Functional MRI reveals frequency-dependent responses during deep brain stimulation at the subthalamic nucleus or internal globus pallidus

Hsin-Yi Laia,b,1, John R. Youncea,b,e,1, Daniel L. Albauhtagh,1, Yu-Chieh Jill Kaoa,b, Yen-Yu Ian Shihacb,d,*

© 2013 Elsevier Inc. All rights reserved.

Abstract

Deep brain stimulation (DBS) represents a widely used therapeutic tool for the symptomatic treatment of movement disorders, most commonly Parkinson’s disease (PD). High frequency stimulation at both the subthalamic nucleus (STN) and internal globus pallidus (GPI) has been used with great success for the symptomatic treatment of PD, although the therapeutic mechanisms of action remain elusive. To better understand how DBS at these target sites modulates neural circuitry, the present study used functional blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) to map global brain responses to DBS at the STN and GPI of the rat. Robust activation centered in the ipsilateral motor cortex was observed during high frequency stimulation at either target site, with peak responses observed at a stimulation frequency of 100 Hz. Of note, frequency tuning curves were generated, demonstrating that cortical activation was maximal at clinically-relevant stimulation frequencies. Divergent responses to stimulation were noted in the contralateral hemisphere, with strong cortical and striatal negative BOLD signal during stimulation of the GPI, but not STN. The frequency-dependence of the observed motor cortex activation at both targets suggests a relationship with the therapeutic effects of STN and GPI DBS, with both DBS targets being functionally connected with motor cortex at therapeutic stimulation frequencies.

Introduction

Deep brain stimulation (DBS) is a well-established neurosurgical technique used for the symptomatic treatment of multiple neurological and psychiatric disorders (Wichmann and Delong, 2011). It is most commonly employed in the treatment of Parkinson’s disease (PD), generally in cases where maximal medical therapy has become inadequate or dyskinesia has become intolerable. Both the subthalamic nucleus (STN) and the internal globus pallidus (GPI) are commonly used in modern practice as stimulation targets for the treatment of PD, with further efficacy in the treatment of additional movement and neuropsychiatric disorders (Kringelbach et al., 2007). DBS at either location alleviates many motor deficits in PD patients, including bradykinesia, tremor, rigidity and gait abnormalities. Although generally comparable in efficacy, large-scale clinical studies have revealed subtle differences between DBS at the STN or GPI targets, including variations in side effect profiles and long-term treatment outcomes (Odekerken et al., 2013; Rouaud et al., 2010; Weaver et al., 2012). For example, DBS at the STN results in a greater reduction in dependence on levodopa (Weaver et al., 2012), and may be more effective for severe motor symptoms (Krack et al., 1998) while there is some indication that GPI DBS is associated with fewer adverse cognitive effects than STN DBS (Moro et al., 2010; Odekerken et al., 2013). Given these differences in clinical outcomes, further study comparing DBS effects at both targets is warranted.

Mounting evidence suggests that DBS modulates neural activity in a diverse set of brain regions. DBS at the STN target has been shown to modulate neural activity in the GPI (Hahn et al., 2008), external globus pallidus (McConnell et al., 2012), substantia nigra pars reticulata (McConnell et al., 2012), ventral thalamus (Xu et al., 2008), and primary motor cortex (Li et al., 2012). Furthermore, the efficacy of STN DBS for obsessive-compulsive disorder suggests that non-motor limbic circuits may also be modulated (Le Jeune et al., 2010). Much less is known of the neural circuits modulated by GPI-DBS, although a recent study in pigs has demonstrated an increased activity throughout the sensorimotor network (Min et al., 2012).

Functional magnetic resonance imaging (fMRI) provides a powerful tool for the exploration of neural circuitry on a whole-brain scale,
in vivo, including the modulatory effects of DBS (Angelstein et al., 2007; Canals et al., 2008; Fige et al., 2013; Jech, 2008; Min et al., 2012; Mueller et al., 2004; Shyu et al., 2004; Stefurak et al., 2003; Young et al., 2011). The combination of fMRI with DBS allows for relatively unbiased mapping of large-scale responses to DBS, including areas that have not been adequately explored using other methods, particularly electrophysiology. To date, a limited number of fMRI-DBS studies have been conducted in human PD patients (Fige et al., 2013; Jech et al., 2001; Kahan et al., 2012; Mueller et al., 2013; Stefurak et al., 2003) and animal models (Min et al., 2012). Although such reports have highlighted DBS modulation of a diverse set of brain areas, both within and outside canonical motor circuits, the frequency-dependence of these responses has not yet been explored. Given that a hallmark component of therapeutic DBS is its dependency upon a narrow range of stimulation frequencies, typically at high values, an understanding of frequency-dependent fMRI responses on a global scale may provide important clues into the neuroanatomical mechanisms of DBS action.

In the present study, we used blood-oxygen-level-dependent (BOLD) fMRI to map changes in regional brain activity during DBS of the STN or GPi at multiple frequencies in the normal rat. We chose the rat model for these investigations due to its widespread application in the PD literature, including prior demonstrations that parkinsonian motor deficits can be ameliorated in the rat using high frequency DBS (So et al., 2012). We report robust, frequency-dependent changes in cortical activity during DBS, including convergent positive responses centered in motor cortex at both target sites. Unexpectedly, we also observed a strong inhibitory response in the contralateral hemisphere during GPi DBS. These results may shed light on how DBS alters functional connectivity in a frequency-dependent manner to achieve symptom alleviation.

Materials and methods

Subjects

A total of 14 adult male Sprague-Dawley rats (weighing 300–450 g; Charles River Laboratories, Wilmington, MA) were studied. Rats were individually housed in cages with food and water available ad libitum and 12:12 day–night cycles with control of humidity and temperature. All procedures were performed in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals) and approved by the University of North Carolina Institutional Animal Care and Use Committee.

Animal preparation

For electrode implantation, all subjects were endotracheally intubated and ventilated using a small animal ventilator (CWE Inc., SAR-830/PA, Ardmore, PA) delivering constant 2–2.5% isoflurane mixed with medical air. Animals were placed on a heating pad and physiological parameters were maintained within a normal range by adjusting ventilation volume, rate, and heating level. Animals were placed in a stereotactic frame (Model 962, Kopf Instruments, Tujunga, CA), and a craniotomy was performed over the location of electrode implantation. Twisted bipolar platinum–iridium electrodes (dimensions: 0.075 mm bare electrode diameter, 0.155 mm insulated diameter, 10 mm electrode length, exposed on the tip transection only, approximately 0.08 mm distance between uninsulated electrode tips) (PlasticsOne Inc., Roanoke, VA) were then implanted unilaterally into the STN (3.6 mm posterior and 2.5 mm right-lateral to bregma, 7.8 mm ventral to the cortical surface, n = 6) or GPi (2.4 mm posterior and 3.0 mm right-lateral to Bregma, of midline and 7.4 mm ventral to the cortical surface, n = 6); a rodent neuroanatomical atlas was used as a reference (Paxinos and Watson, 2004). GPi DBS was performed at the entopeduncular nucleus, the rodent analog to the GPI, though we refer to this area as the GPI for consistency with the neuroanatomical atlas used and the human literature. Electrodes were then fixed in place using dental cement (type 1 class 1, Hygenic Corp., Akron, OH) and rats were allowed to recover for at least 48 h prior to any imaging studies. Rats were monitored for complications during the postoperative period and were given topical antiseptics and lidocaine at the surgical site. Additionally, a pilot group of two rats which underwent identical surgical procedure were used to establish MR microscopy protocol for confirmation of electrode implant locations.

Functional and anatomical MRI procedures

On the day of fMRI experiments, rats were endotracheally intubated and ventilated with 1.25–1.75% isoflurane anesthesia. Paralysis was then achieved using pancuronium bromide (0.1 mg/kg/h, i.p. infusion). Heart rate and blood oxygen saturation level were continuously monitored by a MouseOx Plus system (STARR Life Science Corp., Oakmont, PA) and maintained within normal ranges (heart rate of 350–400 bpm and oxygen saturation above 95%). Capnometry and rectal temperature probes were employed to monitor essential physiological parameters during fMRI. Ventilation rate and volume were adjusted to maintain end-tidal CO2 (EtCO2) stable within a range of 2.6–3.2% via a capnometer (Surgivet, Smith Medical, Waukesha, WI). EtCO2 values from this system were previously calibrated against invasive sampling of arterial blood gas, reflecting a pCO2 level of 30–40 mm Hg (Shih et al., 2012a, 2013). The water temperature of a circulating pad was adjusted to maintain body temperature at 37 °C. MRI was performed using a 9.4 T Bruker BioSpec system with a BGA-9S gradient insert (Bruker Corp., Billerica, MA). A custom-made surface coil with an internal diameter of 1.6 cm placed directly over the head was used as a transceiver. Magnetic field homogeneity was optimized using standard FASTMAP shimming with first order shims on an isotropic voxel of 7 × 7 × 7 mm encompassing the imaging slices. A RARE T2-weighted pilot image was taken in the mid-sagittal plane to localize the anatomical position by identifying the anterior commissure at −0.8 mm posterior to the bregma.

T2-weighted anatomical images were acquired in vivo using a RARE sequence with spectral width = 47 kHz, TR/TE = 2500/33 ms, FOV = 2.56 × 2.56 cm, slice thickness = 1 mm, matrix = 256 × 256, RARE factor = 8, and number of averages = 8, to confirm electrode position with reference to the cortical surface, midline, and the anterior commissure. For functional scans, 4-shot double-sampled gradient-echo EPI sequence was used with spectral width = 160 kHz, TR/TE = 750/13 ms, FOV = 2.56 × 2.56 cm, slice thickness = 1 mm, matrix = 128 × 128, providing temporal resolution = 3 s and in-plane resolution = 200 µm. Eight coronal slices were acquired, with the 4th slice from the posterior direction aligned with the anterior commissure as acquired in the previous T2-weighted pilot scan to ensure anatomical consistency of the imaging data. Evoked fMRI scans were acquired for 210 s, or 70 repetitions, during which stimulation was applied in a 60-second OFF, 30-second ON, 120-second OFF pattern followed by a 2-minute inter-scan resting period. DBS was applied with the following parameters: bipolar square-wave current with an amplitude of 1 mA, frequency of 10, 40, 70, 100, 130, 160, 190, 220 and 310 Hz, and pulse width of 7.8/f ms where f is frequency in Hz. Two to five repeated scans were performed as needed in order to improve measurement precision and optimize SNR. Scans were performed in a pseudo-random manner with respect to stimulation parameters. Pulse width was varied in an inversely-proportional manner with frequency in order to isolate the effect of stimulation frequency on fMRI response. This was necessary in order to make the total duration of current delivery over the stimulation period constant, rather than face the major confounder of increasing total duration of current delivery during the stimulation epoch at high frequencies that would occur with fixed pulse width. These parameters were chosen in order to examine frequencies at and around known effective stimulation frequencies around 130 Hz. Although these stimulation parameters may in some cases generate high charge densities, the applied sequences and...
stimulation parameters produced highly durable responses after 10 h, suggesting that local damage from current delivery or harmful electrode heating was absent or insignificant under these conditions. Additionally, no electrical lesion was observed around the electrode tip. However, it should be noted that the stimulation parameters used with an electrode of this diameter have the potential to cause tissue damage (Merrill et al., 2005), though this damage was not observed in the fMRI response to stimulation or postmortem anatomical examination.

To confirm the accuracy of in vivo anatomical scans with respect to the implantation targets, rats with typical fMRI response patterns were fixed in 10% formalin by perfusion through the ascending aorta, and the electrode was removed with extraction of the brain 1 day later. The extracted brain was then placed in perfluoropolyether (PFPE), an aprotic solvent, and scanned with a magnetic resonance microscopy protocol using high-resolution T2-weighted RARE sequence with spectral width = 50 kHz, TR/TE = 2500/12.6 ms, FOV = 1.8 × 1.28 cm, slice thickness = 0.5 mm, matrix = 360 × 256, RARE factor = 8, and averages = 280. This protocol is able to visualize the margins of the internal capsule, providing confirmation of electrode location within the structures of interest (Fig. 1). This electrode location at both targets was well correlated with the anatomical position measured using the internal capsule and positions of the STN and GPi with relation to the thalamus = 280. This protocol is able to visualize the margins of the internal capsule, providing confirmation of electrode location within the structures of interest (Fig. 1). This electrode location at both targets was well correlated with the anatomical position measured using the internal capsule and positions of the STN and GPi with relation to the thalamus = 280.

Data processing and analysis

Image analysis was performed using a custom-written program (Shih et al., 2007, 2008) in Matlab (MathWorks Inc., Natick, MA). Skull stripping was performed manually with a threshold method. Automatic co-registration using SPM codes was applied to realign time-series data within subjects and then across subjects. Data were then averaged across subjects in order to provide group-averaged fMRI maps using correlation coefficient method with reference to the stimulation paradigm. Bonferroni correction was applied to adjust for the multiple comparisons of fMRI maps by dividing the significance level (p < 0.05) by the number of brain voxels. The correlation coefficient was then smoothed by applying a mean filter with a 3 × 3 kernel. Slices which were affected by the electrode’s susceptibility artifact were excluded from the analysis. Regions of interest (ROIs) were defined on an atlas (Paxinos and Watson, 2004) and then applied onto co-registered data, and four ROIs were defined for analysis: ipsilateral motor cortex (M1, M2), ipsilateral somatosensory cortex (S1fl, S1hi, S1D2, S1ULp, S1j, S2), ipsilateral cingulate cortex (Cg1, Cg2), and contralateral motor cortex. The BOLD signal time-course was then calculated for each ROI using the first 20 frames to establish a baseline. The percent BOLD response was defined as the average percent BOLD during the stimulation epoch, with a relatively long temporal delay of 15 s to account for hemodynamic delay to peak BOLD response. A delay of this magnitude was not needed for our previous thalamic DBS studies (Shih et al., 2012b), possibly indicating that the response observed in this study was significantly farther downstream from the stimulated nuclei and might involve polysynaptic neurotransmission. Statistical analysis was performed using SPSS software (IBM SPSS statistics 19, IBM Corp., Armonk, NY). Repeated-measures ANOVA with Fisher’s post-hoc test was employed to test the significance of responses at different frequencies with a significance threshold set at p < 0.05. All data are presented as mean ± SEM.

Results

Both STN DBS (Fig. 2) and GPi DBS (Fig. 3) produced significant positive BOLD responses in ipsilateral cortical regions, including motor cortex, somatosensory cortex and cingulate cortex (p < 0.05). The ipsilateral areas of activation between these targets were similar, while no significant ipsilateral subcortical activations were found. The strongest and most consistent areas of activation for both stimulation targets were found in ipsilateral motor cortex, while cingulate and somatosensory cortex activations were of smaller magnitude. The activations in ipsilateral motor cortex were also subject to a sharp frequency-dependent effect, where maximal BOLD response was observed at 100 Hz, and little to no response was found at the frequency extremes of 10 Hz and 310 Hz. For STN DBS, stimulation frequencies between 40 and 130 Hz produced BOLD which was significantly increased from the baseline response at 10 Hz, while for GPi DBS stimulation frequencies between 40 and 100 Hz produced responses which were significantly increased.

Fig. 1. Anatomical images and electrode placements. (A–B) Electrode placement maps at the STN (A) and GPi (B). Blue squares indicate electrode locations where stimulation produced a positive BOLD response in ipsilateral cortex, yellow diamond indicates contralateral negative BOLD, green triangle indicates both response types, and white circle indicates no response. (C–D) High-resolution T2-weighted spin-echo images taken in a brain perfused in formalin with the electrode removed. The electrode tract terminates within the STN (marked in yellow; C) or GPi (marked in red; D).
from baseline. Additionally, the positive BOLD responses in the ipsilateral motor cortex from STN DBS were generally stronger than those from GPi DBS, at approximately 3.1 ± 0.5% for STN DBS to 2.3 ± 0.3% for GPi DBS (Fig. 4).

Responses in both the ipsilateral cingulate and somatosensory cortex were of lower magnitude than those in ipsilateral motor cortex. This held true for both STN DBS and GPi DBS, with similar neuroanatomical response patterns for both targets at these locations. The effect of frequency on responses at cingulate and somatosensory cortex was less clear than that observed in motor cortex (Fig. 4), as while 10 Hz and 310 Hz consistently produced no response at either location, there was also no clear peak frequency for either location under either STN or GPi DBS. With STN DBS, responses in the somatosensory cortex with stimulation frequencies between 40 and 160 Hz were significantly elevated from the baseline of 10 Hz. Neither somatosensory cortex with GPi DBS nor cingulate cortex with either target exhibited a statistically significant frequency dependence above the baseline response at 10 Hz at any stimulation frequency.

GPi DBS additionally produced significant negative BOLD responses in the contralateral hemisphere, extending from multiple cortical regions to subcortical structures including striatal and thalamic regions (Fig. 3). This response was not found at 10 and 310 Hz, but its magnitude peaked in the motor cortex at 40 Hz, and declining responses were then observed with increasing frequency (Fig. 4). This response was less consistent than the responses in ipsilateral cortex. When plotted out by electrode location, the responses involving extensive negative contralateral BOLD along with positive ipsilateral BOLD tended to cluster along the dorsomedial border of the GPi as it meets the white matter structure of the internal capsule (Fig. 1).

**Discussion**

Our results demonstrate functional connectivity of STN-M1 and GPi-M1 networks at therapeutic frequencies. We observed significant BOLD responses to STN and GPi DBS in motor cortex, as well as somatosensory and cingulate cortex. Of note, STN DBS produced higher magnitude responses than GPi DBS, a finding which is consistent with some reports that STN DBS has a larger effect on motor symptoms than GPi DBS (Krack et al., 1998). Our results demonstrate modulation of both motor and non-motor pathways with both STN and GPi DBS, consistent with other reports (Anderson et al., 2003; Karimi et al., 2008; Le Jeune et al., 2010; Li et al., 2012; Min et al., 2012). Interestingly, the positive BOLD responses centered in motor cortex reached peak values during stimulation at high frequencies (100–130 Hz), which are known to be therapeutic for PD. One possible mechanism for this response is antidromic stimulation of cortical afferents, which has previously been demonstrated for STN DBS (Li et al., 2012). Although the therapeutic relevance of the recruited circuit could not be directly tested owing to the use of normal rats, prior work by others using optogenetic tools suggests that the motor cortex may play a critical role in therapeutic DBS independently of stimulation of STN cell bodies and efferent axons (Gradinaru et al., 2009). These results implicate the motor cortex as a central hub in the action of DBS, and this hypothesis is supported by a recent study showing direct modulation of motor cortex neurons during STN-DBS via antidromic potentials (Li et al., 2012).

A major motive for the present study was to compare the functional neuroanatomical responses to DBS at the STN and GPi, both of which are common therapeutic targets for PD treatment, as well as additional neurological and psychiatric disorders. The use of healthy rats in the present
study may thus allow our results to generalize to multiple disorders, for example, targeting of the STN for dystonia or obsessive-compulsive disorder (Ostrem et al., 2011; Rouzaire-Dubois and Scarnati, 1985; Schiefer et al., 2011; Welter et al., 2011). In general, DBS at either target produced similar ipsilateral results, though the STN appeared to produce stronger responses. Together, the STN and GPi provide the principal inhibitory output to motor thalamocortical circuits, with STN providing excitatory glutamatergic input to GPi, and GPi providing inhibitory GABAergic output to motor thalamic nuclei. While STN connections are highly varied, including afferents from motor cortex and efferents throughout the basal ganglia including substantia nigra pars compacta (Nauta and Cole, 1978; Watabe-Uchida et al., 2012), GPi primarily projects to thalamic nuclei (Sidibe et al., 1997). Despite differences between these nuclei in location, connectivity and neurotransmitter content (Graybiel, 2000), therapeutic outcomes following DBS at either target are surprisingly similar (Odekerken et al., 2013). Moreover, neural circuits modulated by stimulation at these targets are generally convergent, as reported here and by another study in normal pigs.
proximity of the internal capsule presents a significant problem for both STN and GPi DBS (Namba et al., 1985; Pollak et al., 2002). In either context, it is unlikely to involve antidromic modulation of motor cortex. Further to this point, minor variations in electrode placement between the GPi and STN—since the GPi is enveloped by the internal capsule, while the STN is only bordered by it ventrally (Paxinos and Watson, 2004)—may also minimize the variation of MR-related heating artifacts at the tip of the electrode for purposes of marking stimulation sites, and no such lesions were visible (unpublished observations). However, the potential for tissue damage under these conditions remains a potential confounder in this study.

To maintain the total duration of current delivery constant at all stimulation frequencies, pulse width was varied in this study as an inverse function of frequency. If constant pulse width were to be used, at high frequencies the total duration of current delivery will be much longer than at low frequencies, causing higher charge delivery and producing a major confounder that would make the isolation of the effect of frequency problematic. By standardizing stimulus duration, this design may also minimize the variation of MR-related heating artifacts at different frequencies (Christie et al., 2012). This choice, however, introduces an additional variable that is generally not present in clinical DBS practice and is a potential confounder in this study.

Conclusions

This study suggests that DBS at either the STN or the GPi modulates a cortical network focused on motor cortex in a frequency-dependent fashion, peaking at 100 Hz. STN DBS is seen to produce a larger BOLD response in ipsilateral motor cortex, while GPi DBS appears more likely to stimulate the internal capsule. Both stimulation targets also modulated non-motor cortex, particularly sensorimotor and cingulate regions, in a similar fashion. The frequency–dependency of our motor cortex results suggests a relationship with the therapeutic effect of STN and GPi DBS.
indicating that both STN-M1 and GPi-M1 networks are functionally connected at DBS therapeutic frequencies. Our future studies will further explore the function of these circuits in an animal model of Parkinson's disease.

References


