

# Deep brain stimulation at the subthalamic nucleus produces fMRI response in the motor cortex

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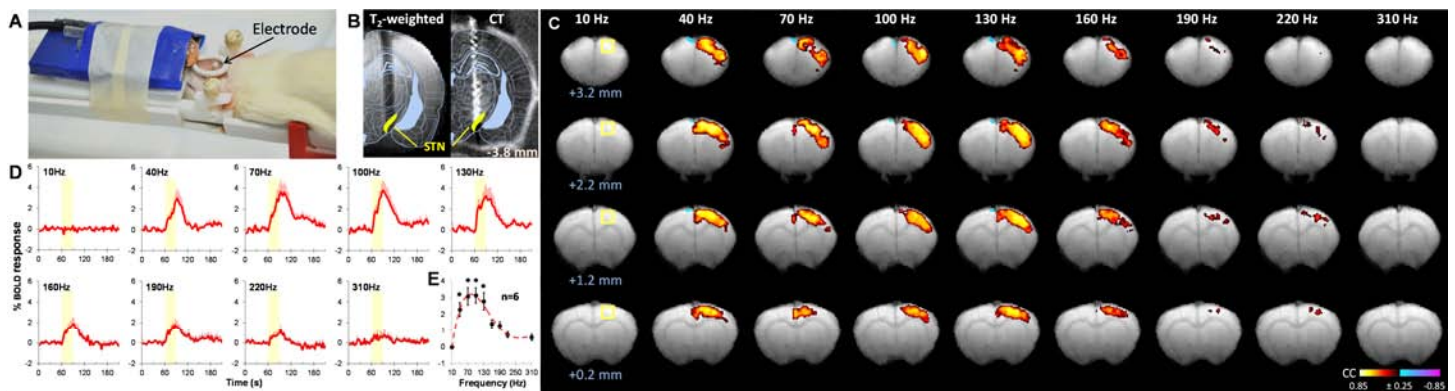
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**INTRODUCTION:** Deep brain stimulation (DBS) is among the most effective clinical treatments of Parkinson's disease (PD), but the underlying mechanisms are not fully understood [1,2]. DBS combined with neuroimaging offers the ability to unambiguously study the functionality of a neuroanatomical circuit, and may be used to quantify and optimize responses to varying therapeutic stimulation parameters and locations. The goal of this study was to characterize fMRI response to DBS at the subthalamic nucleus (STN). STN is the most commonly used DBS target for PD treatment. The classical model of this circuit is that STN projects excitatory ascending efferents to the internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr), and both project inhibitory efferents to the ventral lateral thalamic nucleus that further sends excitatory efferents to the motor cortex [3]. Therefore, a logical hypothesis is that activating STN efferents by DBS inhibits the motor cortex. Our findings challenge this assumption by showing robust frequency-dependent activation in the motor cortex during STN DBS, in accordance with a recent optogenetic study demonstrating that modulation of STN efferents alone does not produce antiparkinsonian effects while high frequency stimulation of STN afferents from motor cortex does alleviate parkinsonian behavior [4].

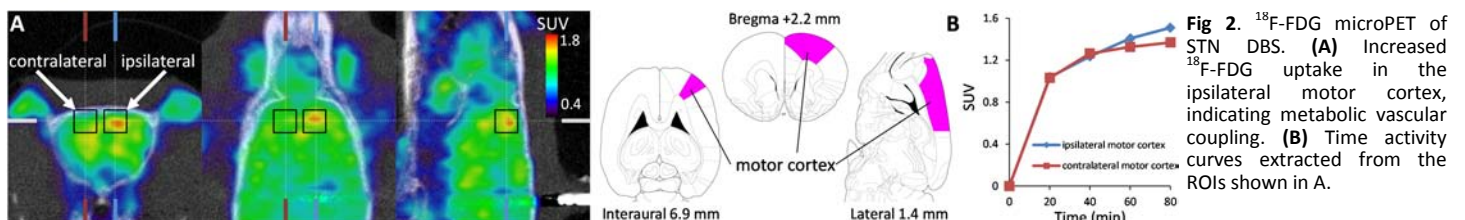
**METHODS:** MRI-compatible two-channel microelectrodes (PlasticsOne, Roanoke, VA) were stereotactically implanted into STN (3 mm posterior to the bregma, 2.5 mm lateral to the midline, 7.8 mm below the cortical surface) [5] in adult male Sprague Dawley rats (300–350 g) under 2–2.5% isoflurane anesthesia (n=6). The electrode was fixed with dental cement and the rats were allowed to recover for at least 5 days before imaging studies. For fMRI experiments, rats were anesthetized with 1.2–1.5% isoflurane, intubated, paralyzed, and ventilated with medical air. The ventilation volume and rate were adjusted to maintain EtCO<sub>2</sub> of 2.8–3.2% and SO<sub>2</sub> above 96%, and a circulated warm water pad was used to maintain rectal temperature at 37±0.5°C. MRI was performed on a Bruker 9.4T system using a home-made surface coil (ID=1.6 cm) and a double-sampled 4-shot gradient-echo EPI sequence (BW=160 kHz, TR=750 ms, TE=13 ms, 128x128 matrix, FOV=2.56x2.56 cm<sup>2</sup>, slice thickness=1 mm, temporal resolution=3 s). Stimulation frequencies of 10, 40, 70, 100, 130, 160, 190, 220 and 310 Hz were used with a bipolar square-wave current of 1 mA and a pulse width of 7.8/f ms where f=frequency in Hz. The stimulation parameters were sequenced in a pseudo-random manner. Two to five repeated trials were performed to improve measurement accuracy and optimize SNR. The stimulation paradigm was 60 s initial rest, 30 s stimulation, followed by 120 s rest and an additional 2 min minimum resting interval between trials. Correlation coefficient (CC) maps were performed by correlating BOLD pixel time courses to the stimulus paradigm with a significance level at p<0.05 (Bonferroni corrected) and a temporal delay of 15 s. The microPET/CT (Explore Vista, GE Healthcare, Chalfont St. Giles, UK) was performed under identical animal preparation in a subject showing robust fMRI responses to corroborate fMRI findings. A dose of 2 mCi <sup>18</sup>F-FDG was administered intravenously to measure glucose metabolism [6]. Stimulation parameters were 1 mA, 100 Hz, and 78 μs pulse-width with a repeating block design of 60 s rest and 60 s stimulation for a total scan time of 80 min. CT and PET images were reconstructed by the MMWKS software provided by the scanner manufacturer, resulting in pixel resolution of 122x122x122 μm<sup>3</sup> for CT and 388x388x775 μm<sup>3</sup> for PET. Statistical analysis employed ANOVA followed by Fisher's post-hoc test, with p<0.05 indicating statistical significance.

**RESULTS & DISCUSSION:** BOLD response in the motor cortex was positive and exhibited a tuning curve shape that peaked at 100 Hz, and frequencies between 40 and 130 Hz evoked significantly higher response than 10 Hz. (Fig. 1). BOLD responses evoked by STN DBS were highly reproducible and produced up to 4% signal change. This pattern of activation correlated to a 10% increase in glucose metabolism as revealed by PET, confirming metabolic-vascular coupling in the motor cortex (Fig. 2). Although the nearby internal capsule (IC) sends projections to many cortical regions and could hypothetically be responsible for a cortical BOLD response, our pilot trial of DBS at the IC under these physiological and stimulation parameters has not evoked a similar BOLD response in motor cortex. The peak response of the tuning curve was observed in a range which is known to be therapeutic for PD symptoms [7]. However, a significant response was also seen at low frequencies (40 Hz) known to be non-therapeutic [7]. This activation may be produced by several hypothetical mechanisms, including direct inhibition of the STN and pallidal output leading to reduced inhibition of thalamic activity [3], as well as antidromic conduction to motor cortex from STN afferents [4]. However, as both electrophysiological and microdialysis data indicate that STN DBS is excitatory to the GPi [8, 9], the latter hypothesis merits further investigation. The comparison of this pattern of fMRI activation to that found in parkinsonian animals will be critical in determining the implications of these findings, and future studies will employ STN DBS fMRI, resting state fMRI, functional track-tracing MEMRI, electrophysiology recording and behavioral tests to examine the therapeutic mechanism of DBS in hemiparkinsonian animal models.

**CONCLUSION:** This study demonstrates unique positive BOLD response in the motor cortex as a result of STN DBS. This fMRI response represents increased cortical metabolism as confirmed by PET, suggesting that STN DBS primarily activates cortical neurons, agreeing with a recent finding [4]. Further investigation of this technique with hemiparkinsonian rats will permit further insight into the therapeutic mechanism of STN DBS for PD.



**Fig 1.** BOLD fMRI of STN DBS (n = 6). (A) DBS fMRI setup. (B) Rat brain atlas overlaid on a T<sub>2</sub>-weighted image (left) and a CT image (right) at bregma -3.8 mm, confirming the position of the electrode. (C) Group-averaged BOLD activation maps (n=6). Responses were mainly located in the motor cortex. Yellow boxes indicate approximate ROIs (8x8 voxels). (D) Grand-averaged BOLD time courses to 9 DBS frequencies. Yellow-shaded area indicates stimulus epoch. (E) BOLD frequency-tuning curve. Error bars are SEM.



**Fig 2.** <sup>18</sup>F-FDG microPET of STN DBS. (A) Increased <sup>18</sup>F-FDG uptake in the ipsilateral motor cortex, indicating metabolic vascular coupling. (B) Time activity curves extracted from the ROIs shown in A.

**REFERENCES:** [1] Kringelbach et al., Nat Rev Neurosci, 2007, 8:623. [2] Wojtecki et al. Front. Integr. Neurosci., 2012, 6:1. [3] Albin et al, Trends Neurosci. 1989, 12:366 [4] Gradinaru et al, Science, 2009, 324:354. [5] Paxinos and Watson, The Rat Brain in Stereotaxic Coordinates (Fifth Edition), 2005. [6] Shih et al., JCBFM, 2011, 31:832 [7] Birdno and Grill, Neurotherapeutics. 2008, 5:14. [8] Hashimoto et al., J Neurosci. 2003, 23:1916. [9] Windels et al., Eur J Neurosci. 2000, 12:4141.