Resonant Antidromic Cortical Circuit Activation as a Consequence of High-Frequency Subthalamic Deep-Brain Stimulation

S. Li, G. W. Arbuthnott, M. J. Jutras, J. A. Goldberg, and D. Jaeger

Biology Department, Emory University, Atlanta, Georgia; Division of Neuroscience, University of Edinburgh, Edinburgh, United Kingdom; and Department of Biology, University of Texas at San Antonio, San Antonio, Texas

Submitted 19 July 2007; accepted in final form 8 October 2007

Li S, Arbuthnott GW, Jutras MJ, Goldberg JA, Jaeger D. Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation. J Neurophysiol 98: 3525–3537, 2007. First published October 10, 2007; doi:10.1152/jn.00808.2007. Deep brain stimulation (DBS) is an effective treatment of Parkinson’s disease (PD) for many patients. The most effective stimulation consists of high-frequency biphasic stimulation pulses around 130 Hz delivered between two active sites of an implanted depth electrode to the subthalamic nucleus (STN-DBS). Multiple studies have shown that a key effect of STN-DBS that correlates well with clinical outcome is the reduction of synchronous and oscillatory activity in cortical and basal ganglia networks. We hypothesized that antidromic cortical activation may provide an underlying mechanism responsible for this effect, because stimulation is usually performed in proximity to cortical efferent pathways. We show with intracellular cortical recordings in rats that STN-DBS did in fact lead to antidromic spiking of deep layer cortical neurons. Furthermore, antidromic spikes triggered a dampened oscillation of local field potentials in cortex with a resonant frequency around 120 Hz. The amplitude of antidromic activation was significantly correlated with an observed suppression of slow wave and beta band activity during STN-DBS. These findings were seen in ketamine-xylazine or isoflurane anesthesia in both normal and 6-hydroxydopamine (6-OHDA)–lesioned rats. Thus antidromic resonant activation of cortical microcircuits may make an important contribution toward counteracting the overly synchronous and oscillatory activity characteristic of cortical activity in PD.

INTRODUCTION

Much research has focused on how an implanted depth electrode to the subthalamic nucleus (STN-DBS) may modulate the output of the basal ganglia (Breit et al. 2004; Dostrovsky and Lozano 2002). Although some proposed mechanisms such as depolarization block of STN projection neurons or stimulation of inhibitory inputs to STN may reduce STN activity, other proposed mechanisms such as the continuous high-frequency output of STN axons (McIntyre et al. 2004) would presumably act in the opposite way. The multiple possibilities—and experimental support—for both increased and decreased neuronal activity caused by STN-DBS have cast doubt on a straightforward explanation of the clinical benefits of STN-DBS mechanism through these effects. Recently, it has become evident that increased synchronization and the appearance of oscillations in the activity patterns of populations of neurons—particularly in the subthalamic nucleus and globus pallidus, but also in motor cortical networks—are salient aspects of Parkinsonism (Goldberg et al. 2002). Pathological synchronization has been observed in human Parkinson’s disease (PD) patients (Priori et al. 2004), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate studies (Raz et al. 2000), and rodents with 6-hydroxydopamine (6-OHDA) lesions (Sharott et al. 2005). STN-DBS has been found to reduce pathological levels of synchronization in PD, and this reduction correlates with improvements of disease symptoms (Silberstein et al. 2005). However, the mechanisms by which STN-DBS is effective in reducing synchronized activity patterns are still unclear.

Some findings suggest that cerebral cortex is closely involved in the mediation of STN-DBS effects. Single pulse stimuli in the STN produce evoked potentials in frontal cortex (Ashby et al. 2001; Baker et al. 2002) and increase the cortical excitability at short latencies (Hanajima et al. 2004). Furthermore, direct motor cortex stimulation was found to alleviate Parkinsonian symptoms (Drouot et al. 2004), although it is generally not as effective as STN-DBS. An effect of STN-DBS not mediated by basal ganglia outflow is also suggested by the effectiveness of STN-DBS in patients with previous thalamotomy (Fraix et al. 2005; Goto et al. 2004). These findings are consistent with a reduction of pathological cortical synchronicity present in PD, particularly in the beta band frequency (Brown and Williams 2005; Kuhn et al. 2006), through direct cortical stimulation or antidromic activation in STN-DBS.

In this study, we pursued the hypothesis that an important functional pathway of STN-DBS may be through antidromic cortical circuit activation. Stimulation electrodes placed in the STN are closely apposed to large cortical axons projecting to the STN, and axons are likely to be stimulated by DBS (McIntyre et al. 2004), resulting in short-latency cortical responses (Baker et al. 2002). In the rat, STN stimulation led to antidromic activation of STN projecting neurons in layer V of prefrontal cortex at a latency of 2–9 ms (Maurice et al. 1998). However, a more dominant projection is given by layer V neurons from motor cortex (Canteras et al. 1990). These neurons are likely to be activated antidromically with STN-DBS, and through their rich axon collaterals in deep and superficial cortical layers (Cowan and Wilson 1994), this activation could affect cortical circuits in complex ways.

To show unequivocally a synaptic activation of cortical neurons through axon collaterals of antidromically activated neurons, intracellular recordings are required. We used ketamine-xylazine–anesthetized rats with or without unilateral
6-OHDA lesions to obtain such recordings, as well as local field potential (LFP) and EEG measurements of population activity. The baseline activity pattern in this preparation has been previously characterized, and consists of slow-wave activity (~2 Hz, which has an intracellular correlate of synchronized up- and down-state transitions (Mahon et al. 2001; Stern et al. 1997), which are shared by striatal (Stern et al. 1998) and pallidal (Goldberg et al. 2003) circuits. We find that antidromic activation of motor cortex is indeed a strong effect of STN-DBS and that this activation engages cortical microcircuits with a resonance frequency around 120 Hz.

METHODS

Electrophysiological recordings were made in 38 male Sprague-Dawley rats (300–380 g). Anesthesia in most animals (n = 32) was induced with halothane (Halocarbon Laboratories, River Edge, NJ) and maintained with a cocktail of ketamine (100 mg/kg, ip), xylazine (5.2 mg/kg, ip), and acepromazine (1 mg/kg, ip). Anesthesia levels were tested by withdrawal reflexes to a pinch of the rear paw and touch-up doses of the same cocktail were given when necessary, usually occurring at intervals of 30–60 min. A smaller set of animals (n = 6) was anesthetized with isoflurane using a vaporizer (901807, VetEquip). During surgery, 3% isoflurane was used, which was lowered to 1–2.5% during recordings, because the EEG was strongly suppressed with 3% isoflurane. A subset of rats (n = 3 for ketamine, and 4 for isoflurane) received a unilateral 6-OHDA lesion 2–4 wk before the recording session. Body temperature was maintained at 36–37°C, and electrocardiographic activity was monitored for changes in heart rate. In these rats, DBS electrodes were always placed in the STN ipsilateral to the lesion. To minimize the number of animals used, we used both hemispheres of most control rats, i.e., first obtained data from one side and subsequently from the other. Data from each hemisphere were considered as separate experiments. All use of rats complied with the National Institutes of Health guidelines on the care and use of laboratory animals and was approved by the local Institutional Animal Care and Use Committee.

In this study all, stereotaxic anterior-posterior (AP) coordinates were determined with reference to bregma, which was identified on the skull surface and calibrated manipulator movements were made to the target coordinates. The EEG was recorded in 15 rats with a 1-mm-diam stainless-steel skull screw inserted above the ipsilateral frontal cortex (AP +4.5; Lat 2.0), which corresponds to an anterior site in motor cortex (Neafsey 1990). EEG recordings were referenced to a chloridized silver wire implanted below the skin behind the neck. In most animals (n = 14), EEG was recorded from four sites simultaneously: frontal (fr), AP +4.5, Lat 2.0; motor cortex (mc1 and mc2), AP +2.0 Lat 3.0 and AP 0, Lat 2.0; and occipital (oc), AP −6.0 Lat 2.0. Recording of LFPs was performed at our mc1 location in a set of 12 animals (AP +2.0 Lat 3.0). In most of these animals (n = 10), EEG was recorded simultaneously. In three animals, LFP recordings were also obtained from our fr and mc2 sites. LFPs were recorded by glass pipette electrodes (TW120F-3, World Precision Instruments) filled with 2% Chicago sky blue (c-8679, Sigma) in 0.5 M NaCl, impedance 1–5 MΩ. EEG and LFP signals were band-pass filtered (1–100 Hz) and amplified (×1,000) before acquisition. All data were digitized at 10 KHz using a PCI-6052E AD card (National Instruments, Austin, TX) and recorded with a LabView interface (National Instruments) for further analysis. Intracellular potentials were recorded from 12 animals without concurrent LFP recordings at the ipsilateral motor cortex at mc1 (AP +2.0; Lat 3.0), which corresponds to the elbow area (Neafsey 1990), using glass electrodes (1B150F-6, World Precision Instruments) filled with 2 M KAc solution. Electrodes were pulled to a resistance between 70 and 100 MΩ.

Two kinds of stimulating electrodes were used for STN-DBS. One was commercially obtained from Rhodes Medical Instruments (SNE-100, 250-µm tip diam, 1-µm tip separation). Shielded custom-made bipolar electrodes were made to reduce stimulation artifacts. For these electrodes, two coated stainless steel wires (diameter, 0.081 mm; 3.944/9.70, Johnson Metthey Metals) were threaded through 30-gauge stainless steel tubing (HTX-30X-12, Small Parts) and glued together. The ends of the wires were machined at 45° angles with a dremel tool to form a combined sharp tip (resistance, 20–40 kΩ). Care was taken to avoid direct contact between the laterally exposed tips. STN stimuli were presented in 40- to 160-Hz trains, with pulse amplitudes between 0.08–0.26 mA. One kind of pulse waveform used consisted of biphasic square waves (0.1-ms positive pulses followed by 0.1-ms negative pulses with 0.1-ms intervals between pulses). A second pulse waveform, which is more commonly used in human DBS, was generated by 0.1-ms, 0.2-mA positive square pulses charge balanced by a subsequent 4-ms, 0.005-mA negative current.

6-OHDA lesion protocol

Rats (220–240 g) were anesthetized with isoflurane (1.5–3%) and pretreated with an intraperitoneal injection of 25 mg/kg desipramine (D3900, Sigma-Aldrich) and 50 mg/kg pargyline (P8013, Sigma-Aldrich) to prevent norepinephrine neuron degeneration and to maximize uptake into DA neurons, respectively. Rats were mounted in a stereotaxic frame, and the skull was opened under sterile conditions to inject 3 µl 6-OHDA (3 mg/ml in 0.9% saline and 0.02% ascorbate, H4381, Sigma-Aldrich) over 6 min at coordinates AP = −3.8 and Lat 1.5 at a depth of 8.0 mm to target the median forebrain bundle. Only rats that showed a positive turning test with apomorphine injection (0.1 mg/kg subcutaneously) 2 wk after lesion were taken for recordings. A turning test was judged positive when >50 contralateral turns were observed within 15 min after the apomorphine took effect. The actual numbers of contralateral turns observed in positive tests ranged from 55 to 100, whereas only 0–4 ipsilateral turns were seen. Two rats showed <50 turns and were euthanized.

Histology

After experiments, an injection of 0.6 ml nembutal (50 mg/ml) was given intraperitoneally, and when all deep reflexes were abolished, rats were perfused transcardially with saline followed by 10% buffered formalin (Sigma, St. Louis, MO) with 15% sucrose. The fixed brains were transferred to buffered formalin with 30% sucrose and incubated for 12 h before sectioning. STN stimulation sites were verified by placing lesions through the active sites of the DBS electrode (100 mA for 10 s) and subsequent histological reconstruction (Fig. 5). The locations of the cortical recordings were labeled by electrophoretical ejection of Chicago sky blue from the recording electrode. To visualize nuclei, brain sections were counterstained with cresyl violet. Sections of brains of 6-OHDA–lesioned animals containing the striatum and substantia nigra were also stained with mouse anti-tyrosine hydroxylase (MAB318, Chemicon) to reveal the successful degeneration of the dopaminergic nigrostriatal pathway.

Data analysis

All data analyses were conducted using Matlab (MathWorks, Natick, MA). DBS stimulation artifacts in the signals were removed before further analysis by constructing average artifact waveforms (typically of 1-ms duration) across trials for each stimulus in a train and subtracting these averaged artifacts from each trial. For each intracellular recording, a probability density distribution of the intracellular potential was used to compute up- and down-state transitions in membrane potential. This analysis was performed after removing action potential waveforms from the raw voltage trace by interpolating a straight line from the point at −1 ms to the point at +2 ms relative to each spike peak. A mixture-of-two-gaussian model was fit to this probability density distribution as described previously (Gold-
berg et al. 2003), and the parameters of the Gaussian curves were used to define location and duration of the up- and down-states in the membrane potential distribution. The delay of DBS triggered spike responses was calculated as the period from the onset of the preceding stimulus to the peak voltage of the spike. The size of EEG responses to individual DBS stimuli was calculated as the voltage difference between the peak voltage of the response and the voltage just before stimulus onset. Before this response analysis, a straight line was drawn between the voltages at the onsets of two successive stimuli, and this line was subtracted from the respective response segment to compensate for baseline shifts between stimuli. Power spectral densities (PSDs) of LFP and EEG signals were estimated by Welch’s method after down-sampling the analog data to 400 Hz. The trapezoid rule was used to calculate the integral of the PSD curves for particular frequency bands. Paired \( t \)-tests were used to assess the significance of differences in PSD integrals between baseline EEG (LFP) and EEG (LFP) during STN-DBS. Current source density (CSD) was calculated as the second derivative of the average STN-DBS response in LFPs with respect to electrode depth (Mitzdorf 1987).

**RESULTS**

**STN-DBS antidromically activates cortical neurons**

Successful stable intracellular recordings were obtained from 32 neurons in 12 rats in the motor cortex (AP: +2.0, Lat: 3.0) with sharp electrodes. During recording, 0.8- to 2-s trains of high-frequency STN-DBS were applied through a bipolar stimulation electrode implanted in the ipsilateral STN (AP: −3.3; Lat: 2.6; see METHODS). This stimulation led to an antidromic spike response in a subpopulation of neurons (5 of 32, 15.6%; Fig. 1, A and B). In antidromically activated cells, action potential initiation followed individual stimulation pulses at a latency of 2.0 ± 0.5 ms \( (n = 5 \text{ cells}; \text{Fig. 1C}) \). The

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Subthalamic nucleus deep brain stimulation (STN-DBS) resulted in direct antidromic spiking in 5 of 32 intracellular cortical recordings (top row) and affected the membrane potential dynamics of other neurons not antidromically activated (bottom row). A and I: examples of intracellular recording in the motor cortex with and without antidromic activation. Black bar indicates period of STN-DBS. Stimulation artifacts are removed. B and J: close-ups of A and I, respectively (before artifact removal). Arrow in B shows a spontaneous spike before stimulation with subsequent loss of an antidromic spike caused by collision. C and K: probability density function (histogram with area normalized to 1.0) of spike delays after STN-DBS pulses. In C, histogram shows a prominent peak at 1.6 ms, whereas in K, latencies are uniformly distributed, indicating the lack of antidromic activation. D and L: Normalized interspike intervals (ISIs) histogram during control period. E and M: histogram of ISI during DBS. In E, most ISIs were distributed at interstimulus interval of 8.2 ms. In the cell lacking antidromic spikes, distribution of ISIs was not obviously changed from the control (I), but the firing rate (FR) was increased. F–H and N–P: all-point histogram of membrane potential before, during, and after DBS. Two gaussians were used to fit the up-state and down-state distribution in the cell (solid line).
ISI distribution of the STN-DBS period thus reflected the stimulation frequency (Fig. 1E), whereas the ISI distribution during the control period before STN-DBS stimulation (Fig. 1D) showed lower-frequency irregular spiking. Antidromically activated neurons were all located deep in cortex (0.9–1.7 mm below dura), and were likely pyramidal projection neurons in layers V/VI. Antidromic spiking was only observed for a subset of stimulation pulses in the high-frequency train. In the first second of stimulation, the proportion of stimuli triggering antidromic responses scored over 10 repetitions of stimulus trains ranged from 17 to 96% for the five activated neurons. This low proportion of antidromic spiking at the soma could be caused by branch point failure of the antidromic spike for pyramidal tract neurons collateralizing to the STN or a failure of antidromic invasion of the soma from the axon initial segment (Chomiak and Hu 2007). In fact, spikelets indicative of the latter situation were observed in two antidromically activated neurons (Supplemental Fig. 1). It is important to note that each of the antidromically activated neurons likely makes hundreds of synapses through local axon collaterals, which could lead to a significant boosting of antidromic activity in the local circuitry despite the relatively small proportion of 16% directly activated neurons.

Indeed, the membrane potential and spiking behavior of neurons without direct antidromic activation were also modulated by STN-DBS (Fig. 1, I–P; n = 27 of 32 neurons). We assessed this modulation by constructing all-points histograms that characterize the up- and down-state characteristics of the slow wave activity in anesthesia (Goldberg et al. 2003). The up- and down-state membrane potential distributions of a sample of 15 neurons with a high recording quality (mean separation of up- and down-states in the control period of ≥5 mV) was analyzed for a modulation by STN-DBS (Fig. 1, N–P). We found that the proportion of time spent in the down-state was diminished in 11 and increased in 4 neurons during STN-DBS. The overall effect was a significant reduction of the down-state times during STN-DBS (paired t-test, P = 0.029, n = 15). In addition, the depolarization reached during the up-state as well as the hyperpolarization reached during remaining down-states tended to be diminished. Overall, the distance between mean up-state potential and mean down-state potential across the population of nonantidromically activated neurons was significantly decreased by an average of −2.9 mV (paired t-test, P = 0.0014, n = 15). One hypothesis to explain the underlying mechanism of this result is that antidromically stimulated neurons through their axon-collaterals activated excitatory and inhibitory local cortical networks, which dampened slow-wave activity in the anesthetized rodent. Such dampening of network oscillations through antidromic circuit activation could alter cortical dynamics dramatically, for example, by suppressing pathological synchronized oscillatory activity in PD. To examine this hypothesis, we next turned in our study to a direct assessment of cortical network activity during STN-DBS using EEG and LFP recordings. This technique complements intracellular recordings by assessing the average stimulated population activity in different cortical layers. By monitoring the response of population activity to stimulation across layers, we can address the hypothesis that fast antidromic activation of some deep layer cells spreads to influence cortical activation in cortical columns.

**STN-DBS–elicited EEG responses suggest resonant activation of cortical microcircuits**

Each STN-DBS stimulation pulse resulted in a transient positive deflection of the ipsilateral frontal EEG, which reached its peak at a latency of 2.6 ± 0.5 ms (n = 25; Fig. 2). This latency is slightly longer than that of antidromically evoked spikes, suggesting that the peak reflects postsynaptic currents flowing due to the activation of the rich collateral branch network (Cowan and Wilson 1994) of antidromically activated axons.

STN-DBS of different intensity and frequency was applied to characterize the dependence of the EEG response on stimulus parameters. An increase in stimulus amplitude from 0.08 to 0.24 mA generally led to a smooth increase in the amplitude of the fast EEG response (Fig. 2, A–C). In addition, a stimulus train onset response also became stronger, which consisted of an initial negative deflection spanning several stimulation pulses and a subsequent slowly decaying positive deflection (Fig. 2A). This train-onset response was not further studied because clinically effective STN-DBS typically is continuous. However, the presence of this response suggests that any stimulus designs using applications of multiple short stimulus trains would evoke distinct additional transient train-onset responses.

Stimulation at pulse frequencies increasing from 40 to 140 Hz in 20-Hz steps revealed a dependency of the fast single-pulse response peak on stimulation frequency with maximal response amplitude at 100- and 120-Hz stimulation (Fig. 2, D–F). At low stimulating frequencies, the single pulse response consisted of two separate response peaks at 2.8- and 11.5-ms latency, respectively (Fig. 2E). Thus for any stimulation frequency exceeding 1,000/11.5 ms corresponding to 87 Hz, the second response peak would occur after the subsequent stimulation pulse. A maximal overlap of the first response peak with the second peak of the preceding stimulus would be expected at a stimulation frequency of 1,000/(11.5–2.8), corresponding to 115 Hz. In fact, this frequency led to the maximal peak amplitude of the primary response. Hence, a resonant superimposition of the primary peak of a given stimulus with the secondary peak of the preceding stimulus explains the amplitude profile of the peak responses. The emergent resonant cortical responses at high stimulation frequencies present a candidate mechanism for the observed increased efficacy of STN-DBS in human patients at similar frequencies. It is thus of great interest to determine the intracortical mechanisms that might explain the high-frequency resonant behavior. We approached this question by using CSD analysis across cortical layers.

**CSD analysis reveals interlayer resonance in cortex caused by STN-DBS**

To record the cortical depth profile of the consequences of antidromic activation, a glass electrode was lowered into motor cortex, and LFPs were recorded for a batch of 10 STN-DBS trials every 0.2 mm along the track. A clear relationship between the spontaneous slow-wave activity of the adjacent surface EEG recording and LFPs was visible at all cortical layers (Fig. 3A). However, there was a phase reversal from in-phase to anti-phase in this relationship at 1.0- to 1.2-mm depth, which lies in layer V (Fig. 3D). In addition, fast spikes...
likely denoting thalamic spindle activity were most pronounced between 0.6 and 1.0 mm of depth (layer IV), whereas slow-wave activity showed the largest amplitude in the deepest layers (Fig. 3A).

To examine the responses of STN-DBS at different cortical depths, 2-s stimulation trains of 60 or 120 Hz were repeated 10 times at each depth, and the average response to 1,200 or 2,400 repetitions was calculated. The averaging across repeated stimuli eliminated noise and background large-amplitude slow-wave activity (Fig. 3A) while preserving the elicited smaller-amplitude responses of the EEG and LFP to each STN-DBS (Fig. 3B). A reversal between EEG and LFP waveforms between 1.0- and 1.2-mm depth was seen for stimulated responses (Fig. 3B), which corresponded to the depth of reversal for spontaneous activity described above (Fig. 3A). The change in potential waveform over recording depth was analyzed quantitatively using CSD analysis, which is calculated by the second spatial derivative along the vertical axis of the depth profile of responses. This analysis reveals the current flow between cortical layers and is commonly used to analyze stimulated activity in cortex (Mitzdorf 1987). The contour map depicting the spatial and temporal profile of stimulated activity (Fig. 3C) revealed a reverberating pattern of cortical activity induced by STN-DBS. Around 2.5 ms after the stimulus, deep layers showed a sudden transition from source to sink, which was likely caused by antidromic spike invasion and reflects local cellular depolarization. This depolarization (sink) moved toward more superficial layers over the next 5 ms, whereas the deep layers became a source again. In response to a single stimulus, the cycle of a deep sink moving toward more superficial layers repeated in a weaker form at 11–12 ms, thus indicating a dampened oscillatory activation of cortex. At a stimulation rate of 60 Hz, each subsequent stimulus reinitiated this oscillation on its third peak (Fig. 3C, left). At 120-Hz stimulation, a stronger oscillation was maintained, as each stimulus arrived in phase with the second wave of activity of the induced cortical oscillation (Fig. 3C, right). It is important to note that the early phase of each response is given by a superposition of the oscillatory response to the previous stimulus with a new wave of activation. The cellular elements providing current sources and sinks cannot be uniquely identified with this method and in particular the activity of inhibitory interneurons is usually not visible (Mitzdorf 1987). The high-frequency of this reverberation suggests that intracortical axon collaterals of layer V cells are the likeliest source of the activation patterns.

**Dampening of cortical oscillations in different frequency bands**

The suppression of oscillatory activity in cortex and STN has been hypothesized to be important in the clinical effectiveness of STN-DBS (Brown and Williams 2005; Kuhn et al. 2006). In ketamine-xylazine–anesthetized rats, cortical oscillations are apparent in multiple frequency bands. Most prominent are 1- to 2-Hz oscillations of cortico-thalamic slow-wave activity (Steriade et al. 1993), but frequencies ≈25 Hz are quite prominent in the power spectrum of EEG and LFP (Fig. 4). We analyzed the ability of STN-DBS in the anesthetized rat to dampen the power in different frequency bands: 0.5–4, 4–8, 8–12, and 12–20 Hz (Fig. 4). We found that the power of the layer V LFP in the low-frequency band (0.5–4 Hz) was significantly depressed during ipsilateral 120-Hz STN-DBS compared with the immediately preceding period (paired t-test, P < 0.05) in 7 of 13 hemispheres (typical example shown in Fig. 4A). Similarly, in 9 of 17 surface EEG recordings, this
frequency was significantly suppressed. In two of eight simultaneous LFP and EEG recordings, a significant suppression of power was seen only in either the surface EEG \((n = 1; \text{Fig. 4B})\) or the layer V LFP \((n = 1)\), suggesting a certain amount of layer specificity in the dampening of low-frequency activity. The power in the next higher-frequency bands was also reduced significantly in several animals in the LFP \((4–8 \text{ Hz}, 3 \text{ of 9 animals}; 8–12 \text{ Hz}, 4 \text{ of 9 animals}; 12–20 \text{ Hz}, 3 \text{ of 9 animals})\) and the EEG \((4–8 \text{ Hz}, 10 \text{ of 17 hemispheres}; 8–12 \text{ Hz}, 6 \text{ of 17 hemispheres}; 12–20 \text{ Hz}, 3 \text{ of 17 hemispheres})\). These data indicate that the suppression of oscillatory activity is present across all low frequencies and may be a general outcome of STN-DBS regardless of behavioral or disease state.

We examined the correlation between the size of individual stimulus responses and the suppression of cortical oscillations to determine whether an easily measured single stimulus response could predict functional outcome. We found that indeed there was a significant linear correlation between these two measures and that EEG response size accounted for 45% of the variability in the ability to suppress oscillations with a 120-Hz stimulus train (Fig. 5A). Because our short-latency EEG response peak was directly related to the antidromic cortical activation, this analysis also suggests that the ability to suppress oscillations with high-frequency stimulation is mediated at least in part through this antidromic activation.

The exact location of effective stimulation sites in or around the STN is of obvious clinical interest. When we plotted the location of our DBS sites in STN against EEG response sizes or the ability to suppress oscillations (Fig. 5, D and E), we noted that successful stimulation sites were present throughout the rostro-caudal extent of the STN and surrounding zona incerta. This is again consistent with an antidromic corticothalamic stimulation, which would strongly antidromically activate motor or premotor cortical areas from all of these locations (Nambu et al. 1996). Likewise, cortical antidromic activation shown with intracellular recordings (Fig. 5D) was found for stimulation sites in and outside of the STN proper. These sites also all showed significant EEG responses.

Multisite cortical recordings reveal widespread antidromic effects

To study the spread of the antidromic activation across cortical areas, multisite LFPs and EEGs were recorded simultaneously from four sites across ipsilateral cortex (see METHODS). As expected from earlier studies, spontaneous 1- to 2-Hz slow-wave activity in ketamine/xylazine anesthesia showed widespread synchronization between cortical areas in the LFP (Fig. 6A) and EEG (Fig. 6B) recordings. In cases where stimulation suppressed ongoing oscillations in motor cortex, this suppression was also evident at other sampled areas. A

![Image of current source density (CSD) analysis of the local field potential (LFP) at different depths of motor cortex. A: spontaneous LFP (black) at different depths and surface EEG (red) recorded simultaneously from 1 animal. A representative 3-s segment of spontaneous LFP activity is shown for each recorded depth aligned to simultaneous EEG recording. Recordings at different depths were obtained within a few minutes from each other. B: averaged LFP responses at different depths over 1,200 stimulation pulses (black) and averaged surface EEG from same trials (red). These responses were pooled from individual stimulations during high-frequency trains. Thus response in each trace also contains superposed effects from immediately preceding stimuli in the train. C: CSD depth profile of average LFP responses. Top trace shows a graph of the CSD profile at 1.2-mm depth. Black lines are created by stimulation artifacts. Note that CSD profile during high-frequency trains. Thus response in each trace also contains superposed effects from immediately preceding stimuli in the train. D: histology of 1 experiment showing laminar structure in motor cortex. Arrow points at blue mark resulting from a pontamine sky blue injection at a depth of 2.2 mm after experiment.]
similar spread was also observed for the initial fast positive response after each stimulus (Fig. 6, A2 and B2), suggesting that antidromic activation spread quickly through collaterals of the directly antidromically activated neurons. In addition, direct antidromic activation of less prominent projections from nonmotor cortical areas to STN (Hamani et al. 2004) may also contribute to this fast spread of antidromic activation.

**Maintained effects during long-duration DBS**

In human STN-DBS, the stimulator is on for long periods of time without a reduction of effect. To study whether the results we describe for short stimulation trains of 1- to 2-s duration persist over longer time periods, we applied stimulation trains of 100 s in a few animals (n = 4). These long stimulation trials reveal that the immediate effects did become weaker but did not approach zero. In fact, the surface EEG responses (average of 4 animals; Fig. 7B) to stimulation approached a steady state of 34% of the original response amplitude after an exponential decay with a time constant of 21 s. The firing rate of an antidromically activated deep-layer neuron (Fig. 7A) decayed in a similar manner, indicating that maintained antidromic spiking at an increased failure rate is likely to underlie the time course of EEG responses. Recent evidence of unreliable invasion of cortical pyramidal cell bodies by antidromic action potentials generated in their descending axons supports the concept of increasing failure rates for fast stimulation trains (Chomiak and Hu 2007). Nevertheless, the maintenance of a steady-state response suggests that the observed mechanisms of cortical antidromic activation and suppression of deep-layer oscillations could operate for long periods of time.

**STN-DBS effects in 6-OHDA–lesioned rats and recordings from rats under isoflurane anesthesia confirm antidromic activation and suppression of oscillatory activity in different dynamical states**

Antidromic activation by STN-DBS occurs through direct electrical induction of action potentials and is unlikely to be...
subject to modulation by anesthesia or neuromodulation. In contrast, the spread of antidromic activation through synaptic transmission at collaterals of activated axons and subsequent network effects might well be expressed differently under such different conditions. To determine the antidromic cortical effects of STN-DBS under Parkinsonian conditions, we repeated the manipulations described above for a set of rats that had received unilateral 6-OHDA lesions (n = 3; Fig. 8A). In addition, we tested the effect of a different anesthetic (isoflurane) in normal rats (n = 2), and rats with 6-OHDA lesions (n = 4; Fig. 8B). Under all conditions, we found that the major findings described above for normal rats under ketamine anesthesia still hold: 1) intracellular recordings (2 of 3) showed antidromic spike activation as shown in Fig. 1 for normal rats; 2) individual stimuli triggered an antidromic fast EEG response with a frequency dependent amplitude (Fig. 8, A1 and B1). 3) The low-frequency power of the PSD was suppressed during STN-DBS as in normal rats (Fig. 8, A2–A5 and B2–B5). These experiments failed to show any difference in the EEG patterns between normal and 6-OHDA–lesioned rats for ketamine-xylazine (Fig. 8A2) or isoflurane (Fig. 8B2) anesthesia during control periods or STN-DBS. This result suggests that unilateral 6-OHDA lesions do not significantly impact cortical activity during deep anesthesia and that further work using 6-OHDA animals should be taken to an awake preparation. However, because of the elaborate multisite recording and intracellular setup of this study, such experiments were not feasible using our recording methods.

**DISCUSSION**

We pursued the hypothesis that an important effect of STN-DBS may be occurring through antidromic cortical circuit activation through the axon collaterals of STN projecting pyramidal neurons. We found that during STN-DBS antidromic spikes are observed in some deep-layer neurons in motor cortex in normal and 6-OHDA–lesioned rats. CSD analysis of the LFPs at different depths of motor cortex showed that the excitation after each pulse in STN DBS first reached layer V and then spread immediately toward the cortical surface. CSD analysis does not allow the identification of cellular elements; therefore the observed spread of activation toward the surface could be caused by the activation of apical dendrites of deep cells as well as activation of superficial layer.
neurons through axon collaterals of antidromically activated neurons. The observed dampened reverberation of activity suggests the activation of recurrent excitatory loops within cortex or perhaps within cortico-thalamic circuits. At DBS frequencies >100 Hz, the secondary peak of this reverberation overlaps with the primary response of the next stimulus. Thus resonance in the response to fast stimulation could explain the frequency range most effective in human DBS treatment.

**Antidromic stimulation of cortex with STN-DBS**

Previous observations in human patients implanted with STN-DBS electrodes showed that short-latency responses can be recorded after single pulse and paired-pulse stimulations. The average EEG responses evoked by single pulse STN stimulations (Baker et al. 2002) showed multiphasic components over a large area of cortex starting with a prominent negativity at a latency of 25 ms that were highly similar to our high-frequency stimulus-train onset-responses (Fig. 2A). This response was increased by short-interval (0.5 ms) paired-pulse stimulation, indicating that antidromic stimulation of axons was the origin of this waveform (Baker et al. 2002). Our finding that this response was only seen once at the onset of high-frequency stimulation (HFS) suggests that it is not likely to be involved in ongoing effects of HFS-DBS. A second study (Ashby et al. 2001) showed two short latency peaks in the surface EEG at 3.4 and 7.5 ms after single STN-DBS pulses, which may correspond to our dampened oscillation of cortical activation in the rat. They also suggested antidromic axonal activation as the origin of the short-latency peaks caused by paired-pulse facilitation (Ashby et al. 2001). Overall, the similar surface waveforms found in our study suggest that antidromic activation in human STN-DBS situations is comparable between human patients and our rat model. In both species, responses were also dominant over frontal cortical sites, which is congruent with an antidromic activation of the hyperdirect cortico-SPN pathway, which primarily originates in motor and premotor cortex (Nambu et al. 1996). Our direct recording of intracellular antidromic spikes in deep layer neurons in motor cortex provides the first unequivocal direct evidence of this mechanism. Our antidromic spike latencies, the depth of activated neurons, and the surface EEG recordings closely paralleled those obtained with pyramidal tract stimulation in rats (Porter and Sanderson 1964), suggesting that stimulation of pyramidal tract collaterals could account for the observed findings. A short-latency facilitation of STN-DBS on voluntary EMG also supports a direct activation of PT collaterals by STN-DBS (Ashby et al. 1999). A short latency inhibition of STN-DBS stimuli on EMG was also found in this study for some stimulation sites. This inhibition but not the fast facili-

---

**FIG. 6.** Local field potentials and EEGs from different cortical areas. mc1, motor cortex; fr, frontal cortex; mc2, motor cortex 2; oc, occipital cortex. A1: LFPs recorded at 1.2 mm below at 3 difference sites. To show amplitude of oscillations, traces during STN-DBS were filtered by a 100- to 1,000-Hz bandstop filter to remove evoked responses. Amplitude oscillatory activity in LFP was reduced during STN-DBS. A2: close-up of traces without bandstop filtering shows evoked LFP responses (stimulation artifacts are removed). A3: PSDs, calculated from 10 trials of 2-s signals show that low-frequency power was significantly reduced some stimulation sites. This inhibition but not the fast facilitation of electrical field potentials (EFPs) suggests that it is not likely to be involved in ongoing effects of HFS-DBS. A second study (Ashby et al. 2001) showed two short latency peaks in the surface EEG at 3.4 and 7.5 ms after single STN-DBS pulses, which may correspond to our dampened oscillation of cortical activation in the rat. They also suggested antidromic axonal activation as the origin of the short-latency peaks caused by paired-pulse facilitation (Ashby et al. 2001). Overall, the similar surface waveforms found in our study suggest that antidromic activation in human STN-DBS situations is comparable between human patients and our rat model. In both species, responses were also dominant over frontal cortical sites, which is congruent with an antidromic activation of the hyperdirect cortico-SPN pathway, which primarily originates in motor and premotor cortex (Nambu et al. 1996). Our direct recording of intracellular antidromic spikes in deep layer neurons in motor cortex provides the first unequivocal direct evidence of this mechanism. Our antidromic spike latencies, the depth of activated neurons, and the surface EEG recordings closely paralleled those obtained with pyramidal tract stimulation in rats (Porter and Sanderson 1964), suggesting that stimulation of pyramidal tract collaterals could account for the observed findings. A short-latency facilitation of STN-DBS on voluntary EMG also supports a direct activation of PT collaterals by STN-DBS (Ashby et al. 1999). A short latency inhibition of STN-DBS stimuli on EMG was also found in this study for some stimulation sites. This inhibition but not the fast facilitation of electrical field potentials (EFPs) suggests that it is not likely to be involved in ongoing effects of HFS-DBS.
In recent years, excessive synchronization in the basal ganglia–cortical network has been recognized as a consistent part of Parkinsonian activity patterns (Brown 2003). In particular, increased coherence in the beta-band (13–30 Hz) is correlated with severity of symptoms in humans and is decreased during STN-DBS (Silberstein et al. 2005). An increase in STN–cortical coherence in the beta band is also seen in 6-OHDA–lesioned rats (Sharott et al. 2005). Although beta-band activity is less prominent in the anesthetized rat, we observed a decrease in this frequency band as well as in the slow-wave activity more characteristic of ketamine anesthesia. Our results suggest that a dampening of synchronized oscillations by STN-DBS is not limited to a specific frequency band. Interestingly, both slow-wave and beta band activity seems to have a mandatory cortical component in vivo, (Magill et al. 2000), although an STN-GP feedback loop could in principle also sustain such oscillations (Plenz and Kital 1999; Terman et al. 2002). Differences between normal and 6-OHDA animals were not apparent under our anesthetic conditions, in that the beta-band activity showed running average of 100 subsequent responses. Inset: latency histogram of the last 30 s, indicating that action potentials were still mainly driven by DBS. Curve shows running average of 100 subsequent responses.

De-synchronization in cortical activities

In recent years, excessive synchronization in the basal ganglia–cortical network has been recognized as a consistent part of Parkinsonian activity patterns (Brown 2003). In particular, increased coherence in the beta-band (13–30 Hz) is correlated with severity of symptoms in humans and is decreased during STN-DBS (Silberstein et al. 2005). An increase in STN–cortical coherence in the beta band is also seen in 6-OHDA–lesioned rats (Sharott et al. 2005). Although beta-band activity is less prominent in the anesthetized rat, we observed a decrease in this frequency band as well as in the slow-wave activity more characteristic of ketamine anesthesia. Our results suggest that a dampening of synchronized oscillations by STN-DBS is not limited to a specific frequency band. Interestingly, both slow-wave and beta band activity seems to have a mandatory cortical component in vivo, (Magill et al. 2000), although an STN-GP feedback loop could in principle also sustain such oscillations (Plenz and Kital 1999; Terman et al. 2002). Differences between normal and 6-OHDA animals were not apparent under our anesthetic conditions, in that the beta-band activity showed running average of 100 subsequent responses. Inset: latency histogram of the last 30 s, indicating that action potentials were still mainly driven by DBS. Curve shows running average of 100 subsequent responses.
output activation conveying the clinical effects of STN-DBS than by direct STN stimulation. Thus a clear role for STN likely to be affected quite differently by stimulating in the GPi may also seem puzzling, because the STN output is as the major site of DBS action. However, this similarity in outcomes. A possible direct stimulation of dopamine fibers ganglia output providing a necessary link to clinical STN-DBS effective in patients, who had undergone a previous ablation of superior (Plaha et al. 2006). Observations that STN-DBS is reducing Parkinsonian symptoms, and sometimes are actually contributing to this complexity and underlie some of the ob-
sidered higher than in controls (5/32) and may suggest increased density of cortico-subthalamic axons in the STN in this condition.

Clinical relevance of antidromic cortical stimulation

Clearly, STN-DBS has the ability to activate multiple pathways and structures. A direct stimulation of STN output affecting pallidal and nigral firing has been a strong focus in the examination of DBS mechanisms (Dostrovsky and Lozano 2002). Direct HFS in internal segment of Globus Pallidus (GPi) is of similar clinical benefit as STN-DBS (Benabid et al. 2005; Goto et al. 2004) casts further doubt on basal ganglia as the major site of DBS action. However, this similarity in effect may also seem puzzling, because the STN output is likely to be affected quite differently by stimulating in the GPi than by direct STN stimulation. Thus a clear role for STN output activation conveying the clinical effects of STN-DBS does not emerge from these studies. The detailed effects of STN-DBS on GPi or substantia nigra pars reticulata (SNr) activity have also been controversial, with some authors finding a decrease in basal ganglia output firing (Tai et al. 2003), whereas others see an equal amount of increases (Maurice et al. 2003; Shi et al. 2006). On the other hand, similar antidromic cortical activation by stimulation in either STN or GPi is suggested by a similar short-latency change of cortical excitability after each stimulation pulse (Hanajima et al. 2004). Interestingly stimulation sites outside of either structure, most notably in the zona incerta, have also been successful in reducing Parkinsonian symptoms, and sometimes are actually superior (Plaha et al. 2006). Observations that STN-DBS is effective in patients, who had undergone a previous ablation of the basal ganglia output pathway through a thalamotomy (Fraix et al. 2005; Goto et al. 2004) casts further doubt on basal ganglia output providing a necessary link to clinical STN-DBS outcomes. A possible direct stimulation of dopamine fibers (Lee et al. 2006) coursing through all of these areas may contribute to this complexity and underlie some of the ob-
served clinical effects. However, antidromic cortical activation also provides a common link between these stimulation sites, which are all in proximity with major cortical output pathways. The clinical benefit of epidural cortical stimulation in the MPTP primate model (Drouot et al. 2004) and in some human PD patients (Canavero et al. 2002) support the hypothesis that direct control of cortical activity is an effective mechanism in alleviating PD symptoms. However, other human PD patient pools derived no benefits from epidural stimulation (Cilia et al., 2007), suggesting that this method produces less than robust effects. There are important differences between STN-DBS and the effects of epidural cortical stimulation, however. Our data show a layer-specific cortical circuit activation with STN-DBS starting with deep layer activation, which is unlikely to be replicated with surface stimulation. Evoked cortical dynamics with epidural stimulation are likely to be substantially different. Thus subcortical antidromic activation of cortical efferents may actually be superior over epidural stimulation as a method to obtain desirable changes in cortical activity patterns. Multiple lines of evidence point to the idea that the ultimate benefit may lie in preventing overly synchronous or oscillatory activity patterns (Brown et al., 2004). Synchronous activity patterns are found in the cortex of MPTP primates (Goldberg et al., 2002), and oscillations at multiple frequency bands in the cortex of Parkinsonian patients (Fogelson et al., 2006). Our data provide the first unequivocal intracellular cortical recordings showing antidromic spike invasion as well as the dampening of oscillations in wide areas of cortex in response to STN-DBS. The reverberatory activation of cortical columns with a resonant frequency above 100 Hz furthermore provides to our knowledge the first evidence for a specific effect of such high-frequency stimulation, which is hard to explain with rate models of basal-ganglia activity changes (Albin et al., 1989). Overall, our data point at antidromic cortical activation as a major contributor to STN-DBS effects, and most notable the suppression of oscillations in multiple frequency bands. Future studies will need to address the question as to how altered thalamic input to cortex caused by STN-DBS effects on basal ganglia activity is interacting with antidromically evoked activity. Because of the continuous interaction of all involved structures during STN-DBS, a precise allocation of effects to specific primary pathways may prove daunting.

Acknowledgments

We thank Dr. Cengiz Gunay and D. Kurzyniec for extensive programming help of Labview data acquisition software. Present addresses: J. A. Goldberg, BioControl Medical Ltd., 3 Geron St., Yehud 56100, Israel; G. W. Arbuthnott, Brain Mechanisms for Behavior Unit, Okinawa Institute of Science and Technology, Okinawa 904-2234, Japan.

Grants

This study was supported by a FastTrack Award from the Michael J. Fox Foundation to D. Jaeger and National Institute of Neurological Disorders and Stroke Grant R01-NS-039852.

References


