Short Communication

fMRI of Deep Brain Stimulation at the Rat Ventral Posteromedial Thalamus

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A B S T R A C T

Background: Functional magnetic resonance imaging (fMRI) of deep brain stimulation (DBS) has potentials to reveal neuroanatomical connectivity of a specific brain region in vivo.

Objective: This study aimed to demonstrate frequency and amplitude tunings of the thalamocortical tract using DBS fMRI at the rat ventral posteromedial thalamus.

Methods: Blood oxygenation level dependent (BOLD) fMRI data were acquired in a total of twelve rats at a high-field 11.7 T MRI scanner with modulation of nine stimulus frequencies (1–40 Hz) and seven stimulus amplitudes (0.2–3.6 mA).

Results: BOLD response in the barrel cortex peaked at 25 Hz. The response increased with stimulus amplitude and reached a plateau at 1 mA. Cortical spreading depolarization (CSD) was observed occasionally after DBS that carries >10% BOLD waves spanning the entire ipsilateral cortex.

Conclusion: fMRI is sensitive to the frequency effect of DBS and has potential to investigate the function of a particular neuroanatomical pathway.

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Introduction

Deep brain stimulation (DBS) is clinically used to treat several neurological symptoms such as Parkinson’s disease, tremor and dystonia [1,2]. The underlying mechanisms remain however largely unexplored. DBS efficacy in the brain has been studied primarily by electrophysiology recording, which does not provide depth-resolved mapping of DBS responses on a whole brain scale. Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) provides noninvasive, in vivo measurement of cerebral neurovascular function and has potentials to study whole brain responses to real-time DBS.

In this study, we aimed to demonstrate thalamocortical connectivity under different frequency and amplitude tunings in rats and establish a DBS fMRI protocol under isoflurane anesthesia that allows longitudinal study. BOLD fMRI (n = 12) data were acquired on an 11.7 T MRI scanner with modulation of nine stimulus frequencies (1, 3, 7, 11, 15, 20, 25, 30, and 40 Hz fixed at 1 mA and corresponding pulse widths of 1/frequency ms) and seven stimulus amplitudes (0.2, 0.6, 1, 1.2, 1.4, 1.8, and 3.6 mA fixed at 7 Hz and 1/7 ms pulse width). Ventral posteromedial (VPM) thalamus was selected due to its rich innervation to the cortex. We hypothesized that the barrel cortex can be reliably activated by DBS and the responses should exhibit frequency and amplitude-dependent properties.

Material and methods

Subjects

A total of 12 adult male Sprague Dawley rats (weighing 250–300 g; Charles River Laboratories) were studied. All experimental procedures...
were approved by the Institutional of Animal Care and Utilization Committee, UT Health Science Center at San Antonio.

Animal preparation

Rats were initially anesthetized with 3% isoflurane and orotracheally intubated for mechanical ventilation. After the animal was secured in an MRI compatible rat stereotactic headset, isoflurane was reduced to 1–1.2%. End-tidal CO2 was continuously monitored via a capnometer. Non-invasive end-tidal CO2 values were previously calibrated against invasive blood-gas samplings under identical conditions [3,4]. Rectal temperature was maintained at 37.0 ± 0.5 °C with warm-water circulating pad. Heart rate and blood oxygen saturation level were continuously monitored by a pulse oximeter. All recorded physiologic parameters were maintained within normal ranges as reported previously [5,6]. A two-channel twisted platinum microelectrode (MS303/9C-B, PlasticsOne, Roanoke, VA) with 156 μm wire diameter was implanted into the VPM thalamus at 3 mm posterior to the bregma, 3 mm lateral to the midline, 6 mm below the cortical surface [7,8], and fixed with dental cement.

Stimulation

Stimulation pulses were generated by a custom-written software with a multifunctional USB module (USB-1208HS-2AO, Measurement Computing, Norton, MA) offering a digital-to-analog conversion rate of 1 MS/s to trigger an isolated current stimulator (A365, World Precision Instruments, Sarasota, FL). Stimulus current was carried by a 2 channel twisted cable (305-491/2 with mesh, PlasticsOne, Roanoke, VA) with a home-made silver wire extension to the DBS electrode. In the first study, modulation of nine stimulus frequencies (1, 3, 7, 11, 15, 20, 25, 30, and 40 Hz) at 1 mA and corresponding pulse widths of 1/frequency ms) were studied (number of subjects = 12). The pulse-width was calibrated to ensure the total amount of charge delivered into the brain tissue per second was identical across different frequencies. In the second study, modulation of seven stimulus amplitudes (0.2, 0.6, 1, 1.2, 1.4, 1.8, and 3.6 mA fixed at 7 Hz and 1/7 ms pulse width) were studied (number of subjects = 7). These parameters were chosen based on a pilot study designed by the authors that explored potential frequency and amplitude dependent responses of VPM DBS. Frequency of 10–30 Hz has been widely used to study whisker system [9–11], by which VPM serves as an important relay. The stimulus parameters were randomized for both studies. Stimulation paradigm was OFF-ON-OFF-ON-OFF, where OFF = 100 s and ON = 50 s.

fMRI experiments

fMRI data were acquired on a 11.7 T, 16 cm bore magnet and a 74 G/cm gradient insert (Bruker, Billerica, MA). A custom-made circular surface coil (ID ~ 2 cm) was placed on the rat head. Magnetic field homogeneity was optimized using standard FAST-MAP shimming on an isotropic voxel of 7 × 7 × 7 mm. A T2-weighted pilot image was taken in the mid-sagittal plane to localize the anatomical position by identifying the anterior commissure (bregma ~0.8 mm). BOLD fMRI was acquired with 4-shot gradient-echo EPI sequence using spectral width = 200 kHz, TR/TE = 1250/12 ms, FOV = 2.56 × 2.56 cm, slice thickness = 1 mm, matrix = 128 × 128, and temporal resolution = 5 s. Repeated scans were performed when cortical spreading depolarization (CSD)-like signal occurred.

Data analysis

Image analysis was performed using a custom-written graphic user interface [12,13] in Matlab (Math-Works, Natick, MA). Percent BOLD activation map was generated by comparing the signal before and during stimulation. Region of interests (ROIs) were placed on the barrel cortex to extract fMRI time-course data. Dynamic cine analysis was used to delineate CSD propagation. The color-coded pixel values at each time frame were formed by subtracting the mean map of the pre-stimulus period, and the threshold was set as a 3% BOLD signal increase from the baseline. Repeated-measures ANOVA with LSD post-hoc test was used to compare stimulus-evoked BOLD signal changes at different stimulus conditions. The significance level for all the data analysis was set at P < 0.05.

Results

MR image with an overlay of a brain atlas showed the electrode tract. The size of electrode artifactually appeared larger due to susceptibility effect (Fig. 1A). Representative BOLD fMRI maps evoked by 20 Hz DBS are shown in Fig. 1B. The activations are mainly located in the S1 barrel field and the upper lip region, which are known to be densely innervated by VPM neurons. No apparent spatial shift was observed at different stimulus parameters throughout the study. In the first study (modulation of nine frequencies), averaged BOLD fMRI time courses at 1–40 Hz stimuli are shown in Fig. 1C. BOLD responses in the barrel cortex exhibited a bell-shaped tuning curve peaked at 25 Hz (Fig. 1D). Compared to the 25 Hz data, significantly lower BOLD responses were observed at 1, 3, 7, and 40 Hz (P < 0.05), and no significant differences were detected at 11, 15, 20, and 30 Hz (P > 0.05). BOLD responses showed robust activation with high contrast-to-noise ratio (up to 8% BOLD at 11.7 T). In the second study (modulation of six amplitudes), averaged BOLD fMRI time courses at 0.2–3.6 mA stimuli are shown in Fig. 1E. BOLD responses increased with stimulus amplitude and reached a plateau at 1 mA.

CSD-like signal was observed occasionally after the DBS (12 times out of 151 trials) that carries >10% BOLD waves spanning the entire ipsilateral cortex (Fig. 2), possibly due to hyperexcitation [14]. This propagating wave usually initiated 1 min after the stimulus onset, propagated toward the midline, anterior, and posterior part of the cortex with an estimated speed at ~4 mm/min. Among all trials, the depolarization waves never crossed the hemisphere and only stayed in the cortex.

Discussion

By systematically evaluating 9 frequencies and 7 pulse widths, we showed that up to 8% BOLD signal changes can be detected at 11.7 T. No obvious lesion was observed in the T2*-weighted images up to 3.6 mA. The subsequent trials also evoked robust and repeatable fMRI responses, indicating the VPM neurons were not functionally damaged at this current amplitude when a short pulse width (1/7 ms) was applied. However, a single pulse of 0.1 mA with 5 s pulse width produced observable lesion in the T2*-weighted images (data not shown), suggesting cautious on using DBS with long pulse width and highlighting the importance of DBS parameter calibration. BOLD response in VPM was not observed because of the susceptibility artifact induced by the commercial electrode. With proper selection of electrode materials, local BOLD responses may be seen [15]. Of note, the cortical BOLD response could originate from stimulating thalamocortical cells, or antidromically stimulating cortical thalamic afferent terminals [16]. Further elucidation using c-fos or optogenetics is warranted.
One potential limitation of the present study is that animals were anesthetized during DBS sessions. Anesthesia is known to alter peak frequency when peripheral sensory stimulus was applied [9,17]; however, a recent study showed that animals under α-chloralose and isoflurane anesthesia exhibit similar frequency-dependence and location of BOLD response, indicating that anesthesia may have less impact on DBS-induced fMRI signals [15]. A possible explanation is that DBS bypassed the anesthetic confounds on the peripheral nervous system and ascending neurons in the spinal cord.

In summary, the present study demonstrated thalamocortical connectivity by stimulating VPM thalamus in rats. This fMRI response was highly frequency-dependent. We also demonstrated that DBS can induce CSD-like signals. This study demonstrates the feasibility of measuring DBS frequency-dependent responses using fMRI. The procedure established herein could serve as the basis of a wide variety of system neuroscience applications, such as mapping DBS treatment effect in Parkinsonian animal models when targeting different brain regions [6,18] or evaluating plasticity changes of thalamocortical tract following injury [19]. This work also provides

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**Figure 1.** DBS fMRI with stimulus frequency modulation. (A) Rat brain atlas overlaid on an anatomy (bregma –2.8 mm), showing the position of the microelectrode. (B) BOLD activation maps of a representative subject. Responses were mainly located in the S1 barrel field/upper lip region. ROI was placed at –0.8 mm. (C) Grand-averaged BOLD responses to 9 stimulus frequencies. Yellow-shaded area indicates stimulus epoch. (D) BOLD responses exhibited a frequency-dependent pattern peaked round 25 Hz, *P < 0.05, different from peak value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
important information regarding the selection of stimulus parameters for future DBS fMRI studies in animal models.

References


