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Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge

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Summary. The effects of massive destruction of granule cells of the fascia dentata on the spatial and temporal firing characteristics of pyramidal cells in the CA1 and CA3 subfields of the hippocampus were examined in freely moving rats. Microinjections of the neurotoxin colchicine were made at a number of levels along the septo-temporal axis of the dentate gyri of both hemispheres, resulting in destruction of over 75% of the granule cells. By contrast there was relatively little damage to the pyramidal cell fields. As assessed by three different behavioral tests, the colchicine treatment resulted in severe spatial learning deficits. Single units were recorded from the CA1 and CA3 subfields using the stereotrode recording method while the animals performed a forced choice behavioral task on the radial 8-arm maze. Considering the extent of damage to the dentate gyrus, which has hitherto been considered to be the main source of afferent information to the CA fields, there was remarkably little effect on the spatial selectivity of "place cell" discharge on the maze, as compared to recordings from control animals. There was, however, a change in the temporal firing characteristics of these cells, which was manifested primarily as an increase in the likelihood of burst discharge. The main conclusion derived from these findings is that most of the spatial information exhibited by hippocampal pyramidal cells is likely to be transmitted from the cortex by routes other than the traditional "trisynaptic circuit". These routes may include the direct projections from entorhinal layers II and III to CA3 and CA1, respectively.

Key words: Hippocampus – Single units – Place cells – Colchicine – Spatial behavior

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Introduction

The hippocampal formation in mammals plays an important role in the acquisition of spatial information (e.g., Jarrard 1978; O'Keefe and Nadel 1978; Olton et al. 1978; Nadel and McDonald 1980; Morris et al. 1982; Sutherland et al. 1983). Almost any treatment that damages this structure or interferes with its normal physiological activity results in impaired acquisition on tasks that require the animal to remember either where it has recently been or some relationship between a reward location and the remote sensory features of the environment. Although spatial learning may reflect a particular case of a more general information processing capacity of this structure (e.g. Sutherland and Rudy 1989), the most impressive behavioral correlate of single unit discharge in the hippocampus of freely moving rodents is the close coupling between the activities of single pyramidal cells and the animal's location and orientation within the testing apparatus (O'Keefe and Dostrovsky 1971; O'Keefe 1976; Olton et al. 1978; McNaughton et al. 1983; Christian and Deadwyler 1986; Muller et al. 1987; O'Keefe and Speakman 1987). O'Keefe coined the terms "place cell" to describe this characteristic, and "place field" to designate the restricted portion of the accessible space over which a given cell exhibited elevated firing. One interesting feature of this spatial selectivity is its apparent independence of prior experience of the animal with the relevant space. That is, pyramidal cell place fields are evident on the first exposure of a rat to a novel spatial location (Hill 1978). Thus, in contrast to the behavioral manifestation of spatial learning, spatially selective firing appears to require little or no learning period.

A popular conception of the organization of hippocampal circuitry has been the notion of a

four neuron – trisynaptic loop (Andersen et al. 1966; Andersen et al. 1971). This circuit consists of the massive perforant path projection from the entorhinal cortex to the granule cells of the fascia dentata (Cajal 1911; Lorente de Nó 1934; Blackstad 1958; Hjorth-Simonsen and Jeune 1972; Steward and Scoville 1976), the highly parallel "lamellar" projection of granule cell axons (mossy fibers) to CA3 pyramidal cells (Blackstad et al. 1970; Gaarskjaer 1978; Claiborne et al. 1986), and finally, the Schaffer collateral projection from CA3 to CA1 pyramidal cells (Schaffer 1892; Cajal 1911; Lorente de Nó 1934; Hjorth-Simonsen 1973). A number of recent findings make this simple scheme untenable, although this concept still provides a useful framework for discussion. It is now well established that all three principal subfields receive direct projections from the entorhinal cortex (Steward and Scoville 1976; Witter et al. 1988; Yeckel et al. 1988) and that there are extensive lateral interactions among the principal cells, at least within FD (Hjorth-Simonsen and Laurberg 1977; Laurberg 1979; Laurberg and Sørensen 1981; Swanson et al. 1981) and CA3 (Lorente de Nó 1934; Hjorth-Simonsen 1973; Swanson et al. 1978; Ishizuka et al. 1986). In the former area, the interactions are indirect, being mediated by a relatively small number of large excitatory interneurons (mossy cells) located in the hilus. CA3 pyramidal cells engage extensively in direct excitatory interactions over a considerable proportion of the longitudinal and transverse axes, a feature which is of major computational significance (McNaughton and Morris 1988). The trisynaptic loop concept is correct, however, to the extent that, between areas, the information flow is strictly from FD to CA3 to CA1. There are no reverse projections.

The question arises, then, as to what extent the primary (trisynaptic) pathway is responsible for the transmission and refinement of spatial selectivity that is observed in pyramidal cells of CA3 and CA1. It is known that much of this information does, indeed, arise in the entorhinal cortex, as Miller and Best (1980) have found that extensive damage of this cortical field results in a substantial reduction in the likelihood of finding spatially-selective hippocampal neurons, and an even more pronounced reduction in the stability of these fields following maze rotation. Disconnection of the ascending afferents to the hippocampus via the fimbria-fornix, on the other hand, had relatively little effect on location-specific firing in spite of its severe effect on spatial memory. Comparable findings have recently been reported following reversible inactivation of the medial septal nucleus using local microinjections of tetracaine (Mizumori et al. 1987).

The discovery by Goldschmidt and Steward (1980) that hippocampal granule cells exhibit a supersensitivity to the toxic effects of the antimitotic alkaloid colchicine has provided a useful tool with which to address this question further. Small, local injections of the appropriate concentration of colchicine into the dentate gyrus resulted in a dramatic loss of granule cells with little or no apparent damage to other cell types in the hippocampus. Several groups have made use of this selective neurotoxicity to demonstrate the necessity of an intact granule cell population, and hence the trisynaptic circuit, for normal spatial learning (Sutherland et al. 1982; Sutherland et al. 1983; Jarrard 1983, 1986; Jarrard et al. 1984; Sutherland 1985; Wishaw 1987; Nanry et al. 1988).

Given the dramatic effects of granule cell destruction on spatial learning, the major question addressed in the present report concerns the relative importance of this component of hippocampal circuitry in determining the spatial firing characteristics of pyramidal cells in the CA3 and CA1 subfields. A preliminary report of some of these results has been presented in abstract form (McNaughton et al. 1988).

Methods

Subjects

A total of 18 male Fischer-344 rats were used in this experiment. These animals were obtained, as 9 months old retired breeders, from the Charles River Breeding facilities in Kingston. Prior to surgery, the animals were housed three per 53.5 cm by 29.5 cm plastic colony cage. Following surgery, animals were individually housed in the same type of cage. Weighing and handling were carried out daily for a two week period prior to use of the animals in the experiment. The animal colony room was maintained on a 12/12 h light/dark cycle, and all manipulations were carried out during approximately the second half of the light phase. For logistical purposes, and because some replacement animals were required for the colchicine group (due either to death or to insufficient FD lesions) these experiments included two separate batches of animals.

Colchicine lesions

All surgeries were carried out under deep sodium pentobarbital anesthesia (Nembutal, 33 mg/kg). In the first series of animals, two stages of surgery were employed, one for the colchicine lesions, and another, between one and six months later, for the implantation of the recording electrode assembly. For the six animals in the lesion group, 6 injections of colchicine were made at evenly distributed points along the rostro-caudal axis of the dentate gyrus in each hemisphere. The injections, which consisted of 0.1 µl of colchicine (4 mg/ml, in 0.9% NaCl, pH 7.2), were delivered using pressure injection (Picospritzer) from a glass pipette with a tip diameter of 20–30 µm. Six control

animals underwent sham surgery in which saline-filled pipettes were lowered as far as the corpus callosum at the same rostro-caudal and medio-lateral coordinates as for the colchicine pipettes. No solutions were ejected. Of this batch of animals, one control and one colchicine animal died following surgery, three of the colchicine animals were found to have insufficient granule cell damage (less than 50% loss overall), and two other animals, one from each group, had technical problems leading to an insufficient yield of recorded neurons. This left four control animals and one colchicine animal. Consequently a second series of surgeries was carried out to supplement the colchicine group.

In this second series, six animals were used. The surgery was done in a single stage, and more extensive injections of colchicine were carried out. In this case 14 0.1 µl injections of the same colchicine concentration were made in each hemisphere, with a slightly increased distribution towards the posterior and temporal dentate gyrus, that had been the spared region in the previous group.

In both batches of colchicine animals there were signs of acute toxic reactions for several days following surgery, and the animals exhibited moderate to extreme hyperreactivity to sensory stimulation for a period of 1 to 2 weeks. This hyperreactivity was ameliorated somewhat by oral administration of diazepam (Valium) following surgery. Two of the animals from the second series died from colchicine toxicity within two days of surgery, and one colchicine animal would not perform the required behavioral task used during unit recording, and had to be rejected from the study. Thus the overall subject population contributing data to this study consisted of the 1 colchicine animal and 4 controls from series 1, and 3 colchicine animals from series 2.

Behavioral training

The primary purpose of this study was an electrophysiological analysis, as it has already been well-documented that colchicine damage to the dentate gyrus seriously impairs spatial learning. Nevertheless, we felt it worthwhile to assess the animals on several spatial learning tests prior to electrophysiological recording. Following the colchicine lesions, series 1 animals were tested first on the Barnes (1979) circular platform task, and then on the Morris (1981) water task two weeks following the colchicine lesions. Testing on the circular platform task was essentially as described by Barnes (1979). Animals were motivated by bright lights shining on the platform surface to find the location of a dark escape tunnel located under one of the 18 holes that surrounded the perimeter. An error was scored if the animal investigated a hole that was not over the escape tunnel. The goal tunnel was kept in the same spatial location for 12 consecutive daily trials, after which it was shifted to a location 135° away from the original position, where it remained for the final 6 trials. The procedure for the water task was essentially as described by Morris et al. (1982) and by Sutherland et al. (1983). The animals were randomly placed into one quadrant of the circular pool and allowed to swim to find an escape platform hidden just under the surface of the opaque water in a fixed position. Accuracy at navigation in this task was assessed by total swim distance, as computed by an automated tracking system, and by the escape latency. Series 2 animals were trained to asymptotic performance prior to surgery on a reference memory problem (see below) using an 8-arm radial maze similar to the one used by Olton and Samuelson (1976) to test spatial working memory. Each day a single, randomly selected arm was designated as the reward arm. On the first trial of the day the animal was given access only to the reward arm. On the three subsequent trials, the animals had to choose from all 8 arms after having been placed in a random orientation on the maze center. Approximately 15 min separated each trial. An error was recorded each time an animal entered an incorrect arm. This provided baseline data for within-animal comparisons which were initiated 2 weeks following the colchicine lesions. This task is referred to as a reference memory problem in the sense that information gathered on one trial is required for correct performance on subsequent trials.

Construction and implantation of electrode assembly

Single unit recording was carried out using the stereotrode technique, essentially as described by McNaughton et al. (1983). Closely-spaced pairs of lacquer-coated, 20 µm, tungsten wire (California Fine Wire Co.) were constructed from a 14 cm length of wire which was first given a hairpin bend in the center, and then dipped into Epoxilite insulating compound. Surface tension held the two ends together while the Epoxilite was oven-cured. The two parallel wires were cut at right angles with sharp surgical scissors resulting in two conductors with a center to center spacing of about 35 µm. The stereotrode was mounted in a 24 mm length of 30 ga stainless steel cannula tubing with about 2 mm of tip protruding. The guide cannula was mounted on a microdrive assembly designed to allow fine movements of the electrode during chronic recording.

The microdrive consisted of two support posts made from 30 mm lengths of threaded, stainless steel rod with a 1/72 in pitch (Small Parts Co.). The threads were machined off from 7 mm at each end of the rods, and on one end were replaced with 4 or 5 circular grooves. On the other end, a flat surface was machined to provide a grip for a haemostat or other tool used to rotate the rods. Five mm lengths of black plastic Amphenol connector strips were used as the main electrode support for the microdrive. Two of the socket holes were tapped to accommodate the 1/72 in threads of the support posts. Thirty ga electrical circuit board wire, stripped at one end, was glued to the Amphenol strips to provide leads connecting the stereotrode wires to a connector socket (Molino and McIntyre 1972) that was mounted on the animal's head at the time of surgery. Electrical connections to the stereotrode were made using conductive nickel paint. Two separate stereotrodes were mounted parallel to one another on each microdrive about 1 to 1.5 mm apart. Prior to implantation, the electrical impedance of each independent recording wire was reduced by electroplating the tips with gold. The final tip impedances ranged from about 100 to about 400 KΩ.

One such microdrive was implanted over the hippocampus of each hemisphere (with the most anterior electrode positioned at approximately 3.8 mm posterior to Bregma and 2.0 mm lateral). The parallel pair of stereotrodes was oriented at 45° in the horizontal plane relative to the midline, corresponding to the septo-temporal axis of the dorsal hippocampus as viewed from above. After puncturing the exposed dura mater with a glass needle, the electrode tips were implanted to a depth of about 500 µm into the cortex. The craniotomy was filled with Dow Corning RTV medical grade silastic to a level just above the exit of the stereotrodes from the guide cannulae. After coating the support posts and guide cannulae with a thin film of Vaseline, the entire assembly was embedded in dental acrylic up to the top of the highest circular groove in the support posts, which were thus free to rotate in the hardened acrylic base, driving the stereotrodes forward. The dental acrylic was anchored by 5 or 6 small jewelers screws mounted in the skull prior to positioning the electrodes. The silastic ensured that no acrylic was allowed to flow below the tip of the guide cannula, causing it to jam.

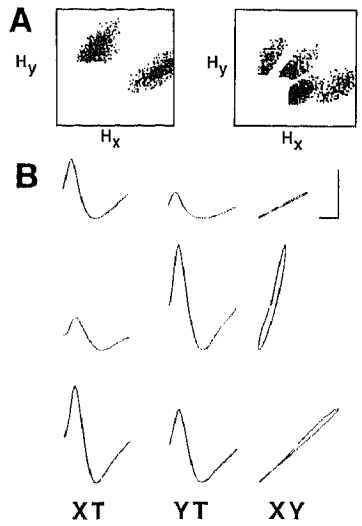

Fig. 1. A Examples of scatter plots of the relative amplitudes of action potentials recorded on the two channels (X, Y) of the stereotrode. Clusters of data points corresponding to putatively isolated single units were extracted on the basis of these and six other waveform parameters using an interactive program that displayed pair-wise scatterplots of parameter values and permitted the assignment of boundary windows. H_X, H_Y refer to the spike amplitudes on the X and Y recording channels. **B** Averaged waveforms of several single units simultaneously recorded on the two channels (X and Y) of the stereotrode in a single recording session. The relative spike amplitudes reflect the relative distances of the single units from the two closely spaced tips of the stereotrode probe. Calibration 200 μsec, 100 μV. At the right of the voltage versus time plots (XT, YT) for each unit is the Lissajous phase plot of voltage in Y versus voltage in X. During data acquisition this type of display proved particularly useful in recognizing the presence of multiple cells

Electrophysiological and behavioral recording

All animals were trained on the radial 8-arm maze before surgery in a room *different* from the one in which electrophysiological recording took place. At least one week following electrode implantation, the animals were again food deprived to 80% of their free feeding weight, and were trained on a forced choice version of the radial 8-arm maze task (Barnes et al. 1983). In this procedure, each arm of the radial maze was made accessible to the animal (by raising a motorized bridge) in random sequence once per trial. Chocolate milk (0.1 ml) contained in a small aluminum cup at the arm-end served as reinforcement for the animal to run to the end, drink, and to return promptly to the central platform, at which time the next arm in the sequence was presented. Typically 10 to 12 such trials (80 to 96 arm traverses) were carried out in a single recording session, with an intertrial interval of about 40 s, during which the animal was placed on a rubber mat in the center of the maze. The overall average number of trials per recording session was 9.8 for controls and 10.6 for colchicine animals. No data were accepted if less than 6 trials were completed.

During recording sessions, the animal's position on the maze was monitored by electronically tracking an infrared-emitting LED attached to the recording preamplifier mounted on the animal's head. The tracking system had a spatial resolution of 1.8 cm and the position was sampled at 20 coordinate pairs per second. The preamplifier consisted of 4 FETs arranged in simple unity gain follower circuits. The wires conveying the necessary 6 V power and electrophysiological signals were suspended from a nine channel commutator (Beila Engineering Corp.) mounted on the ceiling.

The electrical signals were amplified between 5000 and 10000 times (depending on signal amplitude) and band pass filtered between 600 Hz and 6 KHz (first order, one half amplitude frequencies). These signals were then digitally processed. For the first group of animals, the data were collected and analyzed on a PDP-11/23 computer using software developed by the first author. A commercially available version of the same software was developed for the Intel 80386 IBM-compatible processor (Brainwave Systems Inc.). This system was used for the second batch of animals. The essence of the data acquisition involved the logging of the precise time (0.1 ms resolution) of occurrence of each spike event in which the voltage exceeded a predetermined threshold on either channel of the stereotrode pair. For each channel, the signal waveforms were digitized and various diagnostic parameters, including the heights and latencies of the maxima and minima on each channel, were extracted. Two dimensional scatter plots were constructed on the computer display for each pair of the 8 parameters (see Fig. 1). Particularly useful was the scatter plot of the relative heights of the action potentials simultaneously recorded on the two channels, as this information was a reflection of the relative spatial locations of different neuronal generators, thus providing an effective means of spike separation in addition to the usual single channel window technique. The other waveform parameters, such as spike widths (the time differences between the maximum and minimum voltages) also provided additional discrimination power. A more complete description of the spike separation algorithms can be found in the original report (McNaughton et al. 1983), and in Mizumori et al. (1989). Typically two or more cells were isolated with confidence and recorded simultaneously during a given session. Occasionally it was possible to discriminate up to 7 or 8 cells in the same recording session.

Data analysis

A number of analyses were carried out on the temporal and the spatial firing characteristics of each cell, and also on the waveform characteristics (spike height, from maximum to minimum, and the corresponding spike duration). The temporal characteristics included the mean firing rate over the whole session, the peak firing rates attained during any 50 ms interval, the modal frequency of the interspike interval distribution (or the value of the first maximum where the distribution was polymodal), and estimates of the burstiness and/or 7 Hz rhythmicities which were obtained by scoring the autocorrelation functions for each spike train (see Fig. 7). Rhythmicity and burstiness were ranked on a scale of 0 to 5 by an experienced observer who was naive with respect to the treatment group from which the cells were derived. All scoring was done by the same observer. Cells were classified as either complex spike (CS) cells or theta cells according to criteria similar to those of Ranck (1973).

A number of analyses were also performed on the spatial firing characteristics of each cell, some of which have been described previously (McNaughton et al. 1983). The position data were processed so as to assign each sampled point to its corresponding maze arm and radial direction of travel (inward or outward) as inferred from the sequence of position points on either side. Each arm was subdivided into sixteen position bins for each of the inward and outward radial directions (see Figs. 5 and 6). Individual action potentials were assigned to their corresponding position/direction categories, and the sum

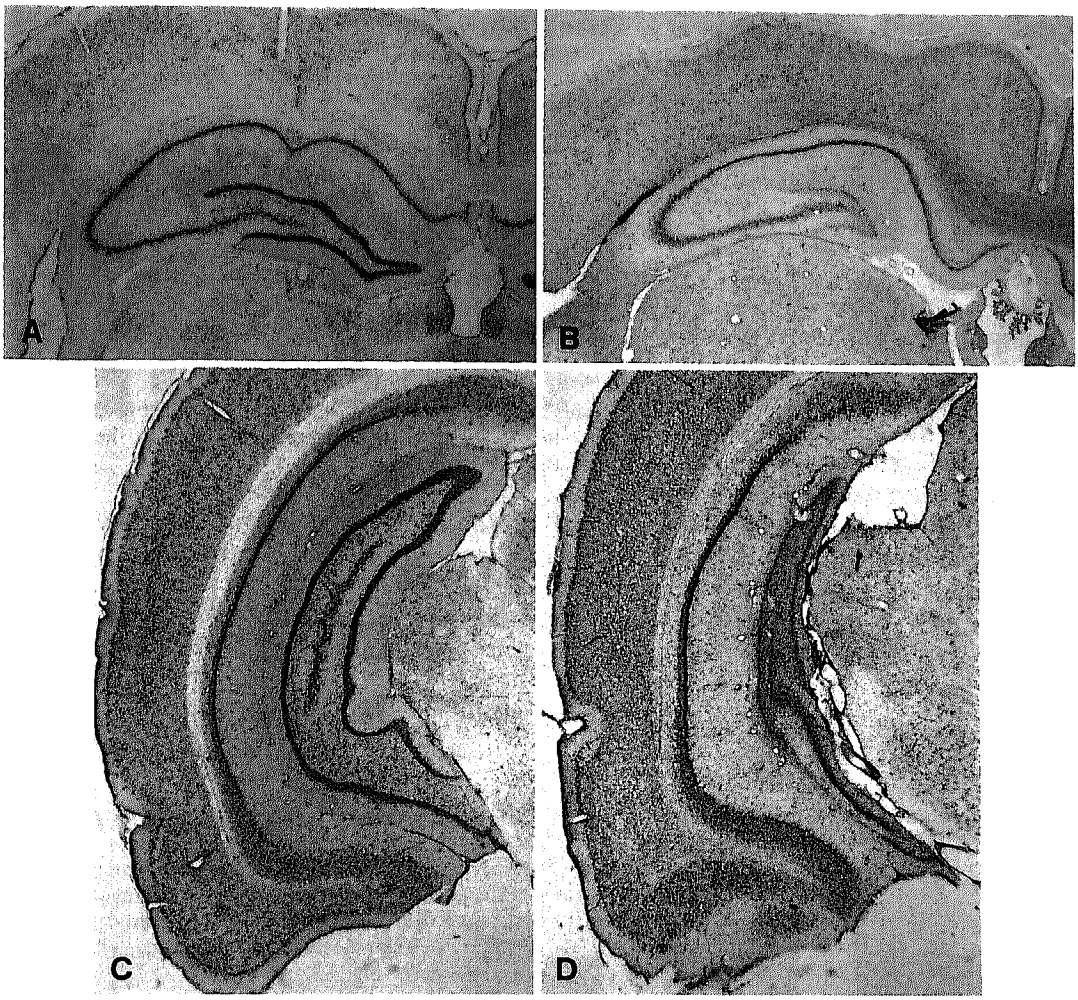

Fig. 2. A, B Photomicrographs from control and colchicine-treated hippocampi, respectively, taken from the dorsal region in the vicinity of the recording site. Note virtual total destruction of dentate granule cells in **B**, with some sparing of large hilar neurons, and some residual molecular layer. CA1 and CA3 are relatively undamaged. The "dimple" in CA1 is a result of tissue drag from the recording electrode. **C, D** Photomicrographs from the same brains as in **A, B** at a more caudal level illustrating the extensive loss of granule cells in the middle and ventral portions of the hippocampus

within a category was divided by the total number of position points therein. A specificity index was defined by integrating the rates for each arm in the two direction categories, providing an inward and an outward rate for each arm. The value of the category with the highest rate over the whole session was divided by the mean of the values of the other 15 categories. A specificity index of 1.0 thus indicated no spatial selectivity. In addition, a subjective estimate of spatial selectivity on a scale of 0 to 5 was also made for each cell on the basis of an examination of spatial firing rate plots illustrated in Figs. 5 and 6, again by a single observer on a blind basis with respect to treatment group. Quantitative studies by Best and Ranck (1982) have shown that such subjective estimates actually provide a reliable measure of spatial selectivity. Two other quantitative measures were derived from the rate distribution data. Reliability was defined as the proportion of trials on which the position/direction category with the highest rate was the maximum, whereas directionality was defined as the ratio of the value of the category with the highest rate divided by the rate for the same arm in the opposite radial direction. In addition, a score of 1 or -1 was assigned, depending on whether the maximum rate was in the outward or inward direction respectively.

Histology

At the end of the experiment, animals were again deeply anesthetized and were perfused transcardially with a 10% formal saline solution. Complete coronal serial sections were cut at 25 μm in order to identify and to trace the electrode track. A one in five series of sections from the medial septum to the back of the entorhinal cortex was mounted and stained for morphometric analysis. Camera lucida drawings were made of the outline of the dentate gyrus, including the molecular and granule layers (see Fig. 2) but not including the subgranular hilar region. These drawings were then digitized using a software package (3DED) kindly provided by Dr. S. Young of the University of California at San Diego. The computed areas of each outline were summed over their corresponding hemi-

spheres to provide a relative estimate of the total volume of dentate gyrus.

The results were evaluated using unpaired t-tests with alpha set at the 0.01 level. The n was taken as the number of cells in all comparisons, except for the anatomy where n corresponded to the number of animals.

Results

Histology

The quantitative results of the morphometric analysis, which can be found in Table 1, indicated a substantial reduction in the volume of the dentate gyrus ($t=5.80$, $p<0.001$). An illustration of the extent of the colchicine-induced damage is provided in Fig. 2. In the colchicine-damaged animals, the area (volume) measure almost certainly overestimated the proportion of remaining granule cells, because of the presence of other cell types (interneurons and glia) which were apparently spared. Even in regions where the granular layer was completely absent, some residual molecular layer often remained and was included in the measurements. In all of the colchicine-treated animals included in this study, we estimate that at least 90% of the granule cells were destroyed for a distance of at

Table 1. Area of fascia dentata in control and colchicine-lesioned animals

Animal no	Treatment	Area left hem	Area right hem	% Area
1713	Control	393.8	457.2	–
1714	Control	318.1	305.0	–
1725	Control	478.7	499.4	–
1726	Control	317.6	330.4	–
1715	Colchicine	136.9	166.0	39
1925	Colchicine	49.5	50.9	13
1926	Colchicine	103.4	173.6	36
1929	Colchicine	68.9	84.7	20

Note: In the last column (% area) total residual fascia dentata area in treated animals is expressed relative to the mean of the control values

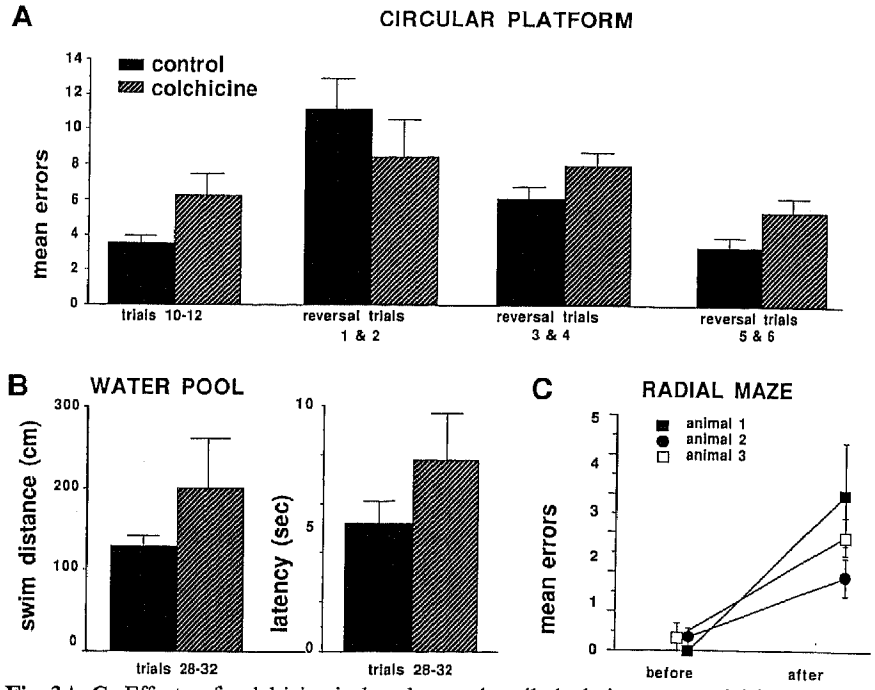

Fig. 3A–C. Effects of colchicine-induced granule cell depletion on spatial learning capacity as assessed by three different tasks. **A** Mean number of errors on the circular platform task averaged across blocks of days. The first histogram pair shows the average for the final 3 days of the initial acquisition. The subsequent histograms show mean errors (in blocks of 2 days) following a change of location of the goal tunnel. Note that the colchicine animals were significantly worse at the end of the initial acquisition, and, unlike control animals, showed little or no increase in errors following the change in goal location. This suggests that the performance of the colchicine animals on the reversal trials was not affected by prior learning of the original goal location. **B** Mean swim distance and escape latencies for the last 4 trials on the Morris water pool (Trials 28–32). Colchicine-treated animals were significantly worse on both measures. **C** Effect of colchicine treatment on spatial reference memory problem using the radial 8-arm maze. Pre- and postlesion scores for the three animals in series 2 are shown. On each day a different arm was assigned as the goal arm, and the animals were given 4 trials separated by about 15 min. The data points represent the average number of errors on the fourth trial, averaged across the last 10 days before colchicine treatment (before) and across the second block of 10 days following recovery from surgery (after). Note that for each animal the performance was substantially and significantly worse following the lesions

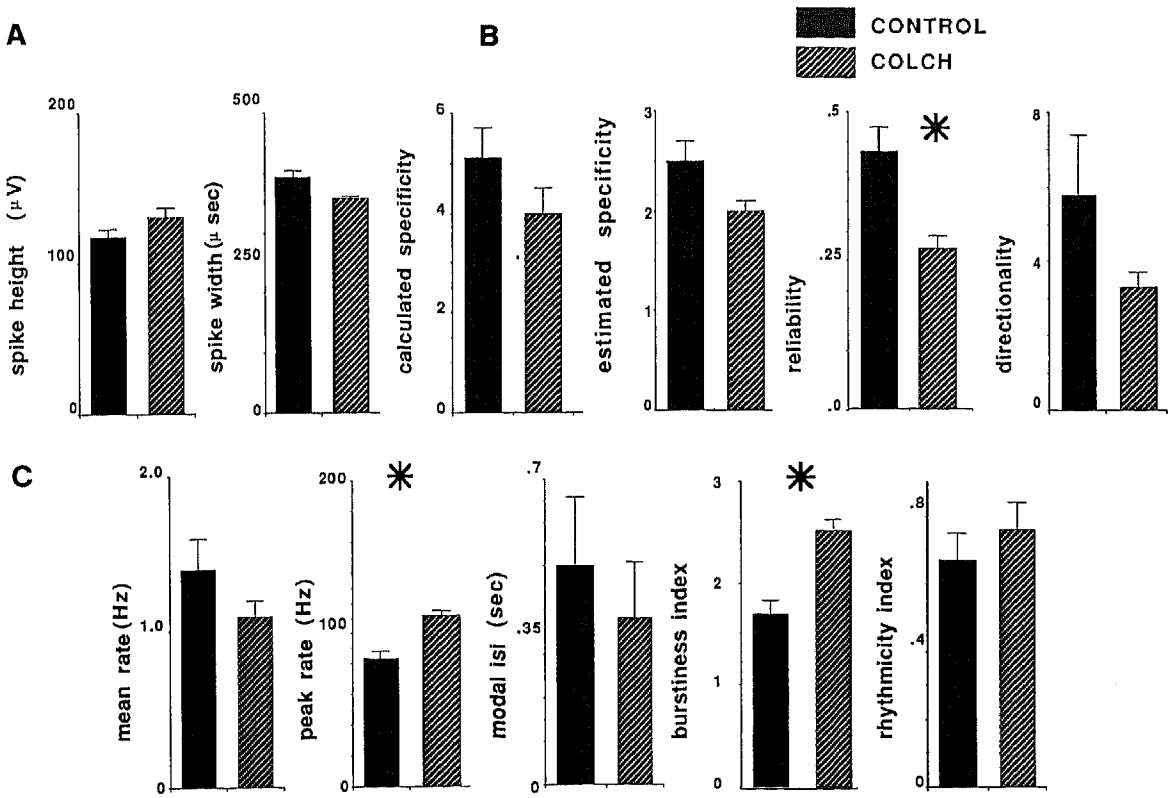

Fig. 4A–C. Effects of colchicine-induced granule cell depletion on electrophysiological parameters of single complex spike (pyramidal) cells recorded from CA1 and CA3. The data are subdivided according to waveshape parameters (**A**), spatial firing characteristics (**B**), and temporal firing characteristics (C). The major finding of interest was that the spatial specificity of pyramidal cell discharge was not significantly affected following substantial destruction of granule cells. There was however, a tendency towards increased variance (burstiness) in the temporal firing characteristics which may have resulted in reduced spatial reliability. Asterisks indicate statistically significant differences ($p < 0.01$)

least 1 mm in the septal and temporal directions from the recording electrode tracks.

Behavior

The results from the 3 behavioral tasks are shown in Fig. 3. Colchicine damage resulted in significant performance deficits in spite of the fact that some of the colchicine animals from the first series (Figs. 3A and B) had less than about 50% actual cell loss, and were thus rejected from the unit recording studies. The data presented in Fig. 3C show the within-animal comparisons for the 3 colchicine-treated animals of series 2.

Unit recording

A total of 244 CS (pyramidal) cells and 53 theta cells (interneurons) were recorded from during a total of 119 recording sessions (i.e. about 2 to 3 cells per session). The distributions of cell yield for each animal are given in Table 2. Data sets were rejected if, upon analysis of the spatial distributions of firing, it appeared as though the same cell was included in the previous day's recording session. This occurred in roughly 5% of cases. In addition, some data sets were discarded either because there were insufficient action potentials recorded on the maze for statistical reliability, or because the cells were judged to be poorly isolated.

Table 2. Distribution of cells recorded in control and colchicine-lesioned animals (CS = Complex Spike, T = Theta)

	CA1 CS	CA3 CS	CA1 T	CA3 T
Control	45	37	9	17
Colchicine	95	67	15	12

The results of the quantitative assessment of waveshape, and temporal and spatial firing characteristics of the CS cells is given in Fig. 4. Surprisingly, neither the spatial selectivity measures nor the mean discharge rates exhibited statistically significant differences between treatment groups.

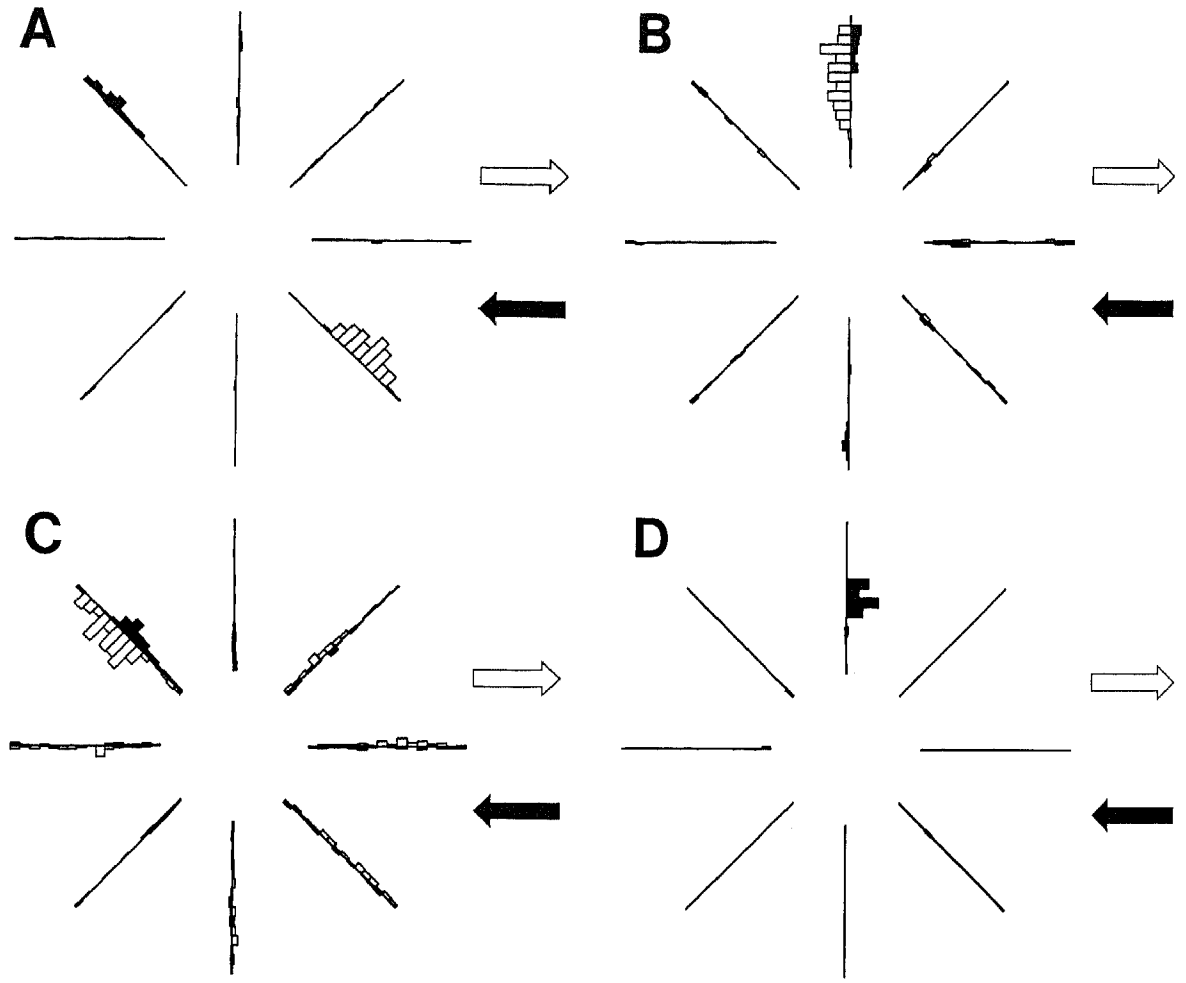

Fig. 5A–D. Spatial firing rate distribution plots on the radial maze. To illustrate the preservation of spatial selectivity, the most selective unit was chosen from each of the four *control* animals. The light and dark histogram bars represent firing in the radially outward and inward directions, respectively, on the corresponding maze arm. The central three histogram bins on each arm correspond to the central platform of the maze and the most peripheral bin represents cells firing at or just over the arm-end

Furthermore, the small difference in actual mean specificity was partly accounted for by an increase, in the lesioned animals, in the proportion of cells with place fields on or near the maze center (control animals 10%, colchicine animals 21%). For example, the firing field located near the center illustrated in Fig. 6C would have been scored by both methods as having a lower specificity because there appears to be approximately equal firing on all 8 arms. This effect would also contribute to differences in the reliability index.

There was, however, a discernable pattern of significant differences in several measures. In particular, colchicine treatment resulted in a significant reduction in the reliability of discharge within the maximum firing field ($t=4.51$, $p<0.0001$), an increase in the peak firing rate measured over 50 ms intervals ($t=2.49$, $p<0.014$), and an increase in the index of "burstiness" ($t=5.44$, $p<0.0001$) from the autocorrelation functions. There was also a substantial, although not statistically significant trend towards a reduced directional bias within the place fields of the colchicine animals. Plots illustrating the spatial selectivity and autocorrelation functions for the two groups are presented in Figs. 5, 6, and 7.

Although there were substantial differences in the mean values for a number of parameters of theta cell discharge, most of these differences were not statistically significant, and because of the relatively small sample size (n for colchicine=27, n for controls=26) we have much less confidence in those differences that were significant. Therefore, only the direction of the apparent trends is

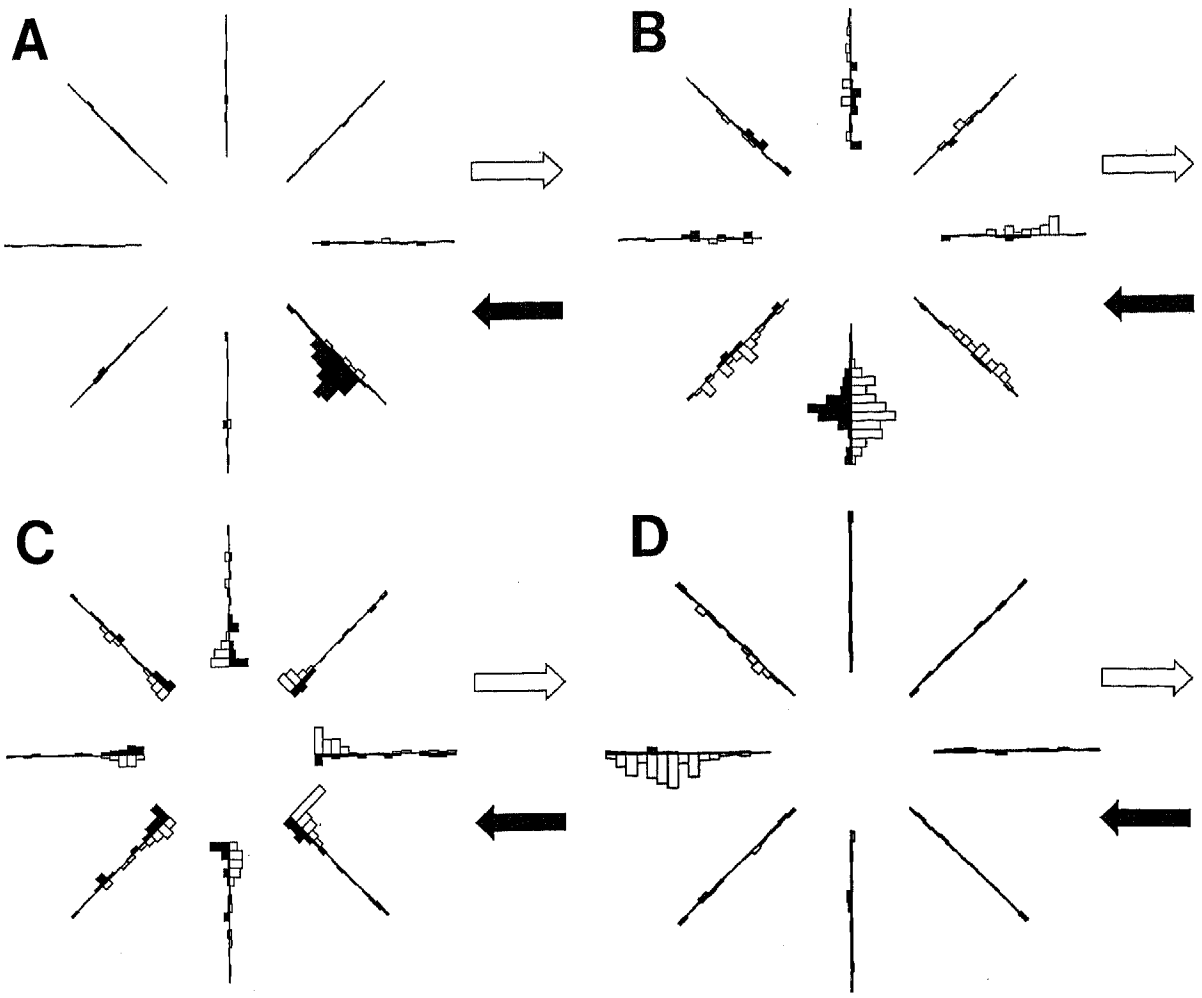

Fig. 6A–D. Spatial firing rate distribution plots on the radial maze. To illustrate the preservation of spatial selectivity, the most selective unit was chosen from each of the four *colchicine* treated animals. The light and dark histogram bars represent firing in the radially outward and inward directions respectively on the corresponding maze arm. The central three histogram bins on each arm correspond to the central platform of the maze and the most peripheral bin represents cells firing at or just over the arm-end

reported here. These trends included a suggestion of an increase in overall mean and peak firing rates as well as 7 Hz rhytmicity in the theta cells from colchicine-treated animals, and a reduced spatial firing bias in the center of the maze, a characteristic feature of theta cell activity (McNaughton et al. 1983) in normal animals.

Discussion

The most interesting and surprising result of these investigations was the failure to find substantial effects of massive depletion of dentate gyrus granule cells on the spatial selectivity of firing in the hippocampal pyramidal cells in spite of a severe effect on spatial learning. The caveat might be raised that spatially selective firing might have been disrupted in the tasks in which the spatial learning deficit was actually determined. This seems rather unlikely in view of the often repeated observation that spatial selectivity is independent of the behavioral requirements of the recording situation (e.g. O'Keefe 1976). In addition, others (Jarrard et al. 1984) have demonstrated cholchicine-induced spatial behavioral deficits using a task with demands similar to those employed in the present situation in which the overall granule cell depletion amounted to *at least* 75%, and the indices of place cell specificity did not differ by more than about 20%. This result strongly suggests that the spatial information on which normal place cell activity depends is not conveyed directly via the granule cells. At the very least it suggests that, in the absence of the granule cells, spatial information is

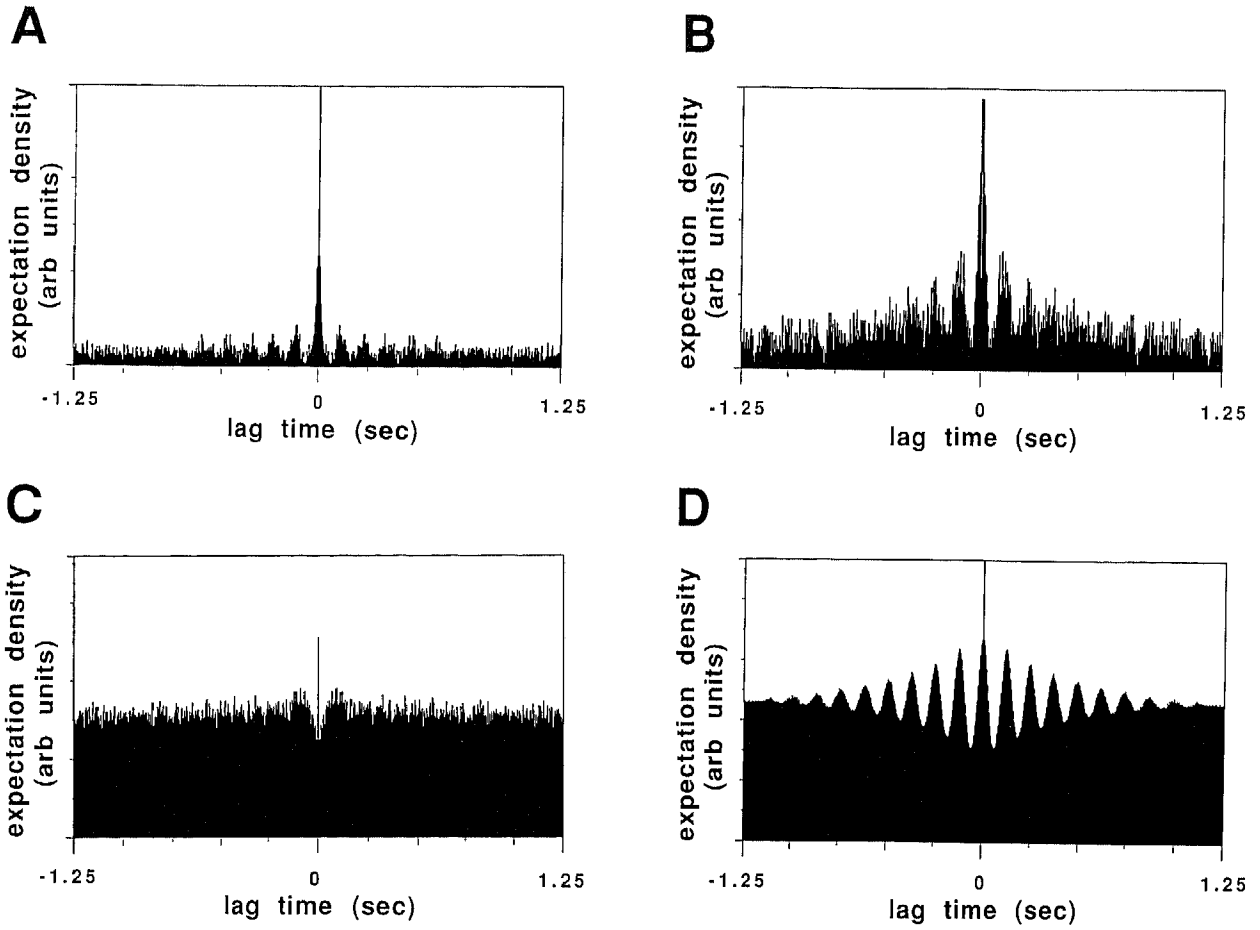

Fig. 7A–D. Autocorrelation functions illustrating the burst discharge and rhythmicity characteristics of hippocampal neurons recorded in this study. The complex spike cells whose data are shown in **A**, **B**, and **C** were classified as highly, moderately, and nonbursty respectively. Both **A** and **B** exhibited moderate rhythmicity at approximately 7 Hz. The highly rhythmic cell whose autocorrelation function is shown in **D** was classified as a theta cell

conveyed via other pathways. One possibility that cannot be entirely excluded is that the residual granule cells towards the septal and temporal poles provided spatial information which was propagated throughout CA3 via the extensive longitudinal excitatory connections within that area, as discussed in the introduction. This seems highly improbable, however, in view of the fact that perfectly normal appearing place fields were observed in one animal in which only about 17% of the total dentate volume remained, and in which over 95% of the total number of granule cells were probably destroyed. It appears much more probable that the spatial selectivity arrives via the direct projection from layer II of the entorhinal cortex to the apical tips of CA3. These are the same axons which form the perforant path to the dentate molecular layer (Witter, personal communication). Additionally some spatial information might reach CA1 via the projection from the deep layers of the entorhinal cortex. However, it seems likely that some spatial information must reach CA3 via the layer II projection, because there is no direct reverse connection from CA1 to CA3, and CA3 has no other major inputs of cortical origin.

While there was no difference in the spatial specificity averaged across trials, there was a reduction in the reliability of this spatial firing from trial to trial. There was also evidence for an increased tendency towards burst discharge in the colchicine-treated animals, as indicated by the larger index of "burstiness" derived from the autocorrelation functions, and by the fact that peak discharge rates were higher while the mean was not changed. This burstiness may partly account for the reduced reliability. Interestingly, the theta interneurons in the colchicine-treated animals exhibited a trend towards higher peak frequencies as well, and also towards higher overall mean rates.

From a functional perspective, these data sug-

gest that normal spatially-selective firing is not sufficient for spatial learning. This conclusion might also have been drawn from Miller and Best's (1980) results with entorhinal lesions. Although they reported a significant reduction in the likelihood of encountering spatially-selective neurons, nevertheless, 50% of their hippocampal neurons in their entorhinal group were classified as place cells. The additional contribution of the present results is that, whereas Miller and Best trained their animals in the test apparatus prior to the entorhinal lesions, the animals in this study had never seen the test room until after the colchicine damage. Given the learning deficit which results from colchicine damage, this result suggests that place-specific firing is not a "learned" phenomenon, but is a relatively "hard-wired" characteristic of hippocampal circuitry. Such a conclusion is consistent with the results of Hill (1978) showing that hippocampal place fields were present upon the first exposure of an animal to a cell's firing field. This conclusion is also consistent with the theoretical notion that the hippocampus implements some form of autoassociative memory that uses a fixed "detonator" or "teaching" input (e.g. Marr 1971; McNaughton and Morris 1988). Following extensive training in the apparatus, hippocampal place fields are remarkably insensitive to the removal of any particular subset of the spatial landmarks (O'Keefe and Conway 1978), a property derivable from the autoassociation hypothesis. Indeed, several studies indicate that spatially-selective firing can be driven by the animal's memory for the spatial characteristics of the apparatus, provided that an initial reference location is given (O'Keefe and Conway 1978; O'Keefe 1983; Jones Leonard et al. 1985; Muller and Kubie 1987; O'Keefe and Speakman 1987). Perhaps it is this associative property, by which a spatial representation can be completed from fragmentary information, that is conferred on the pyramidal place cells by their inputs from the fascia dentata. In the present study, no manipulations of the spatial features of the test environment were carried out. It remains for future work to determine whether the apparently normal spatial selectivity following granule cell destruction breaks down under conditions in which there is a demand for associative memory.

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