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Deep brain stimulation of the ventromedial prefrontal cortex causes reorganization of neuronal processes and vasculature

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ABSTRACT

Background: Chronic high-frequency electrical deep brain stimulation (DBS) of the subcallosal cingulate region is currently being investigated clinically as a therapy for treatment of refractory depression. Experimental DBS of the homologous region, the ventromedial prefrontal cortex (VMPFC), in rodent models has previously demonstrated anti-depressant-like effects. Our goal was to determine if structural remodeling accompanies the alterations of brain function previously observed as a result of chronic DBS.

Methods: Here we applied 6 h of high-frequency bilateral VMPFC DBS daily to 8 9-week old C57Bl/6 mice for 5 days. We investigated the "micro-lesion" effect by using a sham stimulation group (8 mice) and a control group (8 mice with a hole drilled into the skull only). Whole brain anatomy was investigated post-mortem using high-resolution magnetic resonance imaging and areas demonstrating volumetric expansion were further investigated using histology and immunohistochemistry.

Results: The DBS group demonstrated bilateral increases in whole hippocampus and the left thalamus volume compared to both sham and control groups. Local hippocampal and thalamic volume increases were also observed at the voxel-level; however these increases were observed in both DBS and sham groups. Follow-up immunohistochemistry in the hippocampus revealed DBS increased blood vessel size and synaptic density relative to the control group whereas the sham group demonstrated increased astrocyte size.

Conclusions: Our work demonstrates that DBS not only works by altering function with neural circuits, but also by structurally altering circuits at the cellular level. Neuroplastic alterations may play a role in mediating the clinical efficacy of DBS therapy.

Introduction

High-frequency deep brain stimulation (DBS) delivered using a neurosurgically implanted electrode has been applied with great clinical efficacy in the treatment of Parkinson's disease and other movement disorders (Limousin et al., 1995). Given the success of this treatment for movement disorders, the use of chronic DBS has been proposed as a treatment for several other neuropsychiatric conditions as a means of compensating for malfunctioning brain circuitry (Lozano and Lipsman, 2013). Recently, the subcallosal cingulate region has been used as a DBS target in treatment refractory depression (TRD) (Lozano et al., 2008; Mayberg et al., 2005), with patients receiving the therapy demonstrating sustained anti-depressant response in 3–6 year follow-

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up open label studies (Kennedy et al., 2011) in a clinical population at high-risk for suicide (Conwell and Brent, 1995). In patients with TRD, local and remote changes in brain function in response to the focal application of subcallosal DBS have been previously demonstrated and may account, in part, for the therapeutic mechanism of action of DBS in depression (Lozano et al., 2008; Mayberg et al., 2005). Experimental studies involving stimulation of the ventromedial prefrontal cortex (VMPFC; the rodent homologue of the subcallosal cingulate region) in normal wild-type rats demonstrate the alleviation of depressive-like behaviors due to being subjected to a forced swim test (Hamani et al., 2010, 2012). Recent findings from our group show that in addition to functional remodeling, DBS applied in the context of neuropsychiatric disorders may mediate clinical efficacy through neuroanatomical remodeling as well. For example, DBS applied to key nodes in the memory circuit of rodents (Laxton et al., 2010) (such as the entorhinal cortex and the anterior nucleus of the thalamus) induces the generation of new neurons that, once mature, integrate into hippocampal memory circuits responsible for the maintenance of memory (Hamani et al., 2011; Stone et al., 2011). There is mounting evidence that neuroanatomical reorganization is also occurring in patient populations being treated with DBS. In a recent clinical finding, two Alzheimer's disease patients demonstrated hippocampal growth and improvement in their clinical presentation in response to chronic administration of DBS to the fornix (Sankar et al., 2015).

However, in the context of TRD, it is unclear as to whether or not these changes in brain function and behavior are accompanied or mediated by neuroanatomical reorganization, and if so, in what part of the associated network remodeling occurs. There is also recent evidence that the microlesion caused by the implantation of the DBS electrode itself may account for some improvement in symptomatology. We do note, however, that the use of lesion-otomies has been previously proposed in the treatment of major depression, often with mixed results. These include lesions throughout the cingulate, the internal capsule, and the tracts adjacent to the caudate nucleus (Shah et al., 2008); therefore there may be a neuroanatomical basis for remodeling observed due to microlesions and we speculate that neuroanatomical remodeling may be further modulated by chronic deep brain stimulation (possibly leading to therapeutic efficacy). While the microlesion phenomenon has been receiving some attention in the Parkinson's disease literature (Borden et al., 2014; Le Goff et al., 2014; Maltete et al., 2008, 2009), it has been relatively understudied.

Given the emerging interest in the subcallosal cingulate with respect to other neuropsychiatric disorders, such as treatment-refractory anorexia nervosa (Lipsman et al., 2013), it is critical to determine whether and to what extent neuroanatomical remodeling, and more specifically, if changes in neuronal processes, glial cells, and vasculature occur in response to DBS of this region. In this manuscript, we tested this open question by applying DBS to the ventromedial prefrontal cortex (VMPFC; the rodent homologue to the human subcallosal cingulate (Hamani et al., 2010, 2012)) of 8 9-week-old male C57Bl/6 mice. We used whole-brain high-resolution magnetic resonance imaging (MRI) data to analyze the neuroanatomical effects on the stimulated groups. To test the micro-lesion effect we also analyzed the neuroanatomy of a group receiving sham stimulation (i.e., stimulator implanted but no active stimulation received). Volumetric changes observed using MRI analysis were then used to guide histological and immunohistochemical analyses.

Materials and methods

Surgeries, electrode implantation and deep brain stimulation

To test how DBS may impact neuroanatomical networks and brain-wide neuroanatomical remodeling we applied DBS to 8 C57Bl/6 mice (~25 g; Charles River, Wilmington, Massachusetts, USA; all males; 9 weeks old). Mice were anesthetized with ketamine/xylazine (75/10 mg/kg i.p.) and had their heads fixed to a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA). Electrodes with a diameter of 125 μm and a 0.5 mm^2 of exposed surface were connected to a plastic pedestal (Plastics One; Roanoke, VA, USA) and bilaterally implanted in the VMPFC (anteroposterior +1.7, lateral ±0.2, depth 1.2 mm) and used as cathodes (Franklin and Paxinos, 2004). A screw implanted over the somatosensory cortex was used as the anode. Two additional screws were attached to the skull for better securing the cap in place. Electrodes and screws were fixed to the skull with dental acrylic cement. A week after surgery, the DBS group received stimulation for 6 h a day over five days (similar to the design previously used from our group in the study of rats (Hamani et al., 2010, 2012); stimulation parameters: 50 μA, 130 Hz, 90 μs pulse width). These settings were selected as they have been previously effective in improving memory performance in the Morris water maze (Stone et al., 2011) and approximate those used in most clinical applications of DBS if the charge density is taken into account.

To test the influence of the so-called "micro-lesion effect", where symptom improvement is noted shortly after electrode implantation and before DBS has been delivered or optimized (Lozano et al., 2008; Mayberg et al., 2005; Cersosimo et al., 2009; Granziera et al., 2008), we studied an additional group (n = 8) receiving sham stimulation (i.e., where the stimulator is implanted and not turned on). A third control group of 8 mice had holes drilled in the skull but no electrodes implanted (total: 24 mice total, all same background strain, males, and age as the DBS group). The control group was handled over 5 days in order to mimic the handling of both sham and DBS groups.

Magnetic resonance imaging (MRI)

Three days after the end of stimulation mice were perfused through the *left* cardiac ventricle with 30 mL of phosphate-buffered saline (PBS) (pH 7.4) containing 2 mM of ProHance® (Bracco Diagnostics Inc., Princeton, NJ) at room temperature (25 °C). This was followed by infusion with 30 mL of 4% paraformaldehyde (PFA) and 2 mM of ProHance® (Bracco Diagnostics Inc., Princeton, NJ) in PBS at room temperature. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures containing the brain were allowed to postfix in 4% PFA and 2 mM of ProHance® at 4 °C for 12 h and was stored in this fashion for 72 h prior to imaging. A multi-channel 7.0 T MRI scanner (Agilent, Palo Alto, CA) was used to acquire anatomical images of brains within skulls (Nieman et al., 2007). Prior to imaging, the samples were removed from the contrast agent solution, blotted and placed into 13-mm-diameter plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3 M Corp., St. Paul, MN). An array of 16 custom built solenoid coils was used for high-throughput scanning of the specimens using a T2-weighted 3D Fast Spin Echo (FSE) sequence, with TR = 2000 ms, echo train length = 6, TE_{eff} = 42 ms, FOV of 25 mm × 28 mm × 14 mm, and a matrix size of 450 × 504 × 250. This yields an isotropic (3D) resolution of 56 μm. In the first phase encode dimension, consecutive k-space lines were assigned to alternating echoes to move discontinuity related ghosting artifacts to the edges of the FOV. This sequence involves oversampling in the phase encoding direction by a factor of 2 to avoid the interference of these artifacts. This FOV direction was subsequently cropped to 14 mm after reconstruction. Total imaging time was ~12 h.

Image processing for MRI data

Anatomical changes accompanying DBS were estimated using deformation-based analysis of the T2-weighted MRI data (as described in previous publications from our group (Lerch et al., 2008)). All image processing was carried out with images in minc format using the minc suite of software tools (http://www.bic.mni.mcgill.ca/ServicesSoftware/ServicesSoftwareMincToolKit). All MRI volumes were rigidly rotated and translated (3 rotations and 3 translations) to match an initial atlas. All possible pair-wise 12-parameter transformations (3 rotations, translations,