Effects of Repeated Deep Brain Stimulation on Depressive- and Anxiety-Like Behavior in Rats: Comparing Entopeduncular and Subthalamic Nuclei

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**Abstract**

Background: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) or internal globus pallidus (GPi) has been routinely used for the treatment of some movement disorders. However, DBS may be associated with adverse psychiatric effects, such as depression, anxiety and impulsivity. Objective: To compare DBS applied to the entopeduncular nucleus (EPN; the rodent homolog of the GPi) and STN in terms of their effects on depressive- and anxiety-like behavior in rats. Methods: DBS was applied for 21 days (4 h a day) to either the STN or EPN. Rats then underwent behavioral testing on learned helplessness and elevated plus maze tasks before being sacrificed for brain analyses of zif268, BDNF and trkB mRNA as well as BDNF protein levels. Results: Repeated DBS of the STN, but not of the EPN, led to impaired performance in the learned helplessness task, suggesting that STN-DBS induces or potentiates depressive-like behavior. There was no effect of DBS on elevated plus maze or on open field behavior. Repeated STN-DBS, but not EPN-DBS, led to decreased levels of BDNF and trkB mRNA in hippocampus. Acute stimulation of the STN or EPN resulted in similar changes in zif268 levels in several brain areas, except for the raphe where decreases were seen only after STB-DBS. Conclusions: Together these results indicate that the effects of STN- and EPN-DBS differ in behavioral and neurochemical respects. Results further suggest that the EPN may be a preferable target for clinical DBS when psychiatric side effects are considered insofar as it may be associated with a lower incidence of depressive-like behavior than the STN.

**Introduction**

Deep brain stimulation of the subthalamic nucleus or internal globus pallidus has been routinely used for the treatment of movement disorders, including Parkinson’s disease, dystonia and tremor [1–4]. While the therapeutic effects of DBS in these conditions are clear, clinical trials have also indicated that DBS can be associated with adverse effects, such as depression, apathy, anxiety, impulsivity and increased risk of suicide [5–9]. The mechanism(s) underlying these effects remain(s) unclear, and there is as yet no definitive consensus as to which target, the STN or GPi, has a more benign side effect risk profile.

In clinical populations, it is difficult to directly assess which target has a more favorable effect profile [10–12]. The overwhelming majority of studies assessing psychiatric side effects have been performed in patients with Parkinson’s disease (PD), a condition where underlying motor or psychiatric pathology may obscure comparisons between STN and GPi as DBS targets. Moreover, STN-DBS has been more widely applied than GPi-DBS for the treatment of PD and as a result a larger volume of psychiatric effects that have been reported after STN-DBS than after GPi-DBS. In studies designed to directly compare DBS targets, it is emerging that STN-DBS may be associated with more affective side effects than GPi-DBS [13], although this finding has not been consistently reported in all clinical trials [7,14].
The objective of the present study was to compare psychiatric-type effects of DBS applied to the STN and entopeduncular nucleus (EPN, the rodent homolog of the GPi), in the absence of motor disturbances or neurological pathology. We used a learned helplessness protocol to model depressive-like behavior and an elevated plus maze task to assess anxiety-like behavior in otherwise healthy rats that had undergone daily DBS for three weeks. Since hippocampal brain-derived neurotrophic factor (BDNF) has been implicated in depressive syndromes [15–17], we also measured BDNF levels as well as gene expression of its receptor, \( \text{trkB} \), in the hippocampus of repeatedly stimulated rats. Finally, it has been hypothesized that STN-DBS may have a greater effect than EPN-DBS on brain regions distal from the target site [18], which may in turn contribute to their different symptom profiles. To address this possibility we used expression the early gene \( \text{zif268} \), a marker of neuronal activity, to compare the effects of acute STN-vs. EPN-DBS on several brain areas that have been generally implicated in affective processes and emotional reactivity.

Methods

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

Surgery

An initial cohort of 112 male Sprague-Dawley rats weighing 400–450 g were anesthetized with ketamine/xylazine (100/7.5 mg/kg i.p.) and had polymide-insulated stainless steel monopolar electrodes (250 µm in diameter with 0.6 mm of surface exposed) bilaterally implanted into the STN (AP −3.8 mm; ML + 3.5 mm; DV −8.0) or EPN (AP −3.6 mm; ML + 3.6 mm; DV −7.8 mm) according to the atlas of Paxinos and Watson [19]. Anodes were connected to a bone screw over the somatosensory cortex. Sham surgery controls were anesthetized and had holes drilled into the skull but were not implanted with electrodes. Electrode placement was confirmed for each subject using cresyl violet staining. Only subjects with verified electrode placements were included in the analyses. Representative electrode placements pictomicrographs are shown in Fig. 1.

DBS protocol

Starting 1 week after surgery DBS was applied using a portable stimulator (Model 6510, St. Jude Medical, Plano, Texas) set to deliver 100 \( \mu \text{A} \), 90 µs pulse width at 130 Hz. Choice of these stimulation settings was guided by two considerations. First, when electrode diameter and exposed surface are taken into account, we estimated that current intensities in the 100–300 \( \mu \text{A} \) range would generate a charge density that approximates that which is used in humans [20–22]. Second, preliminary observations indicated that currents higher than 100 \( \mu \text{A} \) induced motor effects in some animals (i.e. forelimb dyskinesias in the STN group and motor contractions in the EPN group). DBS was applied for 4 h per day, for twenty-one consecutive days.

Learned helplessness

To assess potential depressive-type behavior we have chosen the learned helplessness (LH) paradigm, which is one of the better studied and validated preclinical models of depressive disorders [23]. In this paradigm, an initial exposure to uncontrollable stress disrupts the ability to acquire escape responses when animals are later placed in an escapable stress situation. The LH protocol began on the 16th day of DBS and was performed over 5 days [24,25]. On Day 1 half of the rats, referred to as the “stressed” groups (\( N = 8 \) Shams, 8 STN-DBS, 8 EPN-DBS), were subjected to an inescapable stress session where they received 0.8 mA inescapable footshocks in sound-attenuated operant boxes (Med Associates, St. Albans, VT). Both the duration (1.5–60 s) and the interval between shocks (1–30 s) were programmed to result in a total shock exposure of 25 min per animal. The other half, the “non-stressed” rats (\( N = 8 \) Shams, 8 STN-DBS, 8 EPN-DBS), were placed in the inescapable shock boxes for 25 min but did not receive any shock. On Day 2 all rats underwent 4 h DBS, as in the previous 3 weeks. On Day 3–5 all animals underwent escape sessions, one session per day, following 4 h DBS [24,25]. Testing was conducted in automated shuttle boxes (Med Associates, St. Albans, VT). A central

Figure 1. Representative pictomicrographs showing localization of electrode tips (arrows). Cresyl violet staining was used to localize electrodes in the EPN (panel A) or STN (panel B).
divider provided passage between two equal size compartments. Each session consisted of 30 trials of escapable footshock (0.80 mA, 5 s duration, 90 s average intertrial interval). Each trial started with visual (houselights on) and auditory (85 dB white noise) cues beginning 5 s before shock onset. Rats could avoid or escape the shock by moving between compartments once the trial started. For each trial, latency to cross over was recorded by floor sensors and the rat’s response was classified as avoidance (latency < 5 s), escape (latency > 5 s) or failure to move between compartments for the 10 s duration of the trial.

**Elevated plus maze**

In a separate cohort of rats (N = 10 sham controls, 11 STN-DBS, 9 EPN-DBS), elevated plus maze testing occurred immediately following stimulation on the twentieth day of DBS. The apparatus consisted of two open arms and two enclosed arms at right angles (arms: 10 cm wide, 15 cm high, 50 cm long) raised 1 m above the floor. Rats were placed in the center of the maze, facing an open arm and allowed to explore for 5 min, during which time behavior was recorded. The number of entries as well as total time spent in open vs. closed arms was quantified by a trained observer blind to treatment group.

**Open field assessments**

As a control for the possibility that repeated DBS might induce motor effects that could affect learned helplessness and/or plus maze results, rats were tested on an open field arena. On the 20th day of DBS, locomotor activity was assessed in an open field (Sham controls = 10, STN-DBS = 10, EPN-DBS = 10). Following DBS and plus maze tests rats were placed in automated activity chambers (Med Associates, St. Albans, VT) and locomotor activity was recorded for 20 min by photobeam breaks. Animals without electrodes were also monitored in the activity chamber for 20 min, to coincide with the timing of behavioral testing relative to DBS.

**BDNF protein measurements**

At the conclusion of twenty-one days of DBS and learned helplessness testing, rats were sacrificed (N = 8 per group), brains were removed, sectioned along the midline and frozen over dry ice and then stored at −80 °C for quantification of BDNF protein and trkB and BDNF in situ hybridization (see below). Quantification of hippocampal BDNF levels was done using an ELISA kit (Promega, Madison, WI), according to manufacturer’s instructions with modified lysis buffer. Briefly, dorsal hippocampus was dissected from frozen tissue and lysed in 1:100 volumes of modified lysis buffer (100 mM PIPES, pH 7, 500 mM NaCl, 0.2% Triton X-100, 0.1% NaN3, 2% BSA, 2 mM EDTA-Na2, 2H2O, 200 mM PMSF frozen in iso-propanol, 10 mM leupeptin frozen separately in deionized, 0.3 maprotinin frozen separately in 0.01 M HEPES pH8 and 1M sodium thiosulfate, dipped in water, dehydrated in 70% ethanol, and air-dried. The slides were exposed to Kodak BioMax film for 6 days at 4 °C along with calibrated radioactivity standards. Probe specificity was confirmed by testing labeled sense and scrambled probes, both of which produced no measurable signal on film. Films were analyzed using an MCID system (Interfocus, Leiton, UK). After normalizing film background, grey levels were converted to μCi/g per gram of tissue using previously calibrated radioactivity standards. Brain regions were analyzed across 8 sections per animal, delineation of the structures was done visually, according to the atlas of Paxinos and Watson [19].

**zi268 mapping**

To obtain comparative information on anatomical targets affected by STN- vs. EPN-DBS, a separate group of stimulation-naïve rats (300–325 g) underwent one hour of continuous DBS (N = 6 shams, 6 STN-DBS, 6 EPN-DBS), after which they were sacrificed. Brains were removed and hybridized performed as described above, using [35S]UTP labeled riboprobes complementary to zi268 (according to Genbank # NM_012551, (bases 660-679), 5'-ttctatactacggcgcttc-3' and (bases 1062–1043) 5'-aggctctctctgtg ttgctg-3').

**Statistical analyses**

All statistical analyses were performed using SPSS version 19. Learned helplessness data were analyzed with repeated measures ANOVAs using Stimulation target (Sham Controls, STN-DBS or EPN-DBS) and Stress as between-subject factors and Test day as a within-subjects factor. Data for the elevated plus maze, open field and BDNF protein levels were analyzed with one-way ANOVAs with Stimulation target as the independent variable. zi268 in situ hybridization data were analyzed with repeated measures ANOVAs, with Brain area as the within-subject factor and Stimulation target as the between-subject factor. The same type of analysis was conducted for BDNF and trkB data, with the addition of a second between-subject factor (Stress). Tukey’s post-hoc tests were performed to determine between-group differences where appropriate.

**Results**

**Learned helplessness**

As expected, animals that were previously exposed to inescapable footshock showed impaired performance in the avoidance task relative to non-stressed controls (Fig. 2). A three-way ANOVA on number of failures over the three days indicated significant main effects of Inescapable Stress (F1,42 = 20.63, P < 0.001) and brain manipulation (sham surgery, STN DBS or EPN DBS; F2,42 = 123.04,
Repeated DBS had no effect on elevated plus maze performance (Fig. 3). The proportion of time spent in open arms ($F_{2,27} = 1.03, P > 0.37$) and the proportion of entries into open arms ($F_{2,27} = 1.13, P > 0.338$) were not different between treatment groups. Similarly, total number of entries made into arms was not affected by DBS ($F_{2,27} = 2.05, P > 0.148$). The observation that open and closed arm values were similar in all groups suggested that extensive daily handling (>12 weeks) could have been a factor. A separate group of rats handled for only one week was added to confirm that test conditions could indeed produce the expected differences between open and closed arm values (Fig. 3).

Open field behavior

As shown in Fig. 4, repeated DBS applied to either the STN or the EPN had no effect on locomotor activity. Total activity, as measured by ambulatory counts was not different between stimulated and un-stimulated groups, as revealed by one-way ANOVA ($F_{2,25} = 1.85, P > 0.177$).

BDNF and trkB gene expression

BDNF and trkB gene expression were measured in hippocampal subdivisions (CA1, CA3, dentate gyrus and subiculum), amygdala
(combined basolateral and basomedial subdivisions), and motor cortex (Figs. 6 and 7).

For BDNF (Fig. 6), two-way repeated measures ANOVA indicated an overall effect of brain area ($F_{6,186} = 88.42, P < 0.001$), no main effect of target, stress, or target × stress interaction (all $P > 0.05$) and a significant target × area interaction ($F_{12,186} = 5.273, P < 0.01$). In the medial blade of the dentate gyrus (DGm), levels of BDNF mRNA were significantly lower in STN-DBS animals relative to both EPN-DBS animals ($P = 0.002$) and sham-operated controls ($P = 0.034$) (Fig. 6).

Likewise, for trkB (Fig. 7) there was an overall effect of brain area ($F_{6,186} = 258.98, P < 0.001$), no main effect of target, stress, or the target × stress interaction (all $P > 0.05$) and a significant target × area interaction ($F_{12,186} = 2.127, P < 0.05$). Post-hoc Tukey tests revealed that both STN-DBS groups had significantly lower trkB expression than sham operated animals in the DGm ($P = 0.019$) (Fig. 7). In addition, in comparison to unstressed controls, STN-DBS rats exposed to stress showed lowered trkB levels in the subiculum ($P = 0.04$) and the amygdala ($P < 0.04$) (Fig. 7). In comparison to EPN rats, both STN groups had significantly lower levels of trkB mRNA on the medial portion of the dentate gyrus $P (< 0.05)$ (Fig. 7).

Discussion

The main finding of this study was that DBS applied repeatedly to the STN, but not to the EPN, impaired performance in the learned helplessness model of depression, and was associated with lower levels of gene expression of BDNF and its receptor trkB. DBS applied to either target had no effect on performance in the elevated plus maze (Fig. 3) and did not affect general locomotor activity (Fig. 4).

In the LH paradigm STN DBS but not EPN had a significant worsening effect on escape performance in previously stressed rats. Interestingly however, STN DBS also produced significant performance deficits in non-stressed rats whereas EPN-DBS unstressed rats performed essentially like controls (Fig. 2). STN-DBS also worsened performance in stressed rats, to an extent similar to its effect in unstressed animals, thus suggesting an additive effect. The effects of STN-DBS in unstressed rats suggest the intriguing possibility that repeated STN-DBS may itself be acting as a stressor. This is not however the only possibility. While the open field data suggest that these effects were not due to general motoric deficits, it would be important for further studies to evaluate whether repeated STN DBS potentially affects important variables such as pain sensitivity, motor learning ability, attention, or other sensorimotor or cognitive functions.
To the extent that LH performance is thought to reflect indices relevant to depressive-type behaviors, the observed pattern of results is consistent with clinical reports suggesting that STN-DBS is more strongly associated with affective symptoms than is GPi-DBS. However, these clinical studies have been carried out in patients with Parkinson’s disease and therefore it was not possible to rule out a possible effect of the underlying neuropathology in the affective symptoms associated with DBS. A further potential difficulty in interpreting clinical studies is the possibility of psychiatric effects associated with dopamine agonists or dopamine replacement therapy in PD patients [27,28]. STN- but not GPi-DBS is often associated with reductions in dopaminergic medication [29], which could potentially also affect the psychiatric side effect profile of STN-DBS relative to EPN-DBS. Due to the compact size of the STN, stimulation delivered at parameters optimized for reduction of motor symptoms may result in current spread to both limbic sub-regions of the STN when electrodes are positioned too medially [38]. In contrast, in the case of the GPi the risk of current spread to non-motor sub areas would be less as the GPi is larger. In rats, although the EPN and STN are closer in size, and although the differences in current spread may be less pronounced than in human patients, STN-DBS was still associated with greater adverse effects than EPN-DBS. This suggests that proximity of anatomical subdivisions in the target nuclei may not explain the higher incidence of psychiatric effects of STN-DBS relative to EPN-DBS.

The results from immediate early gene mapping also suggest that differences in side effects of STN vs. GPi DBS are probably not accounted for by differential effects on limbic projection areas. If DBS applied to the STN were having a greater effect on activity of limbic subregions and limbic projections of the target nuclei [32,33]. Both the STN and EPN are connected to several limbic areas, and are subdivided into associative, limbic, and motor regions [34–37]. In humans, the GPi (average size 478 mm$^3$) is approximately three times larger than the STN (average size 158 mm$^3$), and it has been suggested that the increased size of the GPi may make it an architecturally safer environment for DBS [18].

The results from immediate early gene mapping also suggest that differences in side effects of STN vs. GPi DBS are probably not accounted for by differential effects on limbic projection areas. If DBS applied to the STN were having a greater effect on activity of
limbic areas connected to the target structure, we would expect pronounced differences in limbic zif268 expression in DBS applied to the two targets. While zif268 expression was generally altered in the same direction and to the same extent by DBS of the STN and EPN, there were no significant differences in zif268 expression in any region of the limbic system between the two DBS targets. We did, however, find a difference between the STN and EPN stimulation in the raphe nucleus, where zif268 expression was significantly decreased by STN- but not by EPN-DBS. It has been previously shown that STN decreases the firing of raphe neurons [39–41]. The raphe provides serotonergic innervation to the forebrain [42,43], and the different effects of STN- and EPN-DBS on raphe neurons may relate to the different depressive-like effects observed in the rodent. In support of this idea, acute high-frequency stimulation of the STN has been shown to impair performance in the forced swim test, a task used to model depressive-like behavior [41], and lesions of the STN impair performance in this learned helplessness model [44]. Impaired performance on the forced swim test following DBS was prevented by pretreatment with the SSRI fluoxetine, further implicating a 5-HT mechanism in these depressive-like effects [41].

Another possible mechanism would involve changes in BDNF regulation induced by DBS at the two targets. The neurotrophic theory of stress-related mood disorders posits that stress negatively regulates BDNF in distinct brain regions including the hippocampus [16,22,45–47]. Decreased BDNF levels have been reported in depressed individuals [48–50] and in chronically-stressed animals [51–54]. This decrease in BDNF is responsive to antidepressant treatment, and is thought to contribute to antidepressant response [55,56]. Here we examined the trkB-BDNF system in three ways, namely expression of trkB and BDNF genes as well as hippocampal levels of the BDNF protein. Consistent with behavioral results from the LH task, there were no differences in trkB or BDNF expression in EPN-DBS treated groups relative to controls. However, rats that underwent STN-DBS exhibited reduced trkB and BDNF expression in the medial blade of the dentate gyrus. This region-specific pattern is consistent with previous studies reporting that decreased neurotrophin expression in response to stress is most pronounced in the dentate gyrus [49], and that BDNF knock-down in the dentate gyrus, but not in other hippocampal regions, induces depressive-like behavior in rats [16]. The regulation of BDNF and trkB can be activity-dependent [57,58]. STN- but not EPN-DBS reduced zif268 expression in the

Figure 6. Effect of repeated DBS on BDNF gene expression. EPN-DBS did not affect BDNF expression in any hippocampal subdivision. Stressed and non-stressed animals that underwent repeated STN-DBS had reduced BDNF expression in the DGm relative to control animals and animals that had undergone EPN-DBS. Abbreviations: DGm = dentate gyrus, medial blade; DGl = dentate gyrus, lateral blade; SUB = subiculum; *P < 0.05 vs. the corresponding sham control group. **P < 0.05 vs. the corresponding EPN group. N = 8 per group.
DGm, suggesting that the observed STN-DBS-induced decreases in trkB and BDNF expression could reflect changes in neuronal activity. Moreover, STN-DBS is also known to reduce 5-HT release in the hippocampus [59]. Consistent with this finding, our zif268 results suggest that STN- but not EPN-DBS is associated with decreased activity of the median raphe nucleus (MRN), which provides serotonergic innervation to the hippocampus. Although the interaction between serotonin and BDNF is not fully understood, it has been reported that serotonin enhances BDNF gene expression [60]. Future studies may determine if decreased 5-HT release in the hippocampus induced by STN-DBS may contribute to lower BDNF expression in this brain region.

Within the limitations of preclinical work, and with the necessary caution in extrapolating from preclinical to clinical setting, we suggest that our results could have implications for the choice of DBS target in patients with movement disorders. To our knowledge, this is the first study to systematically compare the induction of depressive-like symptoms after repeated DBS in an animal model. Our data suggest that in the absence of specific motor pathology, DBS of the STN may be more likely to induce depressive-like effects than EPN-DBS. DBS applied to either target did not appear to affect anxiety behavior. While this study did not define a mechanism whereby DBS may induce psychiatrically relevant effects, we found that depressive-like symptoms were also associated with changes in hippocampal BDNF expression. Our mapping results also suggest that it is unlikely that STN-DBS has more pronounced effects than EPN-DBS on neuronal activity in limbic areas. We also suggest that decreased trkB and BDNF expression in the dentate gyrus after STN-DBS may reflect decreased activity in this area. More studies are needed to further elucidate a mechanism for DBS-induced psychiatric effects.

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References


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