Differential Modulation of CA1 and Dentate Gyrus Interneurons During Exploration of Novel Environments

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Submitted 27 June 2003; accepted in final form 29 September 2003

INTRODUCTION

Many questions remain as to the role that interneurons play in the processing of information in the hippocampus. Interneuron subtypes in the hippocampus are characterized by axon terminal fields overlapping in a highly specific manner with intra- or extra-hippocampal sources of afferent input to principal cells (Freund and Buzsaki 1996). In some cases, interneuron subtypes have been shown to share electrophysiological properties such as degree of theta rhythm discharge and phase relationship to hippocampal sharp waves (Klausberger et al. 2003). Hypothesized functions of interneuron diversity include modulation of long-term potentiation and shunting of specific excitatory pathways afferent to principal cells (Buzsaki et al. 1996; Miles et al. 1996; Tomasulo et al. 1993; Traub et al. 1994).

Because hippocampal principal cell discharge is highly correlated to the rat’s position in space (Jung and McNaughton 1993; O’Keefe and Dostrovsky 1971; O’Keefe and Nadel 1978), determination of the spatial correlates of interneuron firing during the encoding of novel spatial information is an important step in examining the role of interneurons in cognitive functions of the hippocampus. With few exceptions (Fyhn et al. 2002; Kubie et al. 1990; Wilson and McNaughton 1993), the focus of attention concerning hippocampal interneuron activity patterns in the behaving animal has been their relationship to EEG and related sleep/wake states (Buzsaki et al. 1983; Colom and Bland 1987; Kodama et al. 1989; Mizumori et al. 1990). Nevertheless, the spatial correlates of interneuron unit activity have been described for both one-dimensional (track-running; McNaughton et al. 1983) and two-dimensional (open-field) foraging tasks (Kubie et al. 1990; Mizumori et al. 1990), where, independent of previously described movement correlates (Ranck 1973), hippocampal interneurons exhibit statistically significant and reproducible modulation of activity as a function of space.

Wilson and McNaughton (1993) demonstrated that a large subset of CA1 interneurons exhibits substantial decreases in firing rate as rats traverse from familiar into adjacent, but previously unseen, novel environments. In the present work, possible overlaps between basic discharge properties of interneurons and their responses to novelty were examined in simultaneous recordings of hippocampal CA1 and dentate gyrus (DG) principal cells and interneurons obtained as animals traversed familiar and adjacent novel environments (Fig. 1). Basic discharge properties of interneurons were not found to predict their response to novelty. However, firing rates of the DG and CA1 interneuron populations as a whole diverged greatly during exploration of novel environments. The results shed light on the diversity of interneuron unit discharge modulation as a function of the relative novelty of an environment and highlight differences in activity patterns of hippocampal sub-regions during encoding of novel spatial information.

METHODS

Recording techniques

Twelve tetrodes (Wilson and McNaughton 1993), each capable of independent movement, were mounted onto a circular array of 14 30-gauge guide canulles housed in a Delrin plastic “hyperdrive” core. The 14 canulles were bent slightly to form a circular bundle 1.2 mm wide at the outlet of the hyperdrive apparatus. Tetrodes were constructed of four 15-μm nichrome wires, which were twisted together and heat-fused. Tetrode tips were cut flat and gold-plated to produce impedances of 0.3–1.0 MΩ. Each wire was led to a connector board mounted on the drive, which contained an array of pin connections for interface with the recording headstage and cable.

On recording days, a recording headstage and cable were mated to the connector board of the hyperdrive apparatus mounted on the animals. The headstage carried operational amplifiers (unity gain) as well as an array of forward and rear LEDs used for tracking the
position and head orientation of the animals. The rats’ positions and head orientations (in the horizontal plane) were recorded at 20 frames/s using the headstage LEDs in conjunction with an SA-2 Dragon tracker (Dragon Tracker, Boulder, CO).

Signals from the animals were fed, via the headstage and recording cable, to multichannel filter/amplifier modules (Neuralynx, Tucson, AZ) and acquired and digitized (31.25 kHz) by seven synchronized 80486 computers. Voltage thresholds were set independently for each channel (4 each) of a tetrode. When threshold was exceeded on any of the four channels of a tetrode, a 1-ms sample of data beginning 0.25 ms before the threshold crossing was collected from all four channels. Cells were distinguished mainly via the relative amplitudes of their action potentials across the four wires of a tetrode. In two-dimensional plots of peak height (e.g., peak height on wire 1 vs. peak height on wire 3), separate “clusters” of points, corresponding to individual neurons, are apparent (Gray et al. 1995; Jung and McNaughton 1993; McNaughton et al. 1983b). Individual points within such clusters were defined by boundaries drawn with a computer mouse using interactive, custom software (“xclust,” Matt Wilson, MIT). Plots of peak height for each pair of wires (6 per tetrode) were made, and boundaries were drawn to produce, for each discriminated neuron, a multi-dimensional set of boundaries based on peak height across the four wires of the tetrode. The stability of such boundaries was validated by comparing drawn boundaries with those of pre- and postbehavioral time periods lasting 10 min or longer. Other spike waveform parameters such as depth of afterhyperpolarization, spike duration (time between spike peak and peak afterhyperpolarization), and integrated area between spike initiation and afterhyperpolarization zero-crossing were similarly used to fine tune discrimination. More extensive treatment of spike discrimination with tetrodes can be found in Harris et al. (2000), Gothard et al. (1996), and Szymusiak and Nitz (2002).

Prior to collection of data, the dorsal–ventral positions of the individual tetrodes were adjusted to obtain recordings from interneurons throughout subregions of the CA1 and DG areas, as well as principal cells in CA1 stratum pyramidale and stratum granulosum of the DG.

Subregions of the CA1 and DG regions were identified by previously described EEG correlates, proximity to principal cell layers, tracking of dorsal–ventral depth adjustments of individual tetrodes, and histological analysis of recording electrode tracts and marker lesions. Interneurons and principal cells were differentiated by comparison of spike width, average firing rate in quiet waking (<2 Hz for principal cells; >5 Hz for interneurons), and the presence or absence of complex spike discharge (Jung and McNaughton 1993; Ranck 1973). Across days of experimental recordings, the amount that each tetrode was advanced was carefully tracked. Such information was used to gauge the depth of each tetrode relative to the point at which the electrode entered the neocortex and/or the point at which the tetrode penetrated the corpus collosum. The former point is easily determined by attention to the large drops in movement artifact and line noise on entering the brain. Entry into the corpus collosus is associated with a large drop in background neocortical electrical activity as heard on an audio monitor while subsequent entry into stratum oriens is associated with increased background activity emanating from the CA1 pyramidal cell layer. Following entry into the hippocampus, two subsequent points could unambiguously be determined. Using well-established criteria (Jung and McNaughton 1993; Ranck 1973) for distinguishing action potentials from hippocampal principal cells (CA pyramidal cells and DG granule cells), the depth at which tetrodes encountered principal cell layers was noted. These points served to further refine depth estimates based on amount of tetrode advancement. Such information was augmented by careful attention to the electrophysiological properties of local field potentials (LFPs) recorded from each tetrode. Specifically, the presence of tetrodes within stratum oriens was associated with positive-going sharp-wave LFPs regularly occurring during immobility or sleep. The polarity of sharp waves reverses and then gains amplitude when tetrodes enter stratum radiatum (Ylinen et al. 1995). On entry to stratum molecular, sharp-wave amplitude begins to decrease and there is an increase in the amplitude of gamma-frequency LFP activity (Bragin et al. 1995).

Finally, histological analysis of each brain was used to confirm tetrode placement based on the combination of depth-tracking and electrophysiological techniques. The brain of each animal was fixed with recording tetrodes in place to enhance the detection of tetrode tracks in Nissl-stained coronal sections. The expected placement of specific tetrodes within particular subregions could be confirmed by detection of marker lesions made (under anesthesia) just prior to perfusion. To avoid ambiguity, such marker lesions were restricted to one to three tetrodes that had yielded interneuron recordings and that were in different subregions of the hippocampus. Examples of such histological confirmation of tetrode recording sites in two animals are shown in Fig. 2, where, among tetrode tracks in stratum radiatum and stratum oriens (black arrows), tetrode marker lesions were found, as expected, in the upper blade of the DG granule cell layer (bottom-most arrows in each panel).

Surgical preparation

Surgeries were conducted according to National Institutes of Health guidelines with Institutional Animal Care and Use Committee protocol approval. Thirteen Fisher 344 rats were anesthetized with sodium pentobarbitol and placed in a stereotaxic apparatus. Depth of anesthesia was ensured as necessary with metophane inhalation. A round, 2-mm-diam section of skull was removed, exposing the neocortex and dura overlaying the right hemisphere of the hippocampus. The coordinates of the center of this section were as follows: bregma, −3.8; lateral, 2.2. Following excision of the dura, the outlet of the hyperdrive recording apparatus was centered over and lowered to the surface of the exposed neocortex and fixed to the skull with dental cement. Skull screws ($\leq 8$) were inserted across the remaining surface of the skull to aid fixation of the hyperdrive to the skull. Another skull screw was used as ground and was placed over the left frontal cortex.

Antibiotic treatment consisted of a presurgical treatment with bicillin (0.2 ml, im) and a 1- wk postsurgical administration of ampicillin. Analgesic treatment consisted of postsurgical oral administration of 0.8 ml Children’s Tylenol (acetominophen). A 10% solution of Children’s Tylenol was available ad libitum for a period of 3 days following surgery. Recordings began a minimum of 5 days after surgery.

Behavioral protocols

Three different environmental manipulations were used to test the unit discharge characteristics of hippocampal cells during exploration of novel environments. These are depicted in Fig. 1. Animals were motivated to forage by restricting their food intake such that their weight was maintained at between 80 and 90% of their weight measured prior to training, when food was available ad libitum. All foraging tasks were preceded and followed by a 15- to 30-min baseline period during which the animal was placed in a 25-cm dish placed on a pedestal near the familiar environment. Such periods were used to verify stability of the recordings.

Chamber experiments

Prior to surgery, nine animals were trained to forage randomly for chocolate sprinkles in a 62-cm square chamber constructed of walls 62 cm in height and adorned with flat geometric objects of various sizes, shapes, and color. Brown construction paper served as the surface of the chamber and was replaced after each experiment. Animals quickly learned this task and were placed in the same chamber for 20- to 30-min periods each day ($\leq 7$ days) prior to
surgery. Animals were re-trained in the same apparatus beginning 2 days after surgery.

After at least 6 days of postsurgical experience in the chamber, novelty experiments were undertaken. Following 8–10 min of foraging, one wall of the chamber was gently removed to allow passage of the animal to an adjacent, but previously unseen, chamber of the same shape (see Fig. 1). The walls of the new chamber were of different colors than the familiar environment and were adorned with unique, flat, geometric shapes. The animal was permitted to forage freely in both halves of the environment for a period of 10–20 min, after which the wall was reinserted and the animal was permitted to forage only in the familiar environment for another 8–10 min. In one case, a second session of familiar-only foraging was not obtained due to a lack of robust movement, possibly a consequence of either exhaustion, or more probably, satiety. Two animals would not cross the border between the familiar and novel portions of the environment despite robust foraging activity in the familiar chamber; data from these animals are not included.

**Rectangle experiments**

Seven of the animals were trained to run a route along one side of a rectangular figure-eight maze for food reward at two of the corners. Two of these animals also took part in a chamber experiment. Each animal was trained on this apparatus for ≥6 days prior to exposure to the novel environment. The rectangular figure-eight dimensions were 94 × 81 cm. The track was 7.5 cm in width, with a homogenous surface of dark blue carpet. On the day of the novel experience, the animals ran 6–20 laps on the familiar side of the maze before a barrier hiding the opposite (novel) side was lifted, exposing the additional three arms of the figure-eight. The animal was now fed at each of the four corners of the figure-eight, although passes across the middle arm of the maze were permitted and were obtained in four of the five animals. Following 6–20 laps on the figure-eight, the barrier was again inserted, and an additional 6–10 laps were made around the familiar rectangle.

**Triangle experiments**

Three of the animals were trained to run along a triangular track (each edge 76 cm in length) for chocolate pellets delivered at the middle of each arm or to forage for chocolate across the surface of a triangular platform (each edge 69 cm in length). A second triangle maze was placed adjacent to the training maze throughout training, well within the rat’s field of view. The second triangle was offset approximately 10 cm from the training maze to prevent traversal by the rat. Both triangles were open to the entirety of the recording room and the distal cues therein. The animal was made familiar with this environment over the course of ≥6 days prior to test day. On the test day, the animal was first run for 6–10 laps on the triangular track or for 8 min on the triangle platform. Subsequently, the second triangle was moved into contact with the familiar triangle, permitting access to both surfaces. One of these animals also took part in a chamber experiment; another also took part in a rectangle experiment.

**RESULTS**

**Interneurons**

A total of 11 chamber experiments involving exploration of a novel environment were conducted. Seven experiments involving exploration of an adjacent, novel rectangle were conducted. Three experiments were conducted using the triangular environments. A total of 58 interneurons were recorded during exploration of novel environments. Based on electrophysiological...
ical measures and histological evidence, 33 interneurons were identified as CA1 interneurons and 25 as DG interneurons.

Hippocampal interneurons are known to have robust, usually positive, relationships to movement velocity (Czurko et al. 1999; McNaughton et al. 1983a; Nitz and McNaughton 1999). We therefore sought to control for the possibility that changes in movement velocity between familiar and novel environments could bias interneuron firing rate comparisons. For each interneuron, a plot of firing rate as a function of velocity was constructed for both familiar and novel portions of the environment (see Fig. 3). For each bin of this plot, the ratio of the firing rate of the cell in the familiar and novel environments was calculated. These ratios were then averaged to yield a measure of firing rate change from the familiar to the novel environment that is independent of differences in EEG theta amplitude between the two environments. Note that these methods of comparing interneuron firing rates overcome confounds caused by differences in the velocity with which the animal moves in each environment as well as differences in the degree to which hippocampal activity overall is modulated at theta frequencies. The methods also take into account the often nonlinear relationships between velocity/EEG theta amplitude and firing rate for hippocampal interneurons.

Plotted in Fig. 4 is the distribution of velocity × firing rate ratios expressed as percentage change in the novel versus familiar environment for CA1 and DG interneurons. The distribution for CA1 cells contains predominantly negative values, indicating that, as a population, activity of CA1 interneurons is decreased as the animal explores the novel portion of the environment (1-sample t-test, \( n = 33, P < 0.001 \)). The largest decrease observed was a 61% drop in rate in the novel area. Depicted in Fig. 5 (left column) are plots of firing rate across the space of the familiar and novel environment for three CA1 interneurons exhibiting discharge rate reductions in the novel region of the explored environment. Sixty-four percent (21 of 33) of CA1 interneurons exhibited decreases in discharge rate in the novel environment larger than 10% of the familiar-chamber discharge rate. Only one CA1 interneuron exhibited a >10% firing rate increase (13.6%) in the novel environment.

The unit discharge rate of DG interneurons during exploration of the novel portion of the environment is in sharp contrast to that observed for CA1 interneurons (Fig. 4, 1-sample t-test, \( n = 25, P < 0.03 \)). Figure 5 (right column) depicts three DG interneurons that exhibited discharge rate increases in the novel region of the explored environment. In large part, DG interneurons exhibited increases in firing rate relative to the familiar environment while that of dentate gyrus interneurons is increased.

FIG. 3. Familiar and novel environment firing rates as a function of velocity. Top graph: velocity × firing rate curves for an individual CA1 interneuron during time spent within the familiar (black lines) and novel (gray lines) environments. Bottom graph: same information for an individual dentate gyrus interneuron. Ratio of novel to familiar firing rates averaged across all velocity bins is 1.31 for the dentate gyrus interneuron and 0.63 for the CA1 interneuron.

FIG. 4. Summary of interneuron firing rate modulation by novelty. Frequency histograms of percentage changes in firing rate in the novel vs. familiar environments for all CA1 (top) and dentate gyrus (bottom) interneurons recorded. As a population, CA1 interneuron activity is decreased in the novel environment while that of dentate gyrus interneurons is increased.
portion of the environment; the largest increase was 47%. Of 25 DG interneurons, 10 (40%) exhibited increases in activity in the novel area >10%. Two cells exhibited a decrease >10%; the percentage decreases in firing rate for these two cells were modest (11.6% and 12.8%).

The distributions depicted in Fig. 4 were statistically compared by t-test. The mean ratio of discharge rate in familiar versus novel environments across all CA1 interneurons was significantly different from that across DG interneurons ($P < 0.0001$). Because the distribution of values for the population of DG interneurons was somewhat skewed, we also compared the CA1 and DG distributions using the nonparametric Kolmogorov-Smirnov test. By this more stringent test, which does not assume normality of the distributions compared, we still find the difference between CA1 and DG to be significant ($\chi^2 = 18.861, P < 0.002$). The same statistical measures were also applied to novel versus familiar firing rate ratios determined from plots of EEG theta amplitude versus interneuron firing rate. Not surprisingly, since movement velocity and EEG theta amplitude are positively correlated (e.g., see Nitz and McNaughton 1999), we again found statistically significant differences between the CA1 and DG interneuron populations ($t$-test, $P < 0.001$; Kolmogorov-Smirnov test, $P < 0.002$).

CA1 and DG interneurons were recorded simultaneously in six different experiments. In one of these experiments, two DG interneurons exhibited novel-environment activity increases of 31.4 and 47.3%, while a CA1 interneuron exhibited a rate reduction of 34.3%. In another, two CA1 interneurons decreased rate by 37.1 and 42.6%, while at the same time, a DG interneuron increased rate by 24.7%. For the entire subset of interneurons, the mean CA1 interneuron percentage change in firing rate (novel vs. familiar environment) was $-16.6 \pm 6.5\%$ (SE; $n = 9$), while that for DG interneurons was $+13.6 \pm 6.3\%$ ($n = 9$). The difference between these subgroups of the overall sample was highly significant ($t = 3.33, P < 0.005$). Thus divergent responses of CA1 and DG interneurons to novel environments occur in parallel (also see Fig. 6, bottom). With the assumption of even sampling of interneuron subtypes, we conclude that the overall activity of interneurons exhibits divergence between CA1 and DG during exploration of novel environments.

The "novelty-on" responses of DG interneurons as well as the "novelty-off" responses of CA1 interneurons were observed in all three experimental conditions. Thus the effect of novelty was independent of differences in random (chamber) versus guided (rectangular) foraging tasks. The effect of novelty was also independent of whether the walking surfaces of the familiar and novel regions were similar or dissimilar, because the effects of novelty were similar in chamber/rectangle (same surface) experiments and in triangle experiments (painted wood vs. rubber surfaces). Most importantly, the alteration of distal visual cues cannot, by itself, explain the response of interneurons in either region, because novelty responses in both regions were observed in triangle experiments where distal cues were unchanged over the course of the experiment.

**Interneuron subtypes**

In an effort to distinguish one or more subtypes of hippocampal interneurons, which exhibit modulation of firing rate as a function of environmental novelty, the correlations between modulation by novelty and anatomical or electrophysi-
ological variables thought to differentiate interneuron subtypes were assessed. Interneurons in different subregions of the hippocampus tend to exhibit divergent patterns in the distribution of dendritic and axonal fields (Han et al. 1993; Lorente de No 1934; Ramon y Cajal 1893; Sik et al. 1996; Somgyi and Freund 1989). Nevertheless, CA1 interneurons in every region examined (stratum oriens, 2/6, 33%; stratum pyramidale 15/24,62%; and stratum radiatum, 2/3, 67%) exhibited decreases in activity specific to the novel environment. Similarly, dentate gyrus interneurons of both stratum moleculare (6/17, 35%) and stratum granulosum (3/7, 43%) exhibited increased discharge rate in the novel region of the environment. Only a single hilar interneuron was recorded; this cell exhibited a small activity decrement in the novel environment. The possibility remains that specific interneuron subtypes sharing histochemical signatures and/or afferent/efferent connectivities will be found to also share similar responses to novelty. Nevertheless, novelty-off and novelty-on responses of, respectively, CA1 and DG interneurons do not appear to be linked in a simple fashion to anatomical subregions of CA1 and DG.

Interneurons also differ widely in the degree to which they are modulated by the hippocampal theta rhythm, their average discharge rate, and the degree to which they tend to exhibit spatially specific increases or decreases in firing rate. To determine each cell’s degree of modulation by the theta rhythm, EEG recordings from the hippocampal fissure were first filtered between 6 and 10 Hz. Determination of each cell’s discharge in relation to the filtered EEG was analyzed as in Skaggs et al. (1996). Briefly, time periods between successive theta peaks were divided into 10 bins, and the proportion of each cells firing in each of these bins calculated. The bin (or phase of EEG theta) containing the highest proportion of total firing was used to determine the degree of theta modulation of the cells activity. Spatial information per spike is a measure describing the efficiency with which the discharge of a cell predicts the animal’s position in an environment (Skaggs et al. 1996). Typically, this measure is used to describe the spatial specificity of principal cell activities; however, interneurons exhibit significant diversity in the degree to which their discharge is modulated by space and this is reflected by the spatial information per spike parameter. Surprisingly, neither average firing rate, degree of theta modulation, nor spatial information prior to exploration of the novel environment were found to correlate significantly with the degree to which cells subsequently exhibited firing rate changes in the novel environment.

Three “theta-off” interneurons were recorded under conditions of novel environment exploration. Typically, interneurons exhibit strong positive correlations of firing rate with the amplitude of the hippocampal EEG theta rhythm. Theta-off interneurons are relatively rarely encountered and have the unusual property of exhibiting a negative relationship of firing rate to the amplitude of the theta rhythm (Buzsaki and Eidelberg 1983; Colom and Bland 1987; Mizumori et al. 1990). Of the three cells, two were located in CA1 and exhibited decreases in discharge rate of 11% and 20% in the novel environment compared with the familiar environment. The other theta-off interneuron, located in the dentate gyrus, exhibited an increase in discharge rate of 40% in the novel environment. Thus theta-off cells exhibited similar responses to the much more common theta-on cells under conditions of novel environment exploration.

Spatial and temporal patterns of interneuron discharge

Modulation of interneuron discharge rate by the theta rhythm was examined as a function of the animal’s position in the familiar versus novel portion of the environment. Both CA1 and DG interneurons exhibited modest but significant increases in degree of theta modulation in the novel compared with the familiar region of the environment (t-test, CA1, P < 0.0001; DG P < 0.05). The average increase was 7 ± 1.9% for CA1 interneurons and 5 ± 2.32% in DG. Correlations between percentage change (novel vs. familiar region) in theta modulation and percentage change in firing rate were very low for both CA1 and DG interneuron populations (Pearson r = −0.012 and −0.024, respectively). Thus the factor(s) controlling changes in interneuron firing rate within the novel environment do not appear to impact the inherent tendency of these cells to oscillate. Instead, the observed changes in theta modulation were most likely linked to differences in the animal’s movement velocity between the two environments. On average, velocity was 11% greater in the novel than in the familiar region, producing an increase in overall EEG theta amplitude of 9% in the novel region. No change in EEG theta amplitude (<1%) was observed when the effect of velocity on theta amplitude was controlled for by comparison of velocity × EEG theta amplitude curves generated separately for time spent in the familiar and novel regions of the environment (data not shown). These results underscore the need to control for the overall activity level of the animal before drawing conclusions concerning alterations in hippocampal EEG as well as interneuron firing rates and patterns.

Interneurons of the hippocampus exhibit a diversity of phase relationships to the EEG theta rhythm. Two cells with similar depth of modulation by the background theta rhythm may nevertheless fire maximally with distinctly different phase relationships (as great as 180° out of phase) to the EEG theta rhythm (Skaggs et al. 1996). Indeed, this characteristic of interneurons has been used as a tool to differentiate interneuron subtypes (Csicsvari et al. 1999; Tsodyks et al. 1997). This characteristic was also unchanged for both CA1 and DG interneurons in comparison with phase firing profiles for the novel and familiar environments. Thus the factor(s) controlling theta phase specificity of interneuron discharge are unchanged by novelty and do not appear to be linked to overall firing rate.

Principal cells

A total of 115 principal cells that exhibited activity during the foraging sessions were recorded. Of these cells, 94 were CA1 pyramidal cells and 21 were dentate gyrus granule cells. Principal cells in both the CA1 and DG areas exhibited spatial firing correlates during each portion of the experiment. Forty-nine percent of all principal cells (CA1 and DG included) were active in both the familiar and novel regions of the environment. Twenty-seven percent of principal cells maintained firing only within the familiar region of the environment. Some cells (24%) that had previously not discharged within the familiar environment did so in the novel portion of the environment. The average pixel-by-pixel correlation (Pearson r) of spatial firing patterns between the initial and postnovelty periods of foraging in the familiar environment was 0.58 ± 0.044 for all principal cells (DG cells and CA1 pyramidal cells)
combined. DG granule cells exhibited a somewhat higher degree of correlation (0.79 ± 0.037) between the firing rate patterns of the two familiar-chamber sessions than did CA1 pyramidal cells (0.50 ± 0.036; \( t \)-test, \( P < 0.001 \)).

During exploration of the familiar + novel environments, the spatial firing pattern of cells with place fields in the familiar portion of the environment was fairly stable; some exceptions to this trend are noted in the subsequent section. The average correlation of firing between the initial, familiar-chamber session and the familiar half of the extended environment was 0.70 ± 0.023 for DG granule cells and 0.48 ± 0.049 for CA1 pyramidal cells. The comparison of granule cells and pyramidal cells was again significant (\( t \)-test, \( P < 0.03 \)).

The firing rates of CA1 principal cells in the novel environment exhibited changes opposite that of the predominant change in interneuron activity. CA1 pyramidal cells discharged at higher rates in the novel than familiar environment, while DG granule cells tended to decrease firing rate in the novel environment (Fig. 6). The comparison of familiar versus novel region firing rates was significant only for the CA1 population (\( t \)-test, \( n = 94, P < 0.01 \)), although the relative differences in rate were not very different between the two cell populations. To ensure that changes in principal cell firing rate in familiar versus novel environments were not biased by the relative number of cells exhibiting discharge only in the familiar or novel environments, comparisons were also made between CA1 and DG principal cells that exhibited within-field discharge of 2 Hz or greater in both the familiar and novel environments. CA1 pyramidal cells of this type exhibited firing rates 43% higher in the novel than in the familiar chamber (familiar rate = 0.88 ± 0.132 Hz; novel rate = 1.26 ± 0.176 Hz, \( n = 45, P < 0.005 \)). Only eight granule cells were recorded with significant (>2 Hz) discharge in both environments. The average firing rate for these cells in the familiar and novel environments was, respectively, 0.48 ± 0.187 and 0.37 ± 0.126 Hz.

**Place field development in the novel environment**

It is clear from some of the firing rate maps in Fig. 4 that the increase or decrease in interneuron discharge within the novel portion of the environment does not form a perfect line at the border between the two environments. In many cases, the area of the extended environment at which the increase or decrease in interneuron discharge rate appeared to begin was biased toward the familiar environment compared with the midline of the environment where the wall was removed. To define more clearly the region of the greatest change in overall interneuron activity, plots of average absolute percentage change in discharge rate as a function of space were constructed for each of the datasets (11) in which three or more interneurons were recorded. For the familiar portion of the environment, absolute differences in firing rate between the familiar-only and familiar + novel sessions were computed on a pixel-by-pixel basis and averaged across the population of recorded interneurons. The plots for three such experiments are depicted in the **leftmost column** of Fig. 7. As can be seen, the point of transition in firing rates lies well within the familiar chamber. Across all recorded interneurons, there was a significantly greater change in discharge rate (37 ± 4.2 vs. 22 ± 2.1%) in the half of the familiar environment bordering the novel environment (paired \( t \)-test, \( n = 11, P < 0.01 \)). In comparing the spatial firing properties of principal cells within and outside of this zone, it became apparent that principal cells of both DG and CA1 having place fields within this zone during the familiar only session were more likely to exhibit significant changes in spatial discharge patterns. Such changes often appeared as “stretching” of a given place field into the novel environment or maintenance of firing in relation to the north wall of the environment as a whole. **Column 2** of Fig. 7 depicts the activity of three such place cells during the initial familiar-chamber session and the subsequent novel chamber session. The differential ornamentation of the familiar and novel chamber walls in the present experiment was apparently insufficient to eliminate the relationship of the cell’s discharge to specific chamber walls. In contrast to cells within the region of greatest change in interneuron activity, cells that had exhibited place-specific firing outside this zone were more likely to exhibit firing restricted to the original field or to develop fields in the novel environment unrelated to that of the familiar environment (Fig. 7, column 3). In support of this observation, a reduced pixel-by-pixel correlation (Pearson \( r \)) of principal cell discharge was found for the half of the familiar environment bordering the novel environment compared with the half of the environment furthest from the novel environment (0.48 ± 0.09 vs. 0.76 ± 0.06, paired \( t \)-test, \( n = 9, P < 0.005 \)). Thus regions of the environment in which a novel pattern of discharge is exhibited by the principal cell population can be predicted by changes in the activity of DG and CA1 interneuron populations.
This study demonstrates that discharge rates of the DG and CA1 interneuron populations as a whole diverge during exploration of novel environments. This pattern of response was independent of the movement characteristics of the animals in familiar and novel environments. The differential response of CA1 and DG interneurons to the presence of the animal in the novel environment suggests a possible divergence in the respective functional roles of the DG and CA1 regions in spatial information processing and/or differences in the type of sensory information contained in afferents to the two regions.

Basic discharge properties of hippocampal interneurons such as firing rate and degree of theta rhythmicity were not found to predict their responses to novelty. Similarly, interneuron responses to novelty were limited to changes in firing rate in effect dissociating firing rate from other properties of discharge such as degree of theta modulation.

A second major finding is that regions of the environment, both familiar and novel, associated with changes in CA1 and DG interneuron discharge, were also associated with the development of novel principal cell spatial firing patterns. Given that hippocampal interneurons discharge in virtually all regions of an environment, this result suggests that the spatial firing patterns of only a few interneurons may be sufficient to map those regions of an environment where rearrangements of principal cell firing patterns occur. The emergence of such changes in hippocampal activity patterns in regions of the familiar environment adjacent to the novel environment suggests that the hippocampus achieves incorporation of novel spatial information into a pre-existing "familiar" representation of the environment.

The relative increase in interneuron activity in DG and decrease in CA1 in the novel environment must reflect alterations in the strength or amount of synaptic input to interneurons of these hippocampal regions. At present, there is no basis to presume that the responses of DG and CA1 interneurons can be attributed to changes in a single afferent system. Since place-related modulation of interneuron discharge is partially explained by principal cell-interneuron synapses (Marshall et al. 2002), changes in overall firing rate of principal cells paralleling those of interneurons within DG or CA1 could explain the observed changes in interneuron rate. However, our data indicate that changes in principal cell discharge rates in CA1 and DG were opposite those of interneurons. Thus feedback control of interneurons by principal cells cannot explain interneuron rate changes in either CA1 or DG.

Changes in feed-forward excitation of CA1 and DG interneurons by entorhinal cortical inputs could potentially explain the observed results. The increased discharge of DG stratum molecular and stratum granulosum interneurons in the novel environment could reflect a feed-forward response to increased input from output cells of the entorhinal cortex coding featural information of the novel environment. In the monkey, it cells of the inferior temporal cortex exhibit response decrements in conjunction with increased familiarity of visual stimuli (Lin et al. 1993). A similar process operating in the entorhinal cortex of the rat could result in greater excitatory feed-forward input to DG interneurons in the novel environment. Such a mechanism may be necessary to maintain sparse spatial coding by DG granule cells. The divergent response to novelty between CA1 and DG may then be explained by differences in the degree to which the two structures are innervated by entorhinal cortex or possibly by the differential sources of entorhinal input to the CA1 and DG regions. In rat and monkey, entorhinal cortical input to CA1 and DG arises from different layers.

The bulk of afferents to CA1 arise from layer III, while those to DG arise from layer II (Steward and Scoville 1976; Witter and Amaral 1991). Interestingly, this connectivity pattern has led to the recent hypothesis that layer II neurons play a role in novelty detection (Lorincz and Buzsaki 2000).

Decreased CA1 interneuron discharge rates in the novel environment could reflect reduced discharge rates of individual CA3 pyramidal cells. Indeed, it has previously been suggested that the level of inhibitory activity in the CA1 region serves to balance the relative amount of excitatory input arising from the CA3 region (Marr 1971; Mizumori et al. 1989). As alluded to above, DG interneurons, under conditions of novelty, may serve to balance increased entorhinal excitatory input to the DG. Clearly, recordings of CA3 and entorhinal cells during exploration of novel environments are necessary to confirm or reject each hypothesis.

Other potential sources of novelty-off and novelty-on responses in interneurons are numerous, since most interneurons receive subcortical noradrenergic and serotonergic inputs, as well as septal cholinergic and GABAergic inputs (Buzsaki 1984; Freund and Antal 1988; Freund et al. 1990; Frotscher and Leranth 1988; Leranth and Frotscher 1987; Rose and Schubert 1977). Of these possibilities, the divergent responses of DG and CA1 interneurons to novel situations are least likely to be explained by alterations in the activity of septal or raphe inputs to the hippocampus. First, previous data suggest that inactivation of septal inputs primarily inhibits the discharge rate of interneurons in both the DG and CA1 regions (Mizumori et al. 1989). Similarly, the predominant response of both DG and CA1 interneurons to pharmacological inactivation of median raphe serotonergic neurons is a velocity-independent increase in discharge rate (Nitz and McNaughton 1999).

Recent findings concerning the effects of novelty on brain stem noradrenergic neurons do suggest a possible mechanism for the predominantly novelty-on responses of DG interneurons (Kitchigina et al. 1997). Noradrenaline is known to increase the firing rates of CA1 and DG interneurons (Bergles et al. 1996; Doze et al. 1991; Pang and Rose 1987), and locus coeruleus (LC) noradrenergic neurons respond to novel stimuli with increased firing rates (Aston-Jones and Bloom 1981; Vankov et al. 1995). In comparison with the CA1 region of the hippocampus, the dentate gyrus receives a greater density of noradrenergic (NA) afferents from the LC (Loy et al. 1980; Morrison et al. 1979; Moudy et al. 1993; Oleskevich et al. 1989). Thus alterations in the modulation of the DG interneuron network by the noradrenergic system could potentially explain the tendency of DG, but not CA1, interneurons to increase discharge rate in the novel environment. In this scheme, the decreased novel-environment discharge exhibited by CA1 interneurons is unexplained. Nevertheless, the preceding argument would predict that interneurons of CA1 having dendritic fields largely restricted to stratum lacunosum-moleculare (not recorded in this study) would have responses to novelty similar to DG interneurons since noradrenergic innervation of this CA1 region is relatively dense and overlaps with
input from the entorhinal cortex (Acsady et al. 1996; Amaral 1993).

An important remaining question is that of the functional role of changes in interneuron activity. How do the observed changes in activity of DG and CA1 interneurons guide what appears to be parallel development of novel patterns of discharge by CA1 pyramidal and DG granule cells?

The respective decreases and increases in CA1 interneuron and principal cell activity could speed the development of synaptic potentiation of afferents to CA1 pyramidal cells and thereby permit development of new associations between self-motion cues and environmental landmark information. Increases in interneuron rate in the DG are more suggestive of an intensification of feed-forward input to this region. In this respect, the functional role of increased inhibition might be to maintain sparsity of patterned activity in the DG. Such a process is consistent with a singular role of the DG in generating orthogonal patterns of output for different environments and/or behavioral contexts. Alternatively, changes in interneuron discharge rate in either CA1 or DG could bias or balance the strength of different afferent inputs to principal cells in the CA1 and DG areas through inhibition or disinhibition at principal cell dendrites. The divergence of CA1 and DG responses might then be explained by differences in the relative strength of cortico–hippocampal versus hippocampo–hippocampal inputs. Despite the parallel development of place-field activities of CA1 pyramidal and DG granule cells, place specific discharge in each region may be differentially influenced by different sources of spatial information such as self-motion versus landmark cues. As such, the divergence of activity in inhibitory networks of the DG and CA1 interneurons may serve to bias the type of information (such as landmark vs. self-motion information) utilized in the development of a spatial map of the novel environment.

Acknowledgments

We thank M. Pauer for valued technical assistance.

Grants

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-20331 and postdoctoral National Research Service Award 10046.

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J Neurophysiol • VOL 91 • FEBRUARY 2004 • www.jn.org


