Calcium-Dependent Spike-Frequency Accommodation in Hippocampal CA3 Nonpyramidal Neurons

RAYMOND A. CHITWOOD AND DAVID B. JAFFE
Division of Life Sciences, University of Texas at San Antonio, San Antonio, Texas 78249

Chitwood, Raymond A. and David B. Jaffe. Calcium-dependent spike-frequency accommodation in hippocampal CA3 nonpyramidal neurons. J. Neurophysiol. 80: 983–988, 1998. Interneurons of the hippocampal formation are traditionally identified electrophysiologically as those cells that fire trains of weakly accommodating action potentials in response to depolarizing current injection. We studied the firing properties of nonpyramidal neurons in the five substrata of the CA3b region of hippocampus. With the use of whole cell recording methods we found that nonpyramidal neurons fired in a range from weak to strong spike-frequency accommodation (SFA) that was calcium dependent. Slow afterhyperpolarizations were not associated with strong SFA. In addition a subset of interneurons (~20%) fired with an irregular firing pattern that was generally calcium independent. These results suggest a calcium-dependent mechanism for SFA in nonpyramidal neurons that is distinct from pyramidal cells and further demonstrates the heterogeneity of hippocampal interneurons.

INTRODUCTION

Inhibitory interneurons, sometimes referred to as nonpyramidal neurons, in the mammalian CNS are thought to be responsible for controlling the overall excitability of neural circuits via widespread inhibition of principal (excitatory) neurons (Freund and Buzsaki 1996). Excitatory neurons in many brain regions, including CA3 pyramidal neurons of the hippocampal formation, make recurrent connections among themselves. Therefore inhibitory neurons are critically important for limiting network firing and preventing epileptiform activity (Connors and Gutnick 1984; Grunze et al. 1993; Hablitz 1984; McNaughton 1989). Synaptic inhibition plays a number of other important computational roles. At the single neuron level, inhibition can modulate the backpropagation of action potentials that may affect associative forms of synaptic plasticity (Buzsaki et al. 1996; Tsubokawa and Ross 1993). In addition, both theoretical and experimental work suggests interneurons may provide a mechanism for controlling network oscillations thought to be important for higher cognitive functions (Cobb et al. 1995; Traub et al. 1996a,b; Wang and Buzsaki 1996; Whittington et al. 1995).

Interneurons of the hippocampal formation are typically characterized electrophysiologically by high input resistances ($R_i$), prominent afterhyperpolarizations (AHPs), and a consistent, weakly accommodating firing pattern in response to somatic depolarization (Lacaille et al. 1987; Schwartzkroin and Kunkel 1985). Such relatively small spike-frequency accommodations (SFAs) may be very important for maintaining fast (~40 Hz) oscillatory network activity (Wang and Buzsaki 1996). Differences in the firing properties of interneurons, such as the expression of SFA, could therefore have significant effects on an inhibitory neural network’s properties. For example, inconsistent synchronization or even multiple frequencies of synchronization might result from networks of accommodating interneurons (unpublished results). Furthermore, differences in the firing properties of nonpyramidal neurons would reflect variations in their active membrane properties and the potential for significant differences in their nonlinear membrane responses.

Here we report that a significant proportion of interneurons of the CA3b region of the hippocampal formation exhibit calcium-dependent SFA in response to depolarizing current injection. In addition, we found that a subset of nonpyramidal cells fire in an irregular pattern that was calcium independent. This work was previously presented in abstract form (Chitwood and Jaffe 1997).

METHODS

Hippocampal slices

Brain slices (300 μm) from 14- to 30-day old Sprague-Dawley rats containing transverse sections of hippocampus were prepared as described previously (Jaffe and Johnston 1990). Coronal sections were vibratome sliced in artificial cerebrospinal fluid (aCSF) at 4°C. The aCSF contained (in mM) 124 choline chloride, 2.5 KCl, 26 NaHCO$_3$, 2 MgCl$_2$, 2 CaCl$_2$, 1.25 NaHPO$_4$, and 10 dextrose. Slices were maintained in a holding chamber at room temperature (~25°C) in oxygenated aCSF (95% O$_2$-5% CO$_2$) in which choline chloride was replaced with NaCl. Slices were transferred as needed to a submersion-type recording chamber perfused with oxygenated aCSF (~1 ml/min), also at room temperature. Where noted, the temperature in the recording chamber was elevated to ~30°C. Finally, when calcium was removed from the extracellular solution, MgCl$_2$ was increased to 6 mM.

Electrophysiology

Whole cell patch-clamp recordings were made from visually identified CA3 nonpyramidal cells with the use of infrared differential interference contrast (IR/DIC) videomicroscopy (Stuart et al. 1993). Cell bodies located 50–100 μm from the surface were patched, and initial micropipette resistance was 3–5 MΩ. Intracellular saline contained (in mM) 120 K-gluconate or KMethSO$_4$, 20 KCl, 0.1 ethylene glycol-bis(β-aminoethyl ether)-N,N',N'',N'''-tetraacetic acid (EGTA), 2 MgCl$_2$, 2 Na$_2$ATP, and 10 N-2-hydroxyethylpiperazine-N''-2-ethanesulfonic acid (HEPES), pH 7.3. Pipettes were backfilled with saline containing 0.5–0.2% biocytin (Sigma, St. Louis, MO) or Neurobiotin (Vector). Electrical recordings were made with an Axoclamp 2b (Axon Instruments, Foster City, CA) in bridge mode and digitized at 1–3 kHz with...
the use of an ITC-16 interface (Instrutech, Great Neck, NY) connected to a Power Macintosh computer running AxoData (Axon Instruments) acquisition software. Analysis of electrical data was performed with custom software written with Igor Pro (WaveMetrics, Lake Oswego, OR).

**Histology**

Cells were filled with biocytin or neurobiotin by passive diffusion during whole cell recordings (30–60 min). After each experiment, slices were immediately fixed in 3% glutaraldehyde at 4°C for ≥24 h. They were processed with an avidin–HRP conjugate (Vector Labs ABC Kit, Burlingame, CA) and reacted with diamobenzidine dihydrochloride (DAB; Cappel, West Chester, PA) and 120 μM nickel ammonium sulfate [adapted from Major et al. (1994) and Kawaguchi (1995)]. Slices were cleared in ascending concentrations of glycerol and stored at room temperature. A computer-assisted cell reconstruction system was used to determine dendritic morphology (Claiborne 1992). Axonal projections were analyzed with the use of camera lucida reconstructions.

**Statistical analysis**

Statistical significance was determined with the use of a two-tailed Student’s *t*-test for paired samples. Numerical values are expressed as means ± SE.

**RESULTS**

Nonpyramidal neurons from all five substrata of the CA3b region were first identified by somatic location, shape, and size with the use of IR/DIC video microscopy. Pyramidal neurons were identified as those cells with classic fusiform somas restricted to stratum pyramidale. Although basket cells in this region also have fusiform- or pyramidal-shaped somas (Gulyas et al. 1993), we specifically chose neurons that appeared round or had characteristic nonpyramidal action potential waveforms (e.g., those with prominent AHPs). All other cells outside the pyramidal cell layer were therefore considered a priori to be nonpyramidal neurons. An $R_n$ larger than 200 MΩ further identified these cells as nonpyramidal neurons. Their mean $R_n$ was 566 ± 24 (SE) MΩ (n = 101), which was significantly greater than the $R_n$ of pyramidal neurons (164 ± 12 MΩ, n = 5). In addition, the dendritic morphology of biocytin-labeled cells was both qualitatively and quantitatively different from pyramidal neurons (Figs. 1, A–C, and 2, A–C).

We examined the firing properties of nonpyramidal neurons in response to 0.5–1 s depolarizing current injections of varying intensities by plotting interspike intervals (ISI) versus interval number (Fig. 1). For all cells, firing frequency increased with current amplitude. The response of most cells (~60%) was a train of weakly accommodating (ISI remained relatively constant) action potentials at all stimulus intensities (Fig. 1A). In contrast, there were also neurons that at submaximal current intensities (a magnitude that triggered 6–10 action potentials) exhibited prominent SFA (Fig. 1B). As current intensity was increased, the slope of the ISI relationship in these cells approached 0. A third group of neurons (~23%) fired in an irregular pattern (Fig. 1C); ISI varied between spike intervals.

Nonpyramidal neuron firing responses were quantified by determining the maximum slope of the normalized (to the 1st interval) ISI relationship. Representative traces and normalized ISI plots are illustrated in Fig. 2, A–C, corresponding to weak SFA, strong SFA, and irregularly firing neurons. A histogram of the normalized ISI relationships for weak SFA (left plot) and strong SFA (middle plot) neurons is illustrated in Fig. 2D. Although this distribution was not well fit by a bimodal Gaussian distribution, we defined weak and strong SFA neurons as those cells with a normalized ISI slope <0.2 or >0.2, respectively, and that were well fit by linear regression (residuals <10%). Additional analysis suggests a continuum of SFA from weak to strong SFA (described below). Irregularly firing cells were readily distinguishable from weak and strong SFA neurons; their normalized ISI relationship (Fig. 2C) was not as well fit by linear regression (residuals >10%).

The three firing patterns were not a result of performing experiments at room temperature. In nine experiments at ~30°C weak SFA (n = 4), strong SFA (n = 2), and irregularly firing cells (n = 3) were still observed.

When we examined the membrane properties of these neurons, we found no significant correlations with their particular firing patterns. For example, there was no correlation between passive membrane properties ($R_n$ and lowest time constant) and the maximum normalized ISI slope. There was also no correlation of spike threshold, amplitude, latency to
the peak of the fast AHP, or the amplitude of the AHP with the maximum ISI slope. The one difference between cells was that the AHP latency for irregularly firing cells (15 ± 2.7 ms) was significantly faster than for weakly and strongly accommodating neurons combined (23.4 ± 1.7 ms; t = 2.33, df = 99, P < 0.05).

Strong SFA depended on extracellular calcium. When calcium was removed and substituted by 6 mM Mg²⁺, strong SFA was reversibly inhibited (Fig. 3A). In eight of nine strong SFA neurons, removal of extracellular calcium reduced the maximum slope from 0.40 ± 0.06 to 0.10 ± 0.05 (Fig. 3B). Removal of calcium had no significant effect on the normalized ISI slope of the subset (n = 15) of weak SFA cells (Fig. 3C). In contrast, irregularly firing neurons did not reliably depend on calcium. In only 27% (3 of 11) of these cells was the normalized ISI slope affected by removing calcium (data not shown).

Given that our definition of weak versus strong SFA neurons was arbitrary, we next compared the change in normalized ISI slope (caused by removal of calcium) to the slope under control conditions, excluding irregularly firing neurons. Combined, there was a significant correlation for both weak and strong SFA neurons (Fig. 3D). When each subset was analyzed individually, we found that there was only a significant correlation for strong but not weak SFA neurons. A comparison of these two regressions indicated that they were not significantly different (t = 1.18, df = 20, P > 0.05, Student’s t-test for difference between 2 regression coefficients). Therefore we cannot rule out the possibility that there is a continuum of calcium-dependent SFA. Finally, we found no significant correlations between changes in active membrane properties (i.e., AHP latency, amplitude, etc.) and the calcium dependence of SFA (data not shown).

Immunohistochemical data indicate that most, if not all,
nonpyramidal neurons are inhibitory (Johansen et al. 1989; Ribak et al. 1978; Somogyi et al. 1983, 1984). Although a remote possibility, if these particular types of cells express autapses or excitatory collaterals onto inhibitory neurons, recurrent inhibition might account for SFA. To control for this, we tested if SFA was sensitive to blockers of excitatory and inhibitory synaptic transmission. Bath application of 1 mM kynureinate and 10 μM bicuculline (n = 2) had no significant effect on strong SFA nonpyramidal neurons (data not shown).

Finally, we attempted to determine if there was a correlation between the firing properties of individual nonpyramidal neurons and specific anatomic cell types. There were no obvious differences in the dendritic morphology of weak SFA, strong SFA, or irregularly firing nonpyramidal neurons (Figs. 1, A–C, and 2, A–C). The continuum of weak to strong SFA cells was observed in all five laminar regions and not correlated with dendritic or axonal morphology, whereas irregularly firing cells were never observed in s. pyramidal. Specific cell characterization with the use of axonal projections was inconclusive because of the significant loss of axon collaterals projecting out of the slice.

**DISCUSSION**

The classic electrophysiological response of an interneuron to depolarizing current steps is a relatively steady, weak-to-nonaccommodating pattern of firing (Lacaille et al. 1987; Schwartzkroin and Kunkel 1985). In this study we found that not all CA3 nonpyramidal neurons fire in this manner. Firing patterns ranged from weak SFA (small changes in frequency with time) to a significant increase in ISI over the course of a depolarizing current injection. In addition, a significant subset of nonpyramidal cells (~20%) exhibited an irregular pattern of firing. There are now a number of studies, including this one, demonstrating SFA of nonpyramidal neurons in the hippocampus, dentate gyrus, and neocortex (Cauli et al. 1997; Han 1996; Mott et al. 1997; Scharfman 1993). In this study we present evidence for a possible continuum from weak to strong SFA that is calcium dependent. This finding is in contrast with a recent study of nonpyramidal neurons in the CA1 region where SFA or irregular firing was not reported (Morin et al. 1996).

The variations in firing properties of CA3 nonpyramidal neurons may result from differences in calcium-associated
mechanisms. Differences in spiking patterns among nonpyramidal neurons might be correlated with particular types of calcium-binding proteins and endogenous calcium buffers (Gulyas et al. 1991, 1992, 1996). SFA in pyramidal neurons results primarily from the activation of slow, calcium-dependent potassium currents that produce the slow AHP (sAHP) (Hotson and Prince 1980; Lancaster and Adams 1986; Lancaster and Nicoll 1987). SFA in CA3 nonpyramidal neurons was also calcium dependent but not associated with an sAHP following a spike train. This suggests that other calcium-dependent mechanisms are responsible for SFA. Other types of outward currents, e.g., strong M-type potassium currents (Kirkwood et al. 1991; Williams and Johnston 1990) or variations in the density of voltage-gated calcium currents (Johnston and Wu 1995), could also underlie the membrane mechanisms responsible for accommodating and irregular firing patterns.

It remains to be determined whether the degree of SFA or irregular firing is correlated with previously characterized types of hippocampal nonpyramidal neurons (Freund and Buzsáki 1996). Given that video microscopy only now allows the identification of interneurons before recording, previous studies may have selected cells on the basis of weak SFA. Neurons that exhibited strong SFA or irregular firing were probably not considered for further anatomic analysis and characterization. Although the preliminary anatomic analysis here was inconclusive, it suggests the potential for further heterogeneity among CA3 interneurons.

SFA as well as the presence of dendritic spines are generally associated with excitatory rather than inhibitory neurons (Freund and Buzsáki 1996). Could strong SFA nonpyramidal cells be excitatory? Scharffman (1993) reported spiny, nonpyramidal neurons within the pyramidal layer of CA3c with firing properties very similar to pyramidal neurons. In this study we found only a few cases (7 of 73 labeled neurons) of spinelike processes on nonpyramidal neurons. However, there was no correlation between their presence and the firing behavior of a particular cell. Dual recordings of the synaptic connections from accommodating nonpyramidal cells onto other CA3 neurons combined with immunohistochemistry will be needed to test directly whether they are in fact excitatory or inhibitory.

We thank A. Hubbard for assistance with histological processing and 3-D reconstructions and Dr. Brenda J. Claiborne for assistance in 3-D reconstructions and critical reading of the manuscript.

This work was supported by National Science Foundation Grant IBN-9511309.

Address for reprint requests: D. B. Jaffe, Division of Life Sciences, University of Texas at San Antonio, 6900 N. Loop 1604 W., San Antonio, Texas 78249.

Received 22 December 1997; accepted in final form 16 April 1998.

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