Activity-dependent differences in function between proximal and distal Schaffer collaterals

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Owen B, Grover LM. Activity-dependent differences in function between proximal and distal Schaffer collaterals. J Neurophysiol 113: 3646–3662, 2015. First published April 8, 2015; doi:10.1152/jn.00446.2014.—Axon conduction fidelity is important for signal transmission and has been studied in various axons, including the Schaffer collateral axons of the hippocampus. Previously, we reported that high-frequency stimulation (HFS) depresses Schaffer collateral excitability when assessed by whole-cell recordings from CA3 pyramidal cells but induces biphasic excitability changes (increase followed by decrease) in extracellular recordings of CA1 fiber volleys. Here, we examined responses from proximal (whole-cell or field-potential recordings from CA3 pyramidal cell somata) and distal (field-potential recordings from CA1 stratum radiatum) portions of the Schaffer collaterals during HFS and burst stimulation in hippocampal slices. Whole-cell and dual-field-potential recordings using 10–100-Hz HFS revealed frequency-dependent changes like those previously described, with higher frequencies producing more drastic changes. Dual-field-potential recordings revealed substantial differences in the response to HFS between proximal and distal regions of the Schaffer collaterals, with proximal axons depressing more strongly and only distal axons showing an initial excitability increase. Because CA3 pyramidal neurons normally fire in short bursts rather than long high-frequency trains, we repeated the dual recordings using 100–1,000-ms interval burst stimulation. Burst stimulation produced changes similar to those during HFS, with shorter intervals causing more drastic changes and substantial differences observed between proximal and distal axons. We suggest that functional differences between proximal and distal Schaffer collaterals may allow selective filtering of nonphysiological activity while maximizing successful conduction of physiological activity throughout an extensive axonal arbor.

high-frequency stimulation; burst stimulation; action potential; Schaffer collateral; hyperexcitability

THE HIPPOCAMPUS IS ESSENTIAL for normal memory function, and long-term potentiation (LTP) of synaptic transmission within the hippocampus serves as a widely used model for examining the mechanisms of memory formation (Bliss and Collingridge 1993; Blundon and Zakharenko 2008). Because LTP induction depends on postsynaptic depolarization, typically resulting from short bursts or longer trains of presynaptic activity, activity-dependent alterations in presynaptic excitability may affect glutamate release, postsynaptic depolarization, and LTP. Previously, we showed that, during trains of continuous high-frequency stimulation (HFS), the Schaffer collateral axons connecting CA3 and CA1 pyramidal neurons undergo biphasic changes in excitability, with an early excitability increase followed by a later decrease (Kim et al. 2012). This later excitability decrease contributes to synaptic depression during HFS (Kim et al. 2012), in turn limiting the magnitude of LTP resulting from HFS (Grover et al. 2009). In addition, our previous findings (Kim et al. 2012) suggested differences between distal and proximal portions of the Schaffer collaterals; antidromic action potentials conducted through proximal axons did not show the initial excitability increase that was observed in fiber volley recordings from distal axons.

Although HFS is frequently used in experimental studies of LTP, the physiological relevance of axon excitability changes during HFS might be questioned because hippocampal pyramidal neurons do not normally fire long trains of action potentials (Albensi et al. 2007). In the intact animal, hippocampal pyramidal neurons often fire in short high-frequency bursts, with bursts repeated at delta-theta frequencies (Kandel and Spencer 1961; Ranck 1973). These endogenous firing patterns are mimicked by burst stimulation protocols like those we have used previously (Grover et al. 2009), which are highly effective for LTP induction (Grover et al. 2009; Larson et al. 1986). In the present study, we examined Schaffer collaterals for excitability changes during delta-theta frequency burst stimulation to determine whether similar excitability changes occur during HFS and more physiological patterns of activity. We also compared responses recorded from proximal and distal portions of Schaffer collaterals during HFS and burst stimulation to determine whether there are functional differences between these axon regions. Our results demonstrate that distal, but not proximal, Schaffer collaterals are capable of activity-dependent increases in excitability. Moreover, we find that increased excitability predominates in distal Schaffer collaterals during burst stimulation at frequencies that are most effective for LTP, whereas decreased excitability predominates during stimulation protocols that are less effective for LTP.

MATERIALS AND METHODS

Slice Preparation

Hippocampal slices were prepared as previously described (Kim et al. 2012). Male and female Sprague-Dawley rats (30–60 days old; Hilltop Lab Animals, Scottsdale, PA) were sedated by CO2-air inhalation and decapitated. The brain was removed and placed into chilled artificial cerebrospinal fluid (ACSF) composed of the following (in mM): 124 NaCl, 26 NaHCO3, 3.4 KCl, 1.2 NaH2PO4, 2 CaCl2, 2 MgSO4, and 10 glucose (pH 7.35, equilibrated with 95% O2-5% CO2). A block containing both hippocampi was glued to the stage of a vibratome (Campden Instruments, Lafayette, IN), immersed in chilled ACSF, and sectioned into 400–500-μm-thick slices in the coronal or horizontal plane. Slices were dissected to remove the
hippocampus from surrounding structures. Hippocampal slices were stored at room temperature (20–22°C) in an interface-holding chamber. For recordings, individual slices were transferred to a small-volume (~200 µl) interface-recording chamber heated to 34.5–35.5°C. Slices were perfused with oxygenated ACSF at a rate of 1.0–1.5 ml/min. All procedures were approved by the Institutional Animal Care and Use Committee at Marshall University.

**Field-Potential Recording**

Extracellular field potentials were recorded through glass micropipettes filled with ACSF (3–5 MΩ); in some recordings, the tip was broken before placement in the slice to lower resistance (to 1–2 MΩ) and reduce noise. Fiber volleys and antidromic population spikes were measured simultaneously by placing two electrodes in the slice, one in CA1 stratum radiatum and one in CA3 stratum pyramidale (Fig. 1A). Field potentials were recorded with an Axoclamp 2B using an HS-2A headstage (Axon Instruments, now Molecular Devices, Sunnyvale, CA) or a DAM50 (WPI) amplifier. The Axoclamp provided a gain of 10, with further amplification (gain of 10–100) from either an Ithaco 4302 dual-filter unit or a WPI FC-23B amplifier; the DAM50 provided a gain of 1,000. Signals were band-pass filtered (0.05–3,000.00 Hz) and digitized (National Instruments PCI-6035E or PCI-1200; Austin, TX) at 50–100 kHz or were band-pass filtered (0.1–10,000.0 Hz) and digitized at 100 kHz. Digitized signals were stored on a personal computer running Windows XP (Microsoft) using WinWCP and WinEDR software (Strathclyde Electrophysiology Software, John Dempster, University of Strathclyde). Fiber volley and population spike amplitude were measured as the difference between the maximum negativity and following positivity (Fig. 1B) and averaged 1.5 ± 0.2 mV (population spike) and 0.6 ± 0.1 mV (fiber volley). Latencies were measured as the time difference between the beginning of the stimulus artifact and the response at 10% of peak amplitude or between stimulus artifact and the response at peak amplitude (see RESULTS). Response areas were calculated as shown in Fig. 1B; a straight line was fitted to connect the first and second positive peaks of the response, and the area between the straight line and the negative-going portion of the response was calculated. Response durations at half-amplitude (half-widths) were calculated as shown in Fig. 1B.

**Whole-Cell Recording**

Somatic whole-cell patch-clamp recordings were obtained from CA3 pyramidal neurons by the method of Blanton et al. (1989). For action potential recordings, patch electrodes (3–5 MΩ) were filled with the following (in mM): 140 potassium gluconate, 10 sodium HEPES (N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]), and 3 MγCl2, adjusted to 285–290 mosM, pH 7.2. Recordings were done in the continuous current-clamp mode of the Axoclamp 2B. Signals were amplified (gain of 10), low-pass filtered (3 kHz), digitized (100 kHz), and stored on a personal computer using the WinWCP or WinEDR programs. Membrane potentials were not corrected for the liquid junction potential. Action potential amplitudes were measured as the difference between the membrane potential immediately before and at the peak of the action potential (Fig. 1B). Latencies were measured as the time difference between the stimulus artifact and the response at 10% of peak amplitude or peak amplitude (RESULTS). Series resistance was required to be <30 MΩ at the start of the recording (mean of 15.5 ± 2.3 MΩ, range 5–26 MΩ) and change by <15% during the recording (at end, mean of 15.4 ± 2.2 MΩ, range 5–26 MΩ). Resting membrane potentials were stable during all

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**Fig. 1.** Illustration of stimulation and recording methods and analysis. A: stimulating electrode was placed in stratum radiatum near the border of areas CA3 and CA1. In some experiments, whole-cell current-clamp recordings of antidromic action potentials were made from CA3 pyramidal neurons; in other experiments, simultaneous extracellular recordings were made from stratum pyramidale in area CA3 (population spike) and stratum radiatum in area CA1 (fiber volley). B: typical whole-cell and field-potential responses. Stimulus artifacts (*) have been partially removed. Top: antidromic action potential recorded from CA3 pyramidal neuron; action potential amplitude was determined as illustrated as the difference in membrane potential immediately before and at the peak of the action potential (dashed lines). Duration at half-amplitude (half-width) is indicated by double-ended arrow. Middle: population spike recorded from CA3 stratum pyramidale. Amplitude was determined as illustrated as the difference between the negative peak and following positive deflection. Half-width is indicated by double-ended arrow. Response area (shaded region) was calculated by fitting a straight line between the first and second positive peaks and integrating the area between the line and the response waveform. Bottom: fiber volley recorded from CA1 stratum radiatum; amplitude, half-width, and area were determined as illustrated. C: illustration of stimulation protocols. High-frequency stimulation (HFS) consisted of 160 consecutive stimuli at 10–100 Hz. Burst stimulation (BS) also consisted of 160 total stimuli, but stimuli were grouped into short bursts of four stimuli, with intervals of 100–1,000 ms between bursts. The relative stimulus number within each burst, or stimulus positions 1–4, is indicated.
potentials were isolated in a blocker cocktail of DNQX (30 μM) or, in some recordings, a blocker cocktail including DNQX, an N-methyl-D-aspartate receptor antagonist (5 μM CGP-37849 or 20 μM MK-801), a GABA<sub>Α</sub> antagonist (10 μM bicuculline methiodide), and a GABA<sub>β</sub> antagonist (1 μM CGP-55845). Antidromic action potentials were isolated in a blocker cocktail of DNQX (30 μM), MK-801 (20 μM), bicuculline methiodide (10 μM), and CGP-55849 (1 μM).

Reagents

Drugs were prepared as concentrated stock solutions. DNQX (30 mM; Tocris, Elllis, MO), CGP-55845 (10 mM; Tocris), and MK-801 (20 mM; Tocris) were dissolved in DMSO. Bicuculline methiodide (10 mM; Tocris or Sigma, St. Louis, MO) and CGP-37849 (5 mM; Tocris) were dissolved in distilled water. Stock solutions were diluted to final concentrations by addition to ACSF perfusing the tissue. Salts and all other reagents were from Sigma or Fisher Scientific (Pittsburg, PA).

Data Analysis and Statistics

Fiber volleys and population spikes were analyzed for latency and amplitude using WinWCP software and for area and half-width using custom routines written in the Python programming language (https://www.python.org/); for comparison among slices, amplitudes were normalized relative to the first response recorded during each round of HFS or burst stimulation. Antidromic action potentials were analyzed for latency, amplitude, and failures, with action potentials ≤5 mV in amplitude counted as failures. Data were analyzed using SPSS (IBM) and Gnuplot (http://gnuplot.org). Repeated-measures ANOVA was used to test for significant main effects (P < 0.05 accepted as significant) with post hoc paired comparisons made using t-tests with the Bonferroni correction (α level adjusted to maintain a per-experiment error rate of 0.05).

RESULTS

Schaffer Collaterals Show Frequency-Dependent Changes in Antidromic Action Potentials During HFS

Previously, we determined that, during continuous HFS, the Schaffer collateral fiber volley, an extracellular population response, is altered in a frequency-dependent manner (Kim et al. 2012). We also made whole-cell recordings of antidromic action potentials during HFS, but we only examined a single stimulation frequency, 100 Hz (Kim et al. 2012). To determine whether similar frequency-dependent changes can be observed in individual neurons as in the fiber volley, we recorded antidromic action potentials from CA3 pyramidal neurons (n = 10) during 10-, 20-, 50-, and 100-Hz HFS; a sample recording from one neuron is shown in Fig. 2. As Fig. 2 illustrates, HFS caused a frequency-dependent increase in action potential failures, with the higher frequencies of stimulation (50 and 100 Hz) causing a greater probability of action potential failure. Quantitative analysis of the effects of HFS on action potentials (amplitude, conduction latency, and half-width) and resting membrane potentials is given in Table 1. When failures were included in the analysis (assigned an amplitude of 0 mV), there were significant differences among the four stimulation frequencies in action potential amplitude during the final 20 stimuli of HFS (P < 0.01 by repeated-measures ANOVA). Although the decrease in action potential amplitude was frequency dependent (greatest decrease during highest frequency of stimulation), action potential amplitudes were significantly decreased by all frequencies of stimulation (all P values <0.001 by paired t-test). The frequency-dependent effect of HFS on action potential amplitudes could be a consequence of increased conduction failure, decreased amplitude independent of failure, or a combination of both. To distinguish among these possibilities, we reanalyzed the amplitude data after removing failures. As shown in Table 1, with failures removed, amplitudes still appeared to decrease during 50- and 100-Hz HFS, but none of the amplitude changes were significant (all P values >0.07). Moreover, when we compared amplitudes during the last 20 responses using repeated-measures ANOVA, there was no longer a significant effect of stimulation frequency (P > 0.2). In contrast, there was a significant difference between stimulation frequencies in the probability of failure during the last 20 stimuli (see Table 1; P < 0.05, repeated-measures ANOVA), although post hoc comparisons did not detect differences between specific frequency pairs.

In addition to amplitude and probability of failure, we analyzed action potential conduction latency (both at 10% of maximal amplitude and at maximal amplitude) and half-width, as well as resting membrane potential during HFS. Results of these analyses are also summarized in Table 1. Although all of these measures appeared to change during HFS, repeated-measures ANOVA failed to find any significant differences among the four stimulation frequencies (all P values between 0.063 and 0.339). In summary, for antidromic action potentials, stimulation frequency principally affected the probability of conduction failure.
Comparison of Distal vs. Proximal Schaffer Collaterals During HFS

Previously, we reported that during HFS, fiber volleys conducted by distal portions of Schaffer collaterals and recorded in area CA1 underwent an initial frequency-dependent increase in amplitude, followed by an amplitude depression, whereas latencies changed in a complementary manner, with an initial decrease followed by increase (Kim et al. 2012). In contrast, antidromic action potentials, conducted by proximal portions of Schaffer collaterals, only decreased in amplitude during the recording, whereas latencies only increased, suggesting differences between distal and proximal axons. To directly compare proximal and distal Schaffer collaterals, we simultaneously recorded responses conducted by distal (CA1 fiber volley) and proximal (CA3 population spike) axons, evoked by a single stimulating electrode placed at the CA1/CA3 border (n = 10 slices).

Amplitude changes. Figure 3 shows sample recordings from a single slice in which proximal and distal Schaffer collateral responses were recorded simultaneously. As Fig. 3 illustrates, by the end of HFS (stimulus 160) both proximal and distal response amplitudes were decreased in a frequency-dependent manner, with the greatest change during 100-Hz stimulation (Fig. 3A) and the smallest change during 10-Hz stimulation (Fig. 3D). However, early during HFS (stimulus 20), when proximal response amplitudes were greatly reduced, distal responses either increased (Fig. 3, A–C) or remained unchanged (Fig. 3D). Analysis of data from all slices (Fig. 4) revealed that these differences were reliable. Distal axon fiber volleys showed the same biphasic response in amplitude at 50–100 Hz that we reported previously (Kim et al. 2012), with a transient increase peaking around stimulus 20 followed by depression. In contrast, CA3 population spikes conducted antidromically over proximal axons showed only amplitude depression.

Table 1. Summary of changes in antidromic action potentials during HFS

<table>
<thead>
<tr>
<th>Stimulation Frequency</th>
<th>100 Hz</th>
<th>50 Hz</th>
<th>20 Hz</th>
<th>10 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Last 20</td>
<td>Initial</td>
<td>Last 20</td>
</tr>
<tr>
<td>Amplitude, mV, failures included†</td>
<td>103.2 ± 2.7*</td>
<td>9.4 ± 3.8*</td>
<td>100.8 ± 3.2*</td>
<td>13.6 ± 5.2*</td>
</tr>
<tr>
<td>Amplitude, mV, failures excluded</td>
<td>105.3 ± 3.9</td>
<td>79.2 ± 8.9</td>
<td>98.6 ± 4.5</td>
<td>73.4 ± 13.4</td>
</tr>
<tr>
<td>Proportion of failures†</td>
<td>0.87 ± 0.06</td>
<td>0.78 ± 0.09</td>
<td>0.63 ± 0.08</td>
<td>0.63 ± 0.09</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>-63 ± 1.6</td>
<td>-60.0 ± 1.7</td>
<td>-61.1 ± 1.6</td>
<td>-61.0 ± 3.5</td>
</tr>
<tr>
<td>Latency change, ms</td>
<td>0.98 ± 0.25</td>
<td>0.98 ± 0.34</td>
<td>0.30 ± 0.11</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>Peak latency change, ms</td>
<td>1.12 ± 0.25</td>
<td>1.17 ± 0.33</td>
<td>0.37 ± 0.11</td>
<td>0.51 ± 0.09</td>
</tr>
<tr>
<td>Half-width change, ms</td>
<td>0.23 ± 0.10</td>
<td>0.29 ± 0.15</td>
<td>0.10 ± 0.02</td>
<td>0.08 ± 0.02</td>
</tr>
</tbody>
</table>

All values are means ± SE. Initial: first response. Last 20: mean of final 20 responses. *Significant paired comparison (initial vs. last 20); †significant difference among stimulation frequencies. HFS, high-frequency stimulation.
Fig. 3. Simultaneous proximal and distal Schaffer collateral field-potential recordings during HFS. In A–D, proximal recordings (CA3 population spikes) are shown on the left, and distal recordings (CA1 fiber volleys) are shown on the right. A: proximal and distal responses to stimuli 1, 20, and 160 during HFS at 100 Hz are shown. Although both proximal and distal responses showed decreased amplitude and increased latency by the final stimulus (160), the distal, but not the proximal, response showed a small increase in amplitude and decrease in latency at stimulus 20. B: proximal and distal responses during 50-Hz HFS resembled those during 100-Hz HFS, except that the final changes in amplitude and latency were smaller at the lower frequency. C: although proximal responses continued to show strong amplitude depression during 20-Hz HFS, distal responses appeared to show minimal change in amplitude (but did continue to show an increase in latency). D: when HFS was given at 10 Hz, amplitude depression of proximal responses was reduced but still apparent, whereas distal responses showed no amplitude depression. Both proximal and distal responses continued to show increased conduction latency even at the 10-Hz stimulation frequency. For clarity, stimulus artifacts (indicated by asterisks) have been partially removed.

We quantified the initial response to HFS by calculating the mean normalized response amplitude during the first 20 stimuli (Table 2). Repeated-measures ANOVA revealed a significant main effect for recording site (proximal vs. distal axon, $P < 0.001$) and a significant interaction between frequency and recording site ($P < 0.001$), indicating that the differences between proximal and distal axons are frequency dependent, with greater differences at higher rates of stimulation. Post hoc analysis revealed significant differences between proximal and distal axon responses for all four stimulation frequencies (all $P$ values $<0.001$). To compare response depression, we calculated the mean normalized response amplitude during the last 20 stimuli (Table 3). On the basis of our previous study, we expected greater response depression at higher stimulation frequency. This expectation was confirmed by repeated-measures ANOVA, which demonstrated a significant main effect for stimulation frequency ($P < 0.001$). We also found a significant main effect for recording site (proximal vs. distal axon, $P < 0.001$) and a significant interaction between frequency and recording site ($P = 0.005$), again indicating that the differences between proximal and distal axons are frequency dependent. Post hoc analysis again showed statistically significant differences between distal and proximal axon responses for all frequencies (all $P$ values $\le 0.002$).

**Latency changes.** In addition to amplitude changes, we measured latency changes in distal and proximal Schaffer collateral responses during HFS at 10–100 Hz. Latency changes were measured at both 10% of maximum (peak) amplitude (Fig. 5, A–D) and at peak (Fig. 5, E–H). In general, latency changes were complementary to the amplitude changes described above, with response latencies steadily increasing during HFS for both proximal and distal Schaffer collaterals and with greater latency increases at higher stimulation frequencies. Comparison of the average latency changes for the first 20 responses (Table 2) revealed a significant main effect for recording site (proximal vs. distal axon) regardless of whether latencies were measured at 10% of peak or at peak amplitude (both $P$ values $= 0.001$). Post hoc tests showed significantly greater latency changes for proximal compared with distal axons at 10–50-Hz stimulation frequencies when measured at 10% of peak and at 20 and 100 Hz when measured at peak (all $P$ values $<0.001$). We also compared latency changes at the end of HFS (mean of last 20 responses), measuring at both 10% of peak and peak amplitude (Table 3). Regardless of the method used to measure latency changes, there was a significant main effect for stimulation frequency ($P < 0.005$ for latency measurements at 10% of peak amplitude, and $P < 0.001$ for measurements at peak), but the interaction between frequency and recording site was significant ($P < 0.01$) only when latencies were measured at 10% of peak amplitude. In summary, analysis of latency changes, like our analysis of amplitude changes, indicated significant effects of stimulation frequency and also significant differences between proximal and distal axons. In addition, there was an overall greater change in latency when measured at peak response amplitude (Fig. 5, A–D) compared with 10% of peak (Fig. 5, E–H), suggesting that response durations might have increased during HFS. To test this, we analyzed response durations at 50% of the peak amplitude (half-width).

**Half-width changes.** Response half-widths increased progressively during HFS in a frequency-dependent manner, with apparent differences between proximal and distal axons (Fig. 6, A–D). Especially at 50 and 100 Hz, half-widths appeared to increase more rapidly in proximal axons than in distal axons (Fig. 6, A and B). Repeated-measures ANOVA of changes in half-width during the first 20 stimuli (Table 2) confirmed this impression, with a significant main effect for location (proximal vs. distal) and a significant interaction between location and stimulation frequency ($P < 0.001$, and $P < 0.02$, respectively). Post hoc paired comparisons indicated significant differences between proximal and distal axon half-width changes during the first 20 stimuli for all frequencies except 10 Hz (all $P$ values $\le 0.002$). Analysis of half-width changes during the final 20 stimuli, however, revealed only a significant main effect for stimulation frequency (Table 3; $P < 0.025$). Together, these results indicate that both proximal and distal Schaffer collateral responses increased in duration during HFS, with significant differences between proximal and distal responses only early during HFS. Because both the proximal (population spike) and distal (fiber volley) responses that we recorded are population responses, the observed increase in response duration could reflect decreased synchronization among the fibers contributing to the response (although at least...
1 other factor could also contribute, see DISCUSSION). If the increase in response duration (half-width) was a result of desynchronized firing, then the amplitude decreases reported above might overestimate the depression of excitability during HFS. Because measurements of response area should be less affected by desynchronization than amplitude measurements, we reanalyzed proximal and distal responses for changes in area.

**Area changes.** Response areas were measured as shown above (Fig. 4) and normalized relative to the area of the first response during HFS. Compared with response amplitudes (Fig. 4), response areas (Fig. 6, E–H) changed similarly in several respects. First, there were substantial differences between proximal and distal axon responses. Second, distal axon response areas increased early during HFS, whereas proximal axon response areas only decreased. Finally, changes in response area, like amplitude, appeared to be frequency dependent. Area measurements differed from amplitude measurements, however, in respect to the amount of response depression. For proximal axons, area measurements indicated a quantitatively smaller magnitude of depression compared with amplitude measurements (Table 3). For distal axon, the differences between area and amplitude measurements were qualitative; whereas distal axon response amplitudes eventually decreased later during HFS, response areas showed a sustained increase, even during the highest frequency of stimulation (contrast Fig. 4A with Fig. 6E). These differences between amplitude and area measurements are discussed later (DISCUSSION). Repeated-measures ANOVA revealed significant main effects for location (distal vs. proximal axon) for both the initial 20 stimuli (Table 2; P < 0.01) and the final 20 stimuli (Table 3; P < 0.02) of HFS and, in addition, a main effect for stimulation frequency during the final 20 stimuli (P < 0.02). Post hoc comparisons showed significant differences between proximal and distal axon responses for 10- and 50-Hz HFS (P < 0.002 and P < 0.01, respectively) during the first 20 stimuli and for 20-Hz HFS for the final 20 stimuli (P < 0.002).

In summary, HFS at 10–100 Hz produced significantly different changes in excitability at distal compared with proximal axon locations. For all frequencies examined, proximal axons showed greater excitability depression during HFS compared with distal axons. At higher frequencies of stimulation (50–100 Hz), distal axon responses displayed a transient increase in response amplitude that peaked around stimulus 20 and persisted to around stimulus 40.

**Excitability Changes During Burst Stimulation**

Burst stimulation more closely resembles the physiological firing pattern of CA3 pyramidal neurons than continuous HFS (Albensi et al. 2007). To determine whether similar differences between distal and proximal Schaffer collateral responses occur during burst stimulation, we recorded simultaneously from distal and proximal locations (n = 10 slices) as in the preceding experiment but delivered stimuli in short bursts (4 stimuli at 100 Hz) repeated at intervals of 100-1,000 ms, rather than as

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**Table 2. Summary of initial changes in field potentials during HFS**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Amplitude, %†‡</th>
<th>Latency change at 10% of peak, ms†</th>
<th>Peak latency change, ms†</th>
<th>Half-width change, ms†‡</th>
<th>Area, %†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 Hz</td>
<td>69.3 ± 4.7*</td>
<td>0.06 ± 0.04</td>
<td>0.27 ± 0.05*</td>
<td>0.63 ± 0.02*</td>
<td>96.4 ± 7.0</td>
</tr>
<tr>
<td>50 Hz</td>
<td>71.7 ± 3.6*</td>
<td>0.04 ± 0.04</td>
<td>0.28 ± 0.06</td>
<td>0.06 ± 0.04</td>
<td>95.9 ± 4.6*</td>
</tr>
<tr>
<td>20 Hz</td>
<td>115.6 ± 5.4*</td>
<td>0.12 ± 0.03*</td>
<td>0.06 ± 0.04</td>
<td>0.43 ± 0.08*</td>
<td>116.2 ± 7.2</td>
</tr>
<tr>
<td>10 Hz</td>
<td>78.1 ± 3.2*</td>
<td>0.01 ± 0.02*</td>
<td>0.31 ± 0.05*</td>
<td>-0.02 ± 0.04*</td>
<td>96.5 ± 3.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE for the first 20 responses. *Significant paired comparison (proximal vs. distal); †significant main effect for recording site (proximal vs. distal); ‡significant interaction between recording site and stimulation frequency.
a continuous HFS train. A representative example of responses simultaneously recorded from both locations in the same slice during burst stimulation is shown in Fig. 7. As this example shows, it was readily apparent that proximal axon responses depressed to a greater extent than distal axon responses, at least at the two shorter burst intervals (100 and 200 ms). We quantified responses during burst stimulation by measuring response amplitudes and conduction latencies at 10% of peak amplitude. We did not assess response areas during burst stimulation because our HFS results indicated similar relative proximal vs. distal differences for amplitude and area measures. Likewise, we omitted analysis of latency changes at peak because our previous recordings indicated little difference in between latency measurements at 10 or 100% of peak amplitude.

Amplitude changes. We observed amplitude changes during burst stimulation at 100-ms intervals that resembled those reported above during 50–100-Hz HFS; for distal axon responses, an initial increase in amplitude was later followed by depression, but, for proximal axon responses, only depression was observed (Fig. 8). Increasing the burst interval from 100 to 500 ms resulted in progressively smaller amplitude changes for

Table 3. Summary of final changes in field potentials during HFS

<table>
<thead>
<tr>
<th>Frequency</th>
<th>100 Hz</th>
<th>50 Hz</th>
<th>20 Hz</th>
<th>10 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>Amplitude, %†‡§</td>
<td>23.7 ± 2.7*</td>
<td>52.3 ± 6.5*</td>
<td>33.1 ± 3.0*</td>
<td>68.1 ± 8.5*</td>
</tr>
<tr>
<td>Latency change at 10% of peak, ms†‡</td>
<td>0.52 ± 0.08</td>
<td>0.77 ± 0.16</td>
<td>0.43 ± 0.05</td>
<td>0.54 ± 0.12</td>
</tr>
<tr>
<td>Peak latency change, ms§</td>
<td>0.93 ± 0.22</td>
<td>0.99 ± 0.15</td>
<td>0.83 ± 0.10</td>
<td>0.75 ± 0.16</td>
</tr>
<tr>
<td>Half-width change, ms§</td>
<td>1.5 ± 0.04</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Area, %†§</td>
<td>42.7 ± 3.5</td>
<td>124.4 ± 37.3</td>
<td>55.1 ± 4.6</td>
<td>155.2 ± 36.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for the last 20 responses. *Significant paired comparison (proximal vs. distal); †significant main effect for recording site (proximal vs. distal); ‡significant interaction between recording site and stimulation frequency; §significant main effect for stimulation frequency.

Fig. 5. Field-potential latency changes for proximal and distal Schaffer collateral axons during HFS. A–D: mean latency changes measured at 10% of peak response amplitude for proximal and distal axons during HFS at 100, 50, 20, and 10 Hz. Proximal and distal axon conduction latencies showed frequency-dependent increases during HFS. For clarity, error bars are shown only for every tenth stimulus. E–H: mean latency changes measured at peak response amplitude for proximal and distal axons during HFS at 100, 50, 20, and 10 Hz. Proximal and distal axon conduction latencies showed a similar pattern of change during HFS regardless of whether measurements were made at 10% of peak amplitude or at peak amplitude. For clarity, error bars are shown only for every tenth stimulus. Data are from the same slices shown in Fig. 4.
both proximal and distal axons, but a further increase to 1,000 ms had no additional effect. For distal axon responses, both the early increase in response amplitude and later depression were blunted as burst interval was increased (Fig. 8, compare A2 to B2, C2, and D2). Similarly, for proximal axon responses, amplitudes showed less depression when the burst interval was increased from 100- to 200-ms burst stimulation, and little depression was observed during stimulation at the longer 500- and 1,000-ms burst intervals (compare Fig. 8, A1 to B1, C1, and D1). For distal but not for proximal axons, we also observed stimulus position-dependent changes within each burst; typically, response amplitudes increased from stimulus positions 1–4 within each burst, with amplitudes largely recovering during the interval between bursts. An exception to this pattern was noted during 100-ms burst stimulation, where distal response amplitudes consistently decreased in amplitude within each burst from around burst 10 (stimulus 40) through the end of stimulation. Position-dependent changes in response amplitude are described in more detail below.

To compare overall (independent of any position effects) initial amplitude changes during burst stimulation, we averaged response amplitudes during the first five bursts (first 20 responses; Table 4). For distal axons, response amplitudes increased during the first five bursts, with the largest increases seen at the 100-ms interval. For proximal axons, response amplitudes decreased during the first five bursts, with the largest decreases seen at the 100-ms burst interval. Repeated-measures ANOVA indicated a significant main effect for recording site (proximal vs. distal, P < 0.01), but also a significant interaction between recording site and burst interval (P < 0.001), indicating that the differences between proximal and distal axons were dependent on the burst interval. Post hoc analysis showed a significant proximal vs. distal difference only for the 100-ms burst interval (P < 0.005).

To assess initial within-burst (stimulus position-dependent) changes, we averaged responses by position number (1–4) across the first five bursts for each burst interval (Fig. 9, A1–A4). Repeated-measures ANOVA was used to test for main effects of recording site (proximal vs. distal) and stimulus position (positions 1–4 within bursts); when ANOVA indicated a significant main effect for recording site or a significant interaction of recording site with stimulus position, we conducted post hoc tests to compare proximal and distal response amplitudes independently at all position numbers. For the 100-ms interval (Fig. 9A1), there was a significant main effect for recording site (proximal vs. distal, P < 0.01) and a significant interaction between recording site and stimulus position (P < 0.025), indicating that differences between...
proximal and distal responses depended on stimulus position within bursts. This was supported by post hoc analysis, which showed significant proximal vs. distal differences for positions 2–4 (P values < 0.01), but not for position 1. For the 200-ms burst interval (Fig. 9A2), there was a significant main effect for recording site (P < 0.05), with distal responses showing greater amplitudes than proximal response, but post hoc analysis did not reveal significant proximal vs. distal differences for any of the individual stimulus positions. For the 500-ms burst interval (Fig. 9A7), there was a significant main effect only for stimulus position (P < 0.005). No significant effects were found for 1,000-ms burst interval (Fig. 9A4). In summary, there were significant proximal vs. distal differences in response amplitude during the first five bursts, but only for the shorter (100 and 200 ms) burst intervals.

To compare the overall final degree of response depression between proximal and distal axons, we calculated mean normalized response amplitudes during the last five bursts (final 20 responses; see Table 4). Repeated-measures ANOVA showed significant main effects for recording site (proximal vs. distal, P < 0.005) and burst interval (P < 0.001) but no significant interaction between the two. Post hoc tests showed significant differences between proximal and distal axon responses for 100-, 200-, and 1,000-ms burst intervals (P values < 0.01), but not for the 500-ms burst interval.

To assess effects of stimulus position within bursts, we used the same procedure described above. For the 100-ms interval (Fig. 9B1), we obtained significant main effects for both recording site (proximal vs. distal axon, P < 0.005) and position number within bursts (1–4, P < 0.001), as well as a significant interaction between the recording site and stimulus position (P < 0.001), reflecting greater proximal vs. distal differences at the beginning compared with the end of each burst. Post hoc analysis revealed significant differences between proximal and distal axon responses at positions 1–3 (P values ≤ 0.011), but not position 4. For the 200-ms interval (Fig. 9B2), ANOVA revealed a significant main effect for recording site (P < 0.01) and a significant interaction between recording site and stimulus position (P < 0.05), reflecting greater proximal vs. distal differences toward the end of each burst. In agreement, post hoc analysis showed a significant difference between proximal and distal axon responses for stimulus position 3 (P < 0.005). Analysis of the 500-ms interval data (Fig. 9B3) showed no significant effects. For the 1,000-ms interval (Fig. 9B4), ANOVA showed a significant main effect for recording site (P < 0.01) but no other significant effects. Post hoc tests showed significant differences between proximal and distal axon responses for stimulus positions 2 and 4. In summary, there were significant proximal vs. distal differences in response amplitude during the last five bursts for all but the 500-ms burst interval.

**Latency changes.** As can be seen in Fig. 10, latency changes during burst stimulation were essentially the inverse of amplitude changes (compare with Fig. 8). Distal axon responses showed a biphasic change in latency, with decreases during the first four to five bursts, followed by a gradual increase. In contrast, proximal axon responses showed latency increases only. In addition, the latency increases appeared to be greater for proximal compared with distal axons. For both proximal and distal axon responses, there were stimulus position-depen-
dent changes in latency within each burst (described in more detail below). Proximal axon responses tended to increase in latency within each burst early during burst stimulation but decrease in latency later, whereas distal axon responses generally decreased in latency within bursts except late during burst stimulation at 100 ms, where response latencies increased within each burst (Fig. 10A2). Response latencies partially recovered from within-burst changes during the intervals between bursts.

To compare latency changes, we followed the same procedures that we used above for analyzing amplitude changes. To assess overall initial changes in latency, we calculated the mean latency change for each burst interval (separately for proximal and distal axon responses) during the first five bursts (first 20 responses, see Table 5). For distal axon responses, there was a small but consistent decrease in mean latency. For proximal axon responses, there was a small but consistent increase in mean latency, with larger changes during burst stimulation at the shorter (100 and 200 ms) intervals. Repeated-measures ANOVA showed a significant main effect for recording site (proximal vs. distal) only. Post hoc tests revealed significant differences between proximal and distal axon latency changes for the 200- and 1,000-ms intervals.

Table 4. Summary of field potential amplitude changes during burst stimulation

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Proximal</th>
<th>Distal</th>
<th>Proximal</th>
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<th>Proximal</th>
<th>Distal</th>
<th>Proximal</th>
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<td>100 ms</td>
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<tr>
<td>First 5 bursts†‡</td>
<td>75.6 ± 7.2*</td>
<td>113.1 ± 6.4*</td>
<td>85.7 ± 6.2</td>
<td>103.7 ± 3.5</td>
<td>92.9 ± 4.5</td>
<td>106.4 ± 3.2</td>
<td>93.0 ± 4.6</td>
<td>106.7 ± 3.7</td>
</tr>
<tr>
<td>Last 5 bursts†§</td>
<td>37.2 ± 2.1*</td>
<td>60.4 ± 4.7*</td>
<td>63.6 ± 6.0*</td>
<td>83.8 ± 4.6*</td>
<td>86.6 ± 3.5</td>
<td>103.1 ± 5.8</td>
<td>91.1 ± 4.8*</td>
<td>102.6 ± 4.5*</td>
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</tbody>
</table>

Values are means ± SE for the first or last 5 bursts (first or last 20 responses). *Significant paired comparison (proximal vs. distal); †significant main effect for recording site (proximal vs. distal); ‡significant interaction between recording site and stimulation frequency; §significant main effect for stimulation frequency.
proximal vs. distal differences being greatest toward the end of each burst. Post hoc tests confirmed that there were significant proximal vs. distal differences for positions 2–4 ($P$ values <0.01) but not for position 1. For the 200-ms interval, there were significant main effects for recording site ($P = 0.001$) and stimulus position ($P < 0.02$). As with the 100-ms interval, there was a significant interaction between recording site and stimulus position ($P < 0.01$), again reflecting an increased difference between proximal and distal latencies from positions 1–4 within bursts. Post hoc tests showed significant proximal vs. distal differences for positions 2–4 ($P$ values ≤0.002) but not position 1. For the 500-ms interval, although proximal vs. distal differences were not as pronounced, statistical analysis revealed the same pattern, namely significant main effects for recording site ($P < 0.03$) and stimulus position ($P < 0.005$) and a significant interaction between recording site and stimulus position ($P < 0.001$), with post hoc tests showing significant proximal vs. distal differences for positions 3 and 4 ($P$ values ≤0.003). Finally, for the 1,000 ms interval, there was a significant main effect for recording site (proximal vs. distal, $P < 0.005$), and a significant interaction between recording site and stimulus position ($P = 0.003$), again attributable to greater differences in proximal vs. distal latencies at later stimulus positions. This was confirmed by post hoc tests, which indicated significant proximal vs. distal differences for positions 2–4 ($P$ values ≤0.005) but not position 1. In summary, there were significant proximal vs. distal differences in latency change during the first five bursts for all four burst intervals.

To compare the overall magnitude of latency change later during burst stimulation, we calculated the mean latency changes during the final five bursts (last 20 responses; see Table 5). Repeated-measures ANOVA showed a significant main effect for burst interval ($P < 0.001$), confirming our observation of smaller latency changes with longer burst intervals, but no other significant effects. We again compared latency changes by stimulus position within bursts, as described above. For the 100-ms burst interval (Fig. 11B1), there were no significant main effects, but there was a significant interaction ($P = 0.002$) between recording site (proximal vs. distal) and position number (1–4), reflecting opposite changes in proximal and distal response latencies from positions 1 to 4. Although the interaction was significant, post hoc tests did not reveal any significant differences between proximal and distal axon latency changes for any of the four stimulus positions. For the 200-ms burst interval (Fig. 11B2), there were significant main effects for recording site ($P < 0.01$) and stimulus position ($P < 0.05$). Although both proximal and distal laten-
cies decreased with stimulus position, there was a greater decrease in latency for proximal axons; this is reflected by the significant interaction between recording site and stimulus position ($P < 0.05$). Post hoc tests showed significant differences between proximal and distal axons for stimulus positions 1 and 4. For the 500-ms burst interval (Fig. 11B3), there was a significant main effect for stimulus position ($P < 0.02$) but no other significant effects. For the 1,000-ms burst interval (Fig. 11B4), we again found a significant main effect for stimulus position ($P < 0.005$) as well as a significant main effect for recording site (proximal vs. distal; $P < 0.02$); post hoc tests showed significant differences between proximal and distal axons at stimulus positions 2 and 3. In summary, there were significant proximal vs. distal differences in latency change during the last five bursts for all burst intervals except 500 ms.

Antidromic vs. Orthodromic Conduction in Proximal Axons

Our analysis of proximal Schaffer collateral function relied on measurement of antidromically conducted action potentials, but antidromic and orthodromic conduction might not be equivalent (see DISCUSSION). We tested orthodromic conduction by proximal Schaffer collaterals during HFS and burst stimulation by placing the stimulating electrode in stratum pyramidale of area CA3 to activate initial segments of CA3 axons and placing the recording electrode in stratum radiatum at the CA3/CA1 border. Orthodromic proximal axon responses re-

Table 5. Summary of field potential latency changes during burst stimulation

<table>
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<th>Proximal</th>
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<td>100 ms</td>
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<tr>
<td>First 5 bursts†</td>
<td>0.12 ± 0.01</td>
<td>−0.07 ± 0.01</td>
<td>0.12 ± 0.04*</td>
<td>−0.06 ± 0.01</td>
<td>0.04 ± 0.03*</td>
<td>−0.05 ± 0.01</td>
<td>0.06 ± 0.03</td>
<td>−0.05 ± 0.01</td>
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<tr>
<td>Last 5 bursts‡</td>
<td>0.59 ± 0.10</td>
<td>0.63 ± 0.04</td>
<td>0.49 ± 0.06</td>
<td>0.28 ± 0.04</td>
<td>0.14 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.02 ± 0.01</td>
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</table>

Values are means ± SE for the first or last 5 bursts (first or last 20 responses). *Significant paired comparison (proximal vs. distal); †significant main effect for stimulation location (proximal vs. distal); ‡significant main effect for burst interval.

Fig. 10. Comparison of field-potential latency changes in proximal and distal Schaffer collateral field potentials during burst stimulation. A–D: latency changes for proximal (1, left) and distal (2, right) axons during burst stimulation at 100-, 200-, 500-, and 1,000-ms intervals. Proximal axons showed monophasic latency increases that were greatest during burst stimulation at the 100-ms interval and that declined as burst interval was increased. In contrast, distal axon responses showed a biphasic change in latency during burst stimulation, with an early decrease in latency followed by a gradual increase; the latency increase was greatest at the 100-ms interval and became less pronounced as burst interval was increased. Data are from the same slices shown in Figs. 8 and 9.
corded in this way also showed only amplitude depression and latency increase during HFS and burst stimulation (Fig. 12). Most importantly, the same pattern of change was seen regardless of the direction of conduction, orthodromic or antidromic (compare Fig. 12 with Figs. 4–5, 8, and 10).

DISCUSSION

In our first experiment, we used whole-cell recordings to examine changes in antidromic action potentials during HFS over a range of stimulation frequencies (10–100 Hz). Our results expand on our previous findings (Kim et al. 2012) to demonstrate activity-dependent depression of antidromic action potentials over a broader frequency range than we previously examined. For all frequencies examined, antidromic action potentials displayed activity-dependent amplitude depression and latency increase. Our analysis indicated that the main effect of stimulation frequency is on probability of conduction failure. In our previous study, we observed an initial period of increased excitability for extracellular recordings from distal Schaffer collaterals (fiber volleys in stratum radiatum of area CA1), but we did not observe a similar excitability increase for antidromic action potentials recorded from single CA3 neurons in either the previous or the present study. To determine whether this discrepancy reflects a difference in function between proximal and distal portions of Schaffer collateral axons, we directly compared responses recorded from both axon regions.

Because whole-cell recording from the very-small-diameter (typically 0.1–0.3 µm; Ishizuka et al. 1990; Shepherd and Harris 1998) distal Schaffer collaterals is not possible, we made simultaneous extracellular recordings from proximal (population spikes in area CA3 stratum pyramidale) and distal (fiber volleys in area CA1 stratum radiatum) regions of the Schaffer collaterals. We found comparable changes in extracellular population spike and whole-cell recordings, validating our extracellular recording method. Direct, simultaneous comparison of proximal and distal axons during both nonphysiological HFS and physiological burst stimulation confirmed that both axon regions undergo activity-dependent amplitude depression, but only the distal Schaffer collaterals show an initial period of increased (hyper) excitability. We observed hyperexcitability during both HFS and burst stimulation, indicating the transient increase in excitability is not an artifact of nonphysiological stimulation protocols.

Comparison of latency changes measured at 10 and 100% of peak amplitude revealed greater latency increases for the latter...
measurement, suggesting that responses increased in duration. This suggestion was confirmed by measurement of response duration (half-width), which revealed increases in duration for both single axon (whole cell) and compound (population spike and fiber volley) responses. Increased duration of compound responses could indicate reduced synchronization of action potential conduction in the population of axons contributing the response, a possibility that would not be surprising given previous reports of functional heterogeneity, namely variability in conduction latency (Soleng et al. 2003b) and in conduction reliability during HFS (Kim et al. 2012; Meeks and Mennerick 2004) among individual Schaffer collateral axons. Alternatively, the increased duration of compound responses could reflect the summed effect of an increase in duration of single axon responses, which we did observe, at least for antidromically conducted (proximal) Schaffer collateral action potentials.

Comparison of amplitude and area measurements revealed comparable differences between proximal (population spike) and distal (fiber volley) responses, with proximal responses decreasing relative to distal responses during HFS, regardless of which measurement was used. There were, however, substantial differences in the absolute degree of response depression during HFS depending on whether amplitude or area was measured. In fact, when response areas were measured, distal (fiber volley) responses no longer appeared to depress. This discrepancy raises the question of whether distal response depression might be an artifact of the measurement technique chosen. Experimental evidence argues otherwise, however. First, response latencies increased during HFS, for both distal and proximal responses, indicating excitability depression for both. Second, recordings from individual axons in CA1 demonstrated conduction failure in a subpopulation of low reliability axons even during short (10 stimuli) 50-Hz HFS (Meeks and Mennerick 2004), directly demonstrating that at least some afferents in CA1 (most likely Schaffer collaterals) do undergo excitability depression during HFS. It seems likely that longer duration HFS, like we used, would have resulted in higher failure rates. What then might have produced the discrepancy between response amplitude and area measurements? An increase in individual axon response areas during HFS might contribute to this discrepancy; this could happen if individual axon action potential durations increased, but amplitudes, when conduction failure did not occur, were unaffected. Regardless of the explanation for the discrepancy between amplitude and area measurements, both measurements revealed
clear functional differences between proximal and distal Schaffer collaterals.

Is Depression of Proximal Axon Responses an Artifact of Antidromic Stimulation?

Our comparisons of proximal and distal Schaffer collateral responses during both HFS and burst stimulation relied on measurements of action potentials conducted in the antidromic direction by the proximal axons. Although action potentials propagate by the same mechanisms in both orthodromic and antidromic directions, the effect of axon branching may not be symmetrical. When action potentials propagate through a branch point, the consequences of branching depend on the relative diameters of the axon on opposite sides of the branch point (Goldstein and Rall 1974). Conduction is more reliable when crossing a branch point from a larger diameter into a smaller diameter and is less reliable when crossing from a small diameter into a larger diameter. In addition, asymmetrical effects are expected depending on conduction direction along tapering axons. Although anatomical studies have demonstrated extensive branching in CA3 pyramidal cell axons and also that these axons taper in diameter from the initial segment (largest diameter) to more distal portions (smaller diameter) (Ishizuka et al. 1990; Li et al. 1994; Wittner et al. 2007), we are not aware of any studies with sufficient detail to predict whether or not there should be significant effects of conduction direction (orthodromic vs. antidromic). In the absence of detailed anatomical information, it seemed possible that conduction by proximal Schaffer collaterals might proceed with different efficacy in antidromic vs. orthodromic directions. Moreover, we were concerned that apparent lack of a hyperexcitable period in our analysis of proximal axon function might be an artifact of our reliance on antidromic conduction. We tested these possibilities by reversing the normal locations of our stimulating and recording electrodes so that we activated Schaffer collaterals near the cell bodies and recorded fiber volleys conducted in the orthodromic direction by the proximal portions of the axons. Extracellular fiber volleys recorded from proximal Schaffer collaterals (orthodromic conduction) showed essentially the same pattern of change during both HFS and burst stimulation as did population spikes recorded from CA3 pyramidal cell bodies (antidromic conduction), indicating that our findings are not an artifact resulting from antidromic conduction.

Possible Mechanisms of Depression and Hyperexcitability

HFS increases extracellular potassium concentration ([K+]/O) and also decreases extracellular sodium and calcium concentrations (reviewed in Heinemann et al. 1990). Manipulation of extracellular potassium, by perfusing slices with ACSF containing altered potassium concentration, alters Schaffer collateral excitability, with small increases in [K+]/O enhancing excitability but larger [K+]/O increases depressing excitability (Meeks and Mennerick 2004; Meeks et al. 2005; Poolos et al. 1987). Because extracellular ion concentrations change slowly during intense activity (Heinemann et al. 1990), it seems possible that the excitability increase we observed in distal Schaffer collaterals occurs as a consequence of an initial, relatively small increase in [K+]/O. A small increase in [K+]/O would slightly depolarize the axon membrane, and this might enhance excitability by reducing the stimulation level needed to reach threshold. With continued stimulation and further increase in [K+]/O, axon membrane potential would be more strongly depolarized, promoting sodium channel inactivation; a reduced rate of spike repolarization and reduced amplitude afterhyperpolarization would contribute to greater sodium channel inactivation.

Although progressive changes in [K+]/O during HFS and burst stimulation could produce biphasic changes in excitability (increase followed by decrease), this does not explain the differences we have observed between proximal and distal portions of the Schaffer collateral axons because similar changes in [K+]/O should occur along the entire axon. Differences in morphology between proximal and distal axon portions might produce a different response to altered ion concentration. Although CA3 pyramidal neuron axon morphology varies with distance from the cell body, with smaller axon diameter at greater distances (Ishizuka et al. 1990), this difference alone should lead to more rapid changes in distal axons attributable to a larger ratio of surface area to volume, rather than to qualitatively different responses (monophasic depression in proximal axons, biphasic hyperexcitability/depression in distal axons). Alternatively, regional variations in ion conductances attributable to differences in channel density or localization could produce differences in sensitivity to small changes in [K+]/O (or small changes in membrane potential) between proximal and distal Schaffer collaterals. For example, Kv2.1 and Kv7.2/7.3 are present at high density in initial segments of hippocampal pyramidal neurons (Klinger et al. 2011; Sarmiere et al. 2008). Similarly, Nav1.6 is concentrated in initial segments, whereas Nav1.2 is more generally distributed (Jarnot and Corbett 2006; Lorincz and Nusser 2010; Royeck et al. 2008).

Studies by Soleng et al. (2003a, 2004) demonstrated that the response of Schaffer collaterals to repetitive activation is shaped by the hyperpolarization-activated current (Ih), suggesting another possible mechanism to explain activity-dependent changes in excitability. Interestingly, our whole-cell recordings revealed hyperpolarization of the resting potential, but the magnitude of change was relatively small (∼5 mV) and was only observed during the lower frequencies that we examined (10 or 20 Hz). The prior studies examined stimulation frequencies an order of magnitude lower than we used (1–5 Hz in Soleng et al. 2003a compared with 10–100 Hz in the present study) or used much shorter stimulation durations (paired pulses in Soleng et al. 2004 compared with trains of 160 stimuli in this study). In addition, the relevance of resting potential changes recorded at the soma for axonal resting potential is unclear because it has not been possible to make membrane potential recordings from Schaffer collaterals for comparison with somatic recordings. Finally, for Ih to explain the differences in function that we observed between proximal and distal regions of Schaffer collaterals, hyperpolarization-activated cyclic-nucleotide-gated channels would need to be differentially distributed, or regulated, along the axon membrane.

Experimental investigation of these possible mechanisms will be difficult because of the very small diameter of distal Schaffer collateral axons. Furthermore, any candidate mechanism must offer an explanation of all experimental observations, including the complex effect of burst interval on within-
burst excitability changes (decreased excitability within bursts during repetitive burst stimulation at a 100-ms interval but increased excitability at longer intervals). Given this complexity, it seems likely that multiple mechanisms, with different kinetics or voltage dependence, must interact to alter excitability.

Possible Functions of Depression and Hyperexcitability

Hippocampal pyramidal neurons do not normally fire long trains of action potentials (Albensi et al. 2007) but rather fire single action potentials at relatively low frequency or fire short, high-frequency bursts that may be repeated at delta-theta frequencies (Kandel and Spencer 1961; Mizuseki et al. 2012; Ranck 1973; Tropp Sneider et al. 2006). We suggest that activity-dependent changes in Schaffer collateral excitability could serve two functions. First, decreased excitability may have a filtering function, acting to prevent successful axonal conduction of abnormal firing, either sustained high-frequency firing or prolonged burst firing. Second, hyperexcitability of distal portions of Schaffer collateral axons could ensure successful conduction of action potentials through the large and elaborately branched axon arbor (Li et al. 1994) formed by the distal processes of CA3 Schaffer collateral axons. Distal Schaffer collaterals are very small in diameter and are therefore vulnerable to depressing effects of activity-dependent increase in [K⁺]₀. In addition, axonal branch points, which are numerous in the Schaffer collateral axon arbor, are likely sites for conduction failure (Goldstein and Rall 1974; Grossman et al. 1979; Stoney 1990). We suggest that hyperexcitability may serve to counteract a tendency to depression and allow successful propagation of short-duration, high-frequency firing as occurs in the normal burst firing of CA3 pyramidal neurons.

Relevance to LTP

The superiority of burst stimulation compared with HFS for LTP induction was demonstrated many years ago (Larson et al. 1986). Among the factors contributing to the superiority of burst stimulation are differences in short-term synaptic plasticity (depression and facilitation) between HFS and burst stimulation (Grover et al. 2009). During HFS, excitatory post-synaptic potentials (EPSPs) depress, resulting in relatively weak postsynaptic depolarization, whereas, during burst stimulation, EPSPs depress very little or may even facilitate, especially as burst intervals are increased from 200 to 1,000 ms (Grover et al. 2009). As we recently showed (Kim et al. 2012), decreased excitability of Schaffer collaterals during HFS contributes to EPSP depression, accounting at least in part for the relative ineffectiveness of HFS for LTP induction. As we show here, Schaffer collateral function is better maintained during burst stimulation compared with HFS. Moreover, Schaffer collateral function is best maintained at the burst intervals (500 and 1,000 ms) that are most favorable for LTP induction (Grover et al. 2009). These differences in Schaffer collateral function may help explain why EPSP amplitudes remain much larger during burst stimulation compared with HFS and help to explain the superiority of burst stimulation for LTP induction. The relevance of activity-dependent Schaffer collateral excitability to LTP is consistent with the functions for these activity-dependent changes that we proposed above. Schaffer collateral depression during non-physiological HFS may act to limit (filter) signal transmission from CA3 to CA1 neurons, resulting in reduced postsynaptic depolarization and relatively poor LTP induction. In contrast, maintenance of Schaffer collateral function during burst stimulation could allow for effective transmission from CA3 to CA1 neurons, greater postsynaptic depolarization, and enhanced LTP induction.


Sarmiere PD, Weigle CM, Tamkun MM. The Kv2.1 K+ channel targets to the axon initial segment of hippocampal and cortical neurons in culture and in situ. BMC Neurosci 9: 112, 2008.


