Published in final edited form as:

Hippocampus. 1995 ; 5(4): 287–305. doi:10.1002/hipo.450050403.

Electrophysiological Diversity of Pyramidal-Shaped Neurons at the Granule Cell Layer/Hilus Border of the Rat Dentate Gyrus Recorded In Vitro

Helen E. Scharfman

Neurology Research Center, Helen Hayes Hospital, West Haverstraw, and Departments of Pharmacology and Neurology, Columbia University, College of Physicians and Surgeons, New York, New York

Abstract

In the rat dentate gyrus, pyramidal-shaped cells located on the border of the granule cell layer and the hilus are one of the most common types of γ -aminobutyric acid (GABA)-immunoreactive neurons. This study describes their electrophysiological characteristics. Membrane properties, patterns of discharge, and synaptic responses were recorded intracellularly from these cells in hippocampal slices. Each cell was identified as pyramidal-shaped by injecting the marker Neurobiotin intracellularly ($n = 17$).

In several respects the membrane properties of the sampled cells were similar to “fast-spiking” cells (putative inhibitory interneurons) that have been described in other areas of the hippocampus. For example, input resistance was high (mean 91.3 megohms), the membrane time constant was short (mean 7.7 ms), and there was a large afterhyperpolarization following a single action potential (mean 10.5 mV at resting potential). However, the action potentials of most pyramidal-shaped cells were not as brief (mean 1.2 ms total duration) as those of most previously described fast-spiking cells. Many pyramidal-shaped neurons had strong spike frequency adaptation relative to other fast-spiking cells. Although these latter two characteristics were apparent in the majority of the sampled cells, there were exceptional pyramidal-shaped neurons with fast action potentials and weak adaptation, demonstrating the electrophysiological variability of pyramidal-shaped cells.

Responses to outer molecular layer stimulation were composed primarily of excitatory postsynaptic potentials (EPSPs) rather than inhibitory postsynaptic potentials (IPSPs), and were usually small (EPSPs evoked at threshold were often less than 2 mV), and brief (less than 30 ms). There was variability, because in a few cells EPSPs evoked at threshold were much larger. However, regardless of EPSP amplitude, suprathreshold stimulation (up to 4 times the threshold stimulus strength) rarely evoked more than one action potential in any cell. The results suggest that stimulation of perforant path axons produces limited excitatory synaptic responses in pyramidal-shaped neurons. This may be one of the reasons why they are relatively resistant to prolonged perforant path stimulation.

The pyramidal-shaped neurons located at the base of the granule cell layer have been associated historically with a basket plexus around granule cell somata, and have been called pyramidal “basket” cells. However, basket-like endings were rare and axon collaterals outside the granule cell layer were common. Many axon collaterals were as far from the granule cell layer as the outer molecular layer and the central hilus, and antidromic action potentials could be recorded in some

cells in response to weak stimulation of these areas. Taken together with the electrophysiological variability, the results indicate that these cells are physiologically heterogeneous.

Keywords

interneuron; inhibition; γ -aminobutyric acid (GABA); granule cell; hippocampus

INTRODUCTION

Inhibition of dentate granule cells has been attributed to various types of inhibitory interneurons located in or near the granule cell layer (Andersen, 1975). Of these, one of the most common types (Seress and Pokorny, 1981) is the pyramidal-shaped cell that is located on the border of the granule cell layer and the hilus. It is oriented so that the apical dendritic tree is in the molecular layer, the basal dendrites are in the hilus, and the soma is usually on the border of the granule cell layer and the hilus (Ramón y Cajal, 1911; Lorenté de Nó, 1934; Amaral, 1978; Seress, 1978; Ribak and Seress, 1983; Seress and Ribak, 1983; Seress and Frotscher, 1991). These cells have several anatomical characteristics of interneurons, such as few spines, an axon that usually arborizes locally, and ultrastructural characteristics such as infolded nuclei and intranuclear rods and sheets (Ramón y Cajal, 1911; Lorenté de Nó, 1934; Ribak and Anderson, 1980; Léranth and Frotscher, 1986). They are thought to be inhibitory because they are immunoreactive for the inhibitory neurotransmitter γ -aminobutyric acid (GABA; Somogyi et al., 1984; Gamrani et al., 1986; Sloviter and Nilaver, 1987; Babb et al., 1988; Woodson et al., 1989; Goodman and Sloviter, 1992) as well as the synthetic enzyme for GABA, glutamic acid decarboxylase (GAD; Ribak et al. 1978; Somogyi et al., 1984; Kosaka et al., 1985; Babb et al., 1988). In addition, they make symmetric synapses on their postsynaptic targets (Ribak and Anderson, 1980). Historically, they have been associated with a basket-like plexus (Ramón y Cajal, 1911; Lorenté de Nó, 1934; Andersen et al., 1963; Blackstad and Flood, 1963). Therefore, it is likely that the pyramidal-shaped neurons play an important role in inhibition in the dentate gyrus. Nevertheless, there has been only one published physiological record from a pyramidal cell in the dentate gyrus (Kawaguchi and Hama, 1987a). Physiological information could be valuable in understanding the principles governing inhibition of granule cells. Therefore, these cells were studied physiologically in hippocampal slices by intracellular recording. The marker Neurobiotin, a compound related to biocytin, was injected into each cell after the recordings were made, and subsequently the slice was reacted histologically to confirm the identity of each cell. In addition, Neurobiotin injection was used to label the axons of each cell to determine if they were exclusively basket cells.

MATERIALS AND METHODS

Preparation and Maintenance of Slices

Animal care and treatment followed the guidelines set by the National Institutes of Health and the New York State Department of Health. Adult (150–250 g) female Sprague-Dawley rats were anesthetized with ether and decapitated. The brain was immediately removed and placed in 4°C buffer (in mM: 126 NaCl, 5 KCl, 2.0 CaCl₂, 2.0 MgSO₄, 26 NaHCO₃, 10 D-glucose). After approximately 30 s, the hippocampus was isolated within a block of tissue from one hemisphere. The block was cut transversely or horizontally in 400- μ m sections with a Vibroslice (Stoelting Instruments). Slices were immediately transferred to a recording chamber (Fine Science Tools) that was modified so that slices could be perfused from below with warmed (33–34°C), oxygenated (95% O₂, 5% CO₂) buffer, and only the upper surface of the slice was exposed to air. Recordings were made 1–8 h after the dissection.

Intracellular Recording and Stimulation of Afferents

Electrodes used for intracellular recording were pulled from borosilicate glass with a capillary fiber in the lumen (0.6 mm inner diameter, 1.0 mm outer diameter) and were approximately 100–150 megohms when filled with 4% Neurobiotin (Vector Labs) in 1 M potassium acetate. The electrode tip was backfilled in the Neurobiotin solution, and then the shaft was filled with 1 M potassium acetate. An intracellular amplifier with a bridge circuit was used for recording (Axon Instruments), and the bridge was balanced whenever intracellular current was passed. An interval generator was used to trigger current pulses or stimuli (World Precision Instruments). Recordings were digitized at 22 kHz and recorded on tape (Neurodata Instruments) for analysis offline. Measurements were made using a digitizing oscilloscope (Nicolet Instruments). Recordings were plotted on an X-Y plotter (Tektronix Instruments).

A monopolar electrode (Teflon-coated stainless steel wire, 50 μm in diameter) was used to stimulate either the molecular layer or the hilus. Stimulation of the molecular layer was used to approximate stimulation of the perforant path (see Discussion). Stimulating electrodes were placed adjacent to the hippocampal fissure, in the outer molecular layer (Fig. 9A, inset). Stimuli were composed of rectangular current pulses, 50–250 μA , 10–250 μs duration.

Dye Injection and Histology

Neurobiotin was injected at the end of the recording session. Depolarizing current pulses (0.5–1.0 nA, 20 ms) were triggered for 3–15 min at 30 Hz. Following dye injection, slices were lifted from the recording chamber with a blunt spatula, placed between filter papers, and immersed in 4% paraformaldehyde (pH 7.6). After 1–10 days, slices were rinsed in Tris buffer (pH 7.4) and covered in hot 3% Agar (Sigma). After cooling, agar blocks were hardened overnight by immersion in 2% paraformaldehyde. Slices were separated from the excess agar and were cut into 50 μm -sections on a vibratome (Lancer).

To react neurobiotin-filled cells, sections were first washed in Tris buffer (twice, 10 min each) and incubated overnight in 0.5% Triton-X 100 (Sigma). Sections were then washed in Tris and placed in 10% methanol in 3% H_2O_2 for 45 min. After washing, sections were transferred to ABC solution (Vector Labs) for 2 h, and subsequently washed, incubated in diaminobenzidine (DAB) for 20 min and then DAB plus 0.0075% H_2O_2 for 45 min. Sections were dehydrated in alcohol and cleared in xylene. They were coverslipped in Permount (Sigma) and viewed with a BH-2 microscope (Olympus). Cells were drawn using enlarged photomicrographs of all focal planes. Cells were photographed with a 35-mm camera system (Olympus). A darkfield condenser (Olympus) and color slide film (Kodak Ektachrome 64T) were used to study the cell in Figure 2.

Data Analysis

Resting membrane potential was defined as the difference between the intracellular potential and the potential measured immediately upon exiting the impaled cell. Action potential amplitude was measured from resting potential to the peak of an action potential evoked at threshold by an intracellular current pulse. Threshold was defined as the current step or synaptic stimulus used to evoke an action potential in approximately 50% of trials. Action potential duration was measured from the start of the action potential to the point where the membrane potential had repolarized. Duration at half-amplitude was measured from the start of the action potential to the point on the rising phase where it reached half of its peak amplitude. The maximum action potential rate of rise was defined as the maximum slope of the rising phase, and the maximum rate of decay was the maximum slope of the repolarizing phase. The ratio (dv/dt ratio) was calculated as (maximum rate of rise)/(maximum rate of

decay). Maximum slope was estimated by digitizing the action potential (22 kHz) and measuring the largest voltage excursion occurring between two points on the rising (maximum rate of rise) or falling phase (maximum rate of decay) of the digitized action potential. Three action potentials were measured in this way, and the results were averaged to yield the final measure of slope for that cell. The membrane time constant was measured from an average of three responses to a hyperpolarizing current pulse sufficient to displace the membrane potential 5–10 mV, and was defined as the time to reach 63% of the steady state voltage change. Input resistance was defined as the slope of the V-I curve that was generated from responses to small current steps (<0.20 nA, 150 ms). Responses to such current pulses were measured at steady state, approximately 145 ms after the start of the pulse. Afterhyperpolarization (AHP) amplitude was measured from the membrane potential immediately before the action potential to the peak of the AHP, using an action potential evoked by a threshold level of intracellular current. AHP half-duration was measured from the end of the action potential (the point where the action potential had repolarized) to the point on the AHP decay where AHP amplitude was half the peak amplitude.

Statistical comparisons were made using PSI-plot software (Polysoft International). Statistical significance was set at $P < 0.05$ prior to all experiments.

Terminology

Pyramidal-shaped cell—The cells that are described as “pyramidal-shaped” had the following characteristics: 1) a soma lying at the border of the granule cell layer and the hilus, 2) a soma shaped in a roughly triangular manner, with the apex of the triangle directed toward the molecular layer, 3) an apical dendritic tree originating from the apex of the triangle, 4) basal dendrites originating at the base of the triangle and extending into the hilus.

Fast-spiking cell—For the purposes of the present study, the term “fast-spiking” refers to cells that have a number of physiological characteristics that can be distinguished from granule cells and area CA1–CA3 pyramidal cells. The primary distinguishing features used were 1) a large AHP following individual action potentials and 2) weak spike frequency adaptation in response to a +0.1–0.5 nA, 150 ms current pulse. In the text, spike frequency adaptation is described as “weak” when the first and last interspike interval differ by less than 5 ms. “Strong” adaptation refers to an increase in interspike interval greater than 20 ms. Interspike interval is defined as the time between the peaks of two action potentials. In addition, these cells are characterized by 1) a short membrane time constant (less than 15 ms), 2) high input resistance (≥ 60 megohms), 3) tonic discharge rather than discharge in bursts, and 4) a “dv/dt ratio” less than 2.5. Dv/dt ratio was defined as (maximum rate of rise)/(maximum rate of decay) of a directly evoked action potential.

In contrast to fast-spiking cells, granule cells have a triphasic AHP after individual action potentials (evoked at -60 mV) and demonstrate strong adaptation for the same range of injected currents. Their dv/dt ratio is high (over 4.0).

Interneuron—The term “interneuron” refers to cells that have fast-spiking physiological characteristics *and* have morphological characteristics of GABA-immunoreactive local circuit neurons, such as few spines, a dendritic tree that is different in orientation from granule cells or pyramidal cells, GABA or GAD immunoreactivity, and an axon that is restricted to the local circuitry. In the past, dentate interneurons that have been identified morphologically have had physiological characteristics that are similar to fast-spiking cells (Kawaguchi and Hama, 1987a; Scharfman 1991, 1992b; Han et al., 1993; Han, 1994), and vice versa. It is acknowledged that this terminology is imperfect, because, for example, some cells that are immunoreactive for GABA or GAD have an axon that projects to the

contralateral hippocampus (Seress and Ribak, 1983; Lranth and Frotscher, 1986; Goodman and Sloviter, 1992).

Classic fast-spiking cell—The phrase “classic fast-spiking” cell defines those cells which, in addition to the above characteristics, also possess a brief action potential (less than 1.0 ms total duration; Schwartzkroin and Mathers, 1978; McCormick et al., 1985), since this was originally how such cells were distinguished.

Possible Sampling Bias

Cells that were encountered in the granule cell layer were filled with Neurobiotin if a stable, high-quality recording was made. Note that implicit in this approach is a possible sampling bias, because cells that are fragile or difficult to impale well would be left out.

Some putative interneurons in this study did not have brief-duration action potentials and therefore were not classic fast-spiking cells. It is important to consider the possibility that the cells actually had brief action potentials, but because of damage to the cell during impalement the action potential was artificially lengthened. This possibility was unlikely because the cells with relatively long-duration action potentials had other characteristics indicative of healthy cells, such as high input resistance and high resting membrane potential. In addition, cells with relatively long action potentials had action potentials with fast rates of rise and long time constants relative to cells with brief-duration action potentials. These latter characteristics suggest that they were recorded as well as cells with brief-duration action potentials.

RESULTS

Anatomy

Seventeen cells that were impaled in the granule cell layer had pyramidal morphology with dendrites that were often beaded and demonstrated few spines (Figs. 1–3, 11). Eight cells were situated in the upper blade, six were located at the “crest” of the dentate gyrus (where the upper and lower blades meet), and the other cells were located between the crest and the upper blade. The cell bodies were located on the border of the granule cell layer and the hilus (Figs. 1–3, 11).

Most anatomical characteristics of these cells were similar to those previously described (Ramn y Cajal, 1911; Lorent de N, 1934; Amaral, 1978; Seress and Ribak, 1983) and therefore will not be described in detail here. For example, the somata of these cells were pyramidal and were large compared to the somata of granule cells stained with similar methods (Figs. 1–3, 11). The dendrites consisted of an apical portion in the molecular layer and a basal portion in the hilus. There were variations in the apical and basal dendritic trees. In four cells, a branch from the apical dendrite passed through the granule cell layer and into the hilus (e.g., Fig. 3A,H). In two cells, basal dendrites entered the molecular layer (e.g., Fig. 11). The dendrites of two cells crossed the hippocampal fissure and entered the subiculum, but this portion of the dendrite was short (less than 100 μ m; e.g., Fig. 2). The basal dendrites included a few simple dendrites (Fig. 3B) or were more elaborate (Fig. 3E), and could extend into the molecular layer (Fig. 11). Pyramidal-shaped cells had dendrites that were smooth, beaded, sparsely spinous, or a combination of the three.

One characteristic has not been noted previously. In several cells, the distal apical dendrites branched more in the layer containing the lateral perforant path (the outer molecular layer) than in other layers (Figs. 1, 2, 11). This suggests that these cells receive input from the lateral perforant path preferentially, because axons from the lateral perforant path form the

bulk of excitatory afferents in the outer molecular layer. Consistent with this possibility, these cells responded to outer molecular layer stimulation (see below). However, the excitatory postsynaptic potentials (EPSPs) evoked by outer molecular layer stimulation were not necessarily larger than EPSPs evoked in cells without such dendritic branching in the outer molecular layer.

Another notable characteristic was the fact that many axons had branches outside of the granule cell layer. Although this has been reported (Léranth and Frotscher, 1986; Deller and Léranth, 1990; Léranth et al., 1990), it has not been emphasized how common it is and how far away from the granule cell layer these axon collaterals extend (Fig. 2). Of the nine cells with labeled axons, basket-like endings in the granule cell layer were observed in only two cells (Figs. 3A, 11). “Basket-like” refers to a portion of an axon with varicosities that forms a relatively small (approx. 10- to 25- μ m-diameter) circular structure within the granule cell layer (Fig. 3A, arrowhead). Both of the cells with basket-like endings had additional axon collaterals in other layers; in one cell the additional collaterals were numerous (Fig. 3A), and in the other cell they were not (Fig. 11). Many axon collaterals were found in the outer molecular layer and the deep hilus (close to area CA3c). Six cells had axon collaterals in the hilus (Figs. 2, 3A,E,G,H), and four cells had axon collaterals in the molecular layer (Fig. 2, 3A,C, 11). There were numerous varicosities on the axon collaterals in both the molecular layer (Fig. 2C) and the hilus, suggesting that the axon collaterals innervated processes of cells in these areas.

Physiology

Intrinsic properties

Membrane properties: The mean values for membrane properties of the interneurons are shown in Table 1. The mean resting membrane potential (RMP) was -62.3 ± 1.3 mV ($\pm$ standard error of the mean, $n = 17$). Action potential amplitude was 70.9 ± 3.7 mV. The membrane time constant was 7.69 ± 0.77 ms, and input resistance was 91.3 ± 9.3 megohms.

Since the membrane properties of granule cells that have been published vary (Brown et al., 1981; Turner and Schwartzkroin, 1983; Fricke and Prince, 1984; Lambert and Jones, 1990; Spruston and Johnston, 1992; Staley et al., 1992), 15 granule cells from the same slices in which the interneurons were impaled were examined for comparative purposes. The mean granule cell RMP was -77.5 ± 1.2 mV (Table 1). Action potential amplitude was 102.9 ± 5.6 mV. Membrane time constant was 15.6 ± 3.1 ms (when granule cells were depolarized to -60 to -65 mV, the approximate RMPs of pyramidal-shaped cells), and input resistance was 75.6 ± 4.4 megohms. RMP and action potential amplitude were significantly different from the values of interneurons (Student's t -tests, $P < 0.05$).

Action potentials: Previous studies have shown that interneurons differ from area CA1–CA3 pyramidal cells or neocortical pyramidal cells in action potential duration and waveform (McCormick et al., 1985; Lacaille and Williams, 1990; Foehring et al., 1991; Scharfman, 1993b). The rate of rise is slower and the decay is usually faster for interneurons. Therefore, the ratio of rise/decay (dv/dt ratio) of pyramidal cell action potentials is usually greater than interneurons (pyramidal cells, 3.0–5.0; interneurons, 1.0–2.0; McCormick et al., 1985; Foehring et al., 1991; Scharfman, 1993b). When this type of comparison was made for pyramidal-shaped cells and granule cells, some pyramidal-shaped neurons were similar to interneurons in that their rate of rise was slower and dv/dt ratio was smaller than granule cells (Fig. 4A1 vs. 4A3; Table 1). However, other pyramidal-shaped cells did not differ greatly from granule cells (Fig. 4A2 vs. 4A3). Cells that were similar and dissimilar to granule cells in their rates of rise and dv/dt ratios were not obviously different morphologically (compare Fig. 1, which shows a cell that was different, and Fig. 2, which

shows a cell that was similar). When quantified, the maximum rate of rise and dv/dt ratio of pyramidal-shaped cells and granule cells were different (Table 1; Student's t -test $P < 0.05$). However, there were no significant differences in the mean action potential total duration, time to half-amplitude, or maximum rate of decay (Student's t -tests, $P > 0.05$; Table 1).

V-I relations: V-I curves of pyramidal-shaped cells were close to linear within 10–20 mV of resting potential (Fig. 5B). This was similar to granule cells (Brown et al., 1981; Fricke and Prince, 1984; Lambert and Jones, 1990; Spruston and Johnston, 1992). If larger currents (> -0.5 nA) were tested in pyramidal-shaped cells, rectification was observed in 12 of 17 cells (Fig. 5). However, granule cells rectified in only five of the 15 cells when large currents were tested.

There was no “sag” evident in the voltage response to hyperpolarizing current steps for 11 of 13 interneurons. In the two exceptions, a 2–4 mV sag occurred in response to 0.5- to 1.0-nA, 150-ms hyperpolarizing currents; larger currents were not tested. There was no sag in any of the granule cell responses to current injection that were tested.

There were no anode break action potentials after termination of hyperpolarizing current steps that were triggered while a cell was at its resting potential. However, when pyramidal-shaped cells or granule cells were depolarized to potentials between -50 and -55 mV, hyperpolarizing current pulses could evoke 1 anode break action potential.

Afterhyperpolarizations—All pyramidal-shaped cells displayed an afterhyperpolarization (AHP) similar to other dentate and hippocampal interneurons. This AHP was a large, brief hyperpolarization after any given action potential (Fig. 4). When a threshold intracellular current step was delivered at resting potential, the AHP peak amplitude was 10.5 ± 0.97 mV and half-duration was 8.02 ± 0.76 ms. AHPs of pyramidal-shaped cells did not have the three phases present in granule cell AHPs when the latter were examined at comparable membrane potentials (approximately -60 mV; Fig. 4B).

AHP amplitude fluctuated, depending on how action potentials were evoked. AHPs were largest when action potentials were evoked by intracellular current injection and when action potential frequency was minimal (Fig. 6). AHP variations were greatest after synaptic stimulation (Fig. 10A). Stimulus frequencies were low in such experiments (≤ 0.1 Hz), so the change in the AHP was probably not due to a high stimulus frequency. In addition to variations in the AHP, synaptically evoked action potentials also fluctuated in duration from stimulus to stimulus (Fig. 9B, compare middle and top records). Most synaptically activated action potentials were longer than action potentials evoked by brief intracellular current injection.

Discharge patterns

Pyramidal-shaped cells discharged tonically: Discharge patterns were determined from responses to a range of injected currents, usually 0.1–0.5 nA, and either 150 or 500 ms duration. All cells discharged in trains of action potentials. Even if a cell was hyperpolarized with DC current, which has uncovered burst-like events in some hippocampal interneurons (Lacaille and Schwartzkroin, 1988), there was no evidence of burst discharge. Bursts at the onset of a current pulse have been observed in other hippocampal interneurons (Buhl et al., 1994b; Han, 1994), and these were not observed in the pyramidal-shaped cells that were sampled. In cells that were tested with a large range of injected currents (0.1–1.0 nA), the primary slope of the f -I curves was not significantly different from the primary slope of granule cell f -I curves (Student's t -test, $P > 0.05$; Table 1; Fig. 6D).

Spike frequency adaptation: When currents up to +0.7 nA were injected for short periods (150 ms; Fig. 7), or when the first 200 ms of a longer current pulse was examined (Fig. 6), adaptation was present in nine of 17 cells. This contrasted with many other fast-spiking cells, which do not appear to adapt during such periods of depolarization. Granule cells always adapted during such periods of current injection, and adapted strongly, as has been shown (Fricke and Prince, 1984; Han, 1994). Adaptation occurred in almost all (15 of 17) pyramidal-shaped cells if the duration of the current pulse was increased to 500 ms. Figure 6A shows the response of a strongly adapting cell to a 500-ms pulse, and Figure 6B shows an example of a weakly adapting cell.

Change in discharge with membrane potential: In eight cells that were examined at multiple membrane potentials, the pattern of discharge changed as cells were hyperpolarized 5–10 mV from resting potential. In these cases, discharge changed from tonic, steady discharge to an irregular pattern (Fig. 7). This irregular firing pattern was robust in that it occurred in response to a range of currents at the hyperpolarized membrane potential (≥ 0.1 – 0.5 nA, 150 ms; Fig. 7A,B). Cells discharged irregularly at approximately -65 to -70 mV, whereas at -50 to -65 mV discharge was regular. Another type of change with hyperpolarization was the appearance of an initial delay or plateau before discharge began (Fig. 7B,C). This delay appeared as the cell was hyperpolarized 5–10 mV from resting potential (Fig. 7B,C). A similar delay has been described in hyperpolarized interneurons in the stratum-lacunosum moleculare region of area CA1 (Williams et al., 1994), and appears similar to the delay produced by an I_D current in hippocampal pyramidal cells (Storm, 1990).

Spontaneous discharge: Spontaneous discharge occurred in only two cells when they were at their resting potentials. These two cells had the most depolarized RMPs (-55 and -56 mV). The frequency of spontaneous discharge varied (0.5–1.0 Hz), although the cells maintained a stable resting potential. When the other cells were depolarized to membrane potentials between -50 and -55 mV, they also fired spontaneously, and did so at similar frequencies. Therefore, the cells that fired spontaneously at their resting potentials were likely to have done so because of their depolarized RMPs, not because they were necessarily more “excitable.” These cells were unlikely to have fired spontaneously due to injury, because their input resistances, time constants, and action potentials were similar to cells that did not discharge spontaneously.

Synaptic potentials and responses to stimulation of the slice

Spontaneous potentials: In nine of the 17 cells, spontaneous potentials were present (Fig. 8). This percentage was low compared to other dentate interneurons impaled in the hilar region (Livsey and Vicini, 1992) and other non-granule cells such as mossy cells (Fig. 8). The amplitudes of spontaneous potentials that were observed in pyramidal-shaped cells at RMP varied in amplitude but were usually small (less than 5 mV; Fig. 8). The frequency of spontaneous potentials varied and was difficult to determine definitively because some events merged or were small enough that they could not be clearly distinguished from “noise” (Fig. 8). Spontaneous potentials were depolarizing when the interneuron membrane potential was depolarized to -70 mV (Fig. 8), so it is unlikely that spontaneous potentials were depolarized inhibitory postsynaptic potentials (IPSPs), as is the case for granule cell spontaneous activity (Otis et al., 1991).

Stimulation of the outer molecular layer: Stimulation of the outer molecular layer was tested in 14 cells. Responses were composed primarily of EPSPs. Small hyperpolarizations (1–3 mV) that could have been IPSPs were present in three cells, but these responses were not robust because they were not evident after each test stimulus. The latencies to onset of

most EPSPs (12 of 14 cells) were 1.2–2.0 ms (measured from the center of the stimulus artifact to the onset of the depolarization). In the two exceptional cells, EPSP latency was 5.2 and 7.2 ms. These data suggest that EPSPs arose from monosynaptic or disynaptic excitatory pathways, such as monosynaptic excitation by perforant path axons, or disynaptic excitation by granule cells that the perforant path excites.

The mean amplitude of EPSPs evoked at threshold at resting potential was 3.21 ± 0.71 mV ($n = 14$). EPSPs were usually small; in 11 of 14 cells the mean amplitude was 1.99 ± 0.33 mV (range, 0.7–4.2 mV; Figs. 9A, 10A). Threshold stimulus strength was defined as the stimulus that evoked an action potential in approximately 50% of trials. In the other three cells, EPSPs with amplitudes larger than 6 mV occurred at threshold (mean of these 3 cells = 7.38 ± 1.04 mV; Fig. 9B). All EPSPs were brief (<45 ms duration). EPSPs displayed simple or complex morphology; when EPSPs were complex, there were inflections on the rising phase (Fig. 9A,B). Small EPSPs (mean 1.99 mV, as described above) occurred at brief or long (<2 ms or >5 ms) latencies, whereas large EPSPs (mean amplitude = 7.38 mV) always had a brief latency. The dendrites in the molecular layer of cells with small EPSPs (e.g., Figs. 2, 11) were not clearly different from the cells with large EPSPs (e.g., Fig. 1), so a difference in morphology does not provide an obvious explanation for the differences in EPSP amplitudes. All EPSPs could evoke discharge, but only one action potential was evoked, even when stimulus strength was raised to $2\text{--}4 \times$ threshold (Fig. 10A). If a cell was depolarized with DC current, two action potentials could be evoked (Fig. 9B).

Antidromic responses: Antidromic action potentials were triggered in three cells. In one cell, an antidromic action potential was evoked by molecular layer stimulation, and an EPSP was recorded after stimulation in another position in the molecular layer. In the second cell, antidromic action potentials followed by depolarizations were evoked either by molecular layer or hilar stimulation (Fig. 9C, cell in Fig. 3H). In the third cell, a 2-mV EPSP (maximum amplitude) was recorded in response to molecular layer stimulation, and an antidromic action potential was triggered by stimulation of the hilus. The axon of that cell entered the hilus and branched there (Fig. 3E). The stimuli used to evoke antidromic responses were weak compared to the stimuli required to evoke action potentials in granule cells, and therefore the responses were unlikely to be due to excessive stimulation and the resultant current spread to a remote location in the slice where the axon might be located. They are likely to reflect the fact that the axon collaterals of the sampled cells are present in the molecular layer and hilus.

Comparison to responses of granule cells and hilar mossy cells: In ten slices, EPSPs of pyramidal-shaped cells were compared to EPSPs of granule cells. In five slices, pyramidal-shaped cells were compared to seven mossy cells. In each slice the stimulus site remained fixed, and several stimulus intensities were tested for each cell at several membrane potentials. Cells were recorded consecutively or simultaneously.

Latency: The latencies of granule cell EPSPs were similar to latencies of most pyramidal-shaped cells (i.e., 1.0–2.0 ms). The latencies of mossy cell EPSPs were variable, but the range of their latencies was similar to the range of latencies of pyramidal-shaped cells (1.0–7.5 ms). These values are consistent with direct or indirect excitation by the perforant path of non-granule cells and direct excitation of granule cells.

Amplitude of EPSPs: EPSPs of nine of ten pyramidal-shaped cells were smaller in amplitude than EPSPs of granule cells in the same slices, when threshold stimulus intensity was used, and cells were manipulated with DC current injection so their membrane potentials were similar. All EPSPs of pyramidal-shaped cells were smaller than those of mossy cells compared in the same slices. Figure 10 shows data from one comparison of the

three cell types in the same slice. The EPSP underlying an action potential at threshold is very small in the case of the pyramidal-shaped cell (Fig. 10A, top left trace), and larger in the case of a granule cell in the same slice and at the same membrane potential (Fig. 10B, top trace). The mean amplitude of EPSPs evoked at threshold at approximately -60 to -65 mV membrane potential was 2.33 ± 0.56 (n = 10) for pyramidal-shaped cells and 4.84 ± 0.44 mV (n = 10) for granule cells, and these means were significantly different (Student's *t*-test, $P < 0.05$). For pyramidal-shaped cells that were compared to mossy cells of the same slices, the mean KPSP amplitude of pyramidal-shaped cells was 2.64 ± 0.98 mV (n = 5) and the mean amplitude of mossy cell EPSPs was 6.84 ± 0.30 mV (n = 7). This difference was significant (Student's *t*-test, $P < 0.05$). Hyperpolarization increased the amplitudes of threshold EPSPs in granule cells and mossy cells greatly, but there was little change in amplitude of threshold EPSPs of pyramidal-shaped cells after similar hyperpolarization (Fig. 10, lower traces). However, EPSPs evoked by stronger stimuli increased in amplitude with hyperpolarization in all cells (Fig. 10). EPSPs of pyramidal-shaped cells were similar to granule cells in that only one discharge was evoked, even at stimulus strengths over threshold (Fig. 10). Pyramidal-shaped cells contrasted with mossy cells in this respect, because most mossy cells can discharge several times after a threshold stimulus (Scharfman, 1993a).

Threshold stimulus strength: The stimulus strength that evoked action potentials in 50% of trials (threshold) was compared across the three cell types. In five of ten comparisons, the threshold for a pyramidal-shaped cell was similar to the threshold for a granule cell. In the other cases, the pyramidal-shaped cell had a lower threshold than granule cells. An example of the latter is shown in Figure 10. In no case was there a granule cell with a lower threshold than a pyramidal-shaped cell.

When compared to mossy cells, pyramidal-shaped cells had similar or higher thresholds than mossy cells. Therefore the mossy cells appear to have the lowest threshold to molecular layer stimulation of these three cell types. These findings should be confirmed by *in vivo* recording, because thresholds may differ when more of the network is intact.

DISCUSSION

Summary

This study examined the membrane properties and synaptic responses of pyramidal-shaped neurons situated at the base of the granule cell layer. The data suggest that the pyramidal-shaped neurons are similar to other hippocampal interneurons physiologically, but that they are not necessarily classic fast-spiking cells, and there is variability among pyramidal-shaped cells.

Responses to molecular layer stimulation, which was used to approximate stimulation of the major afferent input to the dentate gyrus, the perforant path, evoked excitatory potentials primarily. The latencies of these responses suggest that these interneurons could play a role in inhibition of dentate granule cells that occurs after perforant path activation. However, the precise inhibitory roles of these neurons are likely to be diverse, because of variability in the intrinsic and synaptic responses and in the diverse sites where axons were localized.

Anatomy

The results show that many pyramidal-shaped cells innervate areas outside the granule cell layer. Therefore, the term "pyramidal-shaped interneuron" may be more accurate than "pyramidal basket cell" when discussing this population. It is unlikely that intracellular dye injection impaired the ability to stain basket-like endings, because baskets were found in

some labeled cells, and similar techniques have been used to stain the basket plexus of hippocampal interneurons (Scharfman et al., 1989; Seay-Lowe and Claiborne, 1992; Han et al., 1993; Buhl et al., 1994a; Han, 1994).

There is support for this conclusion in the literature. For example, it has been reported that pyramidal-shaped interneurons in the granule cell layer can innervate granule cell proximal dendrites (Seress and Ribak, 1983; Lranth and Frotscher, 1986; Seress and Frotscher, 1991), and a drawing of a pyramidal-shaped cell in a previous study had axon collaterals outside the granule cell layer (Kawaguchi and Hama, 1987a). GABA-immunoreactive cells that innervate the molecular layer include some pyramidal-shaped cells (Soriano and Frotscher, 1993). It is known that the immature dentate gyrus contains pyramidal-shaped cells with axons that arborize outside the granule cell layer (Seress and Ribak, 1990; Seay-Lowe and Claiborne, 1992).

The fact that some pyramidal-shaped cells in this study had axon collaterals in several layers of the dentate gyrus is also significant. It is possible that some pyramidal-shaped cells function in a global sense in that they may inhibit large areas of the local circuit, as opposed to specifically targeting one lamina or one cell type, as has been suggested to be the case for other dentate interneurons (Han et al., 1993; Soriano and Frotscher, 1993). If both possibilities exist, it would provide the capacity for specific and “nonspecific” inhibition. Specific inhibition may be useful in shaping excitatory inputs, whereas global inhibition could be useful in prevention of pathological excitation that might occur during a seizure.

Intrinsic Properties and Patterns of Discharge

Comparison of intrinsic properties of pyramidal-shaped cells to other cortical interneurons—There is a large literature describing the intrinsic properties of other interneurons (Schwartzkroin and Mathers, 1978; McCormick et al., 1985; Kawaguchi and Hama, 1987a,b; Lacaille et al., 1987; Lacaille and Schwartzkroin, 1988; Lacaille and Williams, 1990; Foehring et al., 1991; Scharfman, 1992b; Buhl et al., 1994b; Williams et al., 1994). The first studies of morphologically identified interneurons reported that interneurons were characterized by brief-duration action potentials, and therefore have been referred to as “fast-spiking” cells (Schwartzkroin and Mathers, 1978; Knowles and Schwartzkroin, 1981; McCormick et al., 1985). In addition, interneurons are characterized by a large afterhyperpolarization following action potentials, weak spike frequency adaptation, and short time constants (Schwartzkroin and Mathers, 1978; McCormick et al., 1985; Lacaille et al., 1987; Lacaille and Schwartzkroin, 1988; Lacaille and Williams, 1990; Scharfman, 1992b). Studies in vivo and in vitro have shown that they often have low thresholds for synaptic activation (Buzski and Eidelberg, 1981; Scharfman, 1991). However, some variability has been described, both in area CA1 and elsewhere (Kawaguchi and Hama, 1987b, 1990; Lacaille et al., 1989; Scharfman, 1992b; Han, 1994).

In several ways the intrinsic properties and action potential discharge of pyramidal-shaped cells were similar to other hippocampal interneurons. For example, time constants were brief, input resistances were high, and action potentials were followed by a large, brief AHP that differs from granule cell and area CA1–CA3 pyramidal cell afterpotentials (Storm, 1990; Staley et al., 1992). Action potential rates of rise and decay were similar to other interneurons in that the dv/dt ratio of action potentials of pyramidal-shaped cells (1.9) was similar to other fast-spiking cells (1.4, McCormick et al., 1985; 2.0 Foehring et al., 1991; 1.1, Scharfman, 1993b), but was significantly different from granule cells (5.4).

However, action potential duration of pyramidal-shaped cells was usually long. In this respect they were *unlike* classic fast-spiking cells and most hippocampal interneurons, which have short-duration action potentials (Schwartzkroin and Mathers, 1978; Kawaguchi and

Hama, 1987a; Lacaille et al., 1987; Scharfman et al., 1989). There are exceptions: stratum lacunosum-moleculare interneurons (Lacaille and Schwartzkroin, 1988) and some dentate hilar interneurons (Misgeld and Frotscher, 1986; Scharfman, 1991, 1992b) do have long action potentials. Pyramidal-shaped cells were also distinct in that they often adapted. Many hippocampal interneurons do not appear to adapt (Schwartzkroin and Mathers, 1978; Knowles and Schwartzkroin, 1981) although there are exceptions (Kawaguchi and Hama, 1990; Lacaille and Williams, 1990; Buhl et al., 1994b; Han, 1994). It is difficult to make a rigorous comparison of adaptation among different types of interneurons because previous studies have not necessarily quantified adaptation nor have they examined it in similar ways. Until this is accomplished, one cannot necessarily conclude that pyramidal-shaped cells are unique. They may simply lie within a range that has been reported for other interneurons.

Patterns of discharge and their significance to GABAergic transmission—

Pyramidal-shaped cells changed their pattern of discharge when they were hyperpolarized from their resting potential. Tonic discharge was evoked by intracellular current injection at resting potential, but irregular discharge occurred when the membrane was hyperpolarized approximately 5–10 mV. In addition, a delay occurred before the onset of discharge when pyramidal-shaped cells were hyperpolarized. This information allows one to predict how interneuron discharge may be altered by a hyperpolarization. Such hyperpolarizations might occur, for example, if other GABAergic neurons that innervate pyramidal-shaped cells become active. In such situations, one would expect a disruption in the regular release of GABA that might occur in response to a given depolarizing input. At resting potential, a depolarization would evoke regular discharge and, as a result, regular transmitter release. However, at hyperpolarized potentials, even a strong depolarization would not necessarily evoke regular discharge or regular release of GABA. This may contribute to the decreased inhibition of granule cells that has been noted following activation of septal GABAergic inputs to dentate gyrus interneurons (Bilkey and Goddard, 1985).

Synaptic Responses

Caveats—The outer molecular layer stimulation site was chosen because stimulation of that area would be likely to activate perforant path axons, the major afferent input to the dentate gyrus. How pyramidal-shaped cells respond to the perforant path would be useful in understanding their role in the dentate gyrus. However, stimulation of the molecular layer is not identical with stimulation of the perforant path, because a molecular layer stimulating electrode could directly activate cells with processes in the outer molecular layer, such as granule cell dendrites and interneuron axons or dendrites. Furthermore, stimulation of the outer molecular layer in a slice may be misleading because only a small fraction of perforant path axons could be stimulated at such a site.

Types of responses

Synaptic responses were mostly excitatory but did not evoke multiple action potentials:

Most synaptic responses were dominated by depolarizations, which were likely to be EPSPs because they decreased in amplitude with depolarization, and could trigger action potentials. Inhibitory synaptic responses were not robust in response to molecular layer stimulation. More inhibition might be detected with different methods, such as use of slices cut on a different axis, blockade of excitatory inputs (Scharfman, 1992a), or whole-cell recording (Soltesz and Mody, 1994). The fact that EPSPs were the predominant synaptic responses is consistent with the fact that granule cells are strongly inhibited under normal conditions (Otis et al., 1991), and that the dentate gyrus is a strongly inhibited structure (Lothman et al., 1991).

Despite the observation that EPSPs were the common response to stimulation, these EPSPs were usually small and brief, and only one action potential was evoked even when stimulus intensities were raised far above threshold. Spontaneous EPSPs were also small and brief or were absent altogether. This is very different from many other hippocampal non-principal cells. Many interneurons in area CA1 can have large spontaneous events and can discharge several times in response to a single stimulus (Schwartzkroin and Mathers, 1978; Ashwood et al., 1984; Lacaille et al., 1987). Besides indicating that they are different from other interneurons, these characteristics indicate what type of role pyramidal-shaped cells may play in the dentate gyrus network. For example, they are likely to have a similar inhibitory effect within a wide range of stimulation intensities. Thus, one would expect that they would inhibit their targets (granule cells, other cells) similarly regardless of increasingly strong excitatory inputs from the perforant path. One can also predict that repetitive stimuli would not cause summation of EPSPs (leading to enhanced discharge) unless it is very high in frequency, because the spontaneous and evoked EPSPs are so small and so brief. Indeed, preliminary data from two pyramidal-shaped cells does show that stimulating the molecular layer twice, approximately 10–40 ms apart, does not evoke facilitation of EPSPs or multiple discharges.

Comparison of synaptic responses of pyramidal-shaped cells to other dentate gyrus

neurons: Many pyramidal-shaped cells differed from granule cells in that their EPSPs were smaller in amplitude when evoked at threshold and examined in the same slices. This suggests that the underlying EPSPs of pyramidal-shaped cells and granule cells may differ mechanistically, such as in the receptors that are activated or the ions involved. Livsey and Vicini have identified differences in glutamate receptors on interneurons in the hilar region (Livsey and Vicini, 1992). An alternative explanation is that there could be differences in the number of excitatory synapses on the different cells. There may be differences in the composition of the membrane or axon hillock that would explain how small EPSPs in interneurons can produce discharge so effectively in pyramidal-shaped cells but not granule cells. These possibilities are purely speculative and await experimental evidence.

Pyramidal-shaped cells were similar to granule cells in that the number of discharges evoked by stimulation were limited. This contrasted with mossy cells, which can discharge many times in response to a threshold stimulus (Scharfman, 1993a). It is also different from many hilar interneurons, which can discharge numerous times to a single stimulus of the molecular layer (Scharfman and Schwartzkroin, 1990). These differences may explain why pyramidal-shaped neurons and granule cells are relatively resistant to prolonged perforant path stimulation, whereas many hilar cells are vulnerable (Sloviter, 1987, 1991).

The threshold stimulus strength was similar to granule cells in some cases and lower in others. These data suggest that some pyramidal-shaped interneurons may function as feedforward inhibitory cells. However, the latency to onset of EPSPs was not different from latencies of granule cell EPSPs. Therefore, it is unclear at the present time whether the pyramidal-shaped cells are feedforward or feedback interneurons, as has been hypothesized (Andersen et al., 1963; Blackstad and Flood, 1963; Buzsáki and Eidelberg, 1981).

The pyramidal-shaped cells appear to differ from other dentate gyrus fast-spiking cells. For example, the small responses of pyramidal-shaped cells to stimulation differed from those of a previous *in vivo* study showing that dentate interneurons in or near the cell layer discharge numerous times to stimulation (Buzsáki and Eidelberg, 1981). Axo-axonic cells/chandelier cells are different in several electrophysiological parameters (Buhl et al. 1994a; Han, 1994). Some hilar fast-spiking cells are similar to the pyramidal-shaped cells, but others are distinct (Misgeld and Frotscher, 1986; Scharfman, 1992b). The results indicate that the pyramidal-shaped cells are one of many complex and heterogeneous types of dentate non-granule cells.

The complexity of the system is reminiscent of the diverse types and locations of non-pyramidal neurons in the cerebral cortex.

Acknowledgments

This study was supported by NIH grant NS 30831. I thank Dr. Robert Sloviter for discussions and comments on the manuscript. I also thank Dr. Simon Neubert for assistance with statistical software, Mrs. Annmarie Curcio for technical assistance, and Mrs. Ruth Marshall for secretarial support.

References

- Amaral DG. A Golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol.* 1978; 182:851–914. [PubMed: 730852]
- Andersen, P. Organization of hippocampal neurons and their interconnections. In: Isaacson, RL.; Pribram, KH., editors. *The hippocampus volume I: structure and development.* New York: Plenum Press; 1975. p. 155–175.
- Andersen P, Eccles JC, Løynings Y. Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses. *Nature.* 1963; 198:540–542. [PubMed: 14012800]
- Ashwood TJ, Lancaster B, Wheal HV. In vivo and in vitro studies on putative interneurons in the rat hippocampus: possible mediators of feed-forward inhibition. *Brain Res.* 1984; 293:279–291. [PubMed: 6320970]
- Babb TL, Pretorius JK, Kupfer WR, Jann Brown W. Distribution of glutamate-decarboxylase-immunoreactive neurons and synapses in the rat and monkey hippocampus: light and electron microscopy. *J Comp Neurol.* 1988; 278:121–138. [PubMed: 3209750]
- Bilkey DK, Goddard GV. Medial septal facilitation of hippocampal granule cell activity is mediated by inhibition of inhibitory interneurons. *Brain Res.* 1985; 361:99–106. [PubMed: 4084813]
- Blackstad TW, Flood PR. Ultrastructure of hippocampal axo-somatic synapses. *Nature.* 1963; 198:542–543. [PubMed: 13971406]
- Brown TH, Fricke RA, Perkel DH. Passive electrical constants in three classes of hippocampal neurons. *J Neurophysiol.* 1981; 46:812–827. [PubMed: 7288466]
- Buhl EH, Halasy K, Somogyi P. Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature.* 1994a; 368:823–828. [PubMed: 8159242]
- Buhl EH, Han ZS, Lörinczi Z, Stezhka VV, Karnup SV, Somogyi P. Physiological properties of anatomically identified axo-axonic cells in the rat hippocampus. *J Neurophysiol.* 1994b; 71:1289–1307. [PubMed: 8035215]
- Buzsáki G, Eidelberg E. Direct afferent excitation and long-term potentiation of hippocampal interneurons. *J Neurophysiol.* 1981; 48:597–607.
- Deller T, Léranth C. Synaptic connections of neuropeptide Y (NPY) immunoreactive neurons in the hilar area of the rat hippocampus. *J Comp Neurol.* 1990; 300:433–447. [PubMed: 2266195]
- Foehring RC, Lorenzon NM, Herron P, Wilson CJ. Correlation of physiologically and morphologically identified neuronal types in human association cortex in vitro. *J Neurophysiol.* 1991; 66:1825–1837. [PubMed: 1812219]
- Fricke RA, Prince DA. Electrophysiology of dentate gyrus granule cells. *J Neurophysiol.* 1984; 51:195–209. [PubMed: 6707720]
- Gamrani H, Onteneinte B, Sequela P, Geffard M, Calas A. Gamma-aminobutyric acid immunoreactivity in the rat hippocampus. A light and electron microscopic study with anti-GABA antibodies. *Brain Res.* 1986; 364:30–38. [PubMed: 3512033]
- Goodman JH, Sloviter RS. Evidence for commissurally projecting parvalbumin-immunoreactive basket cells in the dentate gyrus of the rat. *Hippocampus.* 1992; 2:13–22. [PubMed: 1284972]
- Han Z-S. Electrophysiological and morphological differentiation of chandelier and basket cells in the rat hippocampal formation: a study combining intracellular recording and intracellular staining with biocytin. *Neurosci Res.* 1994; 19:101–110. [PubMed: 7516510]

- Han ZS, Buhl EH, Lörinczi Z, Somogyi P. A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurones in the dentate gyrus of the rat hippocampus. *Eur J Neurosci.* 1993; 5:395–410. [PubMed: 8261117]
- Kawaguchi Y, Hama K. Fast-spiking non-pyramidal cells in the hippocampal CA3 region, dentate gyrus and subiculum of rats. *Brain Res.* 1987a; 425:351–355. [PubMed: 3427435]
- Kawaguchi Y, Hama K. Two subtypes of non-pyramidal cells in rat hippocampal formation identified by intracellular recording and HRP injection. *Brain Res.* 1987b; 411:190–195. [PubMed: 3607424]
- Kawaguchi Y, Hama K. Physiological heterogeneity of nonpyramidal cells in rat hippocampal CA1 region. *Exp Brain Res.* 1990; 72:494–502. [PubMed: 3234499]
- Knowles WD, Schwartzkroin PA. Local circuit synaptic interactions in hippocampal brain slices. *J Neurosci.* 1981; 1:318–322. [PubMed: 7264721]
- Kosaka T, Kosaka K, Tateishi K, Hamaoka Y, Yanaihara N, Wu J-Y, Hama K. GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. *J Comp Neurol.* 1985; 239:420–430. [PubMed: 2413092]
- Lacaille J-C, Schwartzkroin PA. Stratum lacunosum-moleculare interneurons of hippocampal CA1 region I. Intracellular response characteristics, synaptic responses, and morphology. *J Neurosci.* 1988; 8:1400–1410. [PubMed: 3357023]
- Lacaille J-C, Williams S. Membrane properties of interneurons in stratum oriens-alveus of the CA1 region of rat hippocampus in vitro. *Neuroscience.* 1990; 36:349–359. [PubMed: 2215928]
- Lacaille J-C, Mueller AL, Kunkel DD, Schwartzkroin PA. Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. *J Neurosci.* 1987; 7:1979–1983. [PubMed: 3612227]
- Lacaille, J-C.; Kunkel, DD.; Schwartzkroin, PA. Electrophysiological and morphological characterization of hippocampal interneurons. In: Chan-Palay, V.; Köhler, C., editors. *The hippocampus—new vistas*. New York: Alan R. Liss, Inc; 1989. p. 287-305.
- Lambert JDC, Jones RSG. A reevaluation of excitatory amino acid-mediated synaptic transmission in rat dentate gyrus. *J Neurophysiol.* 1990; 64:119–132. [PubMed: 1974917]
- Léranth C, Frotscher M. Synaptic connections of cholecystokinin-immunoreactive neurons and terminals in the rat fascia dentata: a combined light and electron microscopic study. *J Comp Neurol.* 1986; 254:51–64. [PubMed: 3027137]
- Léranth C, Malcolm AJ, Frotscher M. Afferent and efferent synaptic connections of somatostatin-immunoreactive neurons in the rat fascia dentata. *J Comp Neurol.* 1990; 295:111–122. [PubMed: 1971287]
- Livsey CT, Vicini S. Slower spontaneous excitatory postsynaptic currents in spiny versus aspiny hilar neurons. *Neuron.* 1992; 8:745–755. [PubMed: 1314622]
- Lorenté de Nó R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J Psychol Neurol (Leipzig).* 1934; 46:113–177.
- Lothman EW, Bertram EH, Stringer JL. Functional anatomy of hippocampal seizures. *Prog Neurobiol.* 1991; 37:1–82. [PubMed: 1947175]
- McCormick DA, Connors BW, Lightall JW, Prince DA. Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *J Neurophysiol.* 1985; 54:782–806. [PubMed: 2999347]
- Misgeld U, Frotscher M. Postsynaptic GABAergic inhibition of non-pyramidal neurons in the guinea-pig hippocampus. *Neuroscience.* 1986; 19:193–206. [PubMed: 3537840]
- Otis TS, Staley KJ, Mody I. Perpetual inhibitory activity in mammalian brain slices generated by spontaneous GABA release. *Brain Res.* 1991; 545:142–150. [PubMed: 1650273]
- Ramón y Cajal, S. *Histologie du système nerveux de l'homme et des vertébrés*. Paris: A. Maloine; 1911.
- Ribak CE, Anderson L. Ultrastructure of the pyramidal basket cells in the dentate gyrus of the rat. *J Comp Neurol.* 1980; 192:903–916. [PubMed: 7419759]
- Ribak CE, Seress L. Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. *J Neurocytol.* 1983; 12:577–597. [PubMed: 6619905]

- Ribak CE, Vaughn JH, Saito K. Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Res.* 1978; 140:315–332. [PubMed: 75042]
- Scharfman HE. Dentate hilar cells with dendrites in the molecular layer have lower thresholds for synaptic activation by perforant path than granule cells. *J Neurosci.* 1991; 11:1660–1773. [PubMed: 2045880]
- Scharfman HE. Blockade of excitation reveals inhibition of dentate spiny hilar neurons recorded in rat hippocampal slices. *J Neurophysiol.* 1992a; 68:978–984. [PubMed: 1359025]
- Scharfman HE. Differentiation of rat dentate neurons by morphology and electrophysiology in hippocampal slices: granule cells, spiny hilar cells, and aspiny “fast-spiking” cells. In: Ribak, CE.; Gall, CM.; Mody, I., editors. *The dentate gyrus and its role in seizures.* Amsterdam: Elsevier; 1992b. p. 93–109.
- Scharfman HE. Characteristics of spontaneous and evoked EPSPs recorded from dentate spiny hilar cells in rat hippocampal slices. *J Neurophysiol.* 1993a; 70:742–757. [PubMed: 8105038]
- Scharfman HE. Spiny neurons of area CA3c of rat hippocampal slices have similar electrophysiological characteristics and synaptic responses despite morphological variation. *Hippocampus.* 1993b; 3:9–28. [PubMed: 8364685]
- Scharfman HE, Schwartzkroin PA. Responses of cells of the rat fascia dentata to prolonged stimulation of the perforant path: sensitivity of hilar cells and changes in granule cell excitability. *Neuroscience.* 1990; 35:491–504. [PubMed: 2381513]
- Scharfman HE, Kunkel DD, Schwartzkroin PA. Intracellular dyes mask immunoreactivity of hippocampal interneurons. *Neurosci Lett.* 1989; 96:23–28. [PubMed: 2927710]
- Schwartzkroin PA, Mathers LH. Physiological and morphological identification of a nonpyramidal hippocampal cell type. *Brain Res.* 1978; 157:1–10. [PubMed: 568020]
- Seay-Lowe SL, Claiborne BJ. Morphology of intracellularly labeled interneurons in the dentate gyrus of the immature rat. *J Comp Neurol.* 1992; 324:23–36. [PubMed: 1401262]
- Stress L. Pyramidal-like basket cells in the granular layer of the dentate gyrus in the rat. *J Anat.* 1978; 127:163–168. [PubMed: 701192]
- Seress L, Frotscher M. Basket cells in the monkey fascia dentata: a Golgi/electron microscopic study. *J Neurocytol.* 1991; 20:915–928. [PubMed: 1722242]
- Seress L, Pokorny J. Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study. *J Anat.* 1981; 133:181–195. [PubMed: 7333948]
- Seress L, Ribak CE. GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons. *Exp Brain Res.* 1983; 50:173–182. [PubMed: 6315469]
- Seress L, Ribak CE. The synaptic connections of basket cell axons in the developing rat hippocampal formation. *Exp Brain Res.* 1990; 81:500–508. [PubMed: 1699778]
- Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science.* 1987; 235:73–76. [PubMed: 2879352]
- Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in that rat: the “dormant basket cell” hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus.* 1991; 1:31–40. [PubMed: 1669342]
- Sloviter RS, Nilaver G. Immunocytochemical localization of GABA-, cholecystokinin-, vasoactive intestinal polypeptide-, and somatostatin-like immunoreactivity in the area dentata and hippocampus of the rat. *J Comp Neurol.* 1987; 256:42–60. [PubMed: 3819038]
- Soltész I, Mody I. Patch-clamp recordings reveal powerful GABAergic inhibition in dentate hilar neurons. *J Neurosci.* 1994; 14:2365–2376. [PubMed: 7908959]
- Somogyi P, Hodgson AJ, Smith AD, Nunzi MG, Gorio A, Wu J-Y. Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *J Neurosci.* 1984; 4:2590–2603. [PubMed: 6149275]
- Soriano E, Frotscher M. GABAergic innervation of the rat fascia dentata: a novel type of interneuron in the granule cell layer with extensive axonal arborization in the molecular layer. *J Comp Neurol.* 1993; 334:385–396. [PubMed: 8376624]
- Spruston N, Johnston D. Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons. *J Neurophysiol.* 1992; 67:508–529. [PubMed: 1578242]

- Staley KH, Otis TS, Mody I. Membrane properties of dentate gyrus granule cells: comparison of sharp microelectrode and whole cell recordings. *J Neurophysiol.* 1992; 67:1346–1358. [PubMed: 1597717]
- Storm JF. Potassium currents in hippocampal pyramidal cells. *Prog Brain Res.* 1990; 83:161–188. [PubMed: 2203097]
- Turner DA, Schwartzkroin PA. Electrical characteristics of dendrites and dendritic spines in intracellularly stained CA3 and dentate hippocampal neurons. *J Neurosci.* 1983; 3:2381–2394. [PubMed: 6631486]
- Williams S, Samulack DD, Beaulieu C, Lacaille J-C. Membrane properties and synaptic responses of interneurons located near the stratum lacunosum-moleculare/radiatum border of area CA1 in whole-cell recordings from rat hippocampal slices. *J Neurophysiol.* 1994; 71:2217–2235. [PubMed: 7931512]
- Woodson W, Nitecka L, Ben-Ati Y. Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. *J Comp Neurol.* 1989; 280:254–271. [PubMed: 2925894]

FIGURE 1.

A pyramidal “basket” cell labeled by intracellular dye injection. A: A montage of a fast-spiking cell that was injected with neurobiotin is shown. The montage was assembled by photographing several focal planes. Recordings from this cell are shown in Figures 4A1, 5B, 6A, 7A, and 9B. Calibration = 50 μm . B: A drawing of the labeled cell in part A is shown. The borders of the granule cell layer (GCL) are indicated. The borders were determined by increasing contrast during microscopy, which makes the outlines of granule cells detectable. Calibration (in A) = 90 μm .

FIGURE 2.

A pyramidal-shaped cell with an axon that arborized in different layers of the dentate gyrus. A: A pyramidal-shaped cell that was filled with Neurobiotin is shown. Several dendrites are cut, or are not shown at all, because the slice was resectioned after recording, and only one 50- μ m-thick section was photographed. Arrowheads mark the main branch of the axon, which originated from the apical dendrite and traveled parallel to the granule cell layer for a short distance, along the border of the granule cell layer and the inner molecular layer. Arrows point to axon collaterals in the molecular layer. All of the dendrites and axon that were found in this and other sections are combined in an illustration of the cell in part D. M, molecular layer; G, granule cell layer; H, hilus. A–D are oriented similarly, with the hippocampal fissure up and the hilus below. This cell was located at the crest of the dentate gyrus, with area CA1 to the right and area CA3 below. Recordings from this cell are shown in Figures 4A2, 5A, 7C, and 8A. Calibration = 40 μ m. B: In an adjacent section to the one

shown in part A, cut dendrites (open arrows) and axons (arrows) in the outer molecular layer are evident. One of the dendrites crosses the fissure and enters the subiculum (arrowheads). F, fissure. Calibration (in A) = 20 μm . C: Numerous varicosities (arrowheads) were present along the axon collaterals in the molecular layer. Open arrows point to dendrites of the cell. Calibration (in A) = 10 μm . D: A drawing of the cell shown in A–C. Left: cell body and dendrites. GCL, granule cell layer. Right: axon. Note that a faintly labeled pyramidal cell was also stained in this slice (this cell lies to the immediate left of the well-stained cell in A), but its dendrites were faint and its axon was not stained, so it is unlikely to have contributed to the axon collaterals that are drawn. Calibration = 70 μm .

FIGURE 3.

General morphology of pyramidal-shaped cells recorded in the granule cell layer (GCL). A–H: Drawings of eight of the 17 pyramidal-shaped cells recorded in this study are shown. Three other cells are shown in Figures 1, 2, and 11. Arrowheads mark the axons. Calibration (in H) = 75 μ m.

FIGURE 4.

Action potentials and afterhyperpolarizations (AHPs) of pyramidal-shaped cells and granule cells. A,B: Action potentials of three different cells are shown with two different time bases. Panel 1: Cell shown in Figure 1, membrane potential, -60 mV. Panel 2: Cell shown in Figure 2, membrane potential -58 mV. Panel 3: Granule cell in the same slice as the cell from panel 2, membrane potential $= -72$ mV. A: Digitized action potentials are shown. B: Action potentials are plotted with a different time base to show their AHPs. Note that the granule cell AHP appears to be triphasic, which is different from the interneuron AHPs. The three phases of the granule cell AHP are indicated.

FIGURE 5.

Voltage-current relations of pyramidal-shaped cells. A: Responses of two pyramidal interneurons to rectangular current pulses injected intracellularly. For both 1 and 2, the top shows superimposed voltage responses and the bottom shows superimposed current pulses. 1. These recordings were taken from the cell in Figure 2. Membrane potential = -60 mV. 2. These recordings were taken from the cell in Figure 1. Membrane potential = -58 mV. B: V-I curves are shown for the cell in A1 (solid circles) and A2 (open circles).

FIGURE 6.

Spike frequency adaptation in pyramidal-shaped cells. As the responses of the same interneuron to increasing depolarizing current steps are shown (1, 0.3 nA; 2, 0.4 nA; 3, 0.5 nA, all 500 ms duration). Voltage responses are shown on the top; current commands are shown below. These recordings were taken from the cell in Figure 1. Membrane potential = -61 mV. B: The response of the pyramidal-shaped cell in Figure 11 to a $+0.6$ nA, 500-ms current step. The response shows that this cell adapted weakly. Similar adaptation was demonstrated in response to other current steps of different amplitudes (0.3–0.7 nA). Same calibration as in part A. C: Interspike intervals occurring at different points during the depolarizing current pulse are plotted for the data shown in A2 (diamonds) and B (circles). The first ten interspike intervals at the onset of the pulses are plotted consecutively, followed by a break in the x axis. After the break, the last ten interspike intervals are plotted consecutively. D: An f - I curve shows action potential frequency as a function of the amplitude of current injected. Action potential frequency was determined from the first interspike interval generated by a 150-ms depolarizing current pulse (up to 1.0 nA). Cell in part A, diamonds; cell in part B, circles.

FIGURE 7.

Discharge patterns of pyramidal-shaped cells change with membrane potential. A–C: Discharge patterns of three different interneurons are shown. 1: The top traces are responses to current steps that were triggered at membrane potentials near resting potential. The current steps (I) are shown below the responses (V). A: –60 mV, cell in Figure 1. B: –59 mV, cell in Figure 3H. C: –65 mV, cell in Figure 2. 2: The same current steps that are shown in part 1 were triggered after the cells were slightly hyperpolarized, using DC current. The break in the record indicates that the cell was hyperpolarized for several seconds before any current pulse was triggered. A: –69 mV; B: –65 mV; C: –72 mV. Note the delay before discharge in B and C. 3: Consecutive interspike intervals are plotted for the data shown in A–C (solid circles correspond to data from traces in row 1; open circles, row 2).

FIGURE 8.

Spontaneous activity of pyramidal-shaped cells. A–C: Continuous recordings are shown from pyramidal-shaped cells (A), a granule cell (B), or a mossy cell (C). A: Top: Recordings from the cell in Figure 3C. Membrane potential = -60 mV. Bottom: Recordings from the cell in Figure 2. Membrane potential = -63 mV. B: Recordings from a granule cell that was impaled in the same slice as the cell in Figure 2. Membrane potential = -81 mV. C: Recordings from a hilar mossy cell in the same slice as the cell in Figure 2. Membrane potential = -68 mV.

FIGURE 9.

Responses of pyramidal-shaped cells to stimulation of the slice. A: Three responses of an interneuron to molecular layer stimulation are superimposed. Stimuli occurred at the dot. Responses were triggered 1) during a depolarizing current pulse, 2) without a pulse, and 3) during a hyperpolarizing pulse. The start and end of the pulses are marked by arrowheads. Stimulus strength was high, at the upper limit of stimulus strengths used in this study ($250\ \mu\text{s}$, $250\ \mu\text{A}$), and evoked a population spike when a recording electrode was placed close to the location of the interneuron (not shown). Nevertheless, only a small response was recorded in the interneuron. Membrane potential = $-60\ \text{mV}$. Inset: The interneuron was located approximately at the "x," and the stimulating electrode was located at the dot. FISS, fissure; GCL, granule cell layer; HIL, hilus; PCL, pyramidal cell layer. B: Responses of the cell in Figure 1 to molecular layer stimulation at different membrane potentials are shown (top, $-52\ \text{mV}$; center, $-63\ \text{mV}$; bottom, $-70\ \text{mV}$). An inflection in the rising phase of the EPSP evoked at $-70\ \text{mV}$ is marked by an arrow. Inset: This cell was located approximately at the "x," and the position of the stimulating electrode is marked by the dot. This cell is shown in Figure 1. C: Responses of an interneuron to stimulation of the CA3c/hilar border (1) and the molecular layer (2). A drawing of this cell is shown in Figure 3H. Note that an antidromic action potential was evoked by stimulation of either site 1 or site 2. Inset: the interneuron was located approximately at the "x," and the stimulation sites are indicated by dots. Membrane potential = $-62\ \text{mV}$.

FIGURE 10.

Synaptic responses of a pyramidal-shaped cell, granule cell, and mossy cell from the same slice. A: Responses of a pyramidal-shaped cell to outer molecular layer stimulation are shown. The cell is shown in Figure 11. Action potentials are inaccurately represented due to digitization of the data (actual amplitudes of this interneuron's action potentials were over 80 mV). Left: Responses of the cell to a 10- μ s stimulus are shown. This stimulus strength evoked an action potential after approximately 50% of stimuli tested (i.e., it was the threshold stimulus). Top: -62 mV (resting potential). Bottom: -75 mV. Right: Responses of the cell to a 40- μ s stimulus (4 $\times$ threshold). Top: -62 mV; Bottom: -75 mV. B: Responses of a granule cell in the same slice to a 40- μ s stimulus of the same stimulation site. The granule cell is shown in Figure 11. Top: The granule cell was depolarized to -60 mV when the stimulus was triggered. At this membrane potential, 40 μ s was the threshold stimulus strength. Bottom: The granule cell was at -75 mV membrane potential. Resting potential, -82 mV. C: Response of a mossy cell in the same slice to a 10- μ s stimulus of the same stimulation site. The cell was at its resting potential, -73 mV. This cell is shown in Figure 11. Inset: An "x" is placed at the approximate locations of the three cells (granule cell, G; interneuron, I; mossy cell, M). The place where the stimulating electrode contacted the slice is marked by a dot. Dorsal is up and area CA3 is to the right.

FIGURE 11.

Morphology of a basket cell, granule cell, and mossy cell recorded from the same slice. A: The three cells from which recordings in Figure 10 were taken are shown. Only a fraction of the dendrites and axon collaterals are shown. The orientation of the cells is shown in part C and the inset of Figure 10. The same orientation (molecular layer up, hilus down) is maintained for parts A–D. Calibration (in part D) = 40 μm . B: A drawing of the three cells in the slice is shown. I, interneuron; G, granule cell; M, mossy cell; GCL, granule cell layer. Calibration (in D) = 30 μm . C: The basket cell is shown at higher power. Arrows indicate the main branches of the axon, and arrowheads mark varicosities along the axon collaterals. Calibration (in D) = 10 μm . D: One section of the slice containing a portion of the axon plexus of the basket cell is shown (arrows). In all sections from the slice, the axon plexus was confined approximately to the local area shown. Arrowheads point to pieces of cut dendrites of the mossy cell. Calibration = 40 μm .

TABLE 1

Electrophysiological Properties of Pyramidal-Shaped Cells and Granule Cells[†]

		Action potential							f-I slope primary (Hz/nA)
		RMP (mV)	Amplitude (mV)	Total duration (ms)	Time to 1/2 amp. (ms)	dv/dt rise (V/s)	dv/dt decay (V/s)	dv/dt ratio	
Pyramidal-shaped cells									
Mean	-62.3 *	70.9 *	1.17	0.214	653.6 *	375.6	1.94 *	7.69	91.3
SEM	2.3	3.7	0.16	0.034	157.8	81.0	0.11	0.77	9.34
n	17	17	17	17	17	17	17	17	17
Granule cells									
Mean	-77.5	102.9	1.205	0.162	1019.4	252.2	5.44	15.6	75.6
SEM	1.2	5.6	0.345	0.021	111.1	13.6	0.65	3.1	4.4
n	15	15	15	15	15	15	15	15	15

Abbreviations: time to 1/2 amp., time to half the maximum amplitude of the action potential; dv/dt rise, maximum rate of rise of the action potential; dv/dt decay, maximum rate of decay of the action potential; dv/dt ratio, (dv/dt rise)/(dv/dt decay); SEM, standard error of the mean. Details of measurements are explained in the Materials and Methods section (see "Data Analysis").

*Time constants of interneurons and granule cells were measured at similar membrane potentials: resting potential for interneurons (approximately -60 mV) and -60 to -65 mV for granule cells. Granule cells were depolarized with DC current to bring their membrane to those potentials; bridge balance was monitored while depolarizing the cells.

*Significantly different from granule cells (Student's *t*-test, *P* < 0.05).