Cellular Mechanisms Preventing Sustained Activation of Cortex During Subcortical High-Frequency Stimulation

Karl J. Iremonger, Trent R. Anderson, Bin Hu, and Zelma H. T. Kiss

Department of Clinical Neurosciences, Hotchkiss Brain Institute, University of Calgary, Calgary, Canada

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INTRODUCTION

High-frequency electrical stimulation of axonal pathways in the CNS has been used chronically to treat neurological and psychiatric disorders (Mayberg et al. 2005; Nuttin et al. 2003) and adopted as a neurophysiological tool to map the brain and probe sensorimotor deficits in the operating room (Garonzik et al. 2002; Thiebaut de Schotten et al. 2005). Although axonal stimulation is thought to elicit repetitive discharges in the pathways stimulated (McIntyre et al. 2004), clinically such high-frequency stimulation instead reduces abnormal function, without obvious influence on normal motor or cognitive functions (Mayberg et al. 2005; O’Suilleabhain et al. 2003). For example, a pilot study of deep brain stimulation (DBS) applied to white matter tracts adjacent to the subgenual cingulate gyrus alleviated refractory depression in some patients, but did not induce other psychomotor symptoms (Mayberg et al. 2005). Furthermore, thalamic DBS for tremor does not interfere with motor control (Chen et al. 2005; Flament et al. 2002; Lee and Kondziolka 2005; Takahashi et al. 1998), even though DBS should produce excitation of thalamocortical axons that project to the motor cortex (McIntyre et al. 2004).

To explain the neural basis of these phenomena, we recently proposed a model of functional axonal deafferentation. High-frequency stimulation (HFS) of the rat motor thalamus invariably caused a strong depression of excitatory glutamatergic synaptic transmission (Anderson et al. 2004, 2006). The synaptic inhibition occurred rapidly, was reversible, and appeared to result from rapid depletion of the neurotransmitter pool in presynaptic terminals, leading to a deprivation of synaptic drive to postsynaptic cells (Anderson et al. 2006).

The main objective of the current study was to verify the axonal deafferentation hypothesis in the neocortex. We were particularly interested in afferents to the motor cortex because the effects of DBS are frequently studied in this fiber pathway both clinically (Haslinger et al. 2003; Molnar et al. 2005; Perlmutter et al. 2002) and with computer simulations (McIntyre et al. 2004). To this end, we prepared rat brain slices that maintain a large part of the subcortical white matter input to the cortex but without direct connections from the thalamus. An advantage of this approach is that the effects of axonal stimulation on cortical neurons can be examined selectively and in the absence of thalamic influences. We hypothesized that high-frequency stimulation of afferent fibers to cortical neurons would not faithfully elicit postsynaptic excitation or affect ongoing cortical network activity. Our results show that in motor neurons, high-frequency axonal stimulation induces an initial postsynaptic depolarization that is followed by synaptic depression. This depression is transient and specific to the stimulated projections. Preliminary results were previously reported in abstract form (Iremonger et al. 2005).

METHODS

Slice preparation

Male Sprague–Dawley rats (p21–p40) (Charles River, Saint-Constant, Quebec, Canada) were anesthetized with halothane and decapitated. All protocols received approval from the University of Calgary Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care. For neocortical recordings, coronal slices containing the primary motor cortex (M1) were prepared, whereas for thalamic recordings, sagittal slices containing the ventrolateral (VL) thalamus were used. Slices (420–450 μm) were cut on a vibrotome (Leica VT 1000S, Leica-Leitz, Wetzlar, Germany) in ice-cold oxygenated artificial cerebrospinal fluid (aCSF), consisting of (in mM): 125 NaCl, 3.25 KCl, 1.5 CaCl2, 1.5 MgCl2, 25 NaHCO3, and 25 d-glucose. Slices were then incubated at 30°C for 1 h, after which they were kept at room temperature.

Whole cell recordings

During experiments, slices were continually superfused with 30–32°C oxygenated aCSF. M1 and VL thalami were identified visually (Paxinos and Watson 1998) under a dissecting microscope and recordings were obtained from either the center of cortical layers (II/III, V, VI) or the center of the nucleus. Whole cell current and single-electrode voltage-clamp recordings were performed with an Axo
and digitized with pClamp 9 (Axon Instruments) at 10 kHz. Cordings were low-pass filtered at 10 kHz (1 kHz for voltage clamp) potentials could be elicited with intracellular current injection. Re-

insic pulses. Clinically, subcortical DBS involves pulse widths of 60 –90 

they had a stable resting membrane potential 

Once in whole cell configuration, cells were kept for analysis only if 

in some experiments to identify the exact location of recorded cells. 

Laboratories, Burlingame, CA) was dissolved in the electrode solution 

about 10 mV and was not subtracted. Neurobiotin (0.1%, Vector 

2 MgCl₂, 4.2 K₂ATP, and 0.4 Na₃GTP (pH 7.2 with KOH), and had a 

resistance of 4–9 MΩ. The measured liquid junction potential was 

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M1, primary motor cortex; S1, somatosensory cortex. Figure adapted from Paxinos and Watson 

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Stimulation applied

For all stimulation paradigms, electrodes were either bipolar tungsten (0.1-mm diameter, 0.75-mm pole separation, 22–27 kΩ) or concentric bipolar (NEX-100, Rhodes Medical Instruments, Woodland Hills, CA). Stimulation was delivered by a constant-current stimulus isolator (A360 or A365, World Precision Instruments, Sarasota, FL) and consisted of 90-μs pulse width, square-wave monophasic pulses. Clinically, subcortical DBS involves pulse widths of 60–90 μs and frequencies of 125–185 Hz. The stimulation frequency used to mimic DBS in this study was 125 Hz and is referred to as HFS. Although we also examined a range of stimulation frequencies (100–300 Hz) in some experiments, the term HFS refers only to 125-Hz stimulation. 

During high-frequency stimulation, “blanking” pulses (0.1–1 ms) were triggered with each stimulation pulse (A-M Systems 2100; A-M Systems, Carlsborg, WA) to reduce stimulus artifact. We previously reported the methods used for “blanking” as well as the current density and spread induced by HFS (Anderson et al. 2004).

For M1 recordings, the stimulating electrode was placed in the external capsule under the recording electrode, similar to subcortical white matter stimulation applied clinically (Abelson et al. 2005; Mayberg et al. 2005; Nuttin et al. 2003) (Fig. 1A). The stimulation intensity used was such as to evoke a reliable excitatory postsynaptic potential (EPSP) or current (EPSC) in the cortical neuron. To avoid compound EPSP responses, we used stimulation currents that produced small-amplitude EPSPs (Deschenes et al. 1982). For characterization of responses to HFS, recordings were performed in layers II/III, V, and VI, layers to which thalamocortical fibers are known to project.

To determine whether axonal conduction failure could contribute to the neuronal responses seen during HFS, different stimulation frequencies (10–300 Hz) were applied to axons of the internal capsule. Action potentials were recorded from VL thalamic neurons and confirmed to be antidromic by collision with somatically evoked spikes. Antidromic responses to different frequencies of stimulation were also recorded from layer V and layer VI cells in M1.

The frequency used for clinical HFS is similar to that used to induce short- and long-term plasticity. To establish whether such clinically utilized thalamic HFS could induce long-term plasticity in M1, EPSCs were recorded in layer V neurons at 1-min intervals after 30 s or 5 min of HFS. Only one train of HFS was delivered per cell per slice. To determine the time course of short-term plasticity, single EPSCs were evoked at different intervals (20 ms to 120 s) after 3-s trains of HFS. At least 3 min was allowed between each 3-s HFS train. The amplitude of all EPSCs were normalized to control EPSCs evoked before stimulation.

Finally, EPSPs were evoked in M1 from intracortical pathways by stimulating the superficial layers of M1 and somatosensory cortex (S1). These afferent pathways were stimulated at 0.2 Hz before, during, and after HFS of the external capsule. Changes in layer V EPSP amplitude were used to determine whether HFS of one pathway altered the response from nonstimulated projections.

FIG. 1. Postsynaptic responses of primary motor cortex (M1) neurons to high-frequency stimulation (HFS). A: schematic of the slice preparation illustrating stimulating and recording electrode locations. Abbreviations: M1, primary motor cortex; S1, somatosensory cortex. Figure adapted from Paxinos and Watson (1998). B: membrane responses to HFS in current- (top) and voltage clamp (bottom), both showing an initial transient depolarization/current that quickly returns to baseline. Traces are from 2 different layer V cells. C: amplitude of initial depolarization in response to HFS increases with stimulation frequency ≥100 Hz, after which increasing the stimulation frequency further does not result in a larger depolarization. Depolarization is normalized to the initial depolarization obtained with 10-Hz stimulation. Fitted curve is a single exponential with a τ ~ 40 Hz. D: HFS-induced initial depolarization was reduced by 91% in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50% in the presence of d-(-)-2-amino-5-phosphonopentanoic acid (AP-5). HFS-induced depolarization was totally abolished when both DNQX and AP-5 were applied together. *P < 0.01.
Pharmacological compounds

All experimental drugs were bath applied. d(-)-2-Amino-5-phospho-
D-(-)-2-Amino-5-phosphonopentanoic acid (AP-5, 50 μM, Sigma-Aldrich) and 6,7-dinitro-
quinoxaline-2,3-dione (DNQX, 10 μM, Sigma-Aldrich) were applied
to determine the relative contribution of N-methyl-D-aspartate
(NMDA) and non-NMDA receptor currents to the observed
responses, respectively. The γ-aminobutyric acid (GABA) antagonists,
picrotoxin (PTX, 50 μM, Sigma-Aldrich) and (2S)-3-[(1S)-1-(3,4-
dichlorophenyl)ethyl][amino-2-hydroxypropyl] (phenylmethyl) phos-
phonic acid (CGP-55845, 1 μM, Tocris), were used to investigate the
degree to which intracortical inhibition shaped the observed re-
sponses. Cyclothiazide (CTZ, 100 μM, Tocris) was used to determine
whether desensitization of α-aminoadonxy-5-methyl-4-isox-
arolepropionic acid (AMPA) receptors limited the excitation resulting
from HFS.

Data analysis

All data were analyzed using Clampfit 9 (Axon Instruments) and
Spike2 (Cambridge Electronic Design). Data are reported as means
SE unless otherwise stated. Statistical significance (P < 0.05) was
determined using ANOVA with Tukey’s multiple-comparison test or
Student’s t-test (Prism, GraphPad software, San Diego, CA).

RESULTS

HFS responses were recorded from a total of 168 neurons in
the primary motor cortex (M1), of which 124 were from layer
V, 20 from layer II/III, and 21 from layer VI. Of these cells, 56
were classified as intrinsically bursting (IB), 93 as regular
spiking (RS), 14 as chattering (Ch), and five as fast spiking
(FS) (Connors et al. 1982; Gray and McCormick 1996). Corti-
cal cells had an average resting membrane potential of
-66.5 ± 0.3 mV.

Characterization of the postsynaptic responses to HFS

Membrane responses to HFS were similar across different
cortical layers and for different cells types. All responses in
this study refer to those of RS, Ch, or IB neurons. Single
stimuli at the intensity used for HFS evoked EPSPs with an
amplitude of 1.6 ± 0.2 mV, similar to that reported by
Deschenes et al. (1982).

At resting membrane potentials, a 30-s train of HFS induced
an initial transient depolarization or inward current (8.9 ± 4.0
mV or 97.1 ± 11.3 pA, respectively; Fig. 1B). The amplitude
of the initial depolarization was larger at higher frequencies
of stimulation, but plateaued at 100 Hz (n = 16, Fig. 1C). In 88%
of cells, spikes were not evoked at the onset of HFS. The mean
stimulation intensity used was 3.1 ± 0.2 mA, although the
current delivered to the tissue was estimated to be closer to 310
μA as previously explained (Anderson et al. 2004).
The input resistance before HFS was 49.6 ± 5.8 MΩ. Input resistance could not be measured during the initial HFS depolarization, although it did not significantly change either during or after the prolonged (steady-state) HFS response (n = 14, P > 0.05; data not shown). In the presence of NMDA blockade, the amplitude of the initial depolarization to 125-Hz stimulation was reduced by nearly 50% (n = 6), whereas in the presence of AMPA and kainate blockade, the initial depolarization was reduced by roughly 91% (n = 7) (Fig. 1D).

Cortical neurons fire normally during HFS

In this set of experiments, we examined whether HFS can influence the overall membrane excitability of cortical motor neurons. Because cortical cells are generally silent in vitro, we depolarized cells with intracellular current to evoke spontaneous firing (n = 45). The initial response to HFS was no change or only a small increase in firing frequency (n = 31) (Fig. 2Ai). In layer V and layer II/III cells, this transiently elevated firing rate lasted from hundreds of milliseconds to about 5 s. After this, the cell returned to prestimulation firing frequency for the remainder of the HFS train. The mean firing frequency of layer V and layer II/III neurons before HFS was 3.8 ± 0.5 and 4.4 ± 0.5 Hz, respectively. When mean firing frequency was compared for the 30 s before, during, and after HFS, no difference was found (P > 0.05, Fig. 2B, i and ii). In layer VI cells, a slightly increased firing frequency was retained for the duration of stimulation (Fig. 2Biit). In 14 of 45 cells, the initial response to HFS was different. At the onset, a small hyperpolarization of the membrane potential and a reduction or cessation of firing lasted briefly (~3 s) before the cell resumed its prestimulation firing rate (Fig. 2Aiit). This initial hyperpolarization was more common in layer II/III and layer VI neurons (12/21) than in layer V neurons (2/24).

GABAergic interneurons limit the initial response to HFS

Thalamocortical projections are known to synapse onto both pyramidal cells and inhibitory interneurons (Agmon and Connors 1991; Gibson et al. 1999). To investigate whether the suppression of M1 excitation was mediated by GABAergic inhibition during the HFS train, whole cell recordings were obtained from layer V cells and HFS was delivered in the presence of GABA_A- and GABA_B-receptor antagonists (PTX and CGP-55845, respectively). With GABA_A blockade, the initial depolarization was much larger and triggered a burst of spikes (n = 6/6, Fig. 3, A, B, and D). However, the membrane potential again repolarized rapidly to baseline. With both GABA_A and GABA_B blocked, HFS induced one to five large depolarizations crowned by action potentials (n = 5/5, Fig. 3, C and D). However, after the cessation of these early events, the membrane potential again repolarized back to baseline levels.

Complete conduction failure does not occur in axons during HFS

In the VL thalamus, HFS responses were recorded from 10 cells, all of which exhibited a low-threshold spike (LTS) on release from a hyperpolarizing current step (Jahnsen and Llinas 1984). These cells had an average resting membrane potential of

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**FIG. 4.** Complete conduction block did not occur in axons during HFS. A: schematic of the slice preparation with location of stimulating and recording electrodes, adapted from Paxinos and Watson (1998). B: when cells were depolarized to evoke somatic spikes, spike collision between somatic and antidromic spikes occurred at short intervals. At stimulation frequencies ≤50 Hz, antidromic action potentials followed 1:1 with extracellular pulses. At higher stimulation frequencies, antidromic spikes could follow 1:1 only at the onset of stimulation. C: at the end of the 30s stimulation train (the last 600 ms duration shown), there was a progressive failure of antidromic spikes with increasing stimulation frequency (Dii), with evoked spike frequency plateauing between 50–70 Hz (Dii, n = 10). Antidromic failure and following frequency was measured as the average failure rate or frequency during the last 10 s of a 30 s train. There was also a broadening of antidromic spikes with increasing stimulation frequency (Ei, ii). Spike width was measured from the spike onset to halfway down the repolarizing side of the spike. All data was taken from the last spike of a 30 s train and normalized to the first spike of that same train.
−63.4 ± 0.7 mV. Antidromic action potentials were evoked by stimulation of the internal capsule (Fig. 4A) and identified by their short, invariable latency and collision with somatically evoked spikes (Fig. 4B). In six cells, synaptic blockers were added to the ACSF to eliminate any synaptic contamination of the antidromic responses (DNQX, PTX, n = 3; DNQX, AP-5, PTX, n = 3). No difference in the antidromic responses were observed, and thus data from experiments with and without synaptic blockers were pooled. The steady-state antidromic response was measured as the average failure rate or firing frequency during the last 10 s of a 30-s train. Antidromic action potentials followed 1:1 for frequencies ≤50 Hz (Fig. 4C). At frequencies >50 Hz there were significantly more failures and a broadening of action potentials (P < 0.05, Fig. 4, D and E). This effect became more pronounced at higher stimulation frequencies.

Subcortical stimulation was also performed to antidromically activate layer V and layer VI neurons in M1 (Fig. 1A). Only two of 14 layer V cells showed antidromic responses, the threshold for which was two- to threefold higher than that required to evoke EPSPs, and often longer stimulus durations were needed. This is consistent with thalamocortical stimulation preferentially evoking orthodromic, not antidromic responses in the cortex (Castro-Alamancos and Connors 1996; Ferster and Lindstrom 1983; Rose and Metherate 2001). In eight of 14 layer VI cells that did exhibit antidromic responses, the percentage of antidromic following to extracellular white matter stimulation decreased with stimulus frequencies >10 Hz. The maximum following frequency of antidromic action potentials peaked at about 30 Hz (data not shown).

Synaptic depression in M1 during HFS

Because conduction failure in thalamocortical axons could not completely explain the limited excitation during prolonged HFS, we next investigated the effect of stimulation frequency on synaptic depression in M1.

Subcortical stimulation applied at 10 Hz for 30 s produced EPSPs in layer V M1 neurons that initially showed a small degree of facilitation and by the end of the stimulus train very little depression. When 20 Hz was applied, there was a small summation of EPSPs at the onset of stimulation and a small depression of the responses by the end of the 30-s train. The membrane responses to ≥50 Hz displayed a transient depolarization at the onset of HFS with the membrane potential returning to baseline by the end of 30 s of stimulation (Fig. 5A). When the size of the 20th and 100th EPSCs was measured in a HFS train, a marked failure/depression of transmission was seen at frequencies ≥50 Hz (n = 9, Fig. 5, B and C). Furthermore, CTZ, which blocks AMPA-receptor desensitization, did not affect responses to HFS (n = 5, data not shown).

Short- and long-term plasticity with HFS

EPSCs were recorded from layer V M1 neurons before and after HFS trains of different duration (30 s and 5 min) were applied to the external capsule. The mean EPSC amplitude before HFS was 22.3 ± 1.6 pA. The responses during and after 30-s (n = 12) and 5-min (n = 8) HFS were similar, except the time course of recovery was slower after 5 min of HFS (Fig. 6, A–C). Generally there was a marked depression of transmission after the stimulus train (P < 0.05). The EPSC was not significantly different from control by 2 min after 30 s of HFS (P > 0.05), but took 6 min before the EPSC was back to control amplitude after 5 min of HFS (P > 0.05). Because depression of transmission during and immediately after HFS may be attributable to depletion of neurotransmitter (Stevens and Wesseling 1999), we plotted the recovery of EPSC amplitude immediately after (20 ms to 2 min) a 3-s HFS train (n = 15). The time course of recovery was well fitted (R² = 0.95) by a two-phase exponential with a fast τ of 120 ms and a slow τ of 8.6 s (Fig. 6D).

Intracortical synaptic responses are unaffected by subcortical HFS

Synaptic depression occurs during HFS of the external capsule, although M1 neurons receive many inputs from other cortical areas. Thus in this set of experiments, we examined
whether synaptic responses evoked from other cortical pathways are affected by subcortical HFS. We evoked EPSPs in layer V M1 neurons from stimulation of either layer I M1 \((n=6)\) or the superficial layers of S1 \((n=9, \text{Fig. 7A})\). These experiments were conducted in current clamp in case subcortical HFS influenced remote synaptic signaling by a voltage-dependent mechanism. EPSPs evoked before HFS from layer I M1 and S1 had an amplitude of 2.2 ± 0.3 and 2.7 ± 0.5 mV, respectively. There was no change in the amplitude of EPSPs evoked from remote locations during or after external capsule HFS (Fig. 7, B and C).

**DISCUSSION**

DBS was first thought to mediate its clinical benefit by inhibiting the stimulated nucleus because the effects of DBS are comparable to lesions (Benazzouz et al. 1995; Schuurman et al. 2000). Recent imaging studies of subcortical white matter DBS applied to the subgenual cingulate for depression have also shown reduced activation in cortical projection sites (Mayberg et al. 2005). However, experimental evidence and models of DBS suggest that efferent axons projecting from the nucleus are preferentially excited (Anderson et al. 2003; Hashimoto et al. 2003; Maurice et al. 2003; McIntyre et al. 2004; Windels et al. 2000). Yet, the hypothesis of efferent excitation cannot be readily reconciled with clinical observations, such as the lack of interference of thalamic DBS on motor control (Flament et al. 2002; O’Suilleabhain et al. 2003; Takahashi et al. 1998).

Responses to HFS are transient

At the onset of HFS, cortical cells showed only a transient depolarization, after which the membrane potential returned to prestimulation levels. When cells were injected with current to produce spontaneous firing, the only change in spike frequency was seen at the onset of HFS in most cases. Both the initial depolarization and change in spike frequency were transient and dependent on the frequency of stimulation applied, consistent with the notion that synaptic transmission shows marked frequency-dependent depression (Abbott et al. 1997). Whereas HFS at resting membrane potentials always evoked a depolarization, during spontaneous spiking HFS often did not produce a detectable depolarization and sometimes evoked a hyperpolarization. This is likely explained by an increased driving force for inhibitory currents and decreased drive for excitatory currents at depolarized membrane potentials.

Several lines of evidence suggest that HFS should result in sustained postsynaptic activation (Haslinger et al. 2003; McIntyre et al. 2004; Perlmutter et al. 2002). Below, we discuss possible reasons why prolonged activation of cortical cells does not occur during long trains of HFS.

**FIG. 6.** HFS-induced synaptic depression. A: overlying traces showing the control EPSC (thick gray trace) and EPSCs evoked at different times (20, 60, and 240 s) after a 30-s train of HFS (thin black trace). B and C: recovery from synaptic depression after 30-s HFS was faster than that after 5-min HFS. Slow time course of recovery of the EPSC after 5-min HFS was fitted \((R^2 = 0.91)\) with a single-phase exponential with a \(\tau\) of about 2.1 min. D: short-term recovery of the mean EPSC after a 3-s HFS train. Recovery was fitted with an exponential with a \(\tau\) of 120 ms and 8.6 s, respectively. Inset: expanded time course of recovery from 0 to 20 s. All cells were voltage clamped at −70 mV and all data were normalized to the mean EPSC amplitude measured before each HFS train.
Mechanisms preventing prolonged cortical excitation during HFS

GABAERGIC INHIBITION. In theory, prolonged excitation could be prevented by increased cortical inhibition through disynaptic activation of inhibitory interneurons. Inhibitory interneurons may sustain higher firing frequencies (Martina and Jonas 1997) and are less susceptible than glutamatergic neurons to conduction failure under conditions of elevated extracellular K⁺ (Meeks and Mennerick 2004). For these reasons we expected that increased intracortical inhibition could provide a plausible explanation for the limited excitation during HFS. However, GABAergic inhibition limited only the initial excitation resulting from HFS (Fig. 3) and did not prevent excitation after the first 8 s of HFS.

AXONAL FAILURE. Conduction failure allows axons to perform signal filtering and mediate plasticity (Debanne 2004). Therefore conduction block of axonal transmission, perhaps arising from elevated extracellular K⁺ (Bikson et al. 2001), could explain the observed responses in cortex to HFS. Antidromically activated thalamocortical fibers failed to faithfully conduct action potentials at stimulation frequencies >50 Hz, although complete conduction block did not occur. When interpreting these data, several caveats must be considered. First, the refractory period of the axon and soma may be different (Swadlow 1982) and, second, axonal action potentials may fail at branch points of thalamocortical fibers as the result of a lower conduction safety factor (Grossman et al. 1979). Finally, changes in spike initiation cannot be distinguished from changes in spike conduction. Our data suggest that complete conduction block does not occur at high frequencies of stimulation, but that some axonal conduction failure does take place.

SYNAPTIC DEPRESSION. Neither GABAergic inhibition nor conduction block can fully explain the lack of prolonged cortical excitation during HFS; thus the most likely alternative for the observed response to HFS is depletion of neurotransmitter. Several lines of evidence indicate that depletion of synaptic vesicles may account for the depression during and after HFS: 1) Recovery from depletion followed a typical time course as previously reported for neurotransmitter depletion (Dittman and Regehr 1998; Sakaba and Neher 2001; Stevens and Wesseling 1999; Wu and Borst 1999). 2) Recovery from depletion was prolonged by longer durations of stimulation (Pyle et al. 2000; Stevens and Wesseling 1999). 3) Depression of transmission during prolonged HFS was not affected by application of CTZ, and thus the observed depression was unlikely attributable to AMPA-receptor desensitization. 4) Depression was limited only to the stimulated pathway.

Although we provide substantial evidence supporting depletion of neurotransmitter during HFS, we cannot definitively

FIG. 7. Synaptic depression was limited to the pathway receiving HFS. A: schematic showing the positions of the stimulating and recording electrodes for dual stimulation experiments (note: only one of the gray stimulating electrodes was used per slice). B: stimulation was applied to layer I of M1 at 0.2 Hz, before, during, and after HFS of the external capsule. Expanded traces show EPSPs evoked from layer I. There was no change in the amplitude of EPSPs recorded in layer V evoked from the second stimulating electrode placed either in layer I M1 or S1 during or after 30 s of white matter HFS (Ci, ii). All EPSPs were normalized to the mean amplitude before HFS.
prove that it occurs as a result of limitations inherent to our methods. Urbano et al. (2002) suggested that neurotransmitter depletion was responsible for shaping the cortical response to ventrobasal thalamic stimulation in slice based on imaging the cortical response to short (600-ms) trains of HFS. Our study provides not only supporting evidence, but also indicates that mechanisms such as receptor desensitization or intracortical inhibition cannot account for the depression seen during HFS.

HFS of the stimulated pathway results in “functional deafferentation” because there is no continued postsynaptic response during prolonged stimulation. Cortical neurons receive many inputs from other cortical areas not activated by the HFS train; however, it is uncertain as to how synaptic responses evoked from other cortical pathways are affected by subcortical HFS. When single EPSPs were evoked from remote sources, responses were not affected by HFS of the external capsule. This suggests that despite homosynaptic depression of subcortical input during HFS, cortical neurons still retain their capacity to respond to other nonstimulated synaptic afferents.

Functional implications

Although axonal stimulation is thought to elicit repetitive discharges in the stimulated pathway (McIntyre et al. 2004), a common outcome of such high-frequency stimulation is often a reduction of abnormal function without obvious influence on other motor or cognitive functions. In this study, we have provided a plausible explanation for this paradox in that prolonged postsynaptic excitation resulting from subcortical axonal HFS is limited by homosynaptic depression. This depression likely explains why thalamic DBS does not produce abnormal movements and may also explain the ability of DBS to normalize the cortical overactivity associated with conditions such as depression (Mayberg et al. 2005) and obsessive–compulsive disorder (Nuttin et al. 2003). Because axons have the lowest excitation threshold in response to extracellular stimulation, it is likely that all forms of therapeutic brain stimulation result in some form of axonal activation. Therefore conduction failure and synaptic depression are likely important contributing factors to DBS in, for example, the subthalamic nucleus, in addition to the reported therapeutic mechanism (Do and Bean 2003; Garcia et al. 2005) that relies on modulation of intrinsic membrane conductances.

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