Title: Modulation of motor cortex neuronal activity and motor behavior during subthalamic nucleus stimulation in the normal primate


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Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a well-established surgical therapy for advanced Parkinson’s disease (PD). An emerging hypothesis is that the therapeutic benefit of DBS is derived from direct modulation of primary motor cortex (M1), yet little is known about the influence of STN DBS on individual neurons in M1. We investigated the effect of STN DBS, delivered at discrete interval intensities (20, 40, 60, 80, and 100%) of corticospinal tract threshold (CSTT), on motor performance and M1 neuronal activity in a naïve non-human primate. Motor performance during a food reach and retrieval task improved during low intensity stimulation (20% CSTT), but worsened as intensity approached the threshold for activation of corticospinal fibers (80% and 100% CSTT). To assess cortical effects of STN DBS, spontaneous, extracellular neuronal activity was collected from M1 neurons before, during and after DBS at the same CSTT stimulus intensities. STN DBS significantly modulated the firing of a majority of M1 neurons, however the direction of effect varied with stimulus intensity such that, at 20% CSTT, most neurons were suppressed, whereas at the highest stimulus intensities the majority of neurons were activated. At a population level, firing rates increased as stimulus intensity increased. These results show that STN DBS influences both motor performance and M1 neuronal activity systematically according to stimulus intensity. In addition, the unanticipated reduction in reach times suggests that STN DBS, at stimulus intensities lower than typically used for treatment of PD motor signs, can enhance normal motor performance.

**Key words:** subthalamic nucleus, deep brain stimulation, primary motor cortex, Parkinson’s disease
Introduction

Changes in neuronal activity in the subthalamic nucleus (STN) are associated with the development of parkinsonian motor signs, while high-frequency deep brain stimulation (DBS) in the area of the STN improves such motor signs (Krack et al. 2003). Although the mechanism underlying its beneficial effects continues to be debated, there is substantial evidence that DBS activates output from the stimulated structure modulates neuronal activity across the pallidothalamocortical circuit (Agnesi et al. 2013)

Given the anatomical and functional connection between the STN and primary motor cortex (M1), activity changes in the STN in PD and during DBS may be mediated in part by changes in neuronal activity at the level of M1. Although anatomical and electrophysiological data support involvement of the STN in modulating activity at the level of the sensorimotor cortex (Israel and Bergman 2008), the effect of STN DBS on motor cortical activity and its relationship to improvements in PD motor signs remains under debate. STN stimulation has been shown to activate M1 neurons antidromically, providing another putative candidate in the search for a therapeutic mechanism(s) (Li et al. 2012). However, positron emission tomography (PET) studies of PD patients suggest that STN DBS may act through a reduction of spontaneous motor cortical activity. (Ceballos-Baumann et al. 1999; Payoux et al. 2004). As a first step towards a better understanding of the physiological relationship between these two key structures, we investigated how STN DBS modulates neuronal activity in the motor cortex and its relationship to motor performance during a reach and retrieval task in the normal non-human primate. We hypothesized intensity-dependent changes in motor performance, with higher intensity stimulation activating corticospinal tract fibers resulting in impaired motor performance and increased movement times, while lower intensity stimulation would have little or no effect on movement times.

Materials and Methods

All surgical and behavioral protocols were approved by the Institutional Animal Care and Use Committee and conducted in accordance with United States Public Health Service policy on the humane care and use of
STN stimulation and M1 activity in normal primate laboratory animals. Data were collected from one naïve, female Rhesus monkey (*Macaca mulatta*, 5.7 kg, 7.5 yrs). Behavior assessments and neuronal recordings were conducted separately, but on the same days, over 2.5 months.

**Surgical procedures and cortical recordings.** Surgical procedures related to the placement of cephalic recording chambers and DBS lead implantation have been detailed previously (Hashimoto et al. 2003). Briefly, a scaled version of the human DBS lead was implanted through a cephalic chamber targeting the STN, which had been previously identified using microelectrode mapping techniques. Neuronal activity was recorded extracellularly using glass-coated platinum-iridium microelectrodes (0.5-1.0 MΩ at 1kHz) advanced using a hydraulic microdrive (Narishige) attached to a chamber targeting motor cortex. Identification of M1 was verified by neuronal responsiveness to passive and active movement and microstimulation-evoked motor responses. Spontaneous activity of M1 neurons was recorded in contiguous 60 second epochs before, during and after STN DBS (Itrel II, Medtronic; 90 μs; 135 Hz; contact 1 cathodal, case anodal). STN DBS was applied over a range of voltage intensities (20, 40, 60, 80 and 100%) relative to the corticospinal tract threshold (CSTT), which was operationally defined as the lowest intensity required to induce muscle contraction in the face or contralateral upper or lower extremity as determined by visual inspection (Tommasi et al. 2008). Throughout the experimental period CSTT threshold was consistently 2.3-2.4 V. At each recording site, the order of stimulus conditions was pseudo-randomized, not allowing for repeated conditions. Random integers 1-5 (coded to the five stimulus conditions) were generated by multiplying the numbers generated by the Random Number (0-1) Function in LabVIEW (National Instruments) by the number of stimulus conditions (five).

**Behavioral assessment and analysis.** Motor behavior was assessed by training the animal to retrieve a series of three food rewards from a modified Klüverboard, which consisted of a 1 x 3 matrix of food wells, 2 cm in diameter and 3 mm deep. The arm ipsilateral to the implanted DBS lead was lightly restrained. Total movement time was defined as the time between the animal’s hand leaving and returning to its mouth with the food reward. Each movement was further sub-divided into three distinct, contiguous epochs: reach, manipulation and retrieval time. Reach time was defined as the time between the animal’s hand leaving its
mouth and reaching the plane of the Klüverboard, with retrieval time defined as the duration of the inverse movement. Manipulation time was defined as the period during which the hand remained beyond the frontal plane of the Klüverboard. An OPTOTRAK 3-D Motion Capture and Analysis System (Northern Digital, Inc.) was used to track limb movement during the food reach/retrieval task performed at the same interval intensities as those used during extracellular recordings. The order of stimulus conditions was pseudo-random as described for neural recordings, and conditions were not repeated until all other conditions were applied. DBS was applied at one intensity level continuously during a behavior recording block, generally with two recording blocks conducted each day. For each condition, behavioral outliers in the movement time data (>1.5×IQR) were excluded from further analysis. Statistical comparisons of total movement time, as well as for each of the three separate epochs, were made as a function of DBS condition using Kruskal–Wallis analysis of variance on ranks, with multiple comparisons corrected using Dunn's method (p<0.05).

**Neuronal Data analysis.** Neuronal recordings were analyzed offline using custom software developed in MATLAB (Mathworks) and Offline Sorter (Plexon Inc.). Stimulus artifacts were removed using template subtraction techniques described previously (Hashimoto et al. 2002). Single units were isolated and sorted using principle component and template-based methods in Offline Sorter. Peri-stimulus time histograms (PSTH, bin size = 0.2ms) of neuronal activity before, during and after DBS were examined, triggered to either the stimulation pulses during DBS or to virtual stimulation epochs before and after DBS (McCairn and Turner 2009); see Fig. 1D, left panel). To test the effects of DBS on neuronal activity, PSTHs were analyzed using a method similar to McCairn and Turner (2009). Briefly, each PSTH was normalized by subtracting the mean firing rate calculated over the individual neuron’s pre-stimulation, baseline period (Fig. 1D, middle panel). The normalized PSTHs reveal changes from baseline activity, referred to here as deviation areas (Fig. 1D, right panel). To determine whether a neuron’s firing was modulated significantly by DBS, each deviation area in the stimulation period was detected and converted to a z-score relative to a control population of deviation areas taken from the baseline, pre-stimulation period. The threshold for significance was corrected for multiple comparisons (alpha = 0.01/(number of deviations detected)). Neuronal responses to DBS fell into four general
STN stimulation and M1 activity in normal primate categories: inhibition, excitation, inhibition followed by excitation, and no significant change (Fig. 1E-F). To assess the effects of DBS on population activity, firing rate PSTHs of each neuron during stimulation were converted to z-scores relative to the baseline PSTH for that neuron and averaged across all other neurons for a given stimulus intensity (Fig. 1G).

**Histology.** After completion of the study, the animal was euthanized with an overdose of pentobarbital. The brain was removed, fixed and sectioned into 50 µm slices, which were stained alternately with parvalbumin, acetylcholinesterase and Nissl to facilitate localization of microelectrode recording sites as well as to identify the position of the DBS lead relative to the STN. The more ventral portion containing the STN was sectioned in the sagittal plane, while the dorsal portion was sectioned in the coronal plane (Fig. 1C).

**Results**

**Changes in behavioral performance during STN DBS**

The total numbers of trials acquired for 0, 20, 40, 60, 80 and 100% CSTT stimulus conditions were 107, 82, 81, 85, and 93, respectively. Figure 1A illustrates the total time required to complete the reach and retrieval behavior task in each stimulation condition. The model revealed a significant main effect for DBS intensity level ($H_5 = 53.87, p < 0.001$), with post-hoc testing using Dunn’s method identifying the changes in total movement time during DBS at 20% ($Q = 3.912, p < 0.05$) and at 100% ($Q = 2.728, p < 0.05$) to be significantly different from the OFF DBS condition. The differences were opposite in direction, with total movement time decreasing (i.e., faster motor performance) when DBS was delivered at 20% of CSTT and increasing (i.e., slower motor performance) during DBS at 100% of CSTT (Figure 1A).

The reach and retrieval task incorporates both gross (reach and retrieval) and fine (grasping the food reward) motor behaviors. To investigate further which components of the overall behavior were influenced by DBS as a function of stimulus intensity, analysis of task performance was extended to each of the three distinct, contiguous epochs: reach, manipulation, and retrieval (Fig. 1B, left, middle, right panels respectively).
The ANOVA model revealed a main effect of DBS condition for both the reach ($H_5 = 41.78, p < 0.001$) and retrieval ($H_5 = 31.903, p < 0.001$) aspects of the behavior, with post-hoc testing identifying as significant the reduction in performance time observed during DBS at 20% of CSTT for both behaviors (Reach: -13%, $Q = 5.516, p < 0.05$; Retrieval: -7%, $Q = 3.894, p < 0.05$). For the manipulation component, the ANOVA again revealed a main effect of DBS condition ($H_5 = 40.454, p < 0.001$), with post-hoc analysis identifying an increase in median manipulation time during DBS at both 80% ($Q = 3.024, p < 0.05$) and 100% ($Q = 4.744, p < 0.05$) of CSTT, respectively, as significant. Overall, these findings were consistent with the effect observed on total movement time, with the improvement in motor behavior during DBS at 20% of CSTT attributable to changes in gross motor behavior (i.e., decreased reach and retrieval times), while the performance degradation observed at higher DBS intensities could be attributed to an increase in the amount of time spent grasping the reward (i.e., increase manipulation time).

**Changes in M1 neuronal activity during STN DBS**

A total of 31 neurons were sampled from M1. Microstimulation-evoked face or contralateral limb movement was observed at each recording site with a mean intensity of 35 µA (range: 17 µA - 100 µA). Given the number of conditions being investigated, it was not possible to maintain stable extracellular recording of a given cell for each planned CSTT voltage level. As such, the data were divided into collection periods across cells, with the activity of 10 M1 neurons collected during DBS at 20% CSTT, 14 at 40%, 12 at 60%, 15 at 80% and 18 at 100%. The majority (N=20) were collected under at least 2 stimulation conditions with twelve collected under 3 or more conditions.

Peri-stimulus time histograms (PSTH, bin size = 0.2ms) of neuronal activity before, during and after DBS were examined (Fig. 1D). As depicted in Figure 1E, there were four primary M1 neuronal response types observed during STN DBS: inhibition, excitation, inhibition/excitation and no change. Figure 1F summarizes the proportion of neurons exhibiting each response type as a function of DBS intensity. Whereas at 20% of CSTT the majority of neurons were inhibited by STN DBS, higher stimulation intensities were associated with a...
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decrease in the proportion of inhibited neurons and a corresponding increase in the proportion of excited
neurons. This overall increase in M1 activity at higher stimulation levels is illustrated more clearly by the
population PSTH plots in Figure 1G, where the PSTH bins of each neuron were converted to z-scores and
averaged across all neurons for a given STN DBS condition. Whereas the mean PSTH at 20% CSTT reflects
decreased activity (negative Z-scores) throughout most of the 7 ms PSTH period, at higher stimulus intensities
there is an overall increase in the neuronal activity (positive Z-scores). As there was no clear pattern of
excitation or inhibition over the PSTH periods, the change in neuronal activity associated with STN DBS was
summarized further by calculating the mean Z-score of the population PSTH as shown in Figure 1H. There was
a positive correlation between stimulation intensity and mean Z-score ($R^2=0.122$, $p < 0.01$). The mean z-scores
at 80 and 100% CSTT were greater than 1 and significantly different from zero (Student’s t-test, $p < 0.01$).

**Correlation of behavior performance with neuronal activities**

When the behavior data were matched with the neuronal changes under the same stimulation conditions,
we observed a strong positive correlation between the change in neuronal activity associated with STN DBS
(mean Z-score) and behavioral performance (total movement time) ($R^2=0.96$, $p < 0.01$).

**Histology**

The DBS lead location is shown to be positioned in the dorsal posterior region of the STN, 6 mm from the
midline (Figure 1C, left panel, red-dashed oval). Recording sites in the motor cortex in the coronal sections
were confirmed to be within the M1 area (Figure 1C, right panel, white-dashed rectangles).

**Discussion**

**Motor performance depends on intensity of STN DBS**

The current study is the first to link motor performance changes with STN DBS in the normal, non-human
primate to changes in extracellular, single-unit neuronal activity in M1. Consistent with our hypothesis, we
observed impaired motor performance as stimulus intensities approached the threshold for overt muscle contractions, attributable to activation of corticospinal fibers in the adjacent internal capsule. An unanticipated behavioral finding was enhancement of motor performance at the lowest intensity of STN DBS (~0.5 V). Notably, these opposing changes in total movement time originated from distinct sub-components of the overall behavior. Whereas the augmented performance at 20% CSTT was associated primarily with an increase in the speed of the more gross aspects of the motor behavior (i.e., reach/withdraw), the increase in total movement time at higher stimulus intensities appeared to relate more to difficulties with fine motor control (i.e., manipulation, grasp). These behavioral findings were found to correlate with changes in mean discharge rate in M1, with the overall population activity reduced at the lowest STN DBS amplitude setting and increased at higher intensities.

The improvement in motor performance at low stimulation intensity is particularly intriguing. Reach and retrieval epochs were completed faster during 20% CSTT DBS but manipulation times were unaffected. Why would 20% CSTT stimulation enable the animal to move more rapidly? One possibility is that this level of stimulation in the naïve animal is analogous to the “therapeutic level” of stimulation used in STN DBS to treat PD motor signs, enabling faster movements in both normal and PD states. Yet in our experience with DBS in parkinsonian animals, therapeutic stimulation levels are typically higher than the 20% CSTT intensity found to improve movement speed in this study. In those models, we assess therapeutic benefit primarily with measures of rigidity, setting stimulation levels slightly below thresholds for corticospinal tract activation and dyskinetic movements (Hashimoto et al. 2003); it is possible that more systematic examination of the relationship between stimulation level and bradykinesia as conducted here could reveal additional benefit at even lower intensities in parkinsonian subjects. Previous studies in patients have found that rigidity improves with increasing intensity up to the level of corticospinal activation, but improvements in bradykinesia are maximal at intermediate stimulation intensities (Butson et al. 2007). Our results raise the question of whether lower-than-normal stimulation levels could still be beneficial in PD patients, particularly those whose primary motor symptom is bradykinesia. This would have the added benefits of increased device battery life and
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reduced motor and neuropsychological side effects caused by sustained high levels of STN stimulation (Castrioto et al. 2014). Alternatively, the mechanisms involved in improved motor performance observed in this study at 20% CSTT in the normal animal could be different from the primary mechanisms involved in improvements in bradykinesia observed in PD at higher stimulation intensities reflecting differences in spontaneous and task related M1 neuronal activity in the parkinsonian state.

**M1 activity depends on STN DBS intensity**

At 20% CSTT, STN DBS inhibited a majority of neurons tested and was associated with a trend towards reduced overall firing rate. Interestingly, this was the only stimulation intensity that significantly improved motor performance. Consistent with these observations, Cilia et al. and Karimi et al. found that therapeutic STN DBS is associated with reduced regional cerebral blood flow in motor and prefrontal cortical areas in PD patients (Cilia et al. 2009; Karimi et al. 2008). Similarly, Payoux et al. have suggested that STN DBS improves parkinsonian motor signs predominately by reducing “abnormal resting overactivity” in the motor system, allowing selective cortical activation during movement (Payoux et al. 2004). DBS of the globus pallidus also has been demonstrated to decrease activity in sensorimotor cortex coincident with improvements in bradykinesia (Valálik et al. 2009). Previous data from our laboratory showed a reduction in M1 activity during pallidal stimulation in the MPTP monkey model and an increase in the signal-to-noise ratio of M1 activity in response to passive limb manipulations (Johnson et al. 2009). These data support the hypothesis that improvements in motor performance during stimulation in the pallidum or the STN in the parkinsonian animal occur in part via suppression of mean discharge rates and/or a change in the proportion of neurons activated and inhibited, together with improvement in processing sensorimotor information (Johnson et al. 2009).

However, this hypothesis is not fully supported by other studies which report increased M1 activity with therapeutic STN DBS in the PD state (e.g. (Li et al. 2012; Walker et al. 2012)). A possible explanation for the different observations may be differences in the relative volume of tissue activated (VTA) by DBS in different animal models, with stimulation methods in rats (e.g. Li et al. 2012) potentially producing larger VTA than in our primate studies leading to increased antidromic activation of M1 corticofugal projections.
We found that overall M1 neuronal activity increased as STN DBS intensity increased (Fig. 1H). It is notable that there is similarity in the percentage of excited and inhibited neurons between the 40% and 100% CSTT categories. However, as illustrated in Figure 1G,H, an important difference between conditions may be in the degree of excitation and inhibition at a population level, rather than solely the percentage of neurons excited or inhibited. The explanation for differential motor cortical activation with stimulus intensity may be differential activation of fiber pathways within and adjacent to the STN with increasing stimulation intensity. STN projects predominantly to the GPi, but also GPe, striatum, and substantia nigra pars compacta, and it receives projections from predominantly the GPe as well as PPN, SNpc, and motor cortex (Temel et al. 2005). The STN is interconnected with the GPe through afferent inhibitory and efferent excitatory projections, and activation of either fiber paths in the STN would result in inhibition of STN cells and decreased activation of GPi. Reduced GPi activity would lead to disinhibition of the thalamus and increased thalamo-cortical activity. Conversely, activation of STN projections to GPi by DBS could increase firing in GPi cells (Hashimoto et al. 2003), thereby increasing GPi inhibitory output to the thalamus and leading to decreased thalamo-cortical activity. STN stimulation could also cause antidromic activation of M1 projections either terminating within the STN or passing adjacent to it in the internal capsule. Either pathway could contribute to excitation of M1 neurons, however we found very few M1 neurons with significant short latency activity that would be indicative of direct antidromic activation. As shown in the mean PSTH plots (Fig. 1G), at 80% and 100% CSTT excitation persisted throughout the 7ms interstimulus interval. This does not rule out antidromic activation per se, just that the M1 neurons sampled were not antidromically activated under these experimental conditions. Effects of STN DBS could be more indirect, with antidromic activation of other M1 neurons influencing the firing probability of the sampled population (Li et al. 2007). Contrary to the hypothesis described above that inhibition of M1 is related to improved motor signs, an alternate hypothesis has been proposed: that the therapeutic effect of STN DBS is derived from antidromic activation of M1. Li et al. showed in 6-hydroxydopamine (6-OHDA)-lesioned rats that antidromic activation corticofugal M1-STN projection neurons is correlated with improved motor performance (Li et al. 2012). Dejean et al. showed that STN
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stimulation that improved motor function in the parkinsonian rat was correlated with the amplitude of a short latency, possibly antidromic, evoked cortical potential (Dejean et al. 2009). Although these studies suggest that antidromic M1 activation may be therapeutic, we found that as stimulation intensity increased toward the threshold for overt muscle contraction, movement performance worsened. One could argue that the different observations represent a difference in the baseline state of the normal versus parkinsonian state; however in a previous study we observed worsening motor performance in the parkinsonian animal during stimulation parameters that lead to activation of the corticospinal tract below thresholds for overt muscle contraction (Xu et al. 2011). Additional studies that assess motor behavior and underlying neural activity at multiple nodes of the basal ganglia-thalamo-cortical motor network are needed to further elucidate the mechanisms underlying the therapeutic effect of STN DBS.

Conclusions

It is well-established that high frequency stimulation of the STN improves motor signs in individuals with PD and animal models of PD, but to our knowledge this is the first demonstration of the capability of DBS to enhance performance of reaching behavior in a normal animal. The data presented suggest that improvement in motor behavior is associated with suppression of activity in the majority of the M1 cells, which shifts to activation with increasing current intensities leading to worsening motor performance. Dissociation between improvement in gross and impairment in fine motor control was observed at low versus high DBS intensities and may reflect the shift in the population of M1 cells that are tonically activated versus suppressed. While these data must be interpreted with caution given the neural and behavior data were collected separately, with neural data obtained during a passive awake state, they support previous studies in parkinsonian subjects demonstrating suppression of M1 activity during therapeutic DBS. Subsequent experiments that record from populations of M1 cells simultaneously during a behavior task will be necessary to more clearly explain how cortical activity is affected by DBS and how it is directly correlated with motor performance.
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**Figure legend**

Behavioral and neurophysiological effects of STN DBS in a healthy non-human primate. Stimulation was delivered at interval intensities (20, 40, 60, 80, and 100%) of corticospinal tract threshold (CSTT). (A) Total movement time of the contralateral hand during a food reach and retrieval task (mean ± SEM shown, *p<0.05). (B) Movement times subdivided into reach, manipulation and retrieval times. (C) (left panel) Sagittal histological section of the basal ganglia area. The DBS lead was located within the dorsal posterior area of the STN (red-dashed circle). (right panel) Coronal section showing M1 recording sites (white-dashed rectangles). (D) (left panel) Example of unit PSTHs before, during and after DBS, triggered to stimulus pulses during DBS or virtual stimulation epochs before and after DBS (left panel). (middle panel) PSTHs normalized by the pre-stimulation period. Significant deviation areas (a,b,c, in middle, right panels) were used to classify response types. (E) Neuronal responses to DBS fell into four general categories. (F) The proportion of cells in each response category according to stimulation intensity. (G) Population average PSTHs. (H) Mean firing rates (converted to z-scores) were significantly different from zero at 80 and 100% CSTT, and there was a positive correlation between stimulation intensity and mean Z-score ($R^2=0.122$, $p < 0.01$), similar to the observed trend of increased total movement time with higher stimulation intensities.
References


