

Deep brain stimulation in rats: Different targets induce similar antidepressant-like effects but influence different circuits



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ABSTRACT

Recent studies in patients with treatment-resistant depression have shown similar results with the use of deep brain stimulation (DBS) in the subcallosal cingulate gyrus (SCG), ventral capsule/ventral striatum (VC/VS) and nucleus accumbens (Acb). As these brain regions are interconnected, one hypothesis is that by stimulating these targets one would just be influencing different relays in the same circuitry. We investigate behavioral, immediate early gene expression, and functional connectivity changes in rats given DBS in homologous regions, namely the ventromedial prefrontal cortex (vmPFC), white matter fibers of the frontal region (WMF) and nucleus accumbens. We found that DBS delivered to the vmPFC, Acb but not WMF induced significant antidepressant-like effects in the FST (31%, 44%, and 17% reduction in immobility compared to controls). Despite these findings, stimulation applied to these three targets induced distinct patterns of regional activity and functional connectivity. While animals given vmPFC DBS had increased cortical *zif268* expression, changes after Acb stimulation were primarily observed in subcortical structures. In animals receiving WMF DBS, both cortical and subcortical structures at a distance from the target were influenced by stimulation. In regard to functional connectivity, DBS in all targets decreased intercorrelations among cortical areas. This is in contrast to the clear differences observed in subcortical connectivity, which was reduced after vmPFC DBS but increased in rats receiving Acb or WMF stimulation. In conclusion, results from our study suggest that, despite similar antidepressant-like effects, stimulation of the vmPFC, WMF and Acb induces distinct changes in regional brain activity and functional connectivity.

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Introduction

Deep brain stimulation (DBS) is currently being investigated as a potential therapy for treatment-refractory depression. Targets commonly studied in different trials include the subcallosal cingulate gyrus (SCG), ventral capsule/ventral striatum (VC/VS) and nucleus accumbens (Acb) (Bewernick et al., 2010; Hamani and Nobrega, 2010; Holtzheimer et al., 2011b; Lozano et al., 2008; Malone et al., 2009; Mayberg et al., 2005). Though no clinical study has been conducted comparing the efficacy of DBS in different targets, approximately 50% of the patients seem to respond to the procedure, independent of the stimulated brain site

(Bewernick et al., 2010; Hamani and Nobrega, 2010; Holtzheimer et al., 2011b; Lozano et al., 2008; Malone et al., 2009; Mayberg et al., 2005).

Cortical regions involved in the pathophysiology of psychiatric disorders include the prefrontal and orbitofrontal cortices (Drevets et al., 2008; Ongur and Price, 2000; Price and Drevets, 2010; Price et al., 1996). These project the ventral striatum and the head of the caudate, which in turn send GABAergic efferents to the limbic and associative regions of the globus pallidus and substantia nigra reticulata. Outflow structures of the basal ganglia innervate the mediodorsal and ventral anterior thalamic nuclei, which in turn project back to the prefrontal and orbitofrontal regions (Drevets et al., 2008; Ongur and Price, 2000; Price and Drevets, 2010; Price et al., 1996). In primates, the main fiber pathways interconnecting the above-mentioned structures are the white matter fibers of the frontal lobe, the anterior limb of the internal capsule and the inferior thalamic peduncle (Lehman et al., 2011; Velasco et al., 2005). Considering the similar antidepressant effects of SCG, Acb and VC/VS DBS in open label trials and the fact that prefrontal

Abbreviations: Acb, nucleus accumbens; DBS, deep brain stimulation; FST, forced swim test; vmPFC, ventromedial prefrontal cortex; WMF, white matter fibers.

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projections to subcortical structures run, at least in part, through the anterior limb of the internal capsule, a plausible hypothesis would be that by stimulating such targets one would just be influencing different relays of the same circuitry.

Animal models have been suggested to have both predictive and construct validity for translational DBS studies in depression (Hamani and Nobrega, 2012; Hamani and Temel, 2012). In rodents, the antidepressant-like effects of DBS in the forced swim test (FST) and chronic unpredictable mild stress (CUS) occur upon stimulation settings that approximate those used in humans and targets analogous to those used in clinical practice (e.g. the Acb or ventromedial prefrontal cortex; vmPFC) (Gersner et al., 2010; Hamani et al., 2010a, 2010b, 2012; Li et al., 2011).

In the present study we tested the hypothesis that DBS in the vmPFC, Acb, or white matter fibers of the frontal region (WMF) induces comparable antidepressant-like effects in rats by modulating a similar set of brain regions. The first two structures are homologous to the subgenual cingulum and nucleus accumbens in humans (Groenewegen et al., 1990; Hamani and Temel, 2012; Hamani et al., 2011; Heimer et al., 1997; Uylings et al., 2003). As the rodent internal capsule does not have an anterior limb, we have applied stimulation to the largest white matter structure in the anterior portion of the rodent brain, namely the forceps minor. The FST was used as the main behavioral paradigm as it has proven to be very sensitive to DBS alone or in combination with other manipulations (Hamani and Nobrega, 2010, 2012; Hamani and Temel, 2012; Hamani et al., 2010c; Temel et al., 2007). To obtain an index of neuronal activity following stimulation at each anatomical site we examined local expression of *zif268*. This immediate early gene (IEG) was chosen as, in addition to being very well characterized, it is known to capture both increases and decreases of regional brain activity (Covington et al., 2005; Creed et al., 2013; Guzowski et al., 2001; Horner and Keefe, 2006; Lonergan et al., 2010; Schiltz et al., 2007; Yano and Steiner, 2005). In a preliminary effort to assess possible changes in functional connectivity, we compared intercorrelation patterns of *zif268* signal following DBS at different targets.

Methods

All protocols were approved by the Animal Care committee of the Centre for Addiction and Mental Health and complied with Canadian Council on Animal Care (CCAC) and NIH standards and guidelines.

Surgical procedures

Male Sprague–Dawley rats (250–300 g) were anesthetized with halothane and had electrodes implanted in one of the following targets (Paxinos and Watson, 1998): 1) vmPFC; anteroposterior (AP) +3.0 mm, lateral (L) \pm 0.4 mm, and depth (D) 5.6 mm; 2) white matter fibers of the frontal region (forceps minor) AP +2.7 mm, L 1.4 mm, and D 5.6 mm; and 3) Acb AP +1.6 mm, L 1.2 mm, and D 8.0 mm. Monopolar stainless steel electrodes (250 μ m in diameter and 0.5 mm of exposed surface) were used as cathodes. An epidural screw placed over the somatosensory cortex was used as anode (AP 0.5, L \pm 1.5, D 1.0 mm) (Paxinos and Watson, 1998). Controls had holes drilled into the skull but were not implanted with electrodes. In behavioral and *zif268* experiments we also tested a sham treated group with electrodes implanted that did not receive stimulation. Results were similar to those recorded in non-stimulated controls (Supplementary Fig. 1 and Supplementary Table 1).

Electrical stimulation

DBS was conducted with a handheld device (ANS model 3510) at 100 μ A, 130 Hz, and 90 μ s. Though we recognize that different targets may require different stimulation amplitudes for a clinical effect, we decided to use a standard current for several reasons. First, with our

electrode system these settings generate a charge density similar to that used in patients undergoing DBS (Hamani et al., 2010b, 2012). Second, by using fixed stimulation parameters we could more easily compare data across groups (as the only different variable was the surgical target). Third, at currents above 200 μ A animals receiving Acb DBS presented stereotypic behavior. Finally, by using 100 μ A the volume of tissue activated by DBS was relatively restricted to the target region (see details below).

Behavioral testing

The FST was conducted one week after electrode implantation. On testing day 1, rats were individually placed for 15 min in a cylinder filled with 25 ± 1 °C water (Detke and Lucki, 1996; Detke et al., 1995). Thereafter, DBS-treated animals received continuous stimulation for 4 h. On the following day, rats in the DBS groups received 2 h of stimulation followed by a 5 min swimming session during which the predominant behavior of the animals was scored every 5 s (immobility, swimming, or climbing) (Detke and Lucki, 1996; Detke et al., 1995). The timeframe of stimulation was selected based on our previous studies (Hamani et al., 2010a, 2010b) and on literature showing that 1–2 doses of antidepressants after swimming on day 1 and one dose before swimming on day 2 reduce immobility (Porsolt et al., 1978). As our systems deliver electrical stimulation, swimming sessions were conducted with the animals disconnected from the swivels (i.e. immediately after receiving DBS). Under these circumstances, it is possible that behavioral responses might have been due to a rebound phenomenon related to the discontinuation of current delivery.

One week after the FST, animals received stimulation for 4 h on day 1 and 2 h on day 2. Thereafter, locomotor activity was assessed in a square 0.49 m² open field (Med Associates) with infrared photo beams placed along the walls. In this study, we subdivided the open field test into two. The first 5 min were used to evaluate an anxiety-like behavioral response to a novel environment. Overall results of the 30 min of testing were considered to measure general locomotion. After the experiments, electrode location was checked in brain slices processed with cresyl violet (Hamani et al., 2010b).

Computational prediction of neural activation

The computational model used to establish the effects of stimulation has been previously described in detail (Butson et al., 2007). The electric field was calculated using a finite element model constructed in COMSOL v4.3 (COMSOL, Lenexa, KS), taking into account the geometry of the stimulation electrode and the configuration of anodes and cathodes. For simplicity, the voltage in the tissue medium was interpolated as if the medium was homogeneous (Holsheimer et al., 2000). The estimated volume of tissue activated was based on the assumption that electrodes were implanted in an ideal location. We note that this approach is somewhat limited, as it does not reflect individual values. It does, however, provide a general sense of the spillover of current as applied in average electrode locations. Simulations were conducted using NEURON (Hines and Carnevale, 1997). Threshold criteria were used to determine the model-predicted volume of tissue activated.

In situ hybridization and histology

One week after surgery, a batch of animals that did not undergo behavioral testing received stimulation for 4 h on day 1 and 2 h on day 2. Sacrifice was carried out immediately after by decapitation following ketamine/xylazine anesthesia. Hybridization was performed using ³⁵S-UTP labeled riboprobes complementary to *zif268*, as previously described (Creed et al., 2012). After hybridization, slides were exposed to Kodak BioMax film for 6 days at 4 °C along with

calibrated radioactivity standards. Film analyses were conducted with an MCID system (Interfocus, UK).

To assess electrode placement brains were stained with cresyl violet (Supplementary Fig. 2).

3D modeling of structures expressing *zif268*

Atlas plates (Paxinos and Watson, 1998) were exported as individual pages in TIFF file format. A custom Matlab script (MathWorks Inc., Natick, MA) was used to position each slice in 3D space according to bregma coordinates (as indicated in the atlas). The script allowed us to trace the boundaries of each nucleus and create a continuous curve. Surface reconstruction was done using Pro/Engineer (PTC, Needham, MA), a parametric solid and surface-modeling package. Once contours were created, the SweptBlend function was used to generate closed surfaces. Files were then exported in IGES format and converted to STL using FreeCad. After surfaces were converted into meshes, duplicate vertices, edges and overlapping surfaces were removed with Meshlab. Finally we used ParaView (Kitware Inc.) an open-source, multi-platform data analysis and visualization application. We imported each structure and gave it a distinct color. Surface normals were applied to give it a smooth appearance. Once an image of the desired nuclei was established, it was saved as a ParaView state file as well as exported as VRML (Virtual Reality Modeling Language). The VRML format allows the entire image to be imported into Adobe Acrobat. Each surface was annotated and the final version exported in PDF format.

Correlation matrices and network construction

Details on the technique used for correlation analyses and network construction may be found elsewhere (Wheeler et al., 2014). Using the *zif268* signal as a dependent variable, all possible pairwise correlations between 98 brain regions expressing this immediate early gene were computed using the Pearson correlation coefficients in controls and the DBS groups separately. High correlation between pairs was noted when *zif268* expression in one structure was strongly related to the other in individuals from the same group. Each complete set of correlations was displayed as color-coded correlation matrices using the Matlab software (Mathworks Inc., Natick, MA).

Networks were constructed by considering the strongest inter-regional correlations in each group of animals (Wheeler et al., 2013, 2014). In selecting a threshold criterion for inclusion in the network, we examined a progressive series of increasing r values and the corresponding series of decreasing numbers of brain areas to be treated as nodes in the network. In an effort to balance a stringent r threshold with a reasonable number of network nodes to be included, the decision was made to retain correlations that correspond to a p value of less than 0.01 in each group. This corresponded to a network $r > \pm 0.83$ for controls, $r > \pm 0.79$ for the vmPFC DBS group, $r > \pm 0.93$ for the WMF DBS group, and $r > \pm 0.93$ for the Acb DBS group. The nodes in the resulting networks represent brain regions and the correlations that survived the threshold criterion were considered to be functional connections. Netdraw software (Analytic Technologies: Lexington, KY) was used to visualize the resulting networks, where node size was set proportional to degree (number of connections) and connection line weights reflect the strength of the correlation.

Statistical analyses

ANOVA (LSD post-hoc) was used to compare behavioral and *zif268* data across groups. Statistical significance was set at $p \leq 0.05$. Correlation analysis was used to measure functional connectivity, as described above.

Results

Forced swim test and open field

Overall, we found a significant treatment effect of DBS in the FST ($p = 0.01$, $F_{3,39} = 6.36$ for immobility; $p = 0.02$, $F_{3,39} = 6.27$ for swimming) (Fig. 1A). This was due to a significant decrease in immobility scores in animals receiving vmPFC ($n = 9$; $p = 0.02$) and Acb stimulation ($n = 10$; $p = 0.001$) as compared to controls ($n = 14$; 31% and 44%, respectively). In contrast, only a non-significant 17% antidepressant-like effect was recorded after WMF DBS ($n = 10$; $p = 0.1$). When the three targets were compared, differences in immobility, swimming and climbing scores were not significant.

In contrast to the FST, no significant treatment effect was recorded in the open field at either the 5 or 30 min interval (Fig. 1B). That being said, DBS in all three targets induced a 20–30% non-significant increase in locomotion in the first 5 min, suggesting a mild anti-anxiety effect. At the 30 min interval, scores of DBS-treated animals were similar to those of non-stimulated controls, suggesting that DBS did not induce a significant increase in locomotion (Fig. 1C).

Volume of tissue activated by DBS

To investigate whether a spillover of current into adjacent structures could have been responsible for our behavioral findings, we calculated the volume of tissue activated by DBS using computer modeling. As can be seen in Fig. 2, current spread with stimulation parameters and electrodes used in our study was limited to the target region. Aside from a small spillover of current to lateral regions of the vmPFC after WMF DBS, no overlap among target structures primarily influenced by stimulation was observed. In other words, current spread after vmPFC or Acb DBS was limited to the vmPFC and Acb, respectively. In animals receiving WMF DBS, in addition to the forceps minor, current has also directly influenced regions of the claustrum and infralimbic cortex. As the exposed surface and length of the tip of our electrodes were relatively large, current delivered to the Acb in our animals has often spread through both core and shell subdivisions.

Brain regions modulated by DBS

To assess the brain circuits modulated by vmPFC, WMF, or Acb DBS, we have studied the expression of *zif268* in regions involved in mechanisms of psychiatric disorders (Supplementary Table 2). Overall, stimulation in each brain site influenced a different set of structures at a distance from the target (Fig. 3, Supplementary Figs. 3–7). As compared to controls, vmPFC DBS increased *zif268* expression exclusively in cortical regions, including the prelimbic, cingulate, lateral entorhinal, piriform, and orbitofrontal cortices, as well as the temporal association area (Fig. 3, Supplementary Fig. 3). In contrast, Acb DBS increased *zif268* expression mainly in subcortical structures (and also the piriform cortex). These included the globus pallidus, reticular nucleus of the thalamus, zona incerta, lateral hypothalamus, red nucleus, and ventral tegmental area (Fig. 3, Supplementary Figs. 3–7). After Acb DBS, decreased *zif268* expression was observed in the pre and prosubiculum. DBS applied to the WMF induced more widespread effect as compared to the other targets, increasing *zif268* expression in the cortical structures (cingulate, piriform and orbitofrontal cortices), basal ganglia (globus pallidus and substantia nigra reticulata), thalamus (mediodorsal and paracentral nuclei), zona incerta, and brain stem (red nucleus, dorsal raphe, median raphe, pedunculo-pontine nucleus and ventral tegmental area) (Fig. 3, Supplementary Figs. 3–7). Levels of *zif268* in the ventral CA1 were decreased after WMF stimulation.

In summary, DBS delivered to different targets influenced different brain structures and circuits. vmPFC stimulation increased the expression of *zif268* in cortical areas, whereas Acb DBS induced changes in

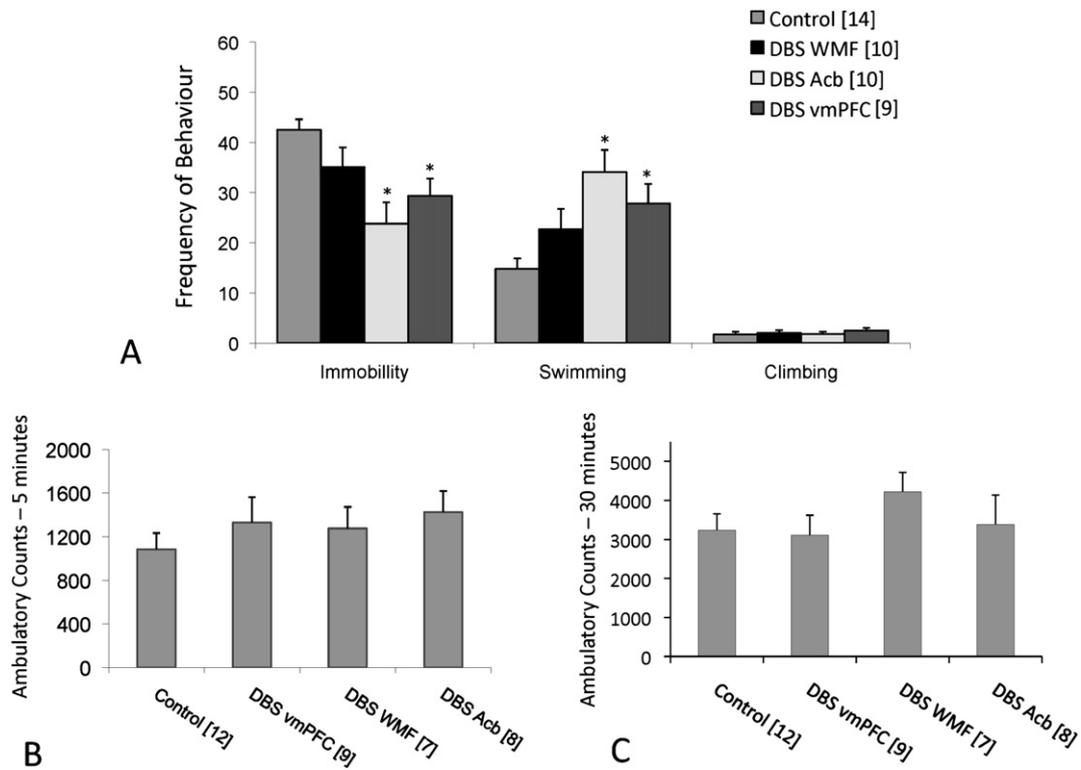


Fig. 1. Outcome of deep brain stimulation (DBS) delivered to the ventromedial prefrontal cortex (vmPFC), nucleus accumbens (Acb) and white matter fibers of the frontal region (WMF) of rats undergoing the forced swim test (FST) and open field. A) During the FST, rats were treated with DBS at 130 Hz, 90 μ s and 100 μ A. For scoring, the predominant behavior (immobility, swimming, or climbing) during the 5 min of testing was recorded every 5 s (maximal score of 60). Animals receiving vmPFC and Acb DBS had a significant reduction in immobility, the hallmark of an antidepressant-like response, as compared to controls ($p = 0.02$ and $p = 0.001$, respectively). In contrast, the antidepressant-like effects of WMF DBS were not statistically significant. B) In the open field, scoring was subdivided into two. The first 5 min of testing were used to evaluate the anxiety of the animals while exploring a novel environment (B). The overall 30 min were considered to be a measure of locomotor activity (C). In neither circumstance, DBS induces significant effect. In all figures of the panel, numbers in parenthesis represent the number of animals per group.

zif268 expression in a subset of subcortical structures. Stimulation of the WMF induced a mixed and more complex pattern of *zif268* expression, altering levels of this immediate early gene in cortical and

subcortical regions. Of note, the piriform cortex was the only region in which *zif268* expression was increased with the delivery of DBS to all targets.

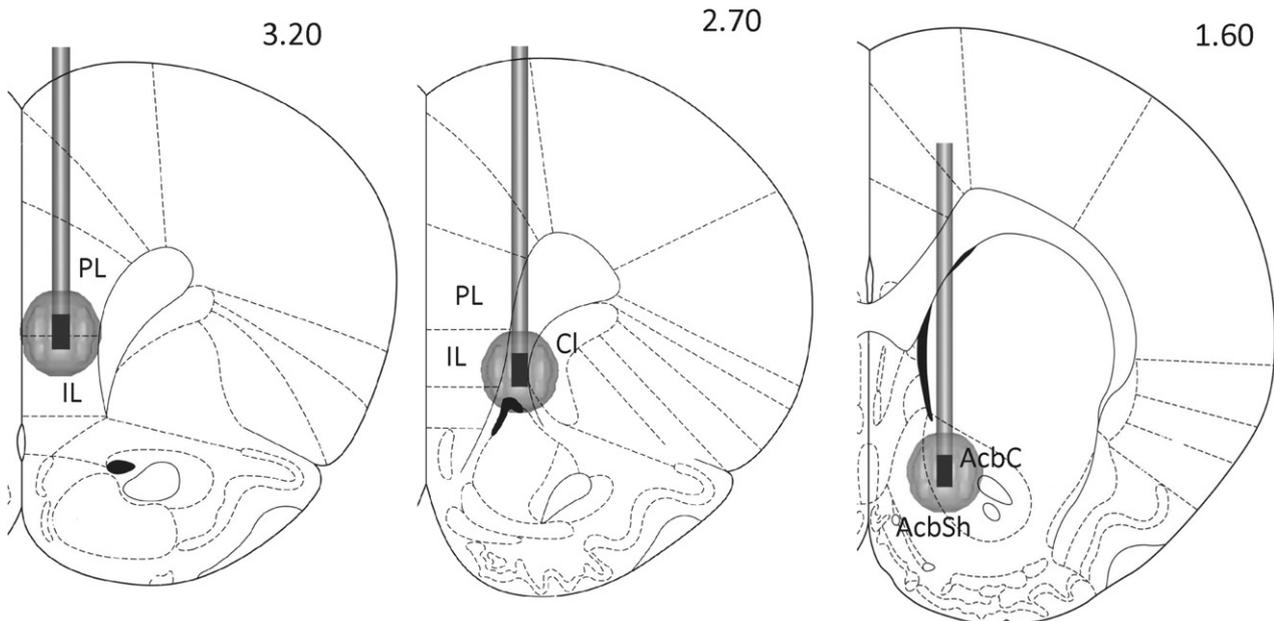


Fig. 2. Volume of tissue activated in animals receiving DBS in the ventromedial prefrontal cortex (vmPFC), nucleus accumbens (Acb) and white matter fibers of the frontal region (WMF). As can be appreciated, with the stimulation settings and electrodes used in our study current spread (gray spheres) was limited to the target region. PL – prelimbic cortex; IL – infralimbic cortex; Cl – claustrum; AcbC – nucleus accumbens core; AcbSh – nucleus accumbens shell. Numbers in the upper right corner of the plates represent distance from bregma (Paxinos and Watson, 1998) (reprinted with permission from Elsevier). Gray bars represent the electrodes while the dark end represents the exposed surface.

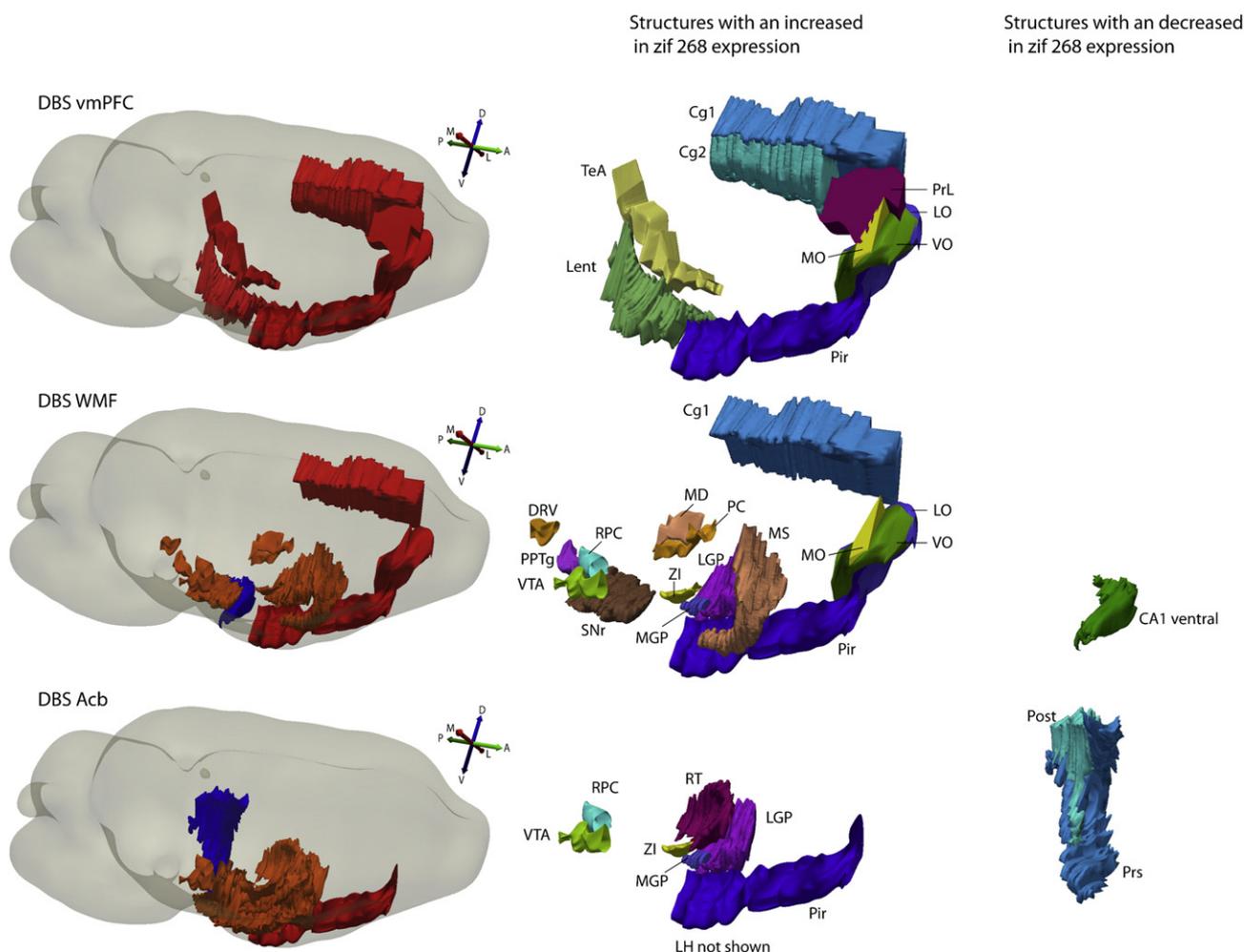


Fig. 3. Differences in *zif268* expression between non-stimulated controls and animals receiving DBS in the ventromedial prefrontal cortex (vmPFC), nucleus accumbens (Acb) and white matter fibers of the frontal region (WMF). In the left, 3D reconstructions give an overview of structures with an increase (red for cortical and orange for subcortical) or a decrease (blue) in *zif268* expression after DBS. In the middle and right columns, these same structures were individually labeled. As can be clearly appreciated, the administration of DBS to different targets influenced different brain structures and circuits. vmPFC stimulation significantly increased *zif268* expression in cortical regions. Acb DBS induced significant changes in *zif268* expression mainly in subcortical structures. Stimulation of the WMF was associated with a more complex pattern of changes in *zif268* expression, altering levels of this immediate early gene in cortical and subcortical regions. CA1 ventral; Cg1 – cingulate gyrus, area 1; Cg2 – cingulate gyrus, area 2; DRV – dorsal raphe, ventral; LGP – lateral globus pallidus; LH – lateral hypothalamus; LO – lateral orbital cortex; MD – mediodorsal nucleus of the thalamus; MGP – medial globus pallidus; MO – medial orbital cortex; MS – medial septum; PC – paracentral nucleus of the thalamus; Pir – piriform cortex; Post – postsubiculum; PPTg – pedunculopontine tegmental nucleus; PrL – prelimbic cortex; Prs – presubiculum; RPC – red nucleus, parvocellular; RT – reticular nucleus of the thalamus; SNr – substantia nigra reticulata; TeA – temporal association cortex; VO – ventral orbital cortex; VTA – ventral tegmental area; ZI – zona incerta.

DBS and functional connectivity

After determining local differences in *zif268* expression, we examined possible changes in functional connectivity in animals receiving vmPFC, WMF or Acb DBS. For this, intercorrelations of *zif268* levels measured in 98 brain regions were computed (for a complete list of structures see Supplementary Table 3). Significant correlations are presented in Figs. 4 and 5. Stimulation of each specific target induced distinct changes in functional connectivity. After vmPFC DBS, correlations across brain regions were globally reduced. Strikingly, this was more pronounced in cortical regions presenting an increase in *zif268* levels after stimulation. Correlations across brain sites were also reduced after Acb DBS, except for the thalamus and brain stem. In contrast, animals given WMF DBS had a widespread increase in functional connectivity in subcortical structures but reduced intercorrelations among frontal cortical regions.

In summary, our findings suggest that DBS in different targets leads to distinct changes in functional connectivity. Interestingly, the highest increases in *zif268* expression and brain connectivity were seen after WMF DBS, a treatment that was associated with the lowest degree of

antidepressant-like effect in the forced swim test. Common to all three targets was a decrease in functional connectivity among frontal cortical regions.

Discussion

The main findings from this study were twofold. First, DBS delivered to the vmPFC and Acb induced comparable antidepressant-like effects in the FST. Second, despite of a lack of overlap in regions directly modulated by DBS, stimulation to each different target influenced activity and connectivity in a distinct set of brain structures and circuits. This suggests that, despite inducing similar antidepressant-like effects, stimulation of the vmPFC, WMF and Acb does not necessarily rely on similar circuits for their mechanisms of action.

Anatomical considerations and stimulation parameters

To mimic the clinical scenario, our choice of target in the region of the Acb was relatively straightforward. Electrodes were placed in the transition between shell and core so that most of the exposed surface

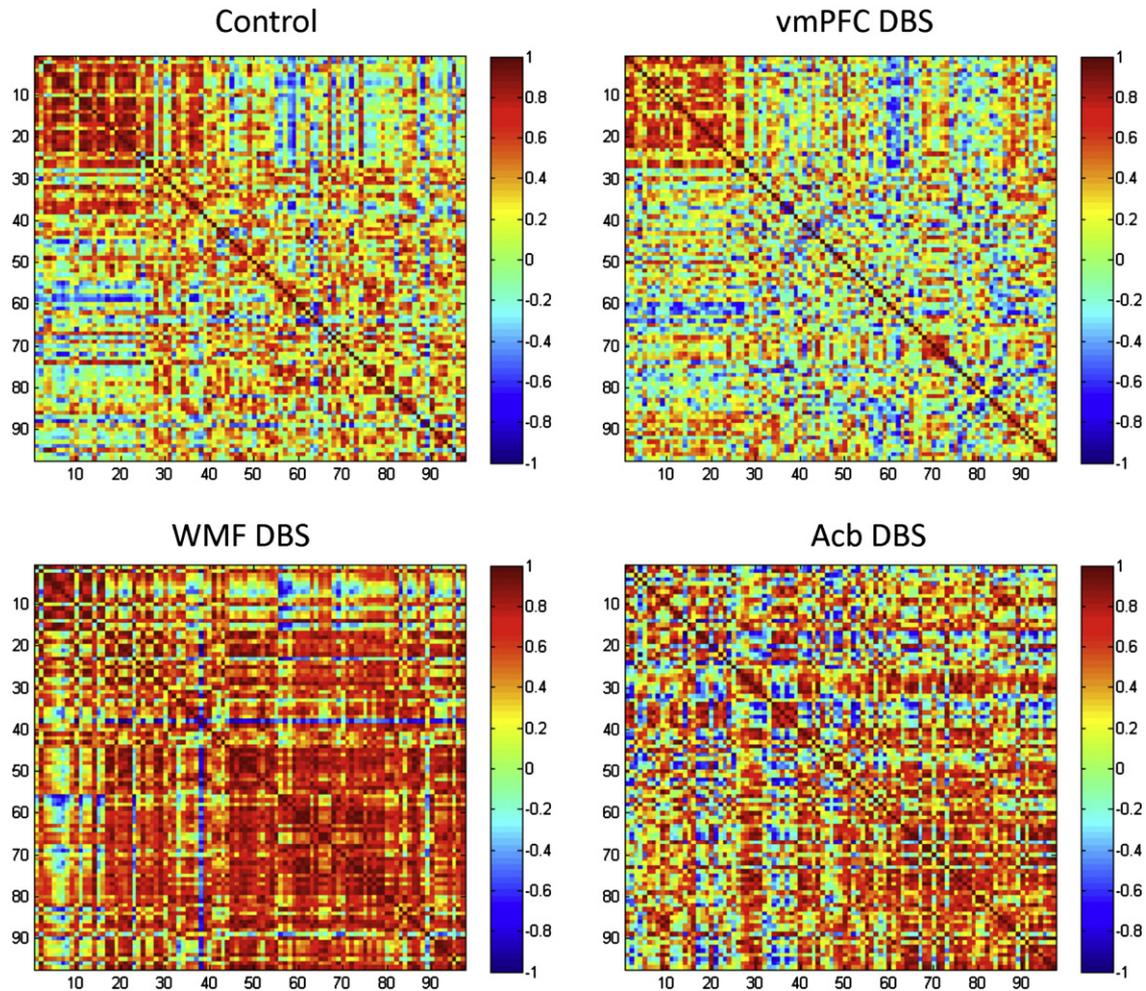


Fig. 4. Correlation matrices in rats given DBS in the ventromedial prefrontal cortex (vmPFC), nucleus accumbens (Acb) or white matter fibers of the frontal region (WMF). Column and row numbers correspond to brain regions indicated in Supplementary Table 3. Permutation testing was used to compare DBS and control matrices. Significant differences are shown graphically. Overall, we found that correlation between *zif268* expressions across brain regions was globally reduced after vmPFC DBS as compared to controls. In contrast, correlation across brain sites after Acb DBS was increased in the thalamus and brain stem (numbers 55–98 in the matrix). In animals given WMF DBS, functional connectivity was increased in most sub-cortical structures (areas 24–98 of the matrix) but reduced in frontal cortical regions (areas 1–23).

would be implanted in the latter (Bewernick et al., 2010; Schlaepfer et al., 2008). In contrast, determining regions homologous to the human SCG or anterior capsule in rodents was far more complex. Based on cytoarchitectural features and anatomical projections, the rodent ventromedial prefrontal cortex (IL and ventral PL) has been suggested to correspond to the human subgenual cingulum (Hamani et al., 2011; Uylings et al., 2003; Vertes, 2004). In rodents, the anterior limb of the internal capsule is not well developed. White matter projections in frontal brain regions comprise the forceps minor of the corpus callosum and the anterior commissure. While acknowledging that none of these structures would resemble the anterior limb of the internal capsule in humans, we chose to implant electrodes in the forceps minor, as this structure contains both commissural and projection fibers. In humans, the anterior limb of the internal capsule is composed in part by cortical projections that innervate the thalamus, basal ganglia and brain stem pontine nuclei (Paxinos and Watson, 1998). Though pathways running through the rodent forceps minor have not been characterized in detail, our stimulation results suggest that they may include projections that innervate the cerebral cortex, basal ganglia, thalamus and brain stem structures. At this point we cannot rule out that some of these structures may have been recruited through polysynaptic circuits. However, we find this unlikely as changes in *zif268* expression and functional connectivity were recorded in numerous brain regions after WMF DBS. Since not many brain structures have such wide patterns of innervation, it

seems more reasonable to think that such changes derived from the activation of a common source (in this case fibers from the forceps minor) rather than multiple brain sites.

As for stimulation parameters, our choice was based on charge density, side effects and the volume of tissue activated by DBS. With similar settings being delivered to each target, we could standardize the delivery of current while minimizing spread to adjacent structures. That being said, we recognize that different parameters may be required for an optimal effect of WMF DBS in the FST. For example, in patients with obsessive–compulsive disorder initial studies used high current intensities during VC/VS stimulation (Nuttin et al., 2003). However, with accumulation of experience and a better characterization of the optimal region for electrode placement, the current required for a good postoperative outcome was dramatically reduced. In depression, stimulation parameters across DBS targets are comparable (high frequency stimulation with current intensities in the order of 3–5 V) and in the range of those used in the current study (Bewernick et al., 2010; Lozano et al., 2008; Malone et al., 2009).

Regional brain differences induced by DBS

One of the main findings of our study was that changes in *zif268* expression after stimulation varied according to target. This suggests that by delivering DBS to the vmPFC, Acb and WMF we were influencing

different sets of structures and not simply simulating relays of the same circuitry. Despite similar behavioral results, levels of *zif268* after vmPFC DBS were only increased in cortical regions, whereas after Acb DBS *zif268* expression was primarily increased in subcortical structures. WMF DBS influenced both cortical and subcortical structures but did not induce a significant antidepressant-like response.

In our study, the only common region presenting increased *zif268* expression after DBS in all three targets was the piriform cortex, a structure primarily involved in processing olfactory information (Isaacson, 2010; Wilson and Sullivan, 2011). Most of the research implicating this cortical region in depression has been conducted after olfactory bulb lesions in rodents (Holmes et al., 1998; Jarosik et al., 2007; Wang et al., 2007). In the FST, studies using microPET and *c-fos* in situ hybridization have shown reduced activity in the piriform cortex of rats treated with fluoxetine or imipramine (Jang et al., 2009; Sairanen et al., 2007). This is in contrast with the increased *zif268* expression recorded after DBS in our study. Though further investigation is needed, these findings suggest that the piriform cortex may be a region in which DBS and medications exert opposite effects.

Distinctions in brain regions expressing *zif268* in animals receiving WMF DBS versus Acb or vmPFC that could explain our behavioral findings include those in the dorsal raphe (DR) and some thalamic nuclei. The DR provides serotonergic innervation to a number of cortical and subcortical structures (Hensler, 2006; Lowry et al., 2008; Michelsen et al., 2007; Molliver, 1987). It has been extensively studied in the context of depression, since a number of commonly used antidepressant medications increase serotonin release in different brain sites (Lowry et al., 2008; Mann, 2005; Michelsen et al., 2007). In previous work, we have shown that the antidepressant-like effects of DBS in the vmPFC are largely dependent on the integrity of the serotonergic system (Hamani et al., 2010b). Bearing this in mind, a question that emerges is why WMF DBS would induce an increase in raphe activity but exert no significant behavioral effects. Though *zif268* is commonly used as a marker of local brain activity, mRNA measures cannot by themselves be used to characterize the cell type involved. Frontal cortex-raphe fibers innervate both serotonergic projection cells and GABAergic interneurons (Hajos et al., 1998; Varga et al., 2001; Warden et al., 2012). One possibility is that WMF stimulation might have predominantly influenced the latter, with a subsequent inhibition of serotonin release. This would be in contrast with Acb or vmPFC DBS, which has been previously shown to induce serotonin release in various brain regions (Hamani et al., 2010b; Juckel et al., 1999; van Dijk et al., 2012). Another unique feature of WMF DBS was that it increased thalamic activity, particularly in the mediodorsal nucleus (MD). The MD is a relay structure that projects to the orbitofrontal and prefrontal cortical regions. It has been largely implicated in mechanisms of stress, impulsivity and addictive behavior. In the clinic, stimulation of the inferior thalamic peduncle (a fiber pathway that interconnects the MD with the orbitofrontal cortex) has been used for the treatment of depression with promising results (Jimenez et al., 2005; Velasco et al., 2005). In rodents, glutamatergic projections from the prefrontal and orbitofrontal regions to the MD innervate primarily glutamatergic neurons (Ray and Price, 1992; Ray et al., 1992). Whether increased MD activity might be responsible for the less pronounced behavioral results of WMF DBS remains to be demonstrated. However, should that prove to be the case, it is possible that focal inactivation of the MD (i.e. through local injections of GABAergic agonists or even DBS) could have antidepressant-like effects.

Interconnectivity patterns

In addition to inducing local changes in *zif268* expression, DBS in the vmPFC, WMF and Acb induced distinct changes in functional connectivity. Common to all three targets, however, was a DBS-induced decrease in the correlation of cortical *zif268* expression. This is in contrast to the differences observed in subcortical connectivity in animals given DBS to the vmPFC, Acb and WMF. Though comparisons of connectivity data between

naïve rodents and humans need to be approached with caution, we find it intriguing that, similar to our DBS findings, electroconvulsive therapy and antidepressant medications also reduce cortical connectivity in patients with depression (McCabe et al., 2011; Perrin et al., 2012; Scheidegger et al., 2012). Results from our study certainly need to be corroborated in other models of depression, in animals undergoing chronic stimulation, and in patients treated with DBS. They do suggest, however, that changes in cortical brain function and connectivity should be further explored as a mechanism of antidepressant therapies.

Limitations

A few limitations of studies such as ours include the use of naïve rats and the translational nature of animal models of psychiatric disorders (Hamani and Nobrega, 2012; Hamani and Temel, 2012). One of the limitations of the forced swim test is the short timeframe required for an antidepressant-like response. In addition, animals do not have genotype and/or phenotype features that may resemble a depressive-like state prior to testing. That being said, the FST is the most commonly used screener for the assessment of the efficacy of antidepressant interventions, having excellent predictive validity (Cryan and Slattery, 2007; Cryan et al., 2002; Krahl et al., 2004; Li et al., 2007; Porsolt et al., 1977, 1978).

Despite the caveats described above, preclinical models seem to have a very good predictive validity for the study the antidepressant-like effects of DBS and may offer a unique contribution to the field (Hamani and Temel, 2012). In humans, the appraisal of the clinical effects of DBS is often carried out through the use of subjective scales. As such, placebo effects are difficult to measure in open label or smaller scale studies. Preclinical research can help us characterize the biological effects of DBS so that clinical trials may be perfected. As an example, we have recently shown that the behavioral and neurochemical effects of DBS may persist after stimulation is discontinued (Hamani et al., 2010b, 2012). This suggests that in clinical trials contemplating a crossover design (i.e. patients receiving active or sham stimulation followed by the inverse treatment) a washout phase is mandatory. Though studies in animal models by all means substitute clinical research, they may certainly provide a guide so that time and resources may be better allocated.

In our study the pattern of immediate early gene expression after DBS has been characterized in naïve rats. Though this does not reflect the scenario observed in “depressive-like states”, our study design may have distinct advantages. In the clinic, it is difficult to appreciate whether particular metabolic changes in patients given stimulation are primarily due to DBS, the clinical effects of stimulation or an interaction of both. By studying naïve rats we were able to dissect the subject and demonstrate specific patterns of activation of DBS related exclusively to treatment.

Finally, structures being investigated as DBS targets in more recent studies are the lateral habenula and medial forebrain bundle (MFB) (Sartorius and Henn, 2007; Sartorius et al., 2010; Schlaepfer et al., 2013). In the former, antidepressant-like effects of stimulation have been described in preclinical models (Li et al., 2011). Whether MFB stimulation induces an antidepressant-like effect in rodents remains to be demonstrated.

Conclusions

In conclusion, we show that DBS delivered to the vmPFC and Acb induced comparable antidepressant-like effects in the FST. Immobility scores in animals that had DBS applied to the WMF were slightly higher than those recorded after vmPFC or Acb DBS but not significantly different from that of non-stimulated controls. Bearing in mind that PFC regions heavily project to the Acb and some of the projections from PFC to subcortical areas run through white matter fibers of the frontal cortex, we expected some degree of overlap in the expression of immediate early genes after DBS in these three different targets. Contrary to our

expectations, however, we found that stimulation of the vmPFC, WMF and Acb influenced activity and connectivity in a distinct set of brain structures and circuits. This suggests that, despite inducing similar antidepressant-like effects, stimulation of these three targets does not necessarily rely on similar circuits for their mechanisms of action. The fact that DBS in different regions induces specific circuitry changes may have important implications to the field. For example, this therapy may be individualized according to the patients' predominant symptoms and the specific circuits that require modulation. In other words, different depressive phenotypes may require DBS in different targets.

At present, DBS for depression remains investigational. Despite the promising results of open label studies, double blind evaluations comparing the effects of active versus sham stimulation in patients treated with VC/VS DBS have been somewhat disappointing (Holtzheimer et al., 2011a). Rather than discouragement, the field should look more into basic research and predictors of a response. Several preclinical studies have shown that DBS does induce antidepressant-like responses and modulates neurochemical substrates involved in depression. At present, we cannot rule out that the antidepressant effects of DBS are due to a placebo response. That said, the fact that electrical stimulation induces concrete biological effects suggests that, rather than a simple placebo effect, we still do not understand who are the ideal candidates, optimal targets and appropriate kinetics of for a DBS response.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nbd.2014.08.007>.

Disclosures

C.H. and C.R.B. are consultants for St Jude Medical. C.R.B. is a consultant for Boston Scientific, Advanced Bionics, Intellect Medical and NeuroPace. The other authors do not have a conflict of interest.

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Fig. 5. Functional networks in rats given DBS in the ventromedial prefrontal cortex (vmPFC), nucleus accumbens (Acb) or white matter fibers of the frontal region (WMF). Networks were constructed by retaining the top 1% strongest correlations for DBS and control groups. This corresponded to a network $r > \pm 0.83$ for controls, $r > \pm 0.79$ for the vmPFC DBS group, $r > \pm 0.93$ for the WMF DBS group, and $r > \pm 0.93$ for the Acb DBS group. Nodes in the resulting networks represent brain regions. Correlations that survived the threshold criterion were considered to be functional connections. Nodes in the networks represent brain regions and the connections reflect super threshold correlations. The number of connections is reflected by the size of the node and the connection strength by line thickness. As in Fig. 4, one can clearly appreciate that after vmPFC DBS, correlation between *zif268* expressions across brain regions was globally reduced. Correlations across brain sites were also reduced after Acb DBS, except for the thalamus and brain stem. In animals given WMF DBS functional connectivity was increased in subcortical structures but reduced in frontal cortical regions.

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