the hippocampus: an experimental analysis, *J. Comp. Neurol.*, 147 (1973) 145–162.
16 Jarrard, L.E., Role of interference in retention by rats with hippocampal lesions, *J. Comp. Physiol. Psychol.*, 92 (1978) 1119–1127.
17 Jarrard, L.E., Selective hippocampal lesions and behavior: effects of kainic acid lesions on performance of place and cue tasks, *Behav. Neurosci.*, 97 (1983) 873–889.
18 Köhler, C., Intrinsic connections of the retrohippocampal region in the rat brain. II. The medial entorhinal area, *J. Comp. Neurol.*, 246 (1986) 149–169.
19 Lee, K.S., Stanford, E.J., Cotman, C. and Lynch, G., Ultrastructural evidence for bouton proliferation in the partially deafferented dentate gyrus of the adult rat, *Exp. Brain Res.*, 29

(1977) 475–485.
20 Maki, W.S., Brokofsky, S. and Berg, B., Spatial memory in rats: resistance to retroactive interference, *Anim. Learn. Behav.*, 7 (1979) 25–30.
21 Malpeli, J.G. and Schiller, P.H., A method of reversible inactivation of small regions of brain tissue, *J. Neurosci. Methods,* 1 (1979) 143–151.
22 Matthews, D.A., Cotman, C. and Lynch, G., An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat. I. Magnitude and time course of degeneration, *Brain Research,* 115 (1976) 1–21.
23 McNaughton, B.L., Barnes, C.A. and O'Keefe, J., The contributions of position, direction and velocity to single unit activity in the hippocampus of freely-moving rats, *Exp. Brain Res.,* 52 (1983) 41–49.
24 McNaughton, B.L., Barnes, C.A., Rao, G., Baldwin, J. and Rasmussen, M., Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information, *J. Neurosci.,* 6 (1986) 563–571.
25 Mizumori, S.J.Y., Rosenzweig, M.R. and Bennett, E.L., Long-term working memory in the rat: effects of hippocampally applied anisomycin, *Behav. Neurosci.,* 99 (1985) 220–232.
26 Mizumori, S.J.Y., McNaughton, B.L. and Barnes, C.A., A comparison of supramammillary and medial septal influences on hippocampal field potentials and single unit activity, *J. Neurophysiol.,* 61 (1989) 15–31.
27 Mizumori, S.J.Y., Barnes, C.A. and McNaughton, B.L., Reversible inactivation of the medial septum: selective effects on the spontaneous unit activity of different hippocampal cell types, *Brain Research,* 500 (1989) 99–106.
28 Mizumori, S.J.Y., McNaughton, B.L., Barnes, C.A. and Fox, K., Preserved spatial coding in hippocampal CA1 pyramidal cells during reversible suppression of CA3c output: evidence for pattern completion in hippocampus, *J. Neurosci.,* 9 (1989) 3915–3928.
29 Molino, A. and McIntyre, D., Another inexpensive headplug for electrical recording and/or stimulation of rats, *Physiol. Behav.,* 9 (1972) 273–275.
30 Morris, R.G.M., Garrud, P., Rawlins, J.N.P. and O'Keefe, J., Place navigation impaired in rats with hippocampal lesions, *Nature,* 297 (1982) 681–683.
31 Muller, R.U., Kubie, J.L. and Ranck Jr., J., Spatial firing pattern of hippocampal complex-spike cells in a fixed environment, *J. Neurosci.,* 7 (1987) 1935–1950.
32 Nadel, L. and MacDonald, L., Hippocampus: cognitive map or working memory?, *Behav. Neural Biol.,* 29 (1980) 405–409.
33 O'Keefe, J. and Conway, D.H., On the trail of the hippocampal engram, *Physiol. Psychol.,* 8 (1980) 229–238.
34 O'Keefe, J. and Dostrovsky, J., The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat, *Brain Research,* 34 (1971) 171–175.
35 O'Keefe, J., Nadel, L., Keightley, S. and Kill, D., Fornix lesions selectively abolish place learning in the rat, *Exp. Neurol.,* 48 (1975) 152–166.
36 O'Keefe, J. and Nadel, L., *The Hippocampus as a Cognitive Map,* Oxford University Press, London, 1978.
37 Olton, D.S. and Feustle, W.A., Hippocampal function required for nonspatial working memory, *Exp. Brain Res.,* 41 (1981) 380–389.
38 Olton, D.S. and Samuelson, R.J., Remembrance of places passed: spatial memory in rats, *J. Exp. Psychol.: Anim. Behav. Proc.,* 2 (1976) 97–116.
39 Olton, D.S., Walker, J.A. and Gage, F.H., Hippocampal connections and spatial discrimination, *Brain Research,* 139 (1978) 295–308.
40 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates,* Academic, Australia, 1982.
41 Schiller, P.H., Malpeli, J.G. and Shein, S.J., Composition of geniculostriate input to superior colliculus of the rhesus monkey, *J. Neurophysiol.,* 42 (1979) 1124–1133.
42 Scoville, W.B. and Milner, B., Loss of recent memory after bilateral hippocampal lesion, *J. Neurol. Neurosurg. Psychiat.,* 20 (1957) 11–21.
43 Steward, O. and Scoville, S.A., Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat, *J. Comp. Neurol.,* 169 (1976) 347–370.
44 Sutherland, R.J. and Rodriguez, A.J., The role of the fimbria/fornix and some related subcortical structures in place learning and memory, *Behav. Brain Res.,* 32 (1989) 265–277.
45 Swanson, L.W. and Cowan, W.M., The connections of the septal region in the rat, *J. Comp. Neurol.,* 186 (1979) 621–656.
46 Swanson, L.W., Wyss, J.M. and Cowan, W.M., An autoradiographic study of the organization of intrahippocampal association pathways in the rat, *J. Comp. Neurol.,* 181 (1978) 681–716.
47 Vanderwolf, C.H., Hippocampal electrical activity and voluntary movement in the rat, *Electroencephalogr. Clin. Neurophysiol.,* 26 (1969) 407–418.
48 Winocur, G., Radial-arm-maze behavior by rats with dorsal hippocampal lesions: effects of cuing, *J. Comp. Physiol. Psychol.,* 96 (1982) 155–169.
49 Winson, J., Loss of hippocampal theta rhythm results in spatial memory deficit in the rat, *Science,* 201 (1978) 160–163.