Deep brain stimulation fMRI in mice

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INTRODUCTION Deep brain stimulation (DBS) is a clinically established procedure to alleviate several neurological and psychological symptoms [1]. Understanding the therapeutic mechanism of DBS is crucial for developing new treatment targets and protocols [2,3]. Mouse model has been used to dissect the DBS effect on Parkinson's disease [4], movement disorders [5], depression [6] and anxiety [7] because of the genetic similarity to humans and availability of transgenic and diseased models. Combined fMRI and DBS in preclinical animal models has proven useful to study functional brain circuitry in normal and diseased states [8-10], but the feasibility of DBS fMRI in mice remains to be demonstrated. fMRI in mice is extremely challenging due to bulk susceptibility artifact, insufficient spatial resolution and unstable physiological conditions [11-15]. This study attempted to demonstrate fMRI activation by DBS in mice. Ventral posteromedial (VPM) thalamus was targeted to probe thalamocortical connectivity. Our hypothesis was that DBS can induce reliable fMRI response in the somatosensory cortex in mice with frequency-dependency similar to that in rats [9]. DBS fMRI creates an opportunity to investigate spatiotemporal characteristic of the fMRI signals as well as functional connectivity of a specific neural circuit in mice which are difficult to assess previously.

METHODS Home-made two-channel microwires with polyimide insulation (tungsten 99.95%, ID = 50μm, California Fine Wire Co., Grover Beach, CA) were stereotactically implanted into the VPM (1.7 mm posterior to the bregma, 1.8 mm lateral to the midline, 3 mm below the cortical surface) and fixed with dental cement in four adult male nude mice (20-25 g) under 1.5-2% isoflurane anesthesia. For fMRI study, isoflurane was adjusted between 0.75-1% to maintain the respiratory rate at ~100 bpm, and a regulated heated pad was used to maintain the body temperature at 37°C. Monocrystalline iron oxide nanoparticle (MION, 50 mg Fe/kg, i.v.) was administered for CBV fMRI [16,17]. MRI was performed on a Bruker 9.4 T Biospec scanner with a home-made surface coil (ID=0.8 cm). fMRI data were acquired with a double-sampled two-shot gradient-echo EPI sequence using spectral width = 150 kHz, TR/TE = 1000/7.5 ms, FOV = 1.28x1.28 cm, slice thickness = 0.75 mm, matrix = 64x64 and temporal resolution = 2 s. Stimulation parameters were 2 mA bipolar square wave with four stimulus frequency (5, 20, 35 and 50 Hz) and pulse width of 1/frequency ms. Stimulation paradigm was OFF-ON-OFF-ON-OFF, where ON = 20 s, OFF = 40 and 80 s for initial and followed rest, respectively, and additional five minute rest was given between scans. The stimulation parameters were performed in a pseudo-random order, and three repeated trials were performed for each stimulus parameter to improve measurement accuracy and optimize SNR. Correlation coefficient (CC) maps were performed by correlating CBV fMRI pixel time courses to the stimulus paradigm after inter-subject coregistration with a significant level at $p<0.05$ (Bonferroni corrected) and a temporal delay of 20 s. Data analysis was similar to that described previously [16,17]. Statistical analysis employed ANOVA followed by Fisher's post-hoc test. Significant level was set at $p<0.05$. Error bars were SEM.

RESULT & DISCUSSION This study demonstrated robust and highly localized cortical CBV fMRI response to DBS at the mouse VPM. CBV response in the sensory cortex was frequency-dependent and peaked at 20 Hz (up to 13% CBV change) (**Fig. A-C**). The optimal frequency of the forepaw stimulation in mice has been reported ranging from 3 to 6 Hz under different anesthetics [13-15]. The peaked response at 20 Hz may be due to the different frequency encoding of neurons in VPM compared to VPL (the sensory relay of forepaw in the thalamus), shorter stimulus pulse-width, or bypassing of the peripheral nervous system and ascending neurons in the spinal cord. The optimal stimulus frequency for mouse DBS was similar to that in rats [9]. With a 20 s stimulus block, the evoked CBV response reported in the present study remained elevated for about 60 s after the stimulus cessation (**Fig. B**), which was not observed in a similar study in rats [9]. The CBV response in the sensory cortex contralateral to the DBS side was also observed, in accordance with a previous report using forepaw stimuli [15]. This could result from interhemispheric connections [18] or innervation from ipsilateral VPM. This study demonstrated for the first time that DBS at the mouse VPM can evoke robust CBV fMRI response in the somatosensory cortex. DBS fMRI has potential to serve as a useful tool to explore neural connectivity and dissect DBS mechanism in normal and genetically engineered mice.

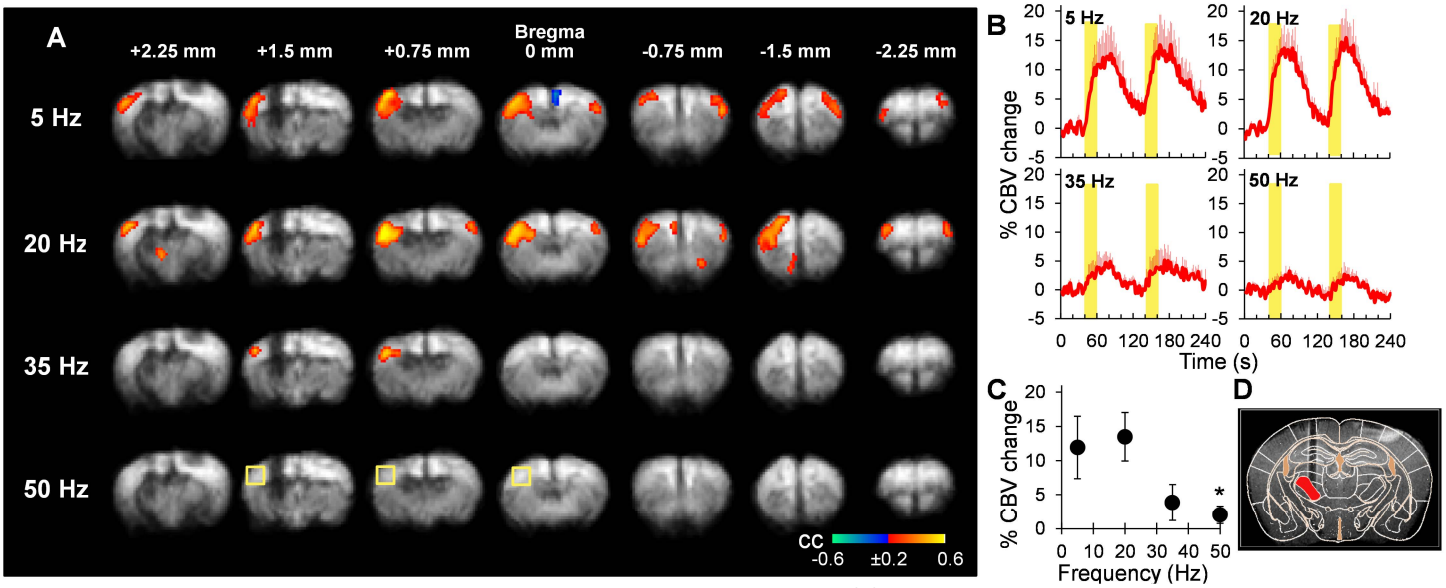

Figure CBV fMRI of deep brain stimulation at the mice ventral posteromedial thalamus (n = 4). **(A)** Group-averaged fMRI activation maps. Responses were mainly located in the barrel field/upper lip of the primary somatosensory cortex. Yellow boxes indicate approximate ROIs (5x5 voxels). **(B)** Group-averaged CBV time courses of four DBS frequencies. Yellow-shaded areas indicate stimulus epochs. **(C)** CBV frequency-dependent response to DBS. *$p<0.05$, different from 20 Hz. **(D)** Mouse brain atlas overlaid on a T_2-weighted image at bregma -1.7 mm, confirming the position of the electrode. Red-colored area indicates VPM. All error bars are SEM.

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