Title

Activation of Subthalamic Neurons by Contralateral Subthalamic Deep Brain Stimulation in Parkinson Disease

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Abstract

Multiple studies have shown bilateral improvement in motor symptoms in Parkinson disease (PD) following unilateral deep brain stimulation (DBS) of the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPI), yet the mechanism(s) underlying this phenomenon are poorly understood. We hypothesized that STN neuronal activity is altered by contralateral STN DBS. This hypothesis was tested intraoperatively in humans with advanced PD using microelectrode recordings (MER) of the STN during contralateral STN DBS. We demonstrate alterations in the discharge pattern of STN neurons in response to contralateral STN DBS including short latency, temporally precise, stimulation frequency-independent responses consistent with antidromic activation. Furthermore, the total discharge frequency during contralateral high frequency stimulation (160 Hz) was greater than during low frequency stimulation (30 Hz) and the resting state. These findings demonstrate complex responses to DBS and imply that output activation throughout the basal ganglia – thalamic – cortical (BG-Th-CTX) network rather than local inhibition is a therapeutic mechanism of DBS.
Introduction

The remarkable success of deep brain stimulation (DBS) surgery when best medical therapy is insufficient or suboptimal demonstrates the importance of electrophysiologically-based treatments for neurological and psychiatric disease (Deuschl et al. 2006; Weaver et al. 2009; Vidailhet et al. 2005; Servello et al. 2008; Visser-Vandewalle 2007). Despite this, little is known about how the response of neurons to DBS improves symptoms.

Although unilateral STN DBS most dramatically reduces the cardinal symptoms of PD in the extremities contralateral to the stimulating electrode, several studies have shown mild but often sufficient improvement in ipsilateral motor function as well (Alberts et al. 2008b; Chung et al. 2006; Nakamura et al. 2007; Tabbal et al. 2008; Walker et al. 2009). Tabbal et al demonstrate with objective kinematic measures and rater blinding that the improvement in ipsilateral motor function from unilateral STN stimulation is present in a relatively large sample of patients whose stimulator had been placed at a median of 8.7 months (range 4 to 76 months) prior to motor testing, and Walker et al. demonstrate bilateral effects from unilateral STN stimulation on timed measures of bradykinesia at up to 12 months postoperatively (Tabbal et al. 2008; Walker et al. 2009). Additionally, the results of Alberts et al. demonstrate that ipsilateral force control is improved by unilateral STN DBS during the performance of bimanual motor tasks (Alberts et al. 2008a). In total, these studies indicate that the mild ipsilateral improvement in motor function from unilateral STN DBS is not merely a transient phenomenon that attenuates during the initial postoperative months.

The mechanism for motor benefit from subcortical brain stimulation is unknown. Investigation of the neuronal activity in the unstimulated STN on the opposite side of the brain may be relevant to ipsilateral clinical benefit and provide a unique perspective to study DBS mechanisms of action. Therefore, we tested the hypothesis that unilateral
STN DBS alters the neurophysiology of the contralateral STN during staged placement of a second DBS electrode on the opposite side of the brain in patients with advanced idiopathic PD.

Recent biochemical, metabolic, and electrophysiological evidence in animal models and patients with movement disorders suggests that a lesion effect or local inhibition alone is unlikely to explain the therapeutic mechanism of DBS (Garcia et al. 2003; Hashimoto et al. 2003; Hilker et al. 2008; Karimi et al. 2008; Windels et al. 2000; Montgomery and Gale 2008). Computer modeling of the biophysical properties of neurons and experimental evidence in biological studies have suggested that DBS results in local inhibition in the cell bodies in direct proximity to the DBS electrode, but that efferent axons, afferent axons, and fibers of passage in the region of stimulation are likely to be activated as well (Hashimoto et al. 2003; Montgomery and Gale 2008; Grill et al. 2008; McIntyre et al. 2004a; McIntyre et al. 2004b; McIntyre et al. 2004c; McIntyre and Grill 2002; Hammond et al. 2008; Anderson et al. 2003; Iremonger et al. 2006; Kita et al. 2005; Maurice et al. 2003; Tai et al. 2003b; Montgomery 2006).

Neurophysiological approaches have related changes in rhythmic neuronal activity in the basal ganglia–thalamic–cortical (BG-Th-CTX) system with the remarkable efficacy of DBS, including antidromic activation, oscillations, resonance, beat amplification, suppression of misinformation by over-writing neuronal activity (misinformation-ablation) and synchronization/desynchronization (McIntyre et al. 2004b; Hammond et al. 2008; Li et al. 2007; Brown 2003; Bar-Gad et al. 2003; Montgomery 2007; Montgomery and Baker 2000). Assuming that changes in the activity of the contralateral STN during unilateral STN DBS are causally related in some way to the ipsilateral improvement in motor function, characterization of such changes in STN activity may provide important insights into the therapeutic mechanisms of action of DBS.
Methods

This study received prior approval by the Institutional Review Board. Inclusion criteria required a diagnosis of idiopathic PD consistent with the UK Brain Bank Criteria. Additional inclusion and exclusion criteria for DBS surgery for PD have been described extensively elsewhere (Deuschl et al. 2003). Patients previously had unilateral STN DBS and were undergoing a second contralateral STN DBS placement. The patients were in the “practically defined off” medication state (following an overnight fast from their anti-parkinson medications) as a matter of routine care for the surgical procedure (Langston et al. 1992).

Microelectrode recordings

All experimental recordings were obtained with the patient awake and at rest. Microelectrode recordings (MER) were recorded through platinum-iridium microelectrodes with impedances between 0.4 and 1 megaOhms (FHC Inc, Bonham, Maine, USA) using standard amplification and filtering (low frequency cutoff 350 Hz, high frequency 6 kHz, gain 25,000) (Alpha Omega Engineering Inc., Jerusalem, Israel) and analog-to-digital conversion occurred at a sampling rate of 24 kHz. MER identified the boundaries of the sensorimotor STN for targeting of the DBS electrode using methods described previously (Baker et al. 2004). Upon encountering typical STN activity, two stimulation paradigms were tested for 120 seconds each. First, the contralateral STN stimulator was activated in continuous mode at high frequency settings (160 Hz) that previously were determined to be clinically effective in the outpatient setting as described in the context of another study (typically monopolar or bipolar configuration, 3.5 Volts, pulse width of 60 microseconds) (Walker et al. 2009). Second, lower frequency stimulation (30 Hz) was delivered from the stimulator with otherwise identical settings. Baseline STN activity was recorded for 120 second intervals before, between,
and following the two stimulation conditions. A schema for the intraoperative STN MER experiments during staged STN DBS surgery is shown in Figure 1.

Stimulation artifact removal and identification of neuronal waveforms

Validated MER analysis software developed by Montgomery et al. was used offline to identify the stimulation artifact peaks (Figure 2A), calculate the mean evoked potential for the stimulus pulse (Figure 2B1), and then subtract this evoked potential from each individual occurrence of the stimulus in the analog data (Figure 2B2). (Montgomery et al. 2005) Because the inherent variability of the immediate fast component of the stimulus artifact made substraction difficult, this brief interval (typically less than 1 ms in duration) was set to zero. A semi-automated parsing routine discriminated the neurons in the deartifacted data based upon waveform morphology using a validated, heuristically driven template-matching algorithm to attribute the neuronal action potential waveforms to individual neurons (Montgomery et al. 2005). Action potential waveforms associated with individual neurons were visually inspected to assure appropriate discrimination. Additionally, neuronal discrimination was verified by constructing an auto-correlogram for each neuron; failure to demonstrate a refractory period in the auto-correlogram indicates that the waveforms of two or more neurons were inappropriately attributed to a single neuron. Similarly, cross-correlograms between all pairs of discriminated neurons at the same recording site demonstrated no evidence of a refractory period and consequently, minimizing a risk of discriminating a single neuron into two or more neurons.

Data analysis

A number of analysis techniques were used to evaluate whether the activity of subthalamic neurons was altered by contralateral STN DBS. Analog raster plots center
epochs of the analog data (the continuous microelectrode recording data) around an
event of interest such as the stimulus pulse or the action potential waveforms associated
with a specific neuron. This allows visualization of the extra-cellular action potentials
attributed to a neuron of interest. These analog data can be can be added together
(averaged) to generate analog histograms (Figures 2C and 2D). These analog
histograms consist of all the action potentials of an STN neuron centered temporally on
the onset of the neuronal discharge with DBS off and during 160 Hz and 30 Hz
contralateral STN stimulation. Alternatively, the neuronal discharges may be viewed as
an analog raster, such that the individual epochs of interest are superimposed
temporally upon one another (Figures 3C1 and 3C2).

Another analysis technique that was employed was the generation of discrete
raster plots. In contrast to the analog rasters, discrete raster plots show the time of
onset of individual neuronal discharges as a point process (a discrete dot) with respect
to the time of an event of interest. In Figure 3A and Figure 4A – 4C, discrete rasters
organize the neuronal activity with respect to the time of the contralateral stimulus pulse,
such that each row in the raster represents the response to a single stimulation pulse.
Note that in the resting state with the DBS off, the rows in the raster are organized
temporally as though the stimulus was being applied ("pseudostimulation,")
(Montgomery et al. 2006). Time-interval histograms are then constructed by summing
across rows in the raster with the stimulator on and off (not shown). To quantitatively
compare the neuronal discharge pattern with DBS on and off, the time interval
histograms are converted into Z-scores by comparing the mean and standard deviations
of the action potential counts in successive time bins. These Z-score adjusted
histograms quantify the degree of statistical difference in the neuronal activity with DBS
on and off (Figure 3B and Figure 4). The bin width in the histograms was 0.3 ms.
Several additional analysis techniques were used to evaluate the short latency
(~1 ms), temporally precise responses observed in the discreet raster plots (Figure 3A
and Figure 4). First, the analog histograms of individual neurons in Figure 2C and 2D
described previously demonstrate the presence of the stimulation artifact at a short
latency prior to the neuronal waveforms. Second, analog raster plots were constructed
conditionally, to only display action potentials from an individual neuron occurring within
1 ms after the contralateral stimulus pulse (Figure 5A). Third, the data was visually
inspected in the continuous data and in the analog rasters to verify the presence of the
action potential waveforms (Figure 3C1 and 3C2 and Figure 5A and 5B). Fourth,
collision analyses were employed, as described by other authors, to evaluate whether
the observed short latency responses are consistent with direct axonal activation by the
stimulus (Li et al. 2007). Collision occurs when a neuron discharges spontaneously
immediately prior to a stimulus pulse and prevents the propagation of the antidromic
response to the recording site because of the absolute refractory period. In the collision
analyses, epochs of data were grouped conditionally based upon whether there was an
action potential within 3 ms prior to the contralateral STN DBS pulse or not. Post-
stimulus histograms of discharge probability were then constructed to characterize the
response of the neuron over time with respect to the stimulus pulse for both conditions
(Figures 5C1 and 5C2). Additionally, the proportion of successful collision events was
calculated by dividing the proportion of the contralateral stimuli that were followed by the
short latency neuronal response in the two conditions. Fifth, the probability of whether a
short latency response occurred after 160 Hz and 30 Hz contralateral STN DBS was
analyzed statistically with one-way Analysis of Variance (ANOVA) with neurons as
subjects and the stimulation conditions as factors (Figure 6B). A test of normality
showed that the probabilities of a short latency response (within 1 ms of the stimulus
The total continuous discharge frequency for each neuron was calculated during the resting state and during 160 Hz and 30 Hz contralateral stimulation. The calculation of total discharge frequency in each of the stimulation conditions takes into account the brief period of flattening during the fastest component of the stimulus artifact transient. The discharge frequencies of each neuron in the stimulation conditions and the resting state were evaluated using one-way analysis of variance (ANOVA) with repeated measures with neurons as subject and the stimulation conditions as factors, and the Bonferroni method was used for pairwise comparison. A test of normality showed that the discharge frequencies were not normally distributed, therefore the data were log transformed to achieve normality. The p value required for statistical significance for this and all other statistical tests in this work was set at 0.01 a priori for multiple comparisons.
Results

Descriptive data for the subjects who participated in the study are contained in Table 1. Age and duration of disease were rounded to the nearest decade to protect subject anonymity. Recordings were obtained from a total of 58 STN neurons.

The discharge pattern of STN neurons is altered by contralateral STN DBS

The responses of a representative individual neuron to contralateral 160 Hz STN DBS are illustrated in the discrete raster plot and histogram in Figure 3A and 3B and in analog rasters in Figures 3C1 and 3C2. There is an absence of neuronal activity immediately during the fastest component of the DBS pulse lasting from approximately 0.2 ms before to 0.8 ms after stimulus onset due to loss of signal from the removal of the brief, fastest component of the stimulation artifact. In general, there are four changes in neuronal activity the post-stimulus rasters and histograms during high frequency DBS illustrated explicitly in Figure 3. First, there is activation of STN neurons that is tightly coupled temporally to the contralateral stimulus at a short latency of approximately 0.8 ms. Although it may appear that a pair of action potentials occur on the same row in the raster, only single action potentials follow any individual DBS pulse. The raster has the appearance of two dots together because of the very large number of rows corresponding to each stimulation pulse. Second, in many neurons there is a reduction of activity compared to baseline following the second response at a latency of approximately 2 ms. Third, there is an increase in neuronal activity approximately 3 ms after the DBS pulse lasting approximately 1.5 ms. In contrast to temporal precision the short latency responses, the Z-score adjusted histogram shows a more sustained increase in neuronal activity during the interval approximately 3 to 5 ms after the stimulus pulse during stimulation. Fourth, in many neurons, following the increased activity 3 ms after the DBS pulse, there is a reduction in neuronal activity. In Figure 3B,
Z-score adjusted histograms quantify each of these changes in the discharge pattern of a neuron with DBS at 160 Hz versus DBS off. **Figure 3** displays analog raster plots of consecutive action potentials of the same neuron in its resting state with DBS on at 160 Hz (**Figure 3C1**) and with DBS off (**Figure 3C2**, "pseudostimulation"). These rasters display the raw data prior to stimulus artifact subtraction to demonstrate the effects of 160 Hz contralateral stimulation on the discharge pattern of the STN neurons. These alterations in the pattern of neuronal discharges by contralateral STN DBS were seen consistently between neurons in individual neurons and across subjects (**Figure 4**).

The first response in the discrete rasters occurs at a short, fixed latency relative to the contralateral stimulus pulse of approximately 1 ms, which suggests that the axons of STN neurons may be activated by the contralateral stimulus pulse. The analog histograms of individual neurons discussed previously in **Figure 2C** and **2D** demonstrate the short latency between the stimulus artifact and the action potentials of these neurons. Note that during 160 Hz and 30 Hz stimulation, the stimulus artifact (gray arrows) appears in the raster at a short latency (~1 ms) prior to the action potential waveform, demonstrating that the action potentials are more likely to occur at a precise short latency with respect to the onset of the contralateral stimulus pulse. Furthermore, the amplitude of the stimulus artifact pulse is smaller relative to the action potential amplitude during 30 Hz stimulation, because the ratio of the number of neuronal discharges to stimulation spikes is smaller when the stimulation frequency is lower.

Finally, the morphologies of the action potentials with DBS off and in the deartifacted data with DBS on are identical. This verifies the consistency of the morphology of the action potential in the various stimulation conditions. If the short latency responses measured during stimulation were artifactual, they would likely manifest unique waveform morphologies that would be present only during the stimulation conditions and not during the resting state. This was not the case – analog rasters demonstrate that the
action potentials measured during stimulation have identical morphologies to the action potentials measured during the resting state when there was no stimulus pulse (Figure 2C and 2D). In order to further evaluate the possibility whether the short latency responses represent axonal activation of STN neurons by contralateral STN DBS, analog rasters centered on the stimulation pulse were generated in Figure 5A to display action potentials occurring within 1 ms of the stimulus pulse. These rasters display epochs where the STN neuronal discharge follows the high frequency contralateral STN stimulus within 1 ms of the onset of the contralateral stimulus pulse. Note that discharges of this individual neuron can be seen in the raw analog data in the data still containing the stimulation artifact (Figure 5A). Similar short latency responses are evident in the discrete rasters for other representative neurons in response to both 160 Hz and 30 Hz contralateral stimulation in Figure 4. Furthermore, collision analyses show a decreased probability of a neuronal discharge in the first millisecond following the DBS pulse occurs in the epochs with an action potential preceding the DBS pulse by 3 ms versus the epochs without an action potential in the 3 ms preceding the DBS pulse (Figure 5C1 and 5C2). The proportion of successful collision events in this example was 0.81. Finally, axonal activation of neurons should be robust and relatively independent of stimulation frequency. This property was examined by calculating the probabilities of these short latency discharges for each neuron in response to both 160 Hz and 30 Hz stimulation. The mean probability of a short latency response following contralateral STN DBS pulse during 160 Hz (0.031 ± 0.038) and 30 Hz (0.036 ± 0.048) stimulation was not significantly different (p = 0.47, Figure 6B, one-way repeated measures ANOVA with Bonferroni pairwise comparison). Given the difference in the mean probability, a power calculation suggests that a type II statistical error is unlikely,
as more than 1,400 neurons would have to be sampled to result in a p value of less than 0.01 using a paired t-test, assuming power of 0.8.

Total discharge frequency is dependent on stimulation frequency

The total discharge frequency over the entire stimulation epoch across all neurons from all subjects was greater during 160 Hz contralateral STN stimulation (12.3 ± 1.7 Hz) than during 30 Hz stimulation (10.6 ± 1.7 Hz, p = 0.006) and the resting state (9.7 ± 1.5 Hz, p < 0.001, one-way repeated measures analysis of variance, with pair-wise comparisons using the Bonferroni method, data expressed as mean ± standard error, Figure 6A). During 160 Hz contralateral stimulation, 42 out of 53 neurons (79.25%) discharged at a higher frequency than during the resting state, and the remaining neurons (19.75%) discharged at a lower frequency. There was a trend towards a higher discharge frequency during low frequency stimulation versus the resting state (p = 0.054). A power calculation using the pair-wise comparison suggests that 10 more neurons with a similar response to low frequency stimulation would be required to achieve a p value less than 0.01 using a paired t-test assuming power of 0.8, supporting a trend towards higher discharge frequencies during low frequency DBS compared to no stimulation.
Discussion

There are two primary findings from this study. First, therapeutic 160 Hz unilateral STN DBS results in increased contralateral STN activity. If one assumes that a change in STN activity is causally related to the contralateral limb improvement (in the limb ipsilateral to the DBS), then these data are consistent with activation and not local inhibition as the therapeutic mechanism of DBS, because the discharge frequency was increased and not decreased during high frequency DBS of the contralateral STN. The observation agrees with the results of Novak et al. who analyzed multiunit STN activity and compared high frequency contralateral STN stimulation with the resting state (Novak et al. 2009). Second, the discharge pattern of STN neurons is altered by contralateral STN DBS, including fixed, short latency responses consistent with antidromic activation. Although the total discharge frequency of STN neurons was greater during contralateral 160 Hz STN DBS, the most prominent change in the discreet data was the alteration of the firing patterns of the neurons in response to stimulation. Combined, these observations demonstrate that both the precise timing and the frequency of STN discharges are altered by contralateral STN DBS, which raises important questions about the BG-Th-CTX system and the therapeutic mechanism of DBS. In particular, our results implicate axonal activation as an important component of the therapeutic response (Baker et. al. 2002, Hammond et al. 2008; Gradinaru et. al. 2009) and suggest that studies restricted to the vicinity of the stimulating electrode may not detect important elements of the response to DBS elsewhere in the BG-Th-CTX network.

Several MER studies have demonstrated reduced STN activity at the stimulation site (Filali et al. 2004; Tai et al. 2003a; Beurrier et al. 2001), while another did not (Carlson et al. 2009). In contrast, recordings from downstream structures show increases in neuronal activity consistent with increased output from the targeted structure (Hashimoto et al. 2003; Tai et al. 2003b; Montgomery 2006; Filali et al. 2004).
Additionally, there are considerable data from computational simulations demonstrating that action potentials in neuronal somas and dendrites can be suppressed by electrical stimulation, yet action potentials can still be generated at the axon hillock or first inter-node (McIntyre et al. 2004a; McIntyre et al. 2004b; McIntyre et al. 2004c; McIntyre and Grill 2002; Johnson and McIntyre 2008). These concomitant findings of both decreased local activity and increased activity in output structures suggests that the therapeutic effect of STN DBS may not be attributed to either mechanism alone. Regarding whether increased efferent activity or decreased local soma activity is causally related to the therapeutic mechanisms of DBS, our data argue that output activation without local inhibition underlies the therapeutic effects of DBS, at least with regard to the ipsilateral improvement in motor function provided by unilateral STN DBS.

A study in a mouse model of Parkinson disease demonstrated with selective expression of optically activated ion channels that the excitation and inhibition of cortex and cortical projection neurons rather than STN neurons themselves was associated with changes in motor function (Gradinaru et al. 2009). Other studies in a rodent model of PD, in non-human primates, and in patients with PD show evidence for antidromic activation of neurons in frontal cortex by STN DBS with intracellular recordings, local field potentials (LFPs), electroencephalography (EEG), and transmagnetic stimulation (TMS) (Montgomery and Gale 2008; Montgomery 2006; Li et al. 2007; Ashby et al. 1999; Baker et al. 2002, Kuriakose et al. 2009). Additionally, functional magnetic resonance imaging (fMRI) studies are most consistent with a local increase in the BOLD signal at the site of the stimulus, whatever implications this may have about the underlying synaptic activity (Hilker et al. 2008; Karimi et al. 2008). At the least, these studies coupled with our findings emphasize the importance of considering axonal activation and other alterations of network activity as an important component of the therapeutic mechanism of DBS.
That the total discharge rate of individual STN neurons is increased during contralateral high frequency STN stimulation versus the resting state is opposite the prediction of the rate model of Albin and DeLong (Albin et al. 1995; Wichmann and Delong 2006). The rate model would instead predict immediate worsening of motor function in the extremities in response to the observed increase in STN neuronal activity, which has not been observed in clinical studies evaluating motor function in the ipsilateral arm. Gradinaru et al. found that both subcortical electrical stimulation and motor cortex activation but not changes in STN neuronal activity improved motor function in a mouse model of PD. (Gradinaru et al. 2009) Additionally, another study showed no difference between the resting STN neuronal activity in PD patients versus patients with epilepsy (Montgomery 2008), and a recent study did not show the predicted decrease in STN discharge frequency during STN DBS in patients with PD (Carlson et al. 2009). Functional imaging studies have shown an increase in metabolic activity in the subthalamic region during STN DBS and decreased metabolic activity in motor cortical areas, findings compatible with STN activation, not inhibition (Hilker et al. 2008; Karimi et al. 2008; Hershey et al. 2003; Haslinger et al. 2005; Arai et al. 2008).

The discharge frequency of individual neurons in this work is lower in general than that reported in some previous studies of STN activity. In our analyses, we used quantitative and objective criteria to discriminate individual neurons, and these criteria were conservative, such that the action potentials of an individual neuron can be confidently felt to represent only one neuron. Any discussion of the frequency of neuronal discharges from extracellular recordings is subject to interpretation with regard to how the neurons are parsed. Regardless, these concerns would not be expected to affect our results, as the findings were consistent across subjects and among individual neurons and statistical analyses used within subject comparisons where each individual neuron served as its own control.
The fixed, short latency responses observed in this study are most consistent with axonal activation of STN neurons by contralateral STN DBS. Prior anatomical studies have demonstrated that STN axons are myelinated, which would be consistent with the short latency responses observed in these subjects (Chang et. al. 1984; Kita and Kitai 1987). In addition to occurring at a fixed, short latency, antidromic responses are characterized by independence from stimulus frequency (Figure 6B) and by the demonstration of collision. Had the antidromic responses been anticipated a priori, further experiments would have been conducted with longer stimulation trains to accumulate a larger number of neuronal discharges. A potential limitation of this study is that we did not have the capability to deliver DBS pulses in response to spontaneous action potentials, because the implanted DBS system is a clinical device. Consequently, in many examples there were a limited number of potential collision events.

The finding of axonal activation of STN neurons by contralateral STN DBS suggests that there are projections of STN neurons that cross the midline in the vicinity of the contralateral (stimulated) STN. Whether these axons terminate in the contralateral STN or are in route elsewhere is unknown. Most studies of the anatomical projections within the basal ganglia do not address contralateral projections or the trajectory of the projections. Consequently, the relative lack of demonstrated projections from the STN to the vicinity of the contralateral STN should not be construed as evidence against this. The results of Parent and Smith indicate that subthalamic neurons project bilaterally to either the substantia nigra pars reticulata or to the pedunculopontine region (Parent and Smith 1987), so it remains possible that the short latency responses we observe result from stimulation of these fibers of passage. Regardless, the demonstration of antidromic activation is in fact evidence in favor of such projections. A prior study showed evidence for antidromic activation of neurons in the thalamus by DBS of the internal segment of the globus pallidus, and anatomical
studies have born out that anatomical projections from the thalamus to the pallidum exist
(Montgomery 2006; Sidibe et al. 1997).

An intriguing aspect of the antidromic responses observed in this study is that they did not occur with high probability following the DBS pulse (typically following less than 5% of stimulation pulses), a phenomenon that has been demonstrated in the thalamus in a human in response to GPi DBS and in studies in animal models (Montgomery 2006; Li et al. 2007; Chomiak and Hu 2007). There are a number of potential explanations for this observation. It is possible that contralateral DBS activates distal elements of axon collaterals beyond branch points, such that the biophysical properties of these branch points decrease the likelihood of generation of an action potential at the soma, as has been described both experimentally and in computer simulations (Grill et al. 2008; Montgomery 2006; Chomiak and Hu 2007; Barron and Matthews 1935; Parnas 1972; Grossman et al. 1979; Beurrier et al. 1999). Additionally, Chomiak and Hu demonstrated that the probability of antidromic potentials invading the soma is also dependent on the resting membrane voltage. This may hold particular relevance in the STN, where fluctuations in the resting membrane potential have been described previously (Beurrier et al. 1999).

An important observation from this study is the change in the discharge pattern of STN neurons during contralateral STN DBS. Following the short latency antidromic response described previously, there is a decrease in activity in many neurons, which most likely represents the refractory period following the action potentials associated with antidromic action potentials. The longer latency responses seen at 3 to 5 ms after the stimulus pulse observed in this study are most suggestive of orthodromic synaptic activation because of their dependence on stimulation frequency, their longer latency relative to the antidromic responses, and their more broad temporal distribution. Since stimulation was delivered continuously and with a constant inter-stimulus interval in
these experiments, we cannot discount the possibility of a “wrapping” effect where the
increased activity actually is due to earlier DBS pulses. Whether the activation of STN
neurons occurs 3 to 5 ms after the preceding stimulus, or whether they are longer
latency responses that occur based on the recent stimulation history in some integer
multiple of the apparent latency is unclear. The fifth and final response observed in the
discrete rasters is a decrease in activity following the broad increase in activity
approximately 3 to 5 ms after the stimulus pulse described previously. This decrease in
activity most likely represents a refractory period following the increase in activity
preceding it, absence of neuronal activity because of the flattening of the subsequent
stimulation artifact, or a combination of both.

The alterations of STN neuronal activity by contralateral DBS observed in this
study adds to considerable experimental evidence and modeling studies suggesting that
DBS has widespread effects in the central nervous system beyond its local effects near
the site of the stimulus. Model simulations of the mechanism of DBS based upon the
biophysical properties of neurons, action potential initiation, and the stimulus pulse
suggest that complex responses to DBS occur, with either excitation or inhibition of cell
bodies proximal to the stimulus and both antidromic and orthodromic activations of
efferent axons and fibers of passage (McIntyre et al. 2004a; McIntyre et al. 2004b).
Additionally, distant effects of DBS have been appreciated in studies using
microelectrode recordings at various anatomical sites, local field potentials, cortical
evoked potentials, and functional imaging (Hashimoto et al. 2003; Karimi et al. 2008;
Anderson et al. 2003; Montgomery 2006; Li et al. 2007; Filali et al. 2004; Baker et al.
findings provide information about the effects of STN DBS on the bilateral BG-Th-CTX
motor system and emphasize that complex alterations in network activity are likely to
underlie the remarkable therapeutic efficacy of DBS. In total, these findings suggest that
the therapeutic mechanism of DBS may have little to do with the STN itself and instead be related to alteration of activity in fibers of passage near the STN or activation of more distant output structures and in turn the entire BG–Th–CTX system.

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Disclosures

Erwin B. Montgomery, Jr. serves as a consultant to Boston Scientific and St Jude Neuromodulation.


**Figure Legends**

**Figure 1.** Experimental schema. (A) Frontal brain MRI with unilateral STN DBS (gray arrow) and a hypothetical contralateral recording tract (dashed line) and STN recording site (black arrow). (B) Intraoperative STN activity in a human with PD. Note the artifact associated with contralateral STN DBS. Abbreviations: hertz (Hz), microvolt (µV), second (sec).

**Figure 2.** Schema for analysis technique used to remove the stimulus artifact. Individual DBS pulses are identified by a voltage amplitude-dependent peak detection algorithm. Note the reproducibility of the stimulus artifact in the superimposed individual traces (A1). The stimulus artifacts identified during peak detection are averaged together into an evoked potential (A2). This evoked potential is then subtracted from each occurrence of the stimulation pulse in the raw data (B1 and B2). Because of the large amplitude and variability of the initial sharp transient in the stimulus pulse, subtraction does not completely eliminate this portion of the stimulus artifact, so the raw data is set to zero or flattened during this brief interval. (C and D) Perineuronal analog histograms from two PD subjects with contralateral STN DBS in various stimulation conditions. The histograms are centered temporally on every occurrence of an individual neuron. Note that the morphology of the action potentials in the DBS off conditions and in the deartifacted data are identical, and that during both high (160 Hz) and low frequency (30 Hz) stimulation epochs, the stimulus artifact (gray arrows) is seen at a short latency (~1 ms) prior to the action potential waveform (black arrows).

Abbreviations: DBS (deep brain stimulation), HF (high frequency stimulation), LF (low frequency stimulation), seconds (sec).
**Figure 3.** (A) Discrete peristimulus raster of the response of an individual neuron to 120 seconds of contralateral 160 Hz STN DBS (gray bar) preceded by 120 seconds of resting STN activity (solid bars). The resting STN data with DBS off is organized in the rows as though a stimulus is being delivered ("pseudostimulation"). Each dot on the raster indicates an action potential from an individual neuron. The letter (A) indicates the period of flattening during the fastest portion of the stimulus artifact, (1) the short latency responses, (2) the diminished activity following the short latency responses, (3) the sustained increase in activity over a more broad latency, and (4) the decrease in activity prior to the subsequent stimulus pulse. (B) The Z-score-adjusted histogram quantitatively compares the pre-stimulus baseline activity to the activity during continuous 160 Hz stimulation, showing highly significant changes in STN activity temporally related to the delivery of the contralateral stimulus (illustrated with the same numbers as in 3A). (C1) Analog raster plots aligned temporally to the onset of the contralateral stimulus, showing consecutive occurrences of the same neuron in the condition where the discharge follows the contralateral 160 Hz STN stimulus. These data display the neuronal waveforms from the 160 Hz stimulation condition in Figure 3A. (C2) This analog raster plot displays the same neuron when the DBS is off. This shows the neuronal waveforms from the DBS off condition in the discrete raster from Figure 3A ("pseudostimulation").

**Figure 4.** (A), (B), and (C) Discrete rasters and Z-score adjusted histograms of 160 Hz and 30 Hz contralateral STN DBS in representative neurons from 3 different subjects undergoing staged STN DBS for PD. In contrast to Figure 3, 120 seconds of contralateral STN DBS (gray bar) are straddled by 120 second epochs of resting STN activity with DBS off (solid bars). Z-score adjusted histograms compare the pre-stimulus baseline activity to the activity during continuous 160 Hz and 30 Hz frequency
stimulation showing significant changes in STN activity temporally related to the delivery of the contralateral stimulus. In addition to the short latency responses described previously in Figure 3, there is an increase in activity at a latency of approximately 3.5 to 5 ms after the stimulus pulse during 160 Hz stimulation but not during 30 Hz stimulation over the same time interval (Figures A2, B2, and C2 versus A4, B4, and C4). Figures A5, B5, and C5 and their accompanying Z-score adjusted histograms contain the same data as that displayed in A3, B3, and C3, except that the response to 30 Hz stimulation is shown over a longer time window. Abbreviations: subthalamic deep brain stimulation (STN DBS), hertz (Hz), seconds (sec).

Figure 5. (A) Analog raster showing consecutive occurrences of the same neuron in the condition where the discharge follows the high frequency contralateral STN stimulus within 1 ms of the stimulus pulse. The analog data contains the stimulus artifact (black arrows). (B) The first column shows epochs of the analog data with neuronal action potentials occurring immediately prior to the contralateral stimulation pulse with no subsequent short latency discharge after the stimulation pulse. The second column displays epochs of the analog data in which the stimulus pulse is followed by a short latency neuronal discharge and not preceded by an action potential. (C1 and C2) Collision analysis compares the probability of a discharge of the neuron when it does not discharge within 3 ms before the stimulus pulse versus when it does discharge within 3 ms before the stimulus pulse. The probability of a short latency action potential is decreased immediately after the stimulation pulse only when the stimulus pulse is preceded by a spontaneous discharge of the neuron. Abbreviations: microvolt (µV), millisecond (ms), second (sec).
Figure 6. (A) The total discharge frequency across all neurons was greater during high frequency contralateral stimulation than during low frequency stimulation or the resting state (p < 0.01, respectively, ANOVA of log transformed discharge frequency). (B) A scatter plot of the probabilities of an antidromic response in each neuron following the contralateral stimulus during high and low frequency stimulation. The probability of an antidromic response was independent of stimulus frequency (Slope 1.19, Spearman correlation 0.89). Abbreviations: subthalamic deep brain stimulation (STN DBS), hertz (Hz), seconds (sec).
<table>
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<th>Age* (years)</th>
<th>Duration of Disease* (years)</th>
<th>Gender</th>
<th>Stimulation site</th>
<th>Recording site</th>
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<th>Medications</th>
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<td>61.1 ± 10.9**</td>
<td>18.3 ± 7.7**</td>
<td>M (0.63)</td>
<td>L (0.5)</td>
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** Abbreviations:** STN (subthalamic nucleus), V (Volts), µsec (microseconds), Hz (hertz)

* rounded to nearest decade

** mean ± standard deviation
A. Bar chart showing discharge frequency (Hz) for contralateral STN DBS stimulation frequency at 0 Hz, 160 Hz, and 30 Hz.

B. Scatter plot showing the probability of antidromic response following 30 Hz contralateral STN DBS pulse vs. the probability of antidromic response following 160 Hz contralateral STN DBS pulse. The slope is 1.19, and the Spearman correlation is 0.89.