ARCHIVAL REPORT

Antidepressant-like Effects of Cortical Deep Brain Stimulation Coincide with Pro-neuroplastic Adaptations of Serotonin Systems

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Background: Cortical deep brain stimulation (DBS) is a promising therapeutic option for treatment-refractory depression, but its mode of action remains enigmatic. Serotonin (5-HT) systems are engaged indirectly by ventromedial prefrontal cortex (vmPFC) DBS. Resulting neuroplastic changes in 5-HT systems could thus coincide with the long-term therapeutic activity of vmPFC DBS.

Methods: We tested this hypothesis by evaluating the antidepressant-like activity of vmPFC DBS in the chronic social defeat stress (CSDS) model of depression (n = 8–13 mice/group). Circuit-wide activation induced by vmPFC DBS was mapped with c-Fos immunolabeling. The effects of chronic vmPFC DBS on the physiology and morphology of genetically identified 5-HT cells from the dorsal raphe nucleus (DRN) were examined with whole-cell recording, somatodendritic three-dimensional reconstructions and morphometric analyses of presynaptic boutons along 5-HT axons.

Results: Acute DBS drove c-Fos expression locally in the vmPFC and in several distal monosynaptically connected regions, including the DRN. Chronic DBS reversed CSDS-induced social avoidance, restored the disrupted balance of excitatory/inhibitory inputs onto 5-HT neurons, and reversed 5-HT hypoexcitability observed after CSDS. Furthermore, vmPFC DBS reversed CSDS-induced arborization of 5-HT dendrites in the DRN and increased the size and density of 5-HT presynaptic terminals in the dentate gyrus and vmPFC.

Conclusions: We validate a new preclinical paradigm to examine cellular mechanisms underlying the antidepressant-like activity of vmPFC DBS and identify dramatic circuit-mediated cellular adaptations that coincide with this treatment. These neuroplastic changes of 5-HT neurons might contribute to the progressive mood improvements reported in patients treated with chronic courses of cortical DBS.

Key Words: Deep brain stimulation, depression, dorsal raphe, prefrontal cortex, neuroplasticity, social defeat

Deep brain stimulation (DBS) of the subcallosal cingulate gyrus (SCG) has demonstrated promise as a somatic therapy for treatment-refractory depression (1–3). Clinical evidence suggests that the antidepressant effects of SCG DBS are reinforced by chronic stimulation, with the fraction of remitters increasing linearly over months of DBS treatment (3), raising the possibility that long-term neuroadaptations are important contributors to its therapeutic activity. However, the existence and neurobiological nature of such changes remains enigmatic.

To date only a handful of studies have examined the behavioral and neurobiological effects of DBS of the ventromedial prefrontal cortex (vmPFC) (the rodent analog of the SCG) in preclinical models. Most of these studies have applied acute stimulation regimens and reported antidepressant-like effects in behavioral screens sensitive to pharmacological antidepressants (4,5). The observation that serotonin (5-HT) depletion blocks the behavioral effects of acute and chronic DBS suggests that engagement of an intact serotonergic (5-HT) system is required for the antidepressant-like activity of DBS in rats (6,7).

Neuroanatomical tracing studies in rodents and primates indicate that the dorsal raphe nucleus (DRN) receives projections from the vmPFC (8–11), and several preclinical studies have reported robust and immediate increases in DRN neural activity and 5-HT output upon electrical or optogenetic stimulation of the vmPFC (6,9,10,12–14). Whether and how chronic regimens of vmPFC DBS induce neuroplasticity in the 5-HT system is not known. Because previous studies have indicated that depression and suicide in humans (15–18) and depressive-like symptoms in animals (19–21) are accompanied by maladaptive plasticity in the 5-HT system, we hypothesized that stable neuroplastic changes in the 5-HT system might coincide with the antidepressant-like effect of chronic vmPFC DBS.

To test this hypothesis we assessed the effects of chronic vmPFC DBS in mice in the context of a chronic social defeat stress (CSDS) paradigm that has face and predictive validity with regard to clinical depression (22,23). We first assessed the ability of chronic vmPFC DBS to suppress CSDS-induced social avoidance and used c-Fos immunolabeling to map the regions in which DBS was inducing neural activity. We then used a transgenic mouse line allowing for genetic identification and conditional viral targeting of 5-HT cells, together with an array of electrophysiological and morphological assays, to investigate whether chronic vmPFC DBS reversed defeat-induced changes in the structure and physiology of DRN 5-HT neurons. We report that chronic vmPFC DBS abolishes social avoidance behavior after CSDS and induces dramatic physiological, dendritic, and axonal neuroplastic adaptations in DRN 5-HT neurons that counteract the maladaptive adaptations induced by CSDS.

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Methods and Materials

Animals

Eight- to twelve-week-old male wild-type (for behavior and c-Fos experiments) or *Pet1-tdTomato* transgenic mice (for electrophysiology and morphology experiments) bred onto a C57BL/6 background were used for all experiments (generation of transgenic mice is described in Supplement 1). Mice were housed on a 12-hour light/dark cycle with food and water available ad libitum, except during DBS or sham stimulation. All studies were conducted strictly according to protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee, and all procedures were performed in accordance with institutional guidelines.

Social Defeat and Social Interaction Testing

Social defeat and social interaction testing were conducted as previously reported (20–23) and described in detail in Supplement 1. Briefly, mice underwent 10 days of aggression stress from trained CD1 aggressor mice. For social interaction tests, mice were allowed to freely explore an arena for 2.5 min in the absence of a novel social target and for 2.5 min in the presence of a novel social target; stress susceptible mice develop a persistent social avoidance, as indicated by a decreased time spent in the "interaction zone" around the social target (22). In all DBS experiments, only defeated mice that expressed social avoidance (i.e., stress-susceptible) were stimulated. Individual mice were assigned randomly to the "Defeat Sham" or "Defeat Stim" conditions on Day 11 such that the two groups had comparable mean avoidance scores before DBS treatment. Control mice were handled identically to defeated mice but were not subjected to aggression stress.

DBS Surgeries

Stainless steel, 125-μm diameter, 4-mm length bipolar electrodes (Plastics One, Roanoke, Virginia) were lowered at a 15° angle and implanted unilaterally into the left vmPFC (1.8 mm anteroposterior, .8 mm mediolateral, −.27 mm dorsoventral) over a 5-min time period. Coordinates were adopted from previous reports such that the electrode tips would be localized to the junction of the prelimbic and infralimbic cortex (21,24). Cyanoacrylate and dental cement were used to affix electrodes to the skull as previously described (25). Sham-stimulated mice were implanted identically. One mouse was removed from all analyses, due to electrode placement outside the vmPFC, and one mouse was removed from all analyses due to excessive tissue damage such that the electrode placement could not be determined.

DBS Experiments

After a 1-week recovery from surgery, implanted electrodes were connected to a programmable stimulator (MED Associates, St. Albans, Vermont). Stimulation was applied at 160 Hz frequency, 60 μs pulse width, and 150 μA current, parameters that are similar to previous DBS studies in rodents and humans (1,6,26–28). For c-Fos expression experiments, mice were stimulated for 1 hour immediately followed by perfusion. For chronic DBS experiments, DBS was applied 5 hours/day for 7 days—a paradigm similar to prior chronic DBS studies for depression that have observed long-lasting antidepressant-like effects (7,29–31)—while mice remained in their home cages. Sham-stimulated mice were handled and connected to the stimulator in an identical manner, but no current was applied. After 7 days of DBS, mice underwent social interaction testing 24 hours after the end of stimulation (mice were not stimulated during testing). To determine electrode placements, mice were perfused 24 hours after the conclusion of social interaction testing. For electrophysiology experiments, mice were sacrificed 24 hours after the final social interaction test. For axonal morphology experiments, mice were perfused 24 hours after the conclusion of DBS.

Histology and Immunohistochemistry

To visualize c-Fos, postsynaptic density protein 95 (PSD-95), and Synaptophysin-Venus (SynP-Venus) by immunohistochemistry, brain slices were processed for immunohistochemistry with standard protocols (20,21). Histology, antibodies, incubation times, and imaging and quantification details are described in Supplement 1.

Whole-Cell Electrophysiology

Coronal slices (200 μm) containing the DRN were used for whole cell recording and prepared as described previously (20,21,32–34). 5-HT cells from the ventromedial DRN (vmDRN) were recorded (32). Detailed electrophysiology and data analysis protocols can be found in Supplement 1.

Morphometric Analyses of Somatodendritic Structure and Axonal Boutons of 5-HT Neurons

To analyze somatodendritic morphology, genetically identified 5-HT neurons from *Pet1-tdTomato* mice were filled with 1% biocytin during whole-cell recording and were analyzed as described previously (20) with Neurolucida software (MBF Bioscience, Williston, Vermont) and described in Supplement 1.

For morphometric analyses of presynaptic boutons along 5-HT axons, we applied a cell-type specific genetic tagging approach that relies on a fluorescently labeled form of the presynaptic protein synaptophysin (Synaptophysin-Venus), which we expressed selectively in DRN 5-HT neurons with a conditional viral vector injected 7 days before the beginning of CSDS. After CSDS, DBS, and perfusion, brain slices were stained with anti-green fluorescent protein antibodies. In addition to the DRN, the dentate gyrus (DG), vmPFC, and basolateral amygdala (BLA) were selected for a subsequent detailed morphometric analysis on the basis of their innervation by DRN neurons and differential activation in response to vmPFC DBS. Bouton length and bouton density were measured as described in Supplement 1 and Figure S3 in Supplement 1. These two variables have been previously validated as sensitive indices of axonal plasticity, including in 5-HT neurons (35–37). A total of 7853 boutons were analyzed of 425 axons representing a cumulated axon length of approximately 30,000 μm.

Statistical Methods

One-way, two-way, or repeated measures analyses of variance (ANOVAs) were performed, followed by post hoc comparisons with Fisher's protected least significant difference test. Comparisons between two groups were performed by two-tailed Student *t* tests. Statistical analyses were performed with Statistica software (StatSoft, Tulsa, Oklahoma). Statistical significance was defined as $p < .05$. All data are presented as the mean ± SEM. Outlying values (3 SDs or greater from the mean) were excluded from group means.

Results

Antidepressant-Like Effect of Chronic vmPFC DBS in Stress-Susceptible Mice

We first investigated the behavioral effect of chronic vmPFC DBS in the CSDS paradigm (Figure 1A). Histological analyses