The electrocorticogram signal can be modulated with deep brain stimulation of the subthalamic nucleus in the hemi-Parkinsonian rat

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Abbreviated title/Running Head:   STN DBS Neuromodulation of Cortical ECoG Signals

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ABSTRACT

Electrocorticogram (ECoG) recordings of the 6-OHDA lesioned Parkinsonian rat have shown an increase in the power of cortical $\beta$ (15-30 Hz) band oscillations ipsilateral to the lesion. The power of these oscillations is decreased with dopamine agonist administration. Here, we demonstrate that stimulation of an electrode implanted in the subthalamic nucleus alters the power of cortical $\beta$ and $\gamma$ oscillations in 6-OHDA lesioned animals. These alterations are dependent on stimulation frequency, charge, and amplitude/pulse width. Oscillations were significantly reduced during 200 and 350 Hz stimulation. A minimum charge of 4 nC was required to elicit a reduction in oscillation power. A number of amplitude and pulse width combinations that reached 4 nC were tested; it was found that only the combinations of 33 $\mu$A-120 $\mu$s and 65 $\mu$A-60 $\mu$s significantly reduced cortical oscillations. The reduction in $\beta$/-$\gamma$ oscillation power due to deep brain stimulation (DBS) was consistent with a significant reduction in the animals’ rotational behavior, a typical symptom of Parkinsonism in the rat. A significant shift from high $\beta$ to low $\gamma$ was observed in the peak frequencies of ECoG recordings while animals were at rest vs. walking on a treadmill. However, DBS exhibited no differential effect on oscillations between these two states. EEG recordings from rodent models of DBS may provide surrogate information about the neural signatures of Parkinson’s disease relative to the efficacy of DBS.

Six keywords: Neuromodulation, DBS, STN, ECoG, EEG
Deep Brain Stimulation is now being used clinically as a treatment for Parkinson's Disease (PD). The subthalamic nucleus (STN) is a frequent target of high-frequency stimulation (HFS) to moderate the symptoms of PD for patients both on and off medication (Obeso et al. 2001). Abnormal oscillations manifest in PD near the β band (~13 – 30 Hz) in humans (Brown et al. 2001; Brown and Williams 2005; Cassidy et al. 2002; Hammond et al. 2007; Levy et al. 2002) and rodent models of PD (Mallet et al. 2008; Sharott et al. 2005). Previous work has correlated cortico-cortical EEG synchrony of β oscillations in humans with the severity of their Parkinsonism (Silberstein et al. 2005) including synchronization and coherence of EEG with the STN field potentials (Williams et al. 2002). The severity of PD symptoms however, was found to have no correlation with the percentage of STN neuronal β oscillations but instead correlated with the magnitude of preoperative motor response to dopaminergic medication (Weinberger et al. 2006). Indeed, administration of levodopa, a precursor of dopamine, has a profound effect upon the motor symptoms of PD (Kuhn et al. 2006) and has been shown to diminish the abnormal β oscillations (Alonso-Frech et al. 2006; Brown et al. 2001; Doyle et al. 2005; Levy et al. 2002; Priori et al. 2004; Williams et al. 2002). However, the degree to which β oscillations correlate to the severity of Parkinsonism is not clear as dopaminergic therapy has been shown to increase cortico-cortical coupling in patients in the early stages of therapy who experience marginal motor improvement (Stoffers et al. 2008).

HFS of the STN has also been shown to reduce STN β oscillations (Wingeier et al. 2006) which corresponds to reduced severity of parkinsonian symptoms, particularly related to akinesia (Kuhn et al. 2008a) and rigidity and bradykinesia (Kuhn et al. 2008b). Yet, during HFS, changes in lower frequency STN oscillations (1 – 7 Hz) have been observed without changes in β oscillations during HFS (Rossi et al. 2008) with β oscillations having no correlation to motor symptom improvement (Foffani et al. 2006). This observation challenges the relevance of β oscillations as an indicator for therapeutic stimulation. Motor improvements brought about by STN DBS however, have been correlated to reduced coherence between multiple EEG electrodes in the 10-35 Hz range in patients with advanced-PD, reflecting a disruption of synchronous β-band oscillations (Silberstein et al. 2005).
The DBS-induced reduction in β oscillation power tends to last longer following longer periods of DBS (Bronte-Stewart et al. 2009). The regions of the STN that exhibit the greatest abnormal β-band oscillations occur at the dorsal STN border (Kuhn et al. 2005; Trottenberg et al. 2007; Weinberger et al. 2006) which corresponds well with models of patient-specific activation patterns that provide maximal clinical response to DBS (Butson et al. 2007). Further, it has been shown in studies combining low-frequency DBS (< 10 Hz) with EEG recordings that motor areas are highly activated during the stimulation (MacKinnon et al. 2005). These studies suggest that the EEG from motor frontal cortex contains a PD signature that is sensitive to STN DBS therapy.

The objective of this study was to investigate the change in cortical β/γ-band power as a function of STN DBS parameters in a rodent surrogate of DBS for alleviating the symptoms of PD. Frequency of stimulation, total charge (a co-variation of current amplitude and pulse width), and behavioral state (resting and walking) were manipulated while continuously recording the electrocorticogram (ECoG). ECoG recordings over the motor cortex of rats have been shown to change in response to a chemical lesion induced by selective destruction of dopamine neurons by 6-Hydroxydopamine (6-OHDA) (Vorobyov et al. 2003), a standard model for PD (Cadet et al. 1992; Perese et al. 1989). The changes in ECoG were, specifically, an increase in the power of β/γ band frequency (20-45 Hz) that could be seen differentially in the same animal (Vorobyov et al. 2003) as the chemical lesion was only created unilaterally (Schwarting and Huston 1996). Previous attempts to reduce the β frequency power on the lesioned side of 6-OHDA treated rats have been made using pharmacotherapy such as dopamine agonists (Sharott et al. 2005). Here we test the hypothesis that abnormal cortical oscillations can be reduced with STN DBS in the hemi-parkinsonian rat and that reducing the power of these oscillations correspond to improved motor symptoms. We demonstrate that β/γ-band power in the ECoG of Parkinsonian rats can be significantly reduced with HFS and that these alterations in the ECoG power may be indicative of the therapeutic effects of STN DBS.
Materials and Methods

Experiments were carried out on 14 male Sprague-Dawley rats (3 normal, and 11 lesioned as described below). All animals were housed in a temperature and humidity-controlled room with a 12-hour light–12-hour dark cycle. All procedures were conducted in accordance with protocols approved by the University of Michigan University Committee on Use and Care of Animals (UCUCA).

Unilateral Dopamine Lesion

Unilateral 6-OHDA lesions were carried out on 11 rats. One hour prior to lesioning, animals were given intraperitoneal injections of Pargyline (50 mg/kg) to amplify the effects on dopaminergic neurons and to help preserve noradrenergic neurons and Desipramine (25 mg/kg), to prevent toxicity to the noradrenergic neurons (Schwarting and Huston 1996). The rats were anesthetized either with inhaled isoflurane or an intraperitoneal injection of a mixture of ketamine (25 mg/kg), xylazine (1.25 mg/kg), and acepromazine (0.1 mg/kg). They were then placed in a stereotactic frame (MyNeuroLab, St. Louis, MO). A 2 mm craniotomy was made. 10 µg of 6-Hydroxydopamine Hydrobromide (6-OHDA, Sigma Chemical Co., St. Louis, MO) stabilized in 0.01% ascorbic acid with 0.9% normal saline (2 µl total volume) was infused at a rate of 0.5 µl/minute through a 26-gauge, 10 µl syringe in the right medial forebrain bundle (MFB) site: 4.4 mm posterior and 1.2 mm lateral to the bregma, and 7.5 mm ventral to the dura mater. All coordinates were obtained from the atlas of Paxinos and Watson (Paxinos and Watson 1998). After the injection of 6-OHDA, the cannula was left in place for 5 minutes before being slowly retracted.

Lesion Testing

Each rat was tested anywhere from 2 to 4 weeks after the 6-OHDA injection. Each test began with an injection of Amphetamine (5 mg/kg, intraperitoneal) followed by placement in a rotometer (Ungerstedt and Arbuthnott 1970). One week later animals were injected with apomorphine (0.025 mg/kg, subcutaneous) and again placed in the rotometer. A lesion was considered successful if the rat managed at least 6 rotations per
minute in the clockwise direction in the time period between 30 to 90 minutes after initial injection during amphetamine challenge and at least 2 rotations per minute in the counter-clockwise direction between 30 to 60 minutes after apomorphine challenge; of the 11 animals initially injected with 6-OHDA, only 7 (64%) met these criteria and were considered for electrode implantation.

Electrode implantation

After demonstrating symptomatic evidence of 6-OHDA lesion, each rat was anesthetized and placed in a stereotactic frame as described above. Two ECoG screws were located at AP 1.7 mm, ML 2.5 mm bilaterally to bregma (supplementary Figure 1). Two additional screws serving as ECoG reference and ground were placed over the parietal lobe and cerebellum. Two types of electrodes were placed in the STN. Initially two animals, one normal and one lesioned, were implanted with a monopolar, stainless steel FHC microelectrode with a tip diameter of 100 μm (FHC instruments, Bowdoin, ME). However, stereotactic implantation of this electrode proved difficult without a guide cannula and histological analysis of the electrode location showed consistent misplacement of these electrodes. All subsequent animals (2 normal, 6 lesioned) received a stainless steel microelectrode with a tip diameter of 100 μm surrounded by a concentric guide cannula (Plastics1, MS308). STN coordinates used were AP -3.6, ML 2.5 to bregma. During surgery, we recorded extracellular action potentials from the stimulating electrode as it was advanced. The electrode was attached via amplifier and audio output (Tucker-Davis Technologies, Alachua, FL). At various points along the tract, electrode advancement was halted and potentials were monitored to determine optimal depth for STN recording and stimulation. Ideal positioning of the electrode would require that it not be retreated after initial advancement as to minimize damage to surrounding tissue. The paradigm developed consisted of advancing the electrode to 1-2 mm proximal of the intended stereotactic depth of DV 7.6 mm. Potentials were monitored for spike activity. When activity decreased, the electrode was assumed to be in the relatively silent Zona Incerta (ZI). Advancing by 50 μm increments, we encountered an increase in activity which was presumed to be the STN and the electrode was fixed at this location.
Electrophysiological Recording and Electrical Stimulation

Each animal was placed in a faraday cage on a pneumatic treadmill while its ECoG was measured and DBS delivered. Left and Right ECoG signals were routed through a commutator before being differentially amplified (10,000x) with respect to a reference bone screw over the parietal lobe, filtered 0.3 – 300 Hz, 6dB/octave rolloff (SR560, Stanford Research Systems, Inc., Sunnyvale, CA) and digitized at 1KHz along with DBS timing event markers (MNAP, Plexon, Inc., Dallas, TX). This configuration allowed recording of robust noise- and artifact-free cortical signals that were stable across days and the behavioral state of the animal. Electrical stimulation was controlled through Matlab (release 7, The Mathworks, Natick, MA) running ActiveX controls that modulated the parameters of the stimulation pulses generated in RPvds software and RP2.1 hardware (Tucker-Davis Technologies, Alachua, FL). Stimuli were then delivered to the animal through an optically isolated, constant current stimulator (Model 2200, A-M Systems, Inc., Carlsborg, WA). Stimulus pulses consisted of square wave biphasic, charge balanced, cathodic-first, constant current pulse pairs delivered at less than 50 μC/cm² charge density, the maximum charge deliverable through stainless steel (Merrell, 2005). This configuration ensured that (1) the amount of charge entering the tissue could be precisely controlled during each phase of pulsing, and (2) a net charge as that was as close to zero as possible was delivered during all stimulation pulses so as to prevent excessive charge buildup that could lead to destruction of the electrode and/or tissue. Additional safety measures were implemented by continuously monitoring the voltage at the electrode such that the voltage did not exceed the water window of stainless steel, (i.e., the voltage at which hydrolysis of water into hydrogen and oxygen occurs, which is approximately +/- 1 V). Trains of pulses were delivered at frequencies of 50, 125, 200, 275 and 350 Hz (Frequency), while current amplitude and pulse width co-varied between 130, 65, 33, 16, and 8 μA (Amplitude), and 30, 60, 120, 240, and 480 μs (Pulse Width), respectively to achieve a maximum charge of 3.9 nC/phase or less (Charge, Figure 1). DBS polarity was essentially monopolar due to much larger surface area (~600x) of the guide cannula that served as the current sink. ECoG measurements during stimulation were achieved by coupling the stimulating mechanism with the blanking input on the ECoG amplifiers that would
reduce the amplifier gain to zero during the brief periods of stimulation to prevent amplifier saturation.

ECoG signals were recorded in two, 76 min, sessions per animal. Each session consisted of alternating 19 min segments of the animal at rest or walking on the treadmill (State) at approximately 14 feet/min. The two sessions were separated by at least three days. A total of 75 possible stimulation combinations of frequency, current amplitude, and pulse width (not including no-stimulation trials) were delivered to each animal at random without replacement. Animals were continuously stimulated for one minute for each stimulation parameter set during each 19 min segment. The animals were not stimulated during the 1-2 minutes to engage or disengage the treadmill at the end of each 19 minute segment.

Behavioral Testing

Animals were placed in a rotometer built in-house that allowed optical encoding of the number of rotations an animal makes in 22.5° increments (Grayhill, Inc., LaGrange, IL) while delivering DBS to the STN through a two-channel mercury commutator (Mercotac, Inc., Carlsbad, CA). Optical encoder information was digitized (Tucker-Davis Technologies, Alachua, FL) and total rotations in clockwise and counterclockwise directions per minute were plotted in Matlab. All electrical stimulation was delivered as described previously. Animals were allowed to rotate freely in either direction while the number of rotations was recorded over a period of eight hours either in the presence or absence of continuous DBS.

Data Analysis

Fast Fourier Transforms (FFT) were made to analyze ECoG data in the frequency domain from 0 – 100 Hz. Power spectral densities (PSD) were estimated from the FFT using 256 Hann window segments based on the Welch method (NeuroExplorer, Littleton, MA) and normalized by $10^{\log_{10}(PSD)}$. Particular attention was focused on data between 20 – 45 Hz (high $\beta$-band, low $\gamma$-band). The mean power of the ECoG PSD over this range was subtracted from the maximum power over this range in order to compare the effects of DBS across animals. This operation accounts for variations in signal power from animal to animal and focuses on absolute
One-sample Kolmogorov-Smirnov test (K-S, $p < 0.05$ to reject) was used to test the null hypothesis that the normalized ECoG power for each comparison could be drawn from a normal distribution. In instances where data passed the K-S test (i.e., assumption of normality), paired samples Student’s t-test were used to assess differences in mean power ($p < 0.05$ to reject). Likewise, one-way ANOVA on related samples were used with Bonferroni-corrected post-hoc tests for repeated measures ($p < 0.05$ to reject). In instances where the distribution of normalized ECoG power did not pass the K-S test, paired-samples Wilcoxon non-parametric tests were used to test rank values ($p < 0.05$ to reject). Likewise, Friedman non-parametric ANOVA on related samples were used with Tukey-Kramer post-hoc tests ($p < 0.05$ to reject). Statistical analyses were made with SPSS release 17 and/or Matlab using the statistics toolbox.

Spectrograms, time-indexed power spectra, were calculated using similar parameters as PSD calculations above in 10 sec bins with 5 sec overlap (Chronux shareware running Matlab, http://chronux.org/). All statistical results can be found in Supplementary Tables 1, 2.

**Histology**

At the end of the study, each animal was deeply anesthetized with urethane (1400 mg/kg, IP) and perfused intracardially with 4% paraformaldehyde. After post-fixation for 24 hours, brains were cryoprotected in a series of graded sucrose washes, cryoembedded, and sectioned at 20 µm thickness. Sections containing the stimulating electrode were stained with cresyl violet (nissl) and imaged with standard light microscopy in order to verify electrode location. Sections posterior to the STN containing the substantia nigra (SNC/SNr) were processed for anti-tyrosine hydroxylase (TH) immunohistochemistry. Briefly, sections were hydrated, blocked in 10% normal goat serum (NGS), and incubated in a primary antibody mixture of TH and NeuN (neuronal nuclei, Chemicon, Billerica, MA) overnight at 4°C. Slides were rinsed, incubated in fluorescent secondary antibodies (Invitrogen, Carlsbad, CA) for one hour at room temperature, and counterstained with Hoechst to label nuclei. Slides were rinsed a final time and coverslipped with ProLong AntiFade reagent. Antibodies were diluted into PBS containing 5% NGS and 0.3% triton X-100 at 1:100, 1:100, and 1:200 ratios for TH, NeuN, and secondaries,
respectively. These slides were then imaged with an Olympus (model BX-51) epifluorescent microscope to visualize unilateral destruction of dopaminergic neurons in the SNc.

Verification of Electrode Location and 6-OHDA Lesion

Histological analysis verified that the DBS electrode was located in the STN in 7/9 animals (2 normal, 5 lesioned, Figure 2a). Only data from these animals were included for subsequent analyses. All lesioned animals that passed the amphetamine/apomorphine challenge (N = 7, including those with misplaced electrodes) showed depletion of TH immunoreactive neurons in the SNc on the animals’ right side (ipsilateral, lesioned) with respect to the left (contralateral, non-lesioned). An example of which can be seen in Figure 2c-d demonstrating the success of the 6-OHDA lesion. Control animals that did not receive the 6-OHDA lesion did not demonstrate this deficit (Figure 2e). In agreement with the immunofluorescence, all lesioned animals passed the amphetamine challenge in the rotometer (Supplementary Figure 2). Animals rotated ipsilaterally (clockwise) on average per animal ranging from 6 to 12.5 times per minute over the course of 90 minutes.
RESULTS

Electrocorticogram Recordings

After recovery, animals were placed in a semi-anechoic chamber that allowed simultaneous recording of ECoG signals and DBS of the STN through a multichannel commutator. Animals were allowed to freely explore their environment during all recording and stimulation sessions. In all subsequent results, the left and right frontal ECoG signals were referenced to the bone screw located over the parietal cortex. The raw ECoG signal from the left and right hemispheres of a single Parkinsonian animal can be seen while the animal was walking on a treadmill in Figure 3a (top two traces) compared to at rest (bottom two traces). The power spectra of the left and right ECoG signals recorded over five minutes were then calculated in the normal animal (Figure 3b) and the hemi-Parkinsonian animal (Figure 3c). As can be seen in Figure 3c, the right-side (6-OHDA lesioned) ECoG exhibits significantly increased power in the $\beta/\gamma$ band frequencies compared to the contralateral (left-side, non-lesioned) ECoG. The distribution of the maximum power (mean subtracted) between 20-45 Hz across all hemi-Parkinsonian animals regardless of animal State exhibited significantly higher power on the 6-OHDA lesioned ECoG as compared to the non-lesioned side ECoG during sham stimulation (paired $t$-test, $t = -3.34$, $p = 0.009$).

The local field potential of the STN was recorded through the stimulating electrode and compared to the ECoG over the right-side motor cortex (Figure 3c). The power in the $\beta$ band frequencies of the STN LFP was less than that recorded from the cortical surface and did not exhibit the decrease in power between 10 – 20 Hz observed in the ECoG.

The abnormal power recorded through the ECoG of Parkinsonian animals shifted from the high $\beta$-band to the low $\gamma$-band as the animals started walking on the treadmill as can be seen in the power spectra of a single animal (Figure 3d). The distribution of frequencies that exhibit maximum power in the power spectrum (peak frequencies) while the animal was at rest was normally distributed ($N = 380$, K-S $Z = 1.00$, $p = 0.268$), however was skewed toward higher frequencies while the animal was walking ($N = 380$, K-S $Z = 3.25$, $p < 0.001$). These distributions were significantly different (Wilcoxon $Z = -13.9$, $p < 0.001$, Supplementary Figure 3). This shift is consistent over time as can seen in a typical Parkinsonian animal’s spectrogram (Supplementary Figure 4).
Deep Brain Stimulation

Electrical stimulation parameters were chosen based on rodent studies that reduced or eliminated amphetamine-induced rotations (Maesawa et al. 2004) but were within safe levels of charge (Temel et al. 2005). The change in the $\beta$ power of the ECoG signal was abrupt following the onset and offset of DBS of the STN as seen in the spectrograms of Figure 4. In this figure, the ECoG was recorded for five minutes without electrical stimulation, followed by five minutes of DBS, followed by no stimulation. During the stimulation, $\beta$ band power was clearly decreased and depended upon the frequency of the DBS as described below. Once the stimulation was removed, the ECoG power reverted to its pre-stimulation, high-$\beta$ (animal at rest) or low-$\gamma$ (animal walking) power signature.

Stimulation parameter sets were varied randomly and the ECoG was recorded for one minute per set. The power spectrum during each one minute recording was calculated and the mean power of the PSD in frequencies from 20 – 45 Hz (high $\beta$ band, low $\gamma$ band) for each stimulation parameter was subtracted from the maximum power of the PSD over this range. Data recorded while DBS was turned on in Parkinsonian animals were not normally distributed (On: $N = 750$, K-S $Z = 2.91$, $p < 0.001$; Off: $N = 10$, K-S $Z = 0.81$, $p = 0.536$). A comparison of ECoG data between DBS on and off, regardless of parameter (e.g., charge, frequency, state, and amplitude), revealed no significant difference (Wilcoxon $Z = -1.89$, $p = 0.059$). However, when DBS delivered at maximum charge (i.e., 4 nC) was tested against no DBS, data were significant (On: $N = 250$, K-S $Z = 2.02$, $p = 0.001$; Off: $N = 10$, K-S $Z = 0.81$, $p = 0.536$; Wilcoxon $Z = -2.70$, $p = 0.007$) in that DBS reduced normalized ECoG oscillation power between 20-45 Hz. Data recorded while DBS was turned on in normal animals were likewise, not normally distributed (On: $N = 300$, K-S $Z = 1.49$, $p = 0.024$; Off: $N = 4$, K-S $Z = 0.4$, $p = 0.998$). A comparison of ECoG data while DBS was turned off with DBS on in normal animals, regardless of parameter (e.g., charge, frequency, state, and amplitude), revealed no significant difference (Wilcoxon $Z = -0.730$, $p = 0.465$). In order to confirm that DBS did not effect normalized ECoG power in normal animals, data were divided by maximum charge. DBS delivered to normal animals at maximum charge were normally distributed (On: $N = 100$, K-S $Z =$
1.21, \( p = 0.108 \), Off: \( N = 4 \), K-S \( Z = 0.4, p = 0.998 \) and no significant difference was found (Student’s \( t \)-test, \( t = -1.24, p = 0.305 \)). No further testing was performed on normal animal ECoG. ECoG data during DBS in Parkinsonian animals at maximum charge were then divided by State (i.e., resting, walking). There was no significant difference in ECoG power while DBS was turned on at maximum charge or turned off while animals were at rest or walking. (resting, On: \( N = 125 \), K-S \( Z = 1.04, p = 0.234 \); Off: \( N = 5 \), K-S \( Z = 0.84, p = 0.474 \); Student’s \( t \)-test, \( t = 2.64, p = 0.058 \); walking, On: \( N = 125 \), K-S \( Z = 1.92, p = 0.001 \); Off: \( N = 5 \), K-S \( Z = 0.45, p = 0.989 \); Wilcoxon \( Z = -1.48, p = 0.138 \)). In subsequent analyses, data from both states were combined.

Frequency of Stimulation

Normalized ECoG data was then divided by the frequency of stimulation (i.e., 0, 50, 125, 200, 275, and 350 Hz) delivered at maximum charge (regardless of State or Amplitude) in order to assess the effects of Frequency of DBS on cortical oscillations. These data passed the K-S test (see Supplementary Table 1) and a one-way repeated measures ANOVA was significant \( (F(5,259) = 3.31, p = 0.007) \). Pair-wise post-hoc comparison of the frequency of stimulation revealed significant decreases from 0 Hz (sham) stimulation in normalized ECoG power for DBS at frequencies of 200 Hz \( (p = 0.021) \) and 350 Hz \( (p = 0.015) \) after Bonferroni corrections for multiple comparisons (Supplementary Table 2). These results suggest that 200 Hz and 350 Hz DBS resulted in the largest decrease in cortical \( \beta/\gamma \) oscillations (Figure 4, 5).

Charge Response

DBS at each frequency was delivered with current amplitude and pulse width combinations that limited the charge per phase to 3.9 nC (50 \( \mu \)C/cm\(^2\)). Current and pulse width combinations that make less charge were also delivered (i.e., 0.2, 0.5, 1.0, and 2.0 nC). According to Figure 1, there are more amplitude and pulse width combinations that make ~4 nC than there are for 2, 1, 0.5, and 0.2 nC, which can result in very large unequal
sample sizes. Unequal sample sizes have implications for the use of the harmonic mean in post-hoc tests. Therefore, one charge was taken from each animal for each Frequency of DBS and State resulting in 50 measurements for each charge (one per animal per frequency per state). In cases where more than one charge of the same value exist (due to amplitude/pulse width combinations), these charges were averaged (i.e., 0 nC, 0 averages; 0.2 nC, 0 averages; 0.5 nC, average of 2 samples; 1.0 nC, average of 3 samples; 2.0 nC, average of 4 samples; and 4.0 nC, average of 5 samples). These data passed the K-S test (see Supplementary Table 1), and a one-way repeated measures ANOVA for Charge (i.e., 0, 0.2, 0.5, 1.0, 2.0, and 4.0 nC) was significant ($F(5,259) = 4.34, p = 0.001$). Pair-wise post-hoc comparison of charge of stimulation revealed significant decreases from 0 nC (sham) stimulation in normalized ECoG power only for DBS delivered at a charge of 4 nC ($p = 0.039$) after Bonferroni corrections for multiple comparisons (Supplementary Table 2). Likewise, the normalized ECoG was significantly reduced by 4 nC DBS compared with 0.2 nC ($p = 0.007$), 0.5 nC ($p = 0.003$), and 1.0 nC ($p = 0.032$) but not for 2.0 nC ($p = 0.422$). Figure 6 demonstrates the effects of increasing the amount of charge delivered to a maximum of 3.9 nC (regardless of State, Frequency or Amplitude) on reducing the abnormal power in the ECoG signal. The results of this analysis suggest that 3.9 nC was the least amount of charge in these experiments required to reduce abnormal oscillations.

**Charge vs. Amplitude**

There are a number of current amplitude and pulse width combinations that deliver 3.9 nC charge (50 μC/cm²). We then determined if it was the current or the maximum charge that was important for reducing abnormal parkinsonian oscillation power by testing current amplitude at maximum charge (i.e., 3.9 nC: 0 μA, 8 μA / 480 μs, 16 μA / 240 μs, 33 μA / 120 μs, 65 μA / 60 μs, and 130 μA / 30 μs). Normalized ECoG data divided by Amplitude at maximum charge was not normally distributed for the combination of 33 μA / 120 μs ($N = 50, K-S Z = 1.41, p = 0.039$). Therefore, the Friedman non-parametric ANOVA on related samples was created and found to be significant ($\chi^2 = 13.83, p = 0.017$). Tukey-Kramer pair-wise post-hoc comparison of the amplitude of stimulation
revealed significant decreases from 0 µA (sham) stimulation in normalized ECoG power for DBS delivered at 33 µA / 120 µs ($p = 0.021$) and 65 µA / 60 µs ($p = 0.007$) (Supplementary Table 2). Likewise, the normalized ECoG was significantly reduced by 65 µA / 60 µs DBS compared with 8 µA / 480 µs ($p = 0.017$). Figure 7 show the Parkinsonian data separated by Amplitude at maximum charge (i.e., 3.9 nC) regardless of Frequency or State. The results of this analysis suggest that DBS at 33 µA / 120 µs and 65 µA / 60 µs is more effective at reducing abnormal cortical oscillations rather than by the charge alone.

Behavior

Animals that received 6-OHDA lesions tend to favor ambulation ipsilateral to the lesion in the absence of amphetamine or apomorphine. Animals were placed in the rotometer for eight-hour sessions in which DBS was delivered continuously either at 200 Hz, 130 µA, 30 µs/phase, or 350 Hz, 65 µA, 60 µs/phase. No stimulation sessions were recorded as well (Figure 8). The rotational behavior of animals was normally distributed (Supplementary Table 1). However, the sample size was very small for the behavioral data set which has implications for the validity of applying Student’s $t$-test on these data. Therefore, Wilcoxon non-parametric tests on related paired samples were performed for all behavioral data. DBS delivered to normal control animals had no effect on the number of rotations the animals made in the rotometer as compared to no stimulation (Wilcoxon $Z = -0.69$, $p = 0.533$) Unstimulated Parkinsonian animals rotated on average close to a ratio of 3:1 ipsilateral to contralateral rotations. Stimulated Parkinsonian animals made fewer ipsilateral rotations than non-stimulated Parkinsonian animals (Wilcoxon $Z = 1.95$, $p = 0.049$); this was a significant reduction in ipsilateral rotations from no stimulation. Only 4/5 parkinsonian animals were tested as one animal, STN8 had died prior to testing. While the $p$ – values of non-parametric tests tend to have low power for small sample sizes, these results suggest that DBS at 3.9 nC (50 µC/cm²) is sufficient to reduce ipsilateral turning, a behavioral symptom of Parkinsonism in the hemi-Parkinsonian rat.
DISCUSSION

**Abnormal Parkinsonian oscillations**

The concept of measuring β oscillation activity as an indirect indicator of the neural Parkinsonian symptoms has been previously proposed (Sharott et al. 2005). It has been demonstrated that the administration of apomorphine, a classic dopamine agonist that has been used as a clinical treatment for PD, can reduce the β power measured in the EcoG over the affected side (Vorobyov et al. 2003). Despite reduction in β oscillation power, rats given apomorphine would continue to rotate away from the lesioned side. In the current study, we applied DBS to the STN in an effort to mimic the effects of dopamine agonist administration on β oscillations and animal behavior. In response to DBS, the rats did exhibit a significant decrease in β oscillation activity similar to an administration of apomorphine. There were some important differences, however. First, DBS stimulation seemed to extinguish the β power peak within a few seconds of activation consistently while apomorphine took longer and lasted for variable extended periods. Second, the β power peak returned shortly after turning off the stimulator. This abrupt change in the EcoG power is similar to quick changes (milliseconds to minutes) observed in human Parkinsonian tremor symptoms following the activation and adjustment of STN DBS (Rodriguez-Oroz et al. 2001). In humans, tremor has been correlated with an increase in γ oscillations (Weinberger et al. 2008) but not β oscillations (Kuhn et al. 2008b). It is not clear how PD tremor symptoms in humans translate to the Parkinsonian rat. Likewise, it is unknown how the changes in oscillatory power are affected by stimulation over longer periods of time (i.e. hours, days, etc.) that affect other Parkinsonian symptoms such as akinesia, bradykinesia, and rigidity. Indeed the return of physical Parkinsonian symptoms such as akinesia, bradykinesia, and rigidity following long-term DBS has been shown to take hours (Temperli et al. 2003).

Recently, magnetoencephalography in humans has demonstrated increased cortico-cortical coupling of β-band activity in patients who demonstrate < ~40% improvement following dopaminergic replacement therapy (Stoffers et al. 2008). Certainly, in rat models of PD, 6-OHDA lesion-related β oscillations have been observed in the STN in single-units, ensembles, and LFPs that manifest over the course of ~4 days (Mallet et al. 2008). Furthermore, these oscillations are profoundly affected by dopaminergic therapy (Sharott et al. 2005). Perhaps...
then, the 6-OHDA lesioned rat model of PD could be a surrogate representative of late-stage Parkinsonism or, possibly represent models of PD symptoms responsive to dopaminergic therapy. Indeed, a predictive indicator of the responsiveness of STN DBS in humans is their response to dopaminergic therapy (Pinter et al. 1999; Welter et al. 2002). Despite mounting evidence that links cortico-cortical and cortico-pallidal oscillations to the severity of Parkinson’s, the extent to which the power of Parkinsonian oscillations reflects underlying Parkinsonian symptoms remains to be seen (Brown 2006; 2003).

Deep brain stimulation of the subthalamic nucleus in the hemi-Parkinsonian rat

Previous studies that examined the behavioral effects of DBS of the STN in the rat model of PD found that stimulation at high amplitudes either reduced or stopped ipsilateral rotations during amphetamine (Maesawa et al. 2004) or apomorphine (Darbaky et al. 2003) challenge or caused contralateral rotations (Meissner et al. 2002). STN-DBS in animals acutely treated with a dopamine receptor antagonist to induce catalepsy improved the level of activity and significantly improved akinesia (Dejean et al. 2008). When stimulating through microelectrodes that approach the size of the STN of rodents, it is crucial to control the amount of charge delivered given the surface area, type of metal, and impedance of the electrode so that stimulating potentials are within the “water window” (i.e., the voltage at which hydrolysis of water into hydrogen and oxygen occurs), thereby preventing irreversible faradaic reactions at the electrode-tissue interface (Merrill et al. 2005) and/or charge/charge densities that exceed safe levels of electrical stimulation (Shannon 1992). Irreversible faradaic reactions at the electrode – tissue interface have been shown to have devastating effects to the electrode material and tissue (Harnack et al. 2004; McCreery et al. 1990; Temel et al. 2004). The absence of our observation of contralateral rotations during DBS could stem from the relative low charge, high charge density used in this study. In this report, both charge and charge density were kept within the charge capacity of stainless steel and the theoretical limits of safe charge injection. Stimulation that elicited dyskinesia was not performed. Additional safety measures were in place to limit the voltage at the electrode to stay within ± 1 V, the approximate water window for stainless steel.
Neuromodulation of β oscillations and motor symptoms with deep brain stimulation

There is much evidence to suggest that STN DBS affects β oscillations. Low frequency STN stimulation (< 10 Hz) in humans has demonstrated evoked potentials recorded from the scalp surface (MacKinnon et al. 2005). Likewise, high frequency stimulation of the rat STN elicits antidromic activation of cortex (Li et al. 2007). Low frequency stimulation (20 Hz) in the range of β oscillations has been shown to impair motor performance in humans suggesting that excessive synchronization between the basal ganglia and cortex contributes to bradykinesia (Chen et al. 2007). Additionally, 20 Hz stimulation increases abnormal synchronization that is reduced with higher frequency stimulation (Brown et al. 2004). Yet, there is evidence in humans from a study that employed simultaneous LFP recordings through unused electrodes during STN DBS that showed little change in β power during clinically effective DBS (Rossi et al. 2007), challenging the relevance of β oscillations with respect to improvements in motor symptoms observed with efficacious DBS. The animal data presented here demonstrate robust high-power oscillations between 20 – 45 Hz present in the ECoG recorded over motor cortex. Furthermore, the power of these oscillations can be modulated by HFS of the STN that corresponds to a decrease in Parkinsonian rotational behavior. The hemi-Parkinsonian rat model of STN-DBS may provide insight into the relationship between abnormal oscillations and Parkinsonian symptoms.

Current amplitude vs charge and charge density

The total charge in the extracellular environment delivered through electrical stimulation has been described as being interrelated between charge per phase (interpreted as the total volume within which neurons are excited) and the charge density (interpreted as the proportion of neurons close to an electrode that are excited) (McCreery et al. 1990). Therefore it would be desirable to maximize charge density (within the limits of the charge capacity of the electrode material) for a given charge. The limitation of the DBS electrodes in these experiments is the charge capacity of stainless steel relative to the geometric surface area of the electrode (50 µC/cm² based on ~7,850 µm²). Although 3.9 nC was enough charge to reduce the abnormal β oscillations, these parameters (current amplitude and pulse width) limited the amount of charge to 3.9 nC/phase and do not
demonstrate the limitations of the amount of charge that can be delivered. Indeed, Dejean and colleagues have
demonstrated that the threshold for eliciting an evoked response in frontal cortex of rat during STN DBS was ~82
µA (at a chronaxie of 40 to 75 µs) (Dejean et al. 2008). While the charge delivered in this study is within the
range of the Dejean study, it is possible that delivering more than 3.9 nC would improve upon the reduction of
the oscillations. The results presented here could be further analyzed with improvements in electrode
technologies to identify how increasing amounts of charge delivered to the STN effects Parkinsonian oscillations
and animal behavior. Furthermore, there are a number of current amplitude and pulse width combinations that
deliver any given amount of charge. Our data demonstrate that current amplitude itself, as proposed in other
studies (Desbonnet et al. 2004; Temel et al. 2005), is not a determining factor in the stimulation-evoked
decrease in the power of β oscillations; rather, this effect can be attributed to an interrelationship with current
amplitude and pulse width.

Oscillations at rest vs during locomotion

In resting animals, the peak power in the abnormal Parkinsonian oscillations was observed in the high β-band;
this power peak shifted to a narrow range of frequencies in the lower γ-band when the animals were walking at
a constant rate, as has been previously demonstrated (Sharott et al. 2005). This shift in the power may be
indicative of a need to alter the parameters of DBS depending upon the state of the animal. Cortical EEG and
local field potentials recorded from the STN in humans have demonstrated coherence between other frequency
bands such as θ (3 – 7 Hz), α (8 – 13 Hz), and low β (14 – 20 Hz) suggesting that several functional loops are
affected by PD (Fogelson et al. 2006). Additionally, γ band energy (65 – 90 Hz), while significantly lower
powered than β band energy (14 – 35 Hz), was found to be significantly increased from rest during a finger
tapping task in both LFP recordings from STN and mesial EEG, whereas no change in power was observed in the
β band energy (Lalo et al. 2008). Our data did not identify a significant effect between animal states. This may
be a limitation of the rat model of DBS for Parkinsonism, possibly underlining the differences between human
and rodent Parkinsonian symptoms. These data did identify a significant effect of 200 & 350 Hz DBS for reducing
the abnormal cortical oscillations, demonstrating the potential utility of the model. Indeed, there is strong
indication that inter-regional EEG coherence changes in the $\beta$ range during high frequency DBS are indicative of
the severity of Parkinsonian symptoms in human subjects at rest (Silberstein et al. 2005) and that local field
potential recordings through the stimulating contact in the STN that produced the best clinical effect also had
the highest coherence with midline EEG oscillations (Marsden et al. 2001). DBS in the hemi-Parkinsonian rat
model may provide the means to rapidly assess new stimulation therapies to inform DBS treatment of the
symptoms of human PD.

In conclusion, the hemi-Parkinsonian rat model of STN-DBS may prove to be a valuable surrogate for human
manifestations of PD. Although there are clear differences between rodent Parkinsonism and human symptoms
of PD, the rat model could be useful for determining the neural mechanisms of Parkinsonism and possibly help
to inform future treatment of human PD with DBS. Future research will be needed to determine the stability,
dynamic range, and long-term effects of DBS on cortical oscillations.
ACKNOWLEDGMENTS

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REFERENCES


Figure Legends

Figure 1: ECoG signals were recorded in two 76 min sessions per animal. Each session consisted of alternating 19 min segments of the animal at rest or walking on the treadmill. A total of 75 possible stimulation combinations of frequency, current amplitude, and pulse width (not including no stimulation trials) were delivered to each animal at random without replacement. Animals were continuously stimulated for one minute for each stimulation parameter set during each 19 min segment. Current amplitude and pulse width combinations are presented as charge (nanoCoulombs, nC).

Figure 2: Verification of DBS electrode location and destruction of dopaminergic neurons. A) DBS electrode relative to the STN. Coronal section is 50 μm thick and shows location of the tip of the DBS electrode (cathode, black arrow) relative to the surrounding guide cannula (anode, white arrow) positioned in the STN (STN 8). B) Magnified image at left. C, D) Immunofluorescence of TH-reactive neurons from lesioned animals. An absence of TH-reactive neurons can be seen in these 20 μm thick sections of different locations in the substantia nigra on the 6-OHDA lesioned side (animal’s right) relative to the intact (non-lesioned) contralateral side (C, STN 2, approx. -5.8 bregma; D, STN 6, approx. -4.8 bregma). E) Immunofluorescence of TH-reactive neurons from a control (non-lesioned) animal (STN 9, approx. -5.3 bregma). VTA, ventral tegmental area; fr, fasciculus retroflexus; SNC, substantia nigra compact; SNr, substantia nigra reticular.

Figure 3: Raw ECoG in the temporal and frequency domains. A) Sample ECoG recorded from the left (normal, non-lesioned) and right (6-OHDA lesioned) hemispheres of a single animal while walking on a treadmill (top two traces) or at rest (bottom two traces). All signals were referenced to a bone screw over the left parietal area and recorded during high-frequency, 50 Hz amplifier blanking (STN 5). B) Power spectral density from a single normal (non-lesioned) animal. The light two traces are the ECoG power while the animal is at rest. The dark two traces are the ECoG power while the animal is walking on a treadmill. C) Power spectrum from a single
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Figure 4: DBS effects Parkinsonian power in the ECoG with frequency dependency. Spectrograms of a sample animal were calculated from ECoG recordings made for two minutes prior to five minutes of DBS delivered at 50 µC/cm², 50 pulses/sec (left column), 125 pulses/sec (center column), or 200 pulses/sec (right column) followed by two minutes of no stimulation while the animal was at rest (top row) or walking on a treadmill (bottom row). Green arrows indicate the start of DBS and red arrows indicate the end of DBS. DBS delivered at 200 Hz caused a greater decrease in abnormal Parkinsonian power in the ECoG than 50 Hz DBS for when the animal was at rest and walking. Note that the effect of the DBS on reduction in the Parkinsonian power is nearly instantaneous. Data are representative of STN 5, 33 µA, 120 µsec pulse width. Units are in decibels.

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Figure 6: DBS dose response. Data are the maximum power in the ECoG between 20-45 Hz (mean power removed) as a function of charge delivered (mean, SD) from all five Parkinsonian animals regardless of frequency and state. For each of the five charges shown, the actual charge delivered (current amplitude X pulse width) was rounded to the nearest nC and grouped according to Figure 1. Only 4 nC charge produced significant decreases in cortical oscillation power suggesting that this is the minimum amount of charge needed to observe this effect in this study.

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### Distribution of Maximum Frequencies

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### Amplitude

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**Supplementary Table 2:** Statistical analyses. Frequency (Hz), Charge (nC), and Current (µA). Probability < 0.05 presented in bold.

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<td>At Max Charge</td>
<td>-2.7</td>
<td><strong>0.007</strong></td>
<td>-1.124</td>
<td>0.305</td>
</tr>
</tbody>
</table>

**Frequency (Hz)**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Bonferroni</th>
<th>Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>one-way ANOVA</td>
<td><strong>0.2</strong></td>
<td>1</td>
</tr>
<tr>
<td>At Max Charge</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>(5,259)</td>
<td><strong>3.31</strong></td>
<td><strong>0.007</strong></td>
</tr>
</tbody>
</table>

**Charge (nC)**

<table>
<thead>
<tr>
<th>Charge</th>
<th>Bonferroni</th>
<th>Current (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>one-way ANOVA</td>
<td><strong>0.2</strong></td>
<td>1</td>
</tr>
<tr>
<td>At Max Charge</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>(5,259)</td>
<td><strong>4.34</strong></td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>

**Amplitude**

<table>
<thead>
<tr>
<th>Friedman ANOVA</th>
<th>Parkinsonian</th>
<th>Tukey-Kramer</th>
<th>Current (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Max Charge</td>
<td><strong>χ</strong>²</td>
<td>p</td>
<td>post-hoc</td>
</tr>
<tr>
<td>13.83</td>
<td><strong>0.017</strong></td>
<td>8</td>
<td>0.561</td>
</tr>
<tr>
<td>16</td>
<td>0.11</td>
<td>0.619</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td><strong>0.021</strong></td>
<td>0.099</td>
<td>0.905</td>
</tr>
<tr>
<td>65</td>
<td><strong>0.007</strong></td>
<td><strong>0.017</strong></td>
<td>0.561</td>
</tr>
<tr>
<td>130</td>
<td>0.056</td>
<td>0.331</td>
<td>0.998</td>
</tr>
</tbody>
</table>

**Behavior**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Parkinsonian</th>
<th>Normal</th>
<th>Parkinsonian</th>
<th>Distribution of Max Frequencies:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotations</td>
<td>Wilcoxon DBS On - DBS off</td>
<td>Wilcoxon DBS On - DBS off</td>
<td>Wilcoxon Walk - Rest</td>
<td>All Parameters</td>
</tr>
<tr>
<td>Z</td>
<td>p</td>
<td>Z</td>
<td>p</td>
<td>Z</td>
</tr>
<tr>
<td>1.95</td>
<td><strong>0.049</strong></td>
<td>-0.69</td>
<td>0.533</td>
<td>-13.9</td>
</tr>
</tbody>
</table>
Random selection within charge limits

Frequency (Hz): 50, 125, 200, 275, 350
Amplitude (µA): 8, 16, 33, 65, 130
Pulse Width (µsec): 30, 60, 120, 240, 480