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Medial septal modulation of entorhinal single unit activity in anesthetized and freely moving rats

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Reversible inactivation of the medial septal area results in a spatial memory impairment and selective disruption of hilar/CA3, but not CA1, location-specific discharge. The present study examined the possibility that such septal deafferentation produces effects on hippocampal function by altering physiological properties of the primary input and output structures for hippocampus, the entorhinal cortex and the subiculum, respectively. Single unit activity of hippocampal, entorhinal, and subicular cells was recorded before, during, and after septal injection of lidocaine in anesthetized rats. When compared to hippocampal cells, relatively few subicular and entorhinal cells showed a change in mean firing rate following septal inactivation. Entorhinal unit responses to septal inactivation (via tetracaine injection) were also examined in freely moving rats performing a spatial maze task. About one-third of entorhinal cells showed enhanced or reduced firing rates of 40% or more. Also, the spatial distribution of cells found in the superficial, but not deep, entorhinal layers became less clear following septal inactivation. Together, these data are consistent with the hypothesis that manipulation of the medial septum affects hippocampal function via its septosubicular and septoentorhinal projections in addition to the more direct septohippocampal pathway. Since entorhinal cortical function was affected by tetracaine injection into the septum, it does not appear that direct entorhinal-CA1 afferents were primarily responsible for the maintenance of CA1 location-specific neural activity in previous septal inactivation experiments. Rather, these data are consistent with the hypothesis that the persistence of CA1 place fields was accomplished by intrahippocampal neural network operations.

INTRODUCTION

Much evidence supports the hypothesis that the hippocampus is essential for normal spatial learning and memory. For example, hippocampal lesions disrupt spatial performance by rodents^{37,39} and humans⁴⁷. Also, the principal type of hippocampal cell (pyramidal cells) selectively discharges when an animal enters discrete locations in its environment^{35,36}. These 'place fields' presumably reflect neural representations of space that facilitate spatial learning by hippocampus.

To better understand the specific computations the hippocampus performs during spatial tasks, it is necessary to consider not only the nature of association formation by intrinsic hippocampal neuronal networks, but also the nature of modulatory influences provided by subcortical afferents. Of the many subcortical nuclei projecting to the hippocampus, the medial septum provides perhaps the most extensive innervation. Medial septal afferents, which are largely cholinergic and γ -aminobutyric acid (GABA)ergic^{4,14}, terminate on primary dendrites or somata of principal cells, or on interneurons^{9,10} which in turn exert powerful regulatory control over the excitatory transmission of principal cells.

Thus, septal terminals are strategically located to provide significant and precise modulatory influences on hippocampal function.

Several studies have examined the contribution of septal afferents to hippocampal function by observing the behavioral and hippocampal electrophysiological consequences of permanent septal lesions^{6,8,25,26,49,50}. Such lesions produce profound spatial memory deficits and eliminate the hippocampal θ rhythm. These findings suggest that cholinergic and GABAergic septal inputs are critical for normal hippocampal function. Consistent with this hypothesis, the cholinergic system is known to play a key role in normal learning (for review, see Ref. 7).

Selective alterations in spontaneous single unit activity have also been observed following septal deafferentation. For example, the effects of reversible inactivation of the septum on specific patterns of hippocampal cellular activity and spatial behavior were recently studied^{28,30-32}. During the period of septal inactivation, animals made more errors on a spatial task and the hippocampal EEG was devoid of movement-sensitive θ . Septal inactivation does not appear to produce a generalized learning deficit since treated animals can learn nonspatial tasks³². The single unit effects of septal inac-

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tivation varied for different populations of hippocampal cells: a significant reduction or enhancement of unit activity was observed for CA3 and dentate gyrus pyramidal cells and interneurons. In contrast, place (pyramidal) cells recorded in the CA1 region retained locationspecific discharge even though the mean discharge rate was reduced by about $10-15\%^{31}$.

The pattern of unit and behavioral changes observed following septal inactivation raised several issues regarding hippocampal function, two of which are addressed in the present study. One issue concerns the mechanism by which CA1 place cells can be maintained despite reduced input from CA3 and dentate gyrus regions. Given that direct entorhinal-CA1 pathways may be more pronounced than previously thought $^{51-53}$, it is reasonable to postulate that the specificity of CA1 place fields are maintained by entorhinal afferents. Alternatively, CA1 place cells might have been maintained by intrahippocampal network properties such as associative recall and pattern completion operations similar to those described by Marr^{16,17}. Marr hypothesized that such network properties rely on feedforward inhibition which effectively divides the excitation of the principle cells by an amount that is proportional to the number of excitatory afferent fibers. Therefore it is possible that spatial coding by CA1 cells was preserved because the decline in feedforward inhibition was proportional to the reduced activation by excitatory CA3 afferents.

A second issue addressed by the previous septal inactivation studies relates to the identification of pathways that importantly contributed to the specific pattern of the hippocampal unit and behavioral changes observed. Perhaps the most parsimonious hypothesis is that lesions of the medial septum eliminate the influence of septohippocampal afferents on hippocampal function. However, given that medial septal cells also project to entorhinal cortex^{1,3,48}, which in turn provides a majority of the cortical input to hippocampus^{15,18}, it could be argued that hippocampal neural activity and associated behaviors were compromised because of reduced neocortical input. Indeed, there are several reports that entorhinal cortex lesions result in spatial memory deficits which are similar to those which occur following hippocampal lesions (e.g. refs. 13, 26, 39, 45, 46). The medial septum also projects to subiculum²⁷, the major output structure for the hippocampus. Since subicular lesions produce significant spatial memory deficits¹², it is also possible that at least part of the spatial memory impairment observed following a septal lesion results from a functional disconnection of the hippocampus from its cortical targets. Of interest in this regard, specific entorhinal and subicular pathology has been reported for Alzheimer's patients, many of whom show severe memory impairments¹¹.

The hypothesis that elimination of septal afferents produces changes in hippocampal single unit activity by disrupting the major cortical input and/or output structures, the entorhinal cortex and subiculum, respectively, was tested in anesthetized and freely behaving rats. During tests with chronically-implanted animals, possible septal inactivation-induced changes in the behavioral correlates of entorhinal activity were examined as rats performed a spatial maze task. Portions of this study have been previously described³³.

MATERIALS AND METHODS

Subjects

Male Fischer-344 rats (9 month old retired breeders; Charles River Laboratories) served as subjects. The animals were allowed at last one week of adaptation to laboratory conditions. During this time, ad libitum access to food and water was provided. Lights were on in the colony room from 07.00 to 19.00. Behavioral and electrophysiological assessments occurred during the light period.

Surgical procedures

Animals were food and water deprived for 24 h prior to surgery. An initial dose of 35 mg/kg Nembutal (50 mg/ml) was administered, followed by supplements of 0.05 ml as needed. For recording in anesthetized rats, small burr holes were drilled to permit localization of recording electrodes in hippocampus, subiculum, and/or the entorhinal cortex, and an injection needle in the medial septum⁴⁰: medial septum - A-P 0.5 mm anterior to bregma, L 0.0 mm (midline), and -5.5 mm ventral to brain surface; dorsal hippocampus - A-P -4.0 mm; L \pm 2.4 mm; subiculum - A-P -6.3 mm; L \pm 3.3 mm; entorhinal cortex - A-P -5.0 to -7.0 mm; L \pm 5.0 to 6.0 mm. Ventral hippocampal units were also tested during the entorhinal passes. The recording depth was initially determined by monitoring the recording electrode aurally, the depth of the electrode, and observing specific patterns of spike discharge. A ground lead was soldered to a small jewelers screw attached to the skull. A reference electrode (114 μ m Teflon-coated stainless steel wire) was placed in the corpus callosum.

For animals tested during the awake state, a guide cannula (25 ga stainless steel tube; 33 ga stylet) was placed about 1 mm dorsal to the medial septum (-4.5 mm from brain surface). The chronic indwelling cannula assembly was constructed as described earlier³¹. Two recording stereotrodes were bilaterally situated 5.0 mm and 7.0 mm posterior to bregma, 5.5 mm lateral of the midsaggital suture, and 0.5 to 1.0 mm below the dural surface. The electrodes entered the brain along a trajectory 15° lateral of the vertical plane. The two stereotrodes entering each hemisphere were mounted on a single moveable microdrive. (Thus, as one turned the drive to lower the electrodes, both stereotrodes were advanced simultaneously). The electrode, ground and reference leads were inserted into a connecting socket³⁴ that was permanently attached to the head with dental acrylic. Following surgery, 0.1 ml Bicillin was administered (i.m.) into each hindleg to guard against infection.

Electrophysiological procedures

A detailed description of the construction of the recording electrodes, or stereotrodes²³, can be found in reports by Mizumori et al.³¹ and McNaughton et al.¹⁹. Briefly, each consisted of a pair of twisted, laquer-coated tungsten wires (20 μ m dia; California Fine Wire Co.) bonded together with epoxylite. Final impedances were 100–200 k Ω (tested at 1 kHz). Stereotrode recording involved first independently monitoring unit activity on each of the two wires (X and Y), then isolating single units by comparing characteristics of the signals received from the two electrodes. For example, comparisons that facilitated unit isolation included the ratios of spike amplitude recorded on the X and Y channels, spike width, and the difference in latency to the first peak from the beginning of the 1 ms sampling period. The sampling frequency was 32 kHz per channel.

Incoming signals were amplified (5-10 K), filtered (at half amplitude) at 600-800 Hz (high pass) and 6 kHz (low pass), then passed through a window discriminator such that a sampling period began when a signal from either channel exceeded a predetermined threshold. Data were acquired by a BrainWave Neuroscience Workstation. Subsequent isolation of single units and data analyses were accomplished with software packages provided by the BrainWave system, Bruce McNaughton, and Carol Barnes.

During experiments with anesthetized animals, an injection needle (33 ga stainless steel tube) was positioned 5.5 mm below the brain surface. Recording stereotrodes were then slowly advanced toward target structures. Although the primary brain regions of interest were the entorhinal cortex and subiculum, cells were also recorded in the CA1 and dentate gyrus areas of the hippocampus for comparison. Unit activity was recorded continuously throughout the different phases of each experiment. Data acquisition began after unit activity was deemed stable for at least 10 min. Baseline discharge rates were recorded for the first 10 min. Then, lidocaine was injected into the medial septal region. The time of injection was logged and unit discharge continued to be monitored for at least 20 min. According to earlier results^{30,31}, inactivation effects should be observed immediately after injection, and last about 15 min in Nembutal-anesthetized rats. When a recording session was complete, the stereotrode was either lowered further into the brain in search of additional cells, or moved to a different stereotaxic location to record from other structures. Thus, an animal served as its own control for each inactivation test and data was acquired from multiple brain regions.

Single units were initially classified according to the depth of the electrode, spike width, and whether they discharged single spikes or high frequency bursts of multiple spikes. This on-line classification was subsequently verified by histological and autocorrelation analyses. Autocorrelation functions represent the expectation density for spike discharge shortly before and after each spike event⁴ Previous research indicates that hippocampal complex-spike cells likely correspond to the population of pyramidal cells while single spiking cells likely correspond to interneurons in CA1, granule cells, and interneuron populations of stratum granulosum^{29,30,31,44}. Within the hilar region of the dentate gyrus, one finds both complexand single spiking cells. Given the diverse array of cell types in this region², it is less certain which morphological cell type best corresponds to complex- and single spiking cells. It is possible that at least some of the complex-spike cells correspond to the variety of pyramidal cells found in this region. For the present experiment, all dentate gyrus cells are considered collectively.

To record unit activity from freely behaving rats, a headstage was attached to a connecting socket mounted on the rat's head. The headstage consisted of 5 FET preamplifiers and an infrared lightemitting diode. The animal's position on the maze was monitored by an automatic tracking system (Dragon Tracker Inc.) which sampled the X-Y coordinates of the diode at a frequency of 20 Hz, then relayed this information to the BrainWave System. The computer then logged the time of each position event.

Reversible inactivation procedure

Neural activity in the medial septal region was temporarily inactivated by microinjection of the local anesthetic lidocaine (anesthetized rats) or tetracaine (freely behaving rats). A 2% solution was prepared by dissolving the drug (Sigma Chemical) in sterile 0.9% NaCl. Each inactivation involved pressure ejection of 0.5 μ l of fluid. Further details of the injection method can be found in Mizumori et al.^{30,31}.

During chronic recording, animals performed daily 15 trials. The tetracaine filled needle was placed in the guide cannula before a recording session. With the needle in place, animals performed the first 5 of the 15 trials. During the intertrial interval between trials 5 and 6, 0.5 μ l tetracaine was pressure injected into the medial

septal area. The rat was subsequently allowed to complete the remaining 10 trials of the session. Since it was not necessary to handle the rat during the injection, it was possible to monitor unit activity and behavior continuously throughout the 15 trial session. The effects of septal inactivation were determined by comparing unit-behavioral correlates during trials 4-5 (baseline period) with similar correlates obtained during trials 6-7 (tetracaine period) and trials 14-15 (recovery period).

Behavioral apparatus

The performance of animals was tested with a black plastic, semi-automated 8-arm radial maze^{31,38}. Eight alleys, or arms (57.5 cm long by 5 cm wide), radiated outward from a round central platform (19.5 cm dia). Rails (0.5 cm high) were attached to the sides of the arms and food cups placed at the distal ends of all arms. The entire maze was elevated 65 cm above the floor. The maze was situated in a room (270 cm by 435 cm) which contained several extramaze items that could serve as distal visual cues. Such items included shelves, tables, chairs, miscellaneous laboratory equipment and the experimenter. The experimenter controlled the presentation of individual arms of the maze to the rat by a remote switch. The room was illuminated by two 60 W incandescent lights placed in the southeast and southwest corners of the room.

Behavioral procedures

During the initial one week laboratory adaptation period, animals were weighed and handled daily. Access to food was then restricted such that animals were maintained at about 80% of their ad libitum body weights. A rat's first exposure to the maze consisted of placement on the central platform with free access to all 8 arms. Chocolate milk was present in the food cups to serve as reward. The optimal strategy for retrieving the reward was to enter each arm only once. Re-entries constituted errors since the food cups were baited only once per trial. To complete this first phase of maze training, rats were required to clear the maze once per day within 15 min for 3 consecutive days.

After this initial training, animals were trained according to a partial forced choice procedure³². Specifically, a rat was first placed on the central platform for about 2 min. Then, 4 different maze arms were individually and sequentially presented to the rat. The location of the arms presented varied from trial to trial. While the rat was drinking chocolate milk from the fourth arm, all 8 arms were presented such that when the rat returned to the central platform, it was required to select those arms not sampled earlier in the trial. When animals performed one trial per day within 15 min for 3 consecutive days, they advanced to the final training phase. That is, they were now required to perform 10 such partial forced choice trials daily (2 min intertiral interval). When a rat completed 10 trials within 1 h for 7 consecutive days, it was given free access to food and water for 2–3 days before surgical impantation of electrodes and cannula.

The animals were allowed to recover from surgery for 7 days during which time they fed ad libitum. During postsurgical training, animals were required to perform 15 daily maze trials within 1 h.

Histological procedures

Following each experiment, injection and recording sites were verified histologically. Animals were perfused transcardially first with 0.9% NaCl, then 10% phosphate buffered formalin. The brains were removed, then allowed to sink in 30% sucrose formalin. Fourty μ m thick frozen sections were mounted on microslides, then stained with Cresyl violet.

RESULTS

Septal inactivation effects in anesthetized animals

A total of 86 cells were recorded from 12 rats. Septal inactivation effects were observed for both dorsal and



Fig. 1. Percent of cells exhibiting more than a 40% change in firing rate following injection of the local anesthetic lidocaine (2%) into the medial septal area. About half of the cells recorded in the fascia dentata (FD) of the hippocampus showed substantially increased or decreased firing. In contrast, such rate changes were observed for only a small number of cells recorded in the CA1 subfield, subiculum (SUB), or the entorhinal cortex (EC).

ventral hippocampal cells. In total, 21 CA1 cells, 19 dentate gyrus cells, 26 subicular cells, and 20 entorhinal cells were tested. All recording and injection sites were verified histologically. For all cell populations, the mean spike width, mean spike amplitude, and mean discharge rates were comparable to those described in earlier reports (e.g. Refs. 5, 30, 31).

The percentage of cells showing changes in firing rate of more than 40% is summarized in Fig. 1. A threshold of 40% was selected because frequency histograms of percent change in rates showed for all cells a normal distribution of rate changes that was centered on 0% and ranged from -40% to +40% change (see Fig. 8 for an example). The distinguishing feature of these response distributions was the number of cells that responded by more than 40%. In accordance with findings of previous studies^{30,31}, about half of the dentate gyrus (i.e. stratum granulosum and hilar/CA3) units were altered while only



Fig. 2. A continuous record of entorhinal cell discharge in response to septal injection of lidocaine. The arrow indicates the time of injection. The bin width is 5 s. The maximum rate per bin is 2.0 Hz. The firing rate of this cell was dramatically but temporarily reduced following septal inactivation.



Fig. 3. Mean (\pm S.E.M.) number of errors made during different phases of the experiment. Animals performed 5 maze trials under normal conditions (BASELINE). During the intertrial interval between trials 5 and 6, tetracaine was applied to the medial septal area. A significant increase in errors was observed during the next two trials (TETRACAINE). The number of errors decreased by the end of the 15 trial recording session (RECOVERY). The number of errors made during the baseline and recovery phases were not significantly different.

4.7% of CA1 complex-spike cells showed rate changes following septal inactivation. About 11.5% and 15% of subicular and entorhinal cells, respectively, showed rate changes of more than 40%.

Those entorhinal and subicular cells which responded to septal inactivation often did so in a dramatic fashion. Fig. 2 illustrates the transient and dramatic nature of change in firing rate by an entorhinal unit.

Septal inactivation effects in freely behaving animals

Behavioral effects of septal inactivation. An average of 23.4 (\pm 0.61) days of training was required for rats to perform 10 partial forced-choice trials daily for 7 days. Following surgery, animals achieved criterion performance within 7 days. As reported earlier, septal inactivation produced a temporary yet significant increase in the number of errors made, $F_{2,40} = 48.81$, P < 0.001. Fig. 3 illustrates that during the baseline period, the rats rarely committed errors. Post-hoc Scheffé tests (α = 0.05) revealed that during the trials immediately following septal injection of tetracaine, the rats made significantly more errors (Fig. 3). This impairment was transient in that significant recovery occurred by the end of the recording session. The number of errors during the baseline and recovery periods were not significantly different. It is worth noting that during the period of septal inactivation, the rat's behavior during maze trials often, but not always, changes. The animals tend to be more reactive to stimuli and increase the speed with which they traverse the maze.



Fig. 4. Frequency distribution of mean discharge rates for entorhinal cells. While several higher rate cells were observed, more than half of the units exhibited very low firing rates (less than 1 Hz). This pattern of distribution is similar to that obtained by Barnes et al.⁵. Movement analyses were performed for cells whose rates were above 1 Hz. Spatially selective discharge patterns were assessed for cells with rates above 0.1 Hz.

The decline in choice accuracy following septal inactivation has been found to correlate with the decline in hippocampal θ^{32} . Therefore, it is reasonable to assume that a reduction in hippocampal θ also occurred during the period of septal inactivation in the present experiment.

Baseline single unit characteristics of entorhinal cortex. A total of 96 entorhinal cells were recorded from 7 rats. Histological analyses revealed that cells were recorded in both superficial and deep layers of lateral and medial entorhinal regions. Since no significant differences emerged following a comparison of lateral and medial entorhinal cells, the data were pooled into superficial and deep entorhinal groups. This allowed us the evaluate whether superficial layers (which receive direct septal innervation) exhibit behavioral properties that were different from deeper layers. Septal cannula tips were observed to be located within the dorsal aspect of the medial septum.

The average (\pm S.E.M.) spike amplitude and spike duration were 102.8 \pm 3.6 μ V and 277.9 \pm 5.7 μ s, respectively. The mean discharge rate during maze performance was 3.38 \pm 0.66 Hz. Examination of the frequency distribution of firing rates (Fig. 4) revealed that a large percent of entorhinal cells fired at rates of less than 1 Hz.

The behavioral correlates of entorhinal cells were assessed in terms of the degree of spatially selective firing, and the extent to which unit discharge was related to translational movements by the animal. These measures were selected because certain hippocampal cell populations appear to be differentially tuned to either the location of an animal in its environment or to movements made by the animal as it moves across the maze. Generally speaking, discharge of the lower rate hippocampal pyramidal cell is associated with the location of the animal, while the higher rate interneuron varies its rate as a function of general movement. To facilitate comparisons, entorhinal cortical cells were analyzed according to the identical routines described for hippocampal place and movement-sensitive cells³¹.

Spatial selectivity refers to the extent to which localized places in space are distinguished by particular patterns of neural discharge. Place-specificity scores were determined by calculating 16 mean firing rates as the rat moved in the inward and outward directions for each of the 8 arms of the maze²⁰. The highest of these rates was divided by the average of the remaining (15) rates. Cell discharge that was perfectly uniform across the maze would be reflected in a score of 1.0. It should be noted that this place specificity measure may underestimate true spatial selectivity if the size of the place field was smaller than the maze arm. If, on the other hand, the field extends across more than one maze arm, the specificity of the field, by definition, will be smaller. The spatial selectivity score may be biased against bidirectional cells that have a specific place field on one arm. However, this situation is rarely observed in this task. To ensure a statistically reliable sample of spikes, place specificity scores were obtained for entorhinal cells with rates greater than 0.1 Hz.

Movement sensitivity was analyzed for those cells with mean firing rates of greater than 1 Hz. Cells whose rates were lower than these values were excluded from this analysis since there would be an insufficient number of spikes available for statistical analyses. To assess the movement correlate of cell discharge, the mean firing rate was calculated during the following distinct behaviors of the animal on the maze: movement radially outward on maze arms, relative immobility while animals drank chocolate milk reward at the ends of the arms, 180° turns at the arm ends, movement radially inward, and movement across the center of the maze. These mean rate values were then subject to statistical analyses. In addition, the firing rates during movements about the maze were compared directly with firing rates obtained while the animal remained still at the arm ends by calculating a movement-to-still ratio.

When compared to hippocampal cells evaluated according to the same algorithm while animals performed the same task³¹, entorhinal cells in both superficial and deep layers showed a moderate spatial bias. The mean place specificity score during the baseline period was 2.41 \pm 0.23 and 2.30 \pm 0.38 for superficial and deep cells, respectively. While these values are similar to those reported for the less spatially selective hippocampal interneurons, the entorhinal place specificity scores stand in contrast to the higher specificity indices observed for different populations of hippocampal place cells (e.g. about 4.3 for CA1 cells and 7.3 for hilar/CA3 cells). The relatively low spatially selective discharge of entorhinal cells is consistent with that recently described by Barnes et al.⁵. However, as reported by Quirk and Ranck⁴³, a

few entorhinal cells showed more clear nonuniform spatial distributions of discharge across the maze (Fig. 5). In addition to these spatial biases, it was noted that entorhinal discharge often had a directional component. The examples at the bottom of Fig. 5 illustrate cells that preferentially fired as the animal moved outward on maze arms.



Fig. 5. Examples of the spatial distribution of entorhinal unit discharge. Dots correspond to position of the animal. The sizes of the circles are proportional to the local firing rate of the cell. Lines radiating from the center of the circle indicate the direction of movement when the cell fired. While most entorhinal cells displayed little reliable place-specific firing, the cells shown here showed consistent firing as the rat traversed a subset of maze arms and little firing on the remaining arms.



Fig. 6. Mean (\pm S.E.M.) firing rate as a function of specific movements by the animal on the maze and phase of the experiment. To assess the movement sensitivity of entorhinal cells, firing rates were determined when the rat remained STILL at the arm ends, during 180° TURNS at the arm ends, as the rat moved INBOUND on a maze arm, moved across the CENTER platform, and finally, as the rat moved OUTBOUND on the arm of the next choice. The movement analysis was conducted on cells whose rates exceeded 1 Hz. In contrast to what is typically observed for hippocampal interneurons, these cells did not show significant changes in firing rates during the baseline period. Furthermore, rates did not change during the subsequent tetracaine and recovery phases.

Many hippocampal cells have been found to be sensitive to voluntary movement (e.g. Ref. 31). Unlike hippocampal cells, the entorhinal cells of this study were not particularly sensitive to an animal's movement during the baseline period (Fig. 6). A repeated measures ANOVA revealed no significant change in firing rate as a function



Fig. 7. Mean (\pm S.E.M.) firing rate of entorhinal cells during different phases of the experiment. Following septal inactivation, during the period when more errors were made, the population of entorhinal cells tested did not appear to be affected.

of movement about the maze, and no differences in the movement-to-still ratio.

Effects of septal inactivation. Eighty-four of the 96 entorhinal units recorded were tested for their response to septal inactivation. During the period of poor choice accuracy induced by septal inactivation (Fig. 3), the mean firing rates of superficial (n = 47) or deep (n = 37)entorhinal cells were not significantly changed (P > 0.6; Fig. 7). Although the group mean rate did not change, individual cells nevertheless may have responded with increased or decreased discharge rates. Indeed, when the percent change in rate was calculated for each cell, it was found that 23.3% of all cells showed rate reductions of more than 40%, and 10.4% of all cells showed rate increases of more than 40% (see Fig. 8). Such rate changes were observed for cells recorded in both superficial and deep layers. Thus, about one third of the cells tested showed substantial, although mixed, rate changes in response to septal inactivation.

No differences in movement sensitivity were observed for either superficial or deep layer entorhinal cells across different phases of the experiment (Fig. 6). This was the case when repeated measures ANOVAs were applied to mean rate values as well as movement-to-still ratios.

Although the place specificity indices of entorhinal cells were not as high as those observed in hippocampus proper, a score of 2.3–2.4 does indicate some spatial bias. Therefore, the specificity index was compared across the different phases of the inactivation experiment for superficial and deep layer entorhinal cells. A significant change was observed in place specific firing by entorhinal units recorded in the superficial layers (Fig. 9), $F_{2,36} = 7.50$, P < 0.01. In contrast, no statistically significant effect was observed for the average place spec-



Fig. 8. Frequency distribution of the percent change in spontaneous discharge rate for entorhinal cortical cells following septal inactivation. While a majority of cells showed moderate changes in firing at best, the discharge rates of several entorhinal cells were dramatically altered.



Fig. 9. The extent to which the spatial distribution of cell discharge was maintained following septal inactivation was assessed. Place specificity scores of entorhinal cells during different phases of the experiment are presented. Although the spatial selectivity scores were not as high as those observed for hippocampal cells³¹, clear nonuniform spatial distributions existed. These spatial biases were significantly reduced following septal inactivation. By the end of the recording period, the specificity scores were no longer significantly different from those during the baseline period.

ificity index of entorhinal units from the deep layers (P > 0.10). Post-hoc Scheffé ($\alpha = 0.05$) analysis revealed that a significant reduction in specificity of superficial layer cells occurred following septal inactivation. By the end of the recording session, the mean specificity index was not statistically different from that obtained during the baseline period.

DISCUSSION

The medial septal area is considered to be one of the primary subcortical afferent structures to the hippocampus. Anatomical data indicate that the septal influence on hippocampal function is likely to be quite complex since septal terminals are found not only in the hippocampus proper, but also in entorhinal cortex and subiculum. Thus, septal deafferentation may produce changes in functional aspects of hippocampus proper via alterations in septohippocampal, septoentorhinal, and/or septosubicular projections. Septal inactivation produces a significant spatial learning deficit. Coincident with this impairment one finds changes in the firing properties of some hippocampal cells³¹. The present study evaluated whether unit changes in entorhinal cortex or subiculum might contribute to the hippocampal functional changes following inactivation of the medial septal area.

Before discussing the unit results, it is worth noting that one might argue that tetracaine injections spread to the recording site and that this was responsible for the observed unit changes. However, as discussed in more detail previously^{30,31}, if this were the case, one would have expected to observe only reduced firing in response to the injection. This did not happen. Rather, several cells responded to septal injection of tetracaine with elevated discharge. That the effects of the tetracaine injection is localized to the medial septal region is also supported by prior control experiments³⁰ which showed that injection of a local anesthetic 1.0 mm above the septum produced no changes in firing for hippocampal cells that were affected when the injection occurred directly into the septal area. It should be noted, however, that tetracaine blocks ion conductance at the cell body and fibers of passage. Thus, it is not yet possible to identify the specific pathway or synaptic mechanism which mediates the unit and behavioral changes.

The results from anesthetized animals revealed that while reversible septal inactivation disrupted spontaneous unit activity for many cells recorded in the dentate gyrus/CA3 region of hippocampus, the discharge rates of relatively few CA1, subicular, and entorhinal single units were altered. Although few entorhinal and subicular cells responded to septal inactivation, Fig. 2 illustrates that the response of these cells could be dramatic. Thus, the overall pattern of effects observed suggest that septal inactivation-induced changes in subiculum or entorhinal cortex are unlikely to fully account for the comparatively large effects observed in the dentate and CA3 regions of hippocampus. The possibility remains, however, that while the mean rates of many cells did not changes significantly following septal inactivation, the representations which may be coded in the patterned neural activity of these cells was altered. To test this hypothesis for entorhinal cortex, septal inactivation tests were also conducted in freely behaving animals.

Septal inactivation produced a spatial memory impairment similar to that observed in previous studies³¹. This behavioral deficit may be attributed to changes in hippocampus proper, subiculum, and/or entorhinal cortex since changes in unit discharge patterns were observed in anesthetized and/or awake animals. Consistent with this hypothesis are previous reports that lesions of these areas produce spatial memory impairments^{13,26,39,45,46}. However, at present, it is difficult to ascribe relative importance to the 11% change in subiculum, the greater than 50% change in hippocampus, and the 15-33% change in entorhinal cortex. This is in part due to the fact that the relative contribution of each population of cells to learning is not yet clear. Nevertheless, one could still argue theoretically that, changes in any of these populations could have resulted in the observed behavioral impairment. For example, without appropriate input from neocortex, hippocampus proper may have insufficient data with which to form the necessary associations

for learning to occur. Without proper subicular output to neocortex, the hippocampus becomes functionally isolated from its neocortical and subcortical targets. Consequently, the hippocampus would not be able to pass on information that is essential for normal learning to be exhibited.

Septal inactivation did not significantly alter the population mean discharge rate of entorhinal or subicular cells. This finding stands in contrast to the preliminary report by Perez et al.⁴¹ who found a 30% reduction in multiple unit discharge rates of entorhinal and subicular cells in anesthetized rats. Given the limited description provided in their report, it is difficult to identify at the present time the relevant sources of variance between studies. In the future, however, it will be important to consider factors such as the differences in the level of analysis (i.e. multiple unit recording vs single unit recording).

Although the mean rates did not significantly change in response to septal inactivation in the present study, a subgroup of cells did respond to septal inactivation by dramatically elevating or reducing firing rates. Thus, it is possible that hippocampal unit responses observed in previous studies were an indirect consequence of changes in entorhinal input to hippocampus, in addition to changes in the septohippocampal pathway itself. The implication for future research is that it is important to evaluate the consequences of afferent lesions by examining the functional integrity of not only the target structure of interest, but also other brain regions whose disruption could potentially (and indirectly) affect the target structure's function.

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The septoentorhinal pathway is likely to be at least in part cholinergic in nature since septal lesions reduce AChE staining in entorhinal cortex²⁴. However, not all septal afferents to entorhinal cortex are cholinergic¹. Given that septal projection cells also contain GABA¹⁴, it is also possible that inhibitory influences in entorhinal cortex arrive from the medial septum. Consequently, one account of the observed unit changes in that following setal inactivation, disinhibition of entorhinal cells which normally receive GABA input resulted is an elevated discharge, while reduced excitation by ACh may have resulted in attenuated discharge of other cells. Since it was not possible to identify the precise morphological type of entorhinal cell being recorded, the exact mechanism of septal inactivation effects in entorhinal cortex remains to be determined.

The finding of attenuated place specificity by entorhinal cells in the superficial, and not deep, layers suggest that input of spatially-relevant information from entorhinal cortex to hippocampus is modulated by the medial septum. Furthermore, this result indicates that entorhinal cortex may not maintain CA1 place-specific firing during periods of septal inactivation³¹. Rather, these data are consistent with the hypothesis that CA1 place fields are maintained because intrahippocampal circuitry engages neural network operations such as pattern completion and associative recall^{16,17,21,22}.

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