Does a unique type of CA3 pyramidal cell in primates bypass the dentate gate?

Paul S. Buckmaster

Departments of Comparative Medicine and Neurology & Neurological Sciences
Stanford University, Stanford, California 94305-5342

Running head: “Dentate” CA3 pyramidal cells in primates

Corresponding author
Paul Buckmaster
Department of Comparative Medicine
300 Pasteur Drive
R321 Edwards Building
Stanford University
Stanford, California 94305-5342
(650) 498-4774
(650) 498-5085 (fax)
psb@stanford.edu

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Abstract

The predominant excitatory synaptic input to the hippocampus arises from entorhinal cortical axons that synapse with dentate granule cells, which in turn synapse with CA3 pyramidal cells. Thus, two highly excitable brain areas – the entorhinal cortex and the CA3 field – are separated by dentate granule cells, which have been proposed to function as a gate or filter. However, unlike rats, primates have “dentate” CA3 pyramidal cells with an apical dendrite that extends into the molecular layer of the dentate gyrus, where they could receive strong, monosynaptic, excitatory synaptic input from the entorhinal cortex. To test this possibility, the dentate gyrus molecular layer was stimulated while intracellular recordings were obtained from CA3 pyramidal cells in hippocampal slices from neurologically normal macaque monkeys. Stimulus intensity of the outer molecular layer of the dentate gyrus was standardized by the threshold intensity for evoking a dentate gyrus field potential population spike. Recorded proximal CA3 pyramidal cells were labeled with biocytin, processed with diaminobenzidine for visualization, and classified according to their dendritic morphology. In response to stimulation of the dentate gyrus molecular layer, action potential thresholds were similar in proximal CA3 pyramidal cells with different dendritic morphologies. These findings do not support the hypothesis that dentate CA3 pyramidal cells receive stronger synaptic input from the entorhinal cortex than do other proximal CA3 pyramidal cells.

Key words: monkey, temporal lobe epilepsy, dentate gyrus
Introduction

The molecular layer of the dentate gyrus is the major site of excitatory synaptic input from the entorhinal cortex to the hippocampus (Ramón y Cajal 1995). Although there is a smaller, direct pathway from entorhinal cortex to the distal dendrites of CA3 pyramidal cells (Blackstad 1958; Yeckel and Berger 1980; Krug et al. 2001), the predominant pathway is through interposed granule cells. This circuitry separates the highly excitable entorhinal cortex and CA3 field with the less excitable dentate gyrus, and previous studies in rodents suggest that the dentate gyrus filters or gates synaptic input from the entorhinal cortical neurons to CA3 pyramidal cells (Collins et al. 1983).

CA3 pyramidal cells proximal to the dentate gyrus display variable dendritic morphology. Rats have classical CA3 pyramidal cells with an apical dendrite that extends into stratum lacunosum-moleculare of the CA3 field, and multipolar CA3 pyramidal cells that lack an apical dendrite (Amaral 1978; Scharfman 1993). In primates the dendritic morphology of proximal CA3 pyramidal cells is even more variable. In addition to classical and nonapical CA3 pyramidal cells, like those found in rats, monkeys have “dentate” CA3 pyramidal cells. Dentate CA3 pyramidal cells have a soma in the pyramidal cell layer like other CA3 pyramidal cells, but their apical dendrite projects through the polymorphic layer (or hilus) and granule cell layer and into the molecular layer of the dentate gyrus (Buckmaster and Amaral 2001). Because of the position of their apical dendrite, dentate CA3 pyramidal cells might receive strong, monosynaptic, excitatory input from entorhinal cortical neurons. If so, they might bypass granule cells and efficiently convey entorhinal input directly to other pyramidal cells in the CA3 and CA1 fields. This primate characteristic might contribute to the apparent predisposition of humans to temporal lobe epilepsy, which is the most common type of epilepsy in adults (Engel et al. 1997). To begin testing this possibility, the action potential threshold for stimulation of the dentate gyrus molecular
layer was measured in proximal CA3 pyramidal cells in hippocampal slices from macaque monkeys, and the biocytin-labeled neurons were visualized to determine their dendritic morphology.

Materials and Methods

All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Stanford University Institutional Animal Care and Use Committee. As described previously, hippocampal slices were prepared from adult, male, neurologically normal monkeys (*Macaca fascicularis*) that were being euthanized for reasons unrelated to this experiment (Austin and Buckmaster 2004). Briefly, after inducing a deep level of anesthesia (25-50 mg/kg pentobarbital, i.v.) the left temporal lobe was resected and 400 µm thick transverse slices of the hippocampus were prepared and maintained until used for recording.

The recording and stimulation methods have been described previously (Kobayashi and Buckmaster 2003). Briefly, recordings of proximal CA3 pyramidal cells were obtained in current clamp mode with sharp electrodes. The molecular layer of the dentate gyrus was stimulated with an electrode (bipolar, 25 µm diameter, stainless steel wires) placed in the outer two-thirds of the molecular layer, approximately 500 µm off-center from the impaled cell. The field potential was recorded with an approximately 15 µm tip diameter pipette filled with 0.9% NaCl positioned at the border of the hilus and granule cell layer, on-center with the impaled cell. Stimuli were delivered (0.1 Hz, 150 µs) at gradually increasing intensities to determine the threshold for the dentate gyrus field potential population spike (1 xT). Then, while simultaneously recording the field potential, the cell’s action potential threshold was measured and standardized by the stimulus intensity for evoking a field potential population spike. Responses to injected current pulses were used to measure input resistance and action potential voltage threshold, amplitude, and duration, and cells were iontophoretically labeled with biocytin as described previously.
Inhibitory postsynaptic potential (IPSP) amplitude was measured from the pre-stimulus resting membrane potential to the maximum negativity occurring at any point within 350 ms after the stimulus. Slices containing labeled neurons were sectioned and processed, cells were reconstructed, and the borders of strata were drawn after thionin-counterstaining of sections as described previously (Buckmaster and Amaral 2001). Results from different types of proximal CA3 pyramidal cells were averaged and compared with P < 0.05 considered significant (one-way ANOVA with post hoc Bonferroni t tests and Chi-squared test, SigmaStat, SYSTAT Software Inc., Richmond, California).

Results

Recordings were obtained from 42 morphologically identified proximal CA3 pyramidal cells from 12 monkeys. All pyramidal cells displayed morphological characteristics of CA3 pyramidal cells including spiny dendrites and axon projections toward the CA1 field (Figure 1). The soma of every pyramidal cell was positioned in the CA3 pyramidal cell layer proximal to the dentate gyrus from a line connecting the ends of the granule cell layer. Based on their dendritic morphology all pyramidal cells were easily classified into one of three groups. Classical CA3 pyramidal cells (n = 12) had an apical dendrite that extended into stratum radiatum (n = 6) or stratum oriens (n = 6) of the CA3 field. Nonapical CA3 pyramidal cells (n = 23) lacked an apical dendrite, and most if not all of their dendrites were confined to the CA3 pyramidal cell layer. Dentate CA3 pyramidal cells (n = 7) had an apical dendrite that extended through the polymorphic and granule cell layers and into the molecular layer of the dentate gyrus.

Molecular layer stimulation evoked a dentate gyrus field excitatory postsynaptic potential (EPSP), which increased in amplitude and duration with higher stimulus intensities (Figure 2 insets). A negative-going population spike arose near the peak of the field EPSP, and its amplitude increased with
increasing stimulus intensities. Population spike threshold (1 xT) was the lowest stimulus intensity that consistently evoked a population spike.

Intracellular responses to molecular layer stimulation consisted of a short latency EPSP followed by a biphasic IPSP (Figure 2). At higher stimulus intensities a single action potential arose from the peak of the EPSP. The average action potential threshold for molecular layer stimulation was slightly higher than the dentate gyrus population spike threshold and similar in all three types of proximal CA3 pyramidal cells (Table 1). In some cells the action potential threshold was less than the population spike threshold (Figure 3), and the percentage of such cells in each CA3 pyramidal cell group was not significantly different (P=0.39, Chi-squared). The maximum IPSP amplitude evoked at the action potential threshold stimulus intensity was not significantly different in dentate CA3 pyramidal cells compared to other CA3 pyramidal cells (Table 1). However, the maximum IPSP amplitude of classical CA3 pyramidal cells was less than that of nonapical CA3 pyramidal cells. Average resting membrane potential, input resistance, and action potential voltage threshold, amplitude, and duration were similar in all three types of proximal CA3 pyramidal cells (Table 1).

Although proximal CA3 pyramidal cells were targeted, in one case recordings were obtained from a mossy cell. The cell displayed a high frequency of large amplitude spontaneous excitatory postsynaptic potentials (data not shown). The soma was in the polymorphic layer, large spines (thorny excrescences) covered the proximal dendrites, and most of the dendrites remained within the polymorphic layer but four projected through the granule cell layer and into the molecular layer of the dentate gyrus (Figure 4). The cell responded to molecular layer stimulation with one or two action potentials at relatively low stimulus intensity levels. The action potential threshold of this cell was 0.3 xT, which was less than that of all of the proximal CA3 pyramidal cells.
Discussion

The action potential threshold for stimulation of the dentate gyrus molecular layer was similar in dentate CA3 pyramidal cells and other types of proximal CA3 pyramidal cells. This finding was surprising, because a previous study in rats found that other cell types (hilar mossy cells and interneurons) had lower action potential thresholds if their dendrites extended into the molecular layer (Scharfman 1991). Consistent with that report, a mossy cell with multiple dendrites in the molecular layer had an action potential threshold much lower than the population spike threshold.

It is unclear why dentate CA3 pyramidal cells do not have a lower action potential threshold for dentate gyrus molecular layer stimulation than other proximal CA3 pyramidal cells. One possibility is that their intrinsic electrophysiological characteristics make them less responsive to excitatory synaptic input than other types of the CA3 pyramidal cells. This seems unlikely, because previous studies found similar resting membrane potentials, input resistances, and other intrinsic properties in proximal CA3 pyramidal cells with different dendritic morphologies in rats (Scharfman 1993) and monkeys (Buckmaster and Amaral 2001). Consistent with previous reports, no significant differences in the intrinsic electrophysiological properties of classical, nonapical, and dentate CA3 pyramidal cells were found in the present study. Another possibility is that dentate CA3 pyramidal cells receive more inhibitory synaptic input, which controls the direct excitatory synaptic input from the entorhinal cortex. This seems unlikely because the maximum IPSP amplitude at the threshold stimulus intensity for evoking an action potential was not significantly different in dentate CA3 pyramidal cells compared to other CA3 pyramidal cells.

In each subclass of proximal CA3 pyramidal cells a minority discharged an action potential at stimulus intensities less than that of the local field potential population spike threshold. There are several possible mechanisms that may account for this. Simultaneous discharge of a few presynaptic
granule cells may cause a depolarization strong enough to evoke an action potential in a postsynaptic CA3 pyramidal cell (Jonas et al. 1993). Simultaneous unit and field potential recordings suggest that in response to stimulation of perforant path fibers some granule cells discharge action potentials at stimulus intensities below the threshold for evoking a population spike (Lomo 1971). Granule cells closer to the stimulating electrode are more likely to discharge action potentials, and they might excite pyramidal cells through their laterally extending axon collaterals. Finally, stimulation of the dentate gyrus molecular layer will activate perforant path fibers, and some may extend to stratum lacunosum-moleculare of the CA3 field where they could synapse with classical CA3 pyramidal cells that have dendritic projections into that region.

Our findings suggest that in response to stimulation of the dentate gyrus molecular layer dentate CA3 pyramidal cells respond like other proximal CA3 pyramidal cells. However, there are caveats to this conclusion. We cannot exclude the possibility that in an intact preparation more physiologically relevant stimuli might selectively activate dentate CA3 pyramidal cells. In the present study only low frequency stimulation was tested, and in future experiments higher frequency stimulation should be used to evaluate the filtering properties of dentate CA3 pyramidal cells. Finally, the sample size is relatively small. However, primate tissue rarely is available for slice experiments, and dentate CA3 pyramidal cells account for only 18% of proximal CA3 pyramidal cells (Buckmaster and Amaral 2001). Despite these caveats, our findings do not support the hypothesis that dentate CA3 pyramidal cells receive stronger excitatory synaptic input from entorhinal cortical neurons than do other proximal CA3 pyramidal cells.
Acknowledgments

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Grants

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Table 1  Action potential threshold for stimulation of the dentate gyrus molecular layer, intrinsic physiological properties, and maximum inhibitory postsynaptic potential (IPSP) amplitude of three types of morphologically defined proximal CA3 pyramidal cells in macaque monkeys

<table>
<thead>
<tr>
<th></th>
<th>Classical CA3 Pyramidal Cell</th>
<th>Nonapical CA3 Pyramidal Cell</th>
<th>Dentate CA3 Pyramidal Cell</th>
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<tbody>
<tr>
<td><em><em>Action potential threshold for molecular layer stimulation (xT</em>)</em>*</td>
<td>1.22 ± 0.36</td>
<td>1.14 ± 0.50</td>
<td>1.14 ± 0.23</td>
</tr>
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<td><strong>Cells with action potential threshold &lt; 1 xT (%)</strong></td>
<td>17</td>
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<td>29</td>
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<td><strong>Resting membrane potential (mV)</strong></td>
<td>-67 ± 4</td>
<td>-66 ± 5</td>
<td>-64 ± 4</td>
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<td><strong>Input resistance (MΩ)</strong></td>
<td>105 ± 39</td>
<td>109 ± 27</td>
<td>99 ± 46</td>
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<td><strong>Action potential voltage threshold (mV)</strong></td>
<td>-50 ± 3</td>
<td>-48 ± 4</td>
<td>-48 ± 4</td>
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<tr>
<td><strong>Action potential amplitude (mV)</strong></td>
<td>71 ± 8</td>
<td>71 ± 9</td>
<td>72 ± 5</td>
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<td><strong>Action potential base width (ms)</strong></td>
<td>1.58 ± 0.15</td>
<td>1.51 ± 0.27</td>
<td>1.57 ± 0.24</td>
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<tr>
<td><strong>Maximum IPSP amplitude (mV)</strong></td>
<td>6 ± 3†</td>
<td>10 ± 4</td>
<td>8 ± 1</td>
</tr>
<tr>
<td><strong>Number of cells</strong></td>
<td>12</td>
<td>23</td>
<td>7</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation. *T = threshold stimulus intensity for evoking the dentate gyrus field potential population spike. There were no significant differences in the evoked action potential threshold or intrinsic electrophysiological properties between the three types of pyramidal cells (ANOVA or Chi-squared). †The maximum IPSP amplitude of classical CA3 pyramidal cells was significantly less than that of nonapical CA3 pyramidal cells (P = 0.036, post hoc Bonferroni t test).
Figure legends

Figure 1  Biocytin-labeled proximal CA3 pyramidal cells in macaque monkeys.  A Classical CA3 pyramidal cell.  A1 Apical dendrite extends into stratum radiatum of the CA3 field and avoids the polymorphic layer (p), granule cell layer (g), and molecular layer (m) of the dentate gyrus.  A2 Axon collaterals (red) primarily extend within the CA3 field, and one projects toward the CA1 field (arrow).  
B Nonapical CA3 pyramidal cell.  B1 Lacks an apical dendrite.  B2 Most dendrites (black) are confined to the CA3 pyramidal cell layer and avoid the polymorphic layer of the dentate gyrus. Some axon collaterals project toward the CA1 field (arrow).  C Dentate CA3 pyramidal cell.  C1 Apical dendrite extends through the polymorphic and granule cell layers and into the molecular layer of the dentate gyrus.  C2 Axon projections resemble those of other types of proximal CA3 pyramidal cells. Scale bar in C1 is for A1, B1, and C1.

Figure 2  Responses to stimulation of the dentate gyrus molecular layer of a classical (A), nonapical (B), and dentate CA3 pyramidal cell (C). Stimulus intensity is indicated and standardized by the threshold intensity for evoking a dentate gyrus field potential population spike (1 xT). Field potentials (insets) were recorded at the border of the granule cell layer and polymorphic layer, on-center with the recorded pyramidal cells.

Figure 3  Action potential thresholds for stimulation of the dentate gyrus molecular layer of morphologically defined proximal CA3 pyramidal cells. Action potential threshold is expressed with respect to the threshold stimulus intensity for evoking the dentate gyrus field potential population spike, which is indicated by the dashed horizontal line.
Figure 4 A mossy cell in a macaque monkey. A The soma and most of the dendrites are in the polymorphic layer (p). Four dendrites (arrowheads) extend from the polymorphic layer, through the granule cell layer (g), and into the molecular layer (m). B Large spines (arrows) cover the proximal dendrites. C Responses to stimulation of the dentate gyrus molecular layer. Stimulus intensity is indicated and standardized by the threshold intensity for evoking a field potential population spike (1 xT). The mossy cell had a low action potential threshold (0.30 xT). Field potentials (insets) were recorded at the border of the granule cell layer and polymorphic layer, on-center with the mossy cell.
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