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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].

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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, *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 *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 μA, 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 μA 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 μA 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

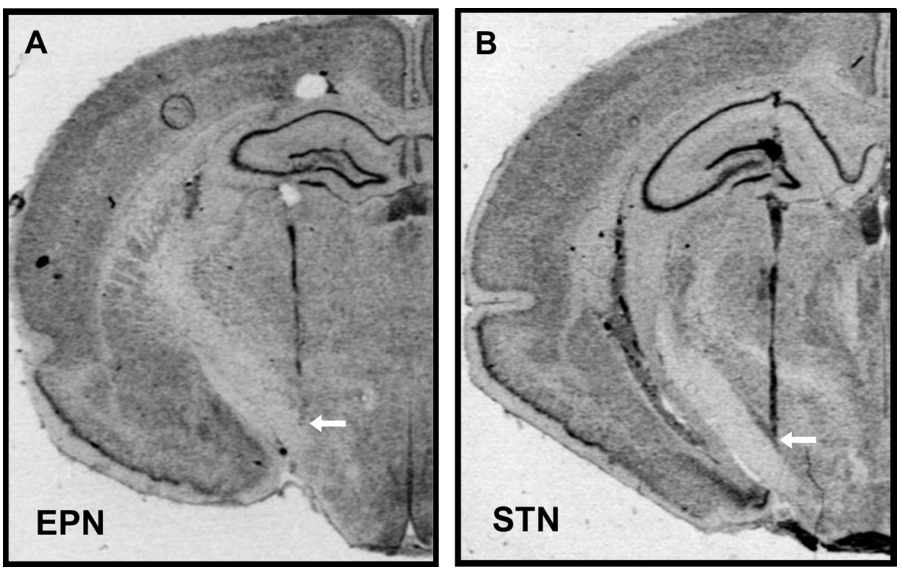

Figure 1. Representative pictomicrographs showing localization of electrode tips (arrows). Cresyl violet staining was used to localize electrodes in the EPN (panel A) or STN (panel B).

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·Na_2·$2H_2O$, 200 M PMSF frozen in isopropanol, 10 M leupeptin frozen separately in deionized water, 0.3 maprotinin frozen separately in 0.01 M HEPES pH 8 and 1 M pepstatin frozen separately in DMSO) [26]. Samples were centrifuged for 30 min at 16,000 × *g* at 4 °C. Supernatants were then removed and frozen at −70 °C until analysis according to kit instructions. Plates were read with an automated plate reader using SPF software.

trkB *and* BDNF in situ *hybridization*

Frozen unperfused brains from rats in the learned helplessness experiment were used. Twenty-micron coronal sections were then prepared on a cryostat. Hybridization was performed using ^{35}S-UTP labeled riboprobes complementary to *trkB* (according to Genbank # NM_012731, (bases 2213-2232) 5′- ggtatcaccaacag ccagct-3′ and (bases 2600–2583) 5′-ggcggtgggcgggttaccct-3′ and *BDNF* according to Genbank # NM_012513.2 (bases 240–259, 5′-gcccaacgaagaaaaccata-3′ and (bases 594–575, 5′-gcagccttccttcgtg taac-3′) and promoter sequences for the T7 RNA polymerase. Using the NCBI BLAST Tool, the sequences were checked for homology with the rat genome and found to be specific for their respective transcripts. Probes were diluted to a concentration of 200,000 cpm/μl in hybridization solution containing: 50% formamide, 35% Denhardt's solution, 10% dextran sulfate, 0.1X SSC, salmon sperm DNA (300 μg/ml), yeast tRNA (100 μg/ml), and DTT (40 μM). Slides were incubated in plastic mailers overnight at 60 °C. After hybridization, sections were rinsed in 4X SSC at 60 °C, treated in RNase A (20 μg/ml) solution at 45 °C for 40 min, washed with agitation in decreasing concentrations of SSC containing 5 g/l 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].

zif268 *mapping*

To obtain comparative information on anatomical targets affected by STN- vs. EPN-DBS, a separate group of stimulation-naive 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 hybridization performed as described above, using [^{35}S]UTP labeled riboprobes complementary to *zif268* (according to Genbank # NM_012551, (bases 660-679), 5′-tcacctatactggccgcttc-3′ and (bases 1062–1043) 5′-aggtctccctgttg ttgtgg-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. *zif268* 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 ($F_{1,42} = 20.63$, $P < 0.001$) and brain manipulation (sham surgery, STN DBS or EPN DBS; $F_{2,42} = 123.04$,

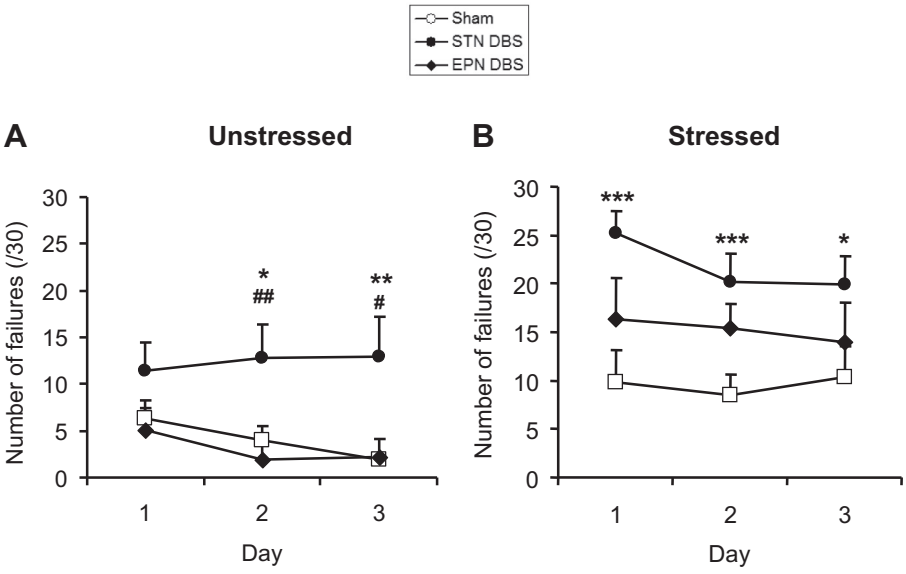

Figure 2. Effect of repeated DBS on learned helplessness performance. DBS affected escape failures in previously unstressed (A) and stressed rats (B). Both stressed and non-stressed animals who underwent repeated STN-DBS (filled circles) exhibited higher failure rates than their respective controls. Repeated EPN-DBS had no effect in non-stressed rats and induced a higher but non significant rates of failure in stressed. $N = 8$ per group. $^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.01$, Bonferroni-adjusted comparisons vs. the respective Sham control mean. $^{\#}P < 0.05$, $^{\#\#}P < 0.02$ Bonferroni-adjusted comparisons vs. the respective EPN-DBS mean.

$P < 0.001$), and no significant interaction among these factors ($P > 0.05$) or between these factors and the Test Days repeated-measures factor ($P > 0.05$). The overall significant effect of stimulation target persisted when the Sham control group was omitted from the analyses ($F_{1,28} = 15.40$, $P = 0.001$), suggesting a reliable difference between STN- and EPN-DBS failure rates in the LH task. Further inspection suggested that STN-DBS groups had higher failure rates than other groups. Therefore, despite the absence of a significant interaction term, we decided to examine these effects further using Bonferroni-adjusted comparisons. As illustrated in Fig. 2 (A and B), STN-DBS resulted in higher failure rates than sham controls ($P < 0.05$). Moreover, this effect was of comparable magnitude in both stressed and non-stressed animals (Fig. 2A and B) ($P < 0.05$). In contrast, performance of animals that underwent repeated EPN-DBS did not differ significantly from that of un-stimulated sham control rats (Fig. 2A and B).

Elevated plus maze

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 ($F_{2,21} = 1.13$, $P > 0.338$) 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 ($F_{2,25} = 1.85$, $P > 0.177$).

zif268 *mRNA expression*

Results are shown in Fig. 5. Repeated measures ANOVA indicated significant main effects of stimulation target ($F_{2,15} = 12.69$, $P < 0.001$), brain region ($F_{15,255} = 241.09$, $P < 0.001$) and their interaction ($F_{30,255} = 8.21$, $P < 0.001$). Relative to sham controls, DBS applied to either the STN or the EPN increased *zif268* expression in CA1 (EPN = 59%, STN = 66%, $P = 0.001$) (Fig. 5A) and perientorhinal cortex (EPN = 97%, STN = 96%, $P = 0.001$) (Fig. 5A), the piriform cortex (EPN = 61%, STN = 51% $P < 0.001$) (Fig. 5B); claustrum (EPN = 66%, STN = 56%, $P = 0.001$) (Fig. 5B), and n. accumbens shell (EPN = 68%, STN = 73%, $P = 0.016$) (Fig. 5B). In addition, STN-DBS, but not EPN-DBS, decreased *zif268* expression in the medial blade of the dentate gyrus (DGm; −17%, $P < 0.04$) (Fig. 5A), median raphe (−35%, $P = 0.035$) (Fig. 5C) and dorsal raphe (−78%, $P < 0.001$) (Fig. 4C). The only significant difference in *zif268* expression between STN-DBS and EPN-DBS animals was observed in the dorsal raphe nucleus ($P < 0.001$) (Fig. 5C).

Hippocampal BDNF levels

There was no main effect of stress ($F_{1,40} = 0.004$, $P > 0.98$) or target ($F_{2,40} = 0.97$, $P > 0.387$) on BDNF protein levels in the dorsal hippocampus, as revealed by two-way ANOVA. Neither was the stress × target interaction significant ($F_{2,40} = 0.219$, $P > 0.80$). While BDNF levels in STN-DBS groups (Stressed: 5259 ± 146 pg/g w/w; Non-Stressed: 5202 ± 107 pg/g w/w) were slightly lower than those in EPN-DBS (Stressed: 5422 ± 74 pg/g w/w; Non-Stressed: 5443 ± 102 pg/g w/w) or non-stimulated groups (Stressed: 5348 ± 106 pg/g w/w; Non-Stressed: 5412 ± 108 pg/g w/w), this difference did not approach statistical significance. Mean BDNF levels for all treatment groups ranged from 4136 to 6234 pg/g w/w hippocampal tissue.

BDNF *and* trkB *gene expression*

BDNF and *trkB* gene expression were measured in hippocampal subdivisions (CA1, CA3, dentate gyrus and subiculum), amygdala

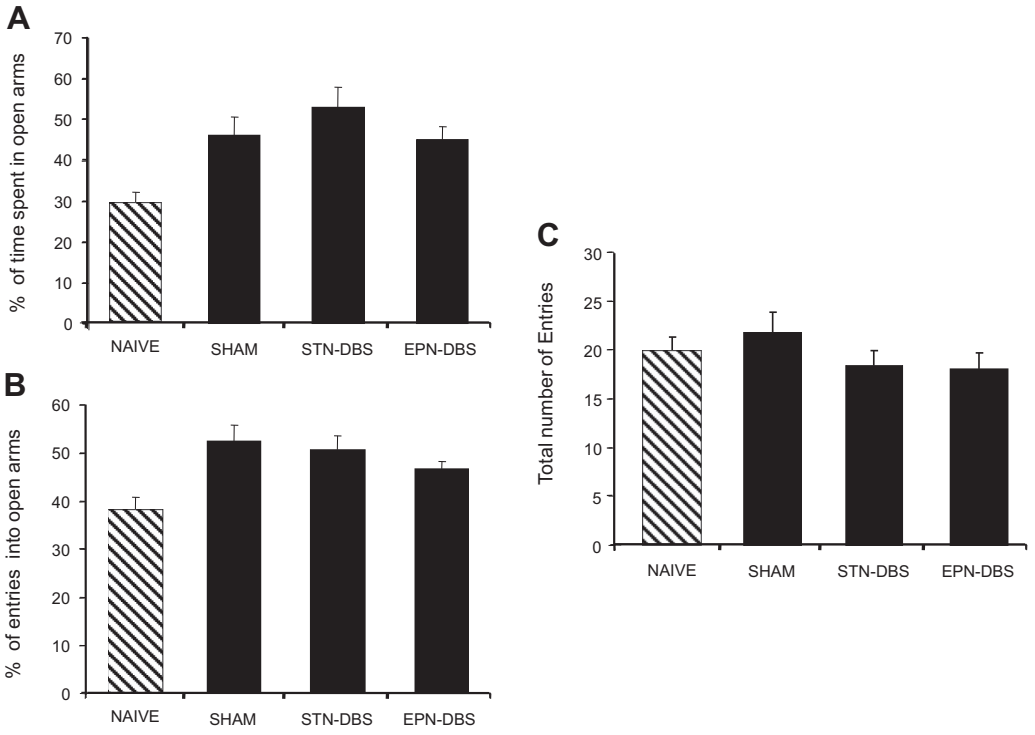

Figure 3. Effect of repeated DBS on elevated plus maze performance. DBS of either the STN or the EPN did not affect percentage of time spent in open arms (A) or percentage of entries made into open arms (B). Total number of entries also did not differ between treatment groups (C). ($N = 10$ sham controls, 11 STN-DBS, 9 EPN-DBS). A separate group of 8 naive rats handled for only one week was subsequently added to verify that test conditions could indeed produce the expected differences between open and closed arm values.

(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).

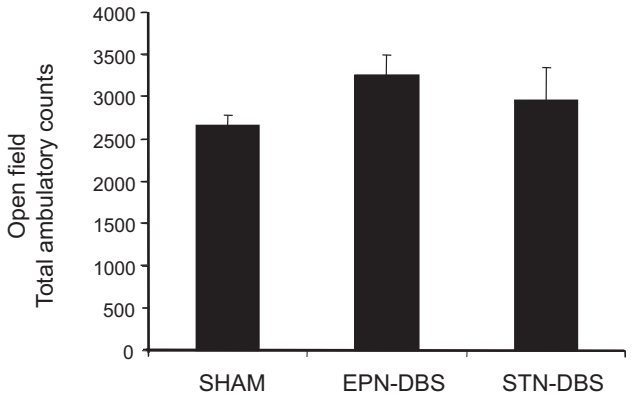

Figure 4. Effect of repeated DBS on open field ambulation. Animals were run in an activity chamber once, following DBS on the 20th day of stimulation. There was no significant effect of DBS on locomotor activity. ($N = 10$ sham controls, 10 STN-DBS, 10 EPN-DBS).

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.

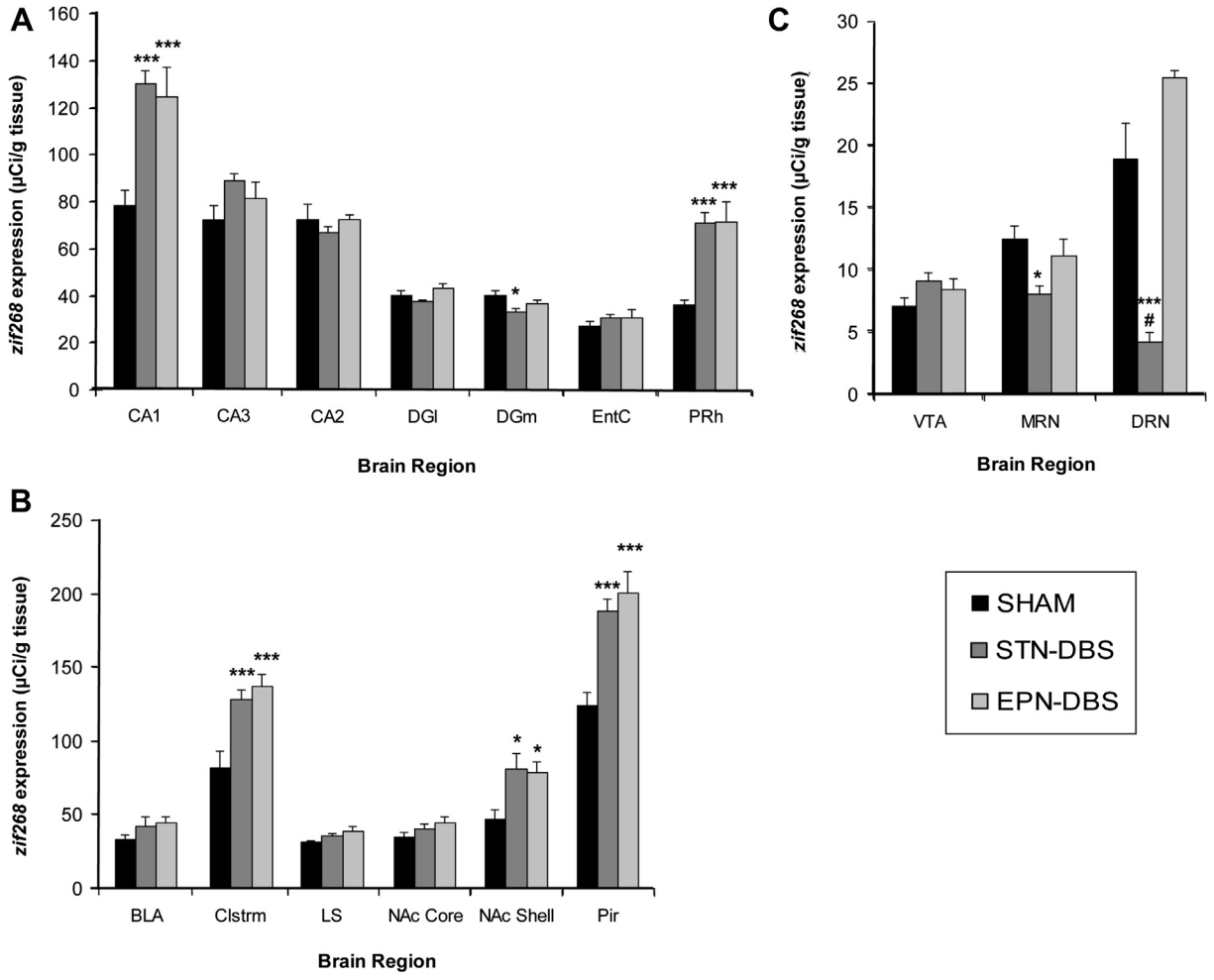

Figure 5. *zif268* expression following acute DBS. Immediate early gene mapping was performed using *zif268* expression as an index of neuronal activation in brain areas implicated in emotional reactivity including hippocampus (A), other limbic areas (B) and brain stem regions (C). Abbreviations: MRN = median raphe nucleus; Pir = piriform cortex; Clstrm = claustrum; NAc = nucleus accumbens; LS = lateral septum; VP = ventral pallidum; DG = dentate gyrus; BLA = basolateral amygdala; EntC = entorhinal cortex; PRh = perientorhinal cortex; VTA = ventral tegmental area. $N = 6$ per group. $^{*}P < 0.05$, $^{***}P < 0.001$, Tukey's post-hoc comparisons against the corresponding sham control group; $^{\#}P < 0.05$ in comparison to the corresponding EPN group.

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 [30,31]. As DBS becomes increasingly used in other movement disorders (e.g. tardive dyskinesias and dystonia) which are not necessarily associated with dopaminergic neuron degeneration, it becomes important to disentangle the contribution of DBS itself from the effects of the underlying neuropathology. The current findings confirm that STN stimulation in the absence of other neuropathology is able to induce or accentuate depressive-type behavior in rodents.

One leading hypothesis for the increased incidence of psychiatric effects with STN as compared to the GPi DBS refers to activation 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]. 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, given the larger total size. 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

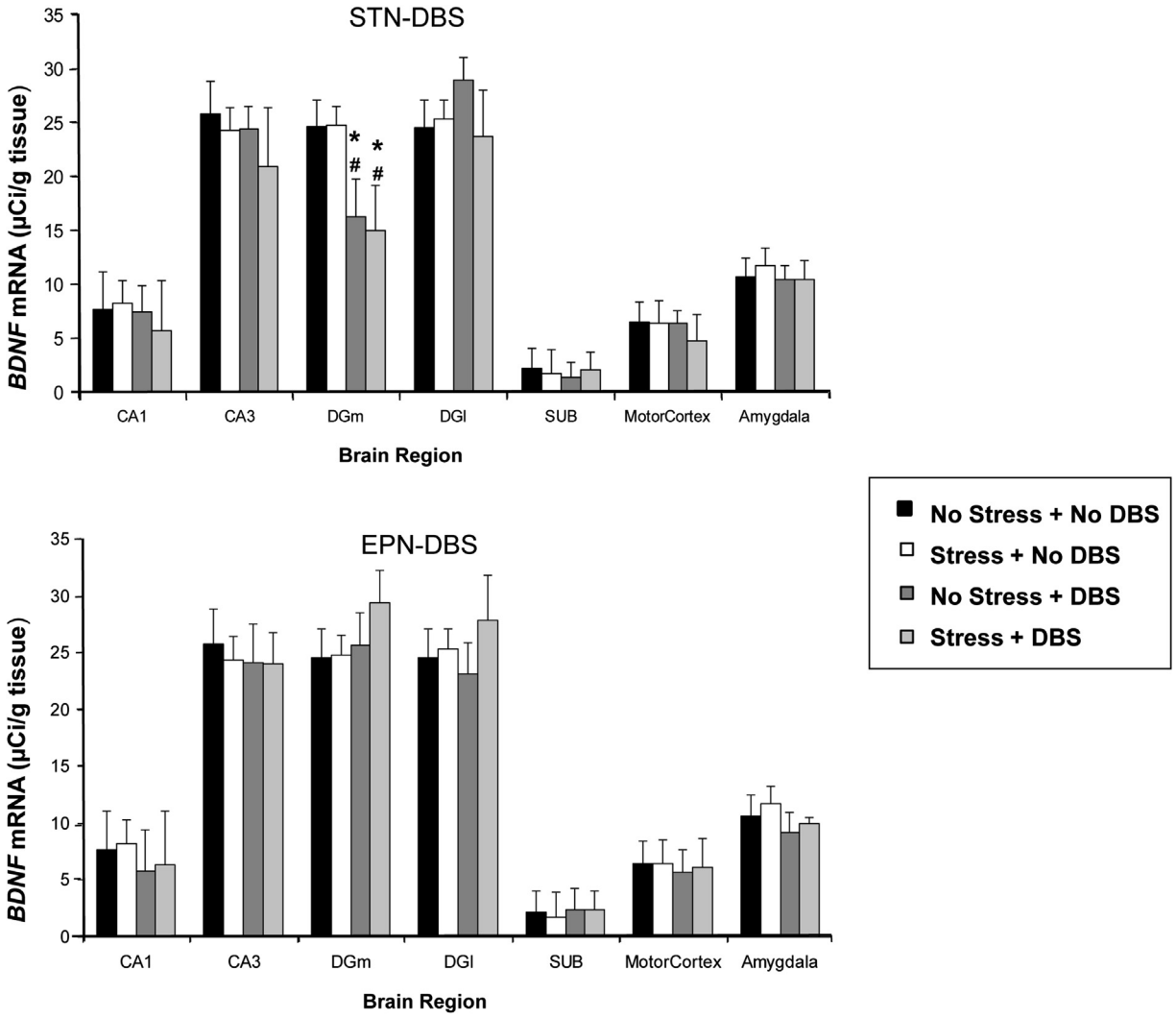

Figure 6. Effect of repeated DBS on *BDNF* gene expression. EPN-DBS did not affect *BDNF* expression in any hippocampal subdivision. Stressed and non-stressed animals that underwent repeated STN-DBS had reduced *BDNF* expression in the DGm relative to control animals and animals that had undergone EPN-DBS. 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.

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 has 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

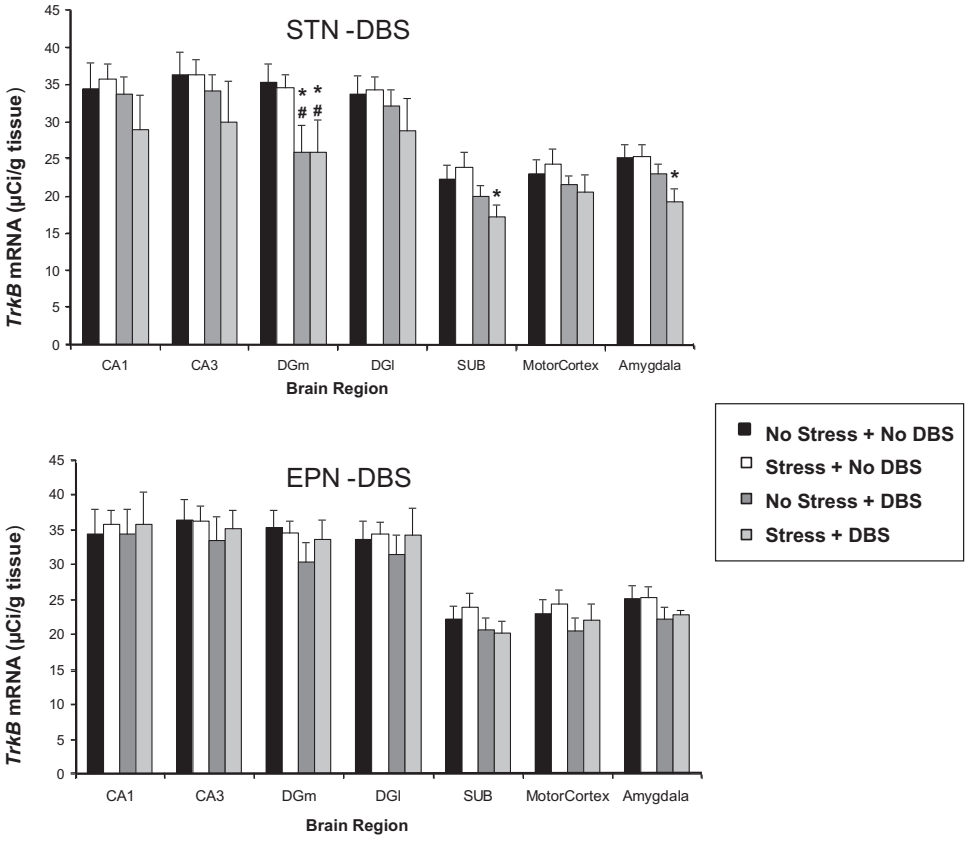

Figure 7. Effect of repeated DBS on *trkB* gene expression. EPN-DBS did not affect *trkB* expression in any hippocampal subdivision. Stressed and non-stressed animals that underwent repeated STN-DBS had reduced levels of *trkB* expression in the DGm, SUB and Amygdala. Abbreviations: DGl = dentate gyrus, lateral blade; DGm = dentate gyrus, medial 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 *BNDF* 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

[1] Blahak C, Bazner H, Capelle HH, Wohrle JC, Weigel R, Hennerici MG, et al. Rapid response of parkinsonian tremor to stn-dbs changes: direct modulation of oscillatory basal ganglia activity? Mov Disord 2009;24:1221–5.
[2] Capelle HH, Blahak C, Schrader C, Baezner H, Kinfe TM, Herzog J, et al. Chronic deep brain stimulation in patients with tardive dystonia without a history of major psychosis. Mov Disord 2010;25:1477–81.
[3] Welter ML, Grabli D, Vidailhet M. Deep brain stimulation for hyperkinetics disorders: dystonia, tardive dyskinesia, and tics. Curr Opin Neurol 2010;23:420–5.
[4] Benabid AL, Benazzouz A, Hoffmann D, Limousin P, Krack P, Pollak P. Long-term electrical inhibition of deep brain targets in movement disorders. Mov Disord 1998;13(Suppl. 3):119–25.
[5] Albanese A, Piacentini S, Romito LM, Leone M, Franzini A, Broggi G, et al. Suicide after successful deep brain stimulation for movement disorders. Neurology 2005;65:499–500 [author reply 499–500].
[6] Castelli L, Zibetti M, Rizzi L, Caglio M, Lanotte M, Lopiano L. Neuropsychiatric symptoms three years after subthalamic dbs in pd patients: a case-control study. J Neurol 2008;255:1515–20.
[7] Okun MS, Fernandez HH, Wu SS, Kirsch-Darrow L, Bowers D, Bova F, et al. Cognition and mood in parkinson's disease in subthalamic nucleus versus globus pallidus interna deep brain stimulation: the compare trial. Ann Neurol 2009;65:586–95.
[8] Temel Y, Kessels A, Tan S, Topdag A, Boon P, Visser-Vandewalle V. Behavioural changes after bilateral subthalamic stimulation in advanced

parkinson disease: a systematic review. Parkinsonism Relat Disord 2006;12:265–72.
[9] Witt K, Daniels C, Reiff J, Krack P, Volkmann J, Pinsker MO, et al. Neuropsychological and psychiatric changes after deep brain stimulation for parkinson's disease: a randomised, multicentre study. Lancet Neurol 2008;7:605–14.
[10] Okun MS, Wu SS, Foote KD, Bowers D, Gogna S, Price C, et al. Do stable patients with a premorbid depression history have a worse outcome after deep brain stimulation for parkinson disease? Neurosurgery 2011;69:357–60 [discussion 360–1].
[11] Rodriguez RL, Miller K, Bowers D, Crucian G, Wint D, Fernandez H, et al. Mood and cognitive changes with deep brain stimulation. What we know and where we should go. Minerva Med 2005;96:125–44.
[12] Vitek JL. Deep brain stimulation for parkinson's disease. A critical re-evaluation of stn versus gpi dbs. Stereotact Funct Neurosurg 2002;78:119–31.
[13] Bronstein JM, Tagliati M, Alterman RL, Lozano AM, Volkmann J, Stefani A, et al. Deep brain stimulation for parkinson disease: an expert consensus and review of key issues. Arch Neurol 2011;68:165.
[14] Kirsch-Darrow L, Zahodne LB, Marsiske M, Okun MS, Foote KD, Bowers D. The trajectory of apathy after deep brain stimulation: from pre-surgery to 6 months post-surgery in parkinson's disease. Parkinsonism Relat Disord 2011;17:182–8.
[15] Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A. Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. J Neurosci 2011;31:4475–83.
[16] Taliaz D, Stall N, Dar DE, Zangen A. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. Mol Psychiatry 2010;15:80–92.
[17] Thompson Ray M, Weickert CS, Wyatt E, Webster MJ. Decreased bdnf, trkb-tk+ and gad67 mrna expression in the hippocampus of individuals with schizophrenia and mood disorders. J Psychiatry Neurosci 2011;36:195–203.
[18] Okun MS, Green J, Saben R, Gross R, Foote KD, Vitek JL. Mood changes with deep brain stimulation of stn and gpi: results of a pilot study. J Neurol Neurosurg Psychiatry 2003;74:1584–6.
[19] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. Acadmic Press; 1986.
[20] Creed M, Hamani C, Nobrega JN. Deep brain stimulation of the subthalamic or entopeduncular nucleus attenuates vacuous chewing movements in a rodent model of tardive dyskinesia. Eur Neuropsychopharmacol 2011;21:393–400.
[21] Hamani C, Diwan M, Macedo CE, Brandao ML, Shumake J, Gonzalez-Lima F, et al. Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. Biol Psychiatry 2009;67:117–24.
[22] Hamani C, Machado DC, Hipolide DC, Dubiela FP, Suchecki D, Macedo CE, et al. Deep brain stimulation reverses anhedonic-like behavior in a chronic model of depression: role of serotonin and brain derived neurotrophic factor. Biol Psychiatry 2012;71:30–5.
[23] Willner P. Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. Prog Neuropsychopharmacol Biol Psychiatry 1986;10:677–90.
[24] Martin P, Soubrie P, Puech AJ. Reversal of helpless behavior by serotonin uptake blockers in rats. Psychopharmacology (Berl) 1990;101:403–7.
[25] Martin P, Soubrie P, Simon P. Shuttle-box deficits induced by inescapable shocks in rats: reversal by the beta-adrenoreceptor stimulants clenbuterol and salbutamol. Pharmacol Biochem Behav 1986;24:177–81.
[26] Szapacs ME, Mathews TA, Tessarollo L, Ernest Lyon W, Mamounas LA, Andrews AM. Exploring the relationship between serotonin and brain-derived neurotrophic factor: analysis of bdnf protein and extraneuronal 5-ht in mice with reduced serotonin transporter or bdnf expression. J Neurosci Methods 2004;140:81–92.
[27] Ceravolo R, Frosini D, Rossi C, Bonuccelli U. Spectrum of addictions in parkinson's disease: from dopamine dysregulation syndrome to impulse control disorders. J Neurol 2010;257:S276–83.
[28] Fenu S, Wardas J, Morelli M. Impulse control disorders and dopamine dysregulation syndrome associated with dopamine agonist therapy in parkinson's disease. Behav Pharmacol 2009;20:363–79.
[29] Evidente VG, Premkumar AP, Adler CH, Caviness JN, Driver-Dunckley E, Lyons MK. Medication dose reductions after pallidal versus subthalamic stimulation in patients with parkinson's disease. Acta Neurol Scand 2010;124:211–4.
[30] Funkiewiez A, Ardouin C, Krack P, Fraix V, Van Blercom N, Xie J, et al. Acute psychotropic effects of bilateral subthalamic nucleus stimulation and levodopa in parkinson's disease. Mov Disord 2003;18:524–30.
[31] Witt K, Daniels C, Herzog J, Lorenz D, Volkmann J, Reiff J, et al. Differential effects of l-dopa and subthalamic stimulation on depressive symptoms and hedonic tone in parkinson's disease. J Neuropsychiatry Clin Neurosci 2006;18:397–401.
[32] Butson CR, Cooper SE, Henderson JM, McIntyre CC. Patient-specific analysis of the volume of tissue activated during deep brain stimulation. Neuroimage 2007;34:661–70.
[33] Le Jeune F, Drapier D, Bourguignon A, Peron J, Mesbah H, Drapier S, et al. Subthalamic nucleus stimulation in parkinson disease induces apathy: a pet study. Neurology 2009;73:1746–51.
[34] Hong S, Hikosaka O. The globus pallidus sends reward-related signals to the lateral habenula. Neuron 2008;60:720–9.
[35] Mallet L, Schupbach M, N'Diaye K, Remy P, Bardinet E, Czernecki V, et al. Stimulation of subterritories of the subthalamic nucleus reveals its role in the integration of the emotional and motor aspects of behavior. Proc Natl Acad Sci U S A 2007;104:10661–6.
[36] Pralong E, Pollo C, Coubes P, Bloch J, Roulet E, Tetreault MH, et al. Electrophysiological characteristics of limbic and motor globus pallidus internus (gpi) neurons in two cases of lesch-nyhan syndrome. Neurophysiol Clin 2005;35:168–73.
[37] Temel Y, Blokland A, Steinbusch HW, Visser-Vandewalle V. The functional role of the subthalamic nucleus in cognitive and limbic circuits. Prog Neurobiol 2005;76:393–413.
[38] Hamani C, Saint-Cyr JA, Fraser J, Kaplitt M, Lozano AM. The subthalamic nucleus in the context of movement disorders. Brain 2004;127:4–20.
[39] Hartung H, Tan SK, Steinbusch HM, Temel Y, Sharp T. High-frequency stimulation of the subthalamic nucleus inhibits the firing of juxtacellular labelled 5-ht-containing neurones. Neuroscience 2011;186:135–45.
[40] Tan SK, Hartung H, Sharp T, Temel Y. Serotonin-dependent depression in parkinson's disease: a role for the subthalamic nucleus? Neuropharmacology 2011;61:387–99.
[41] Temel Y, Boothman LJ, Blokland A, Magill PJ, Steinbusch HW, Visser-Vandewalle V, et al. Inhibition of 5-ht neuron activity and induction of depressive-like behavior by high-frequency stimulation of the subthalamic nucleus. Proc Natl Acad Sci U S A 2007;104:17087–92.
[42] Parent A. Comparative anatomy of the serotoninergic systems. J Physiol (Paris) 1981;77:147–56.
[43] Parent M, Wallman MJ, Gagnon D, Parent A. Serotonin innervation of basal ganglia in monkeys and humans. J Chem Neuroanat 2011;41:256–65.
[44] Klein J, Winter C, Coquery N, Heinz A, Morgenstern R, Kupsch A, et al. Lesion of the medial prefrontal cortex and the subthalamic nucleus selectively affect depression-like behavior in rats. Behav Brain Res 2010;213:73–81.
[45] Schmidt HD, Duman RS. The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behav Pharmacol 2007;18:391–418.
[46] Schmidt HD, Duman RS. Peripheral bdnf produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 2010;35:2378–91.
[47] Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 2008;64:527–32.
[48] Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal bdnf immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001;50:260–5.
[49] Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006;59:1116–27.
[50] Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 2005;136:29–37.
[51] Fumagalli F, Bedogni F, Perez J, Racagni G, Riva MA. Corticostriatal brain-derived neurotrophic factor dysregulation in adult rats following prenatal stress. Eur J Neurosci 2004;20:1348–54.
[52] Koo JW, Park CH, Choi SH, Kim NJ, Kim HS, Choe JC, et al. The postnatal environment can counteract prenatal effects on cognitive ability, cell proliferation, and synaptic protein expression. FASEB J 2003;17:1556–8.
[53] Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA. Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol Psychiatry 2004;55:708–14.
[54] Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA. Early maternal deprivation reduces the expression of bdnf and nmda receptor subunits in rat hippocampus. Mol Psychiatry 2002;7:609–16.
[55] Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, et al. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc Natl Acad Sci U S A 2004;101:10827–32.
[56] Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, et al. Activation of the trkb neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J Neurosci 2003;23:349–57.
[57] Lu B. Bdnf and activity-dependent synaptic modulation. Learn Mem 2003;10:86–98.
[58] Nagappan G, Lu B. Activity-dependent modulation of the bdnf receptor trkb: mechanisms and implications. Trends Neurosci 2005;28:464–71.
[59] Navailles S, Benazzouz A, Bioulac B, Gross C, De Deurwaerdere P. High-frequency stimulation of the subthalamic nucleus and l-3,4-dihydroxyphenylalanine inhibit in vivo serotonin release in the prefrontal cortex and hippocampus in a rat model of parkinson's disease. J Neurosci 2010;30:2356–64.
[60] Martinowich K, Lu B. Interaction between bdnf and serotonin: role in mood disorders. Neuropsychopharmacology 2008;33:73–83.