

Deep Brain Stimulation Reverses Anhedonic-Like Behavior in a Chronic Model of Depression: Role of Serotonin and Brain Derived Neurotrophic Factor

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Background: Deep brain stimulation (DBS) is being investigated as a treatment for major depression, but its mechanisms of action are still unknown. We have studied the effects of ventromedial prefrontal cortex (vmPFC) stimulation in a chronic model of depression and assessed the involvement of the serotonergic system and brain derived neurotrophic factor (BDNF) in a DBS response.

Methods: Rats were subjected to chronic unpredictable mild stress during 4 weeks. Decline in preference for sucrose solutions over water, an index suggested to reflect anhedonic-like behavior, was monitored on a weekly basis. The outcome of chronic vmPFC stimulation alone (8 hours/day for 2 weeks) or combined with serotonin-depleting lesions was characterized. BDNF levels were measured in the hippocampus.

Results: Stress induced a significant decrease in sucrose preference as well as hippocampal BDNF levels as compared with those recorded in control subjects. vmPFC stimulation completely reversed this behavioral deficit and partially increased BDNF levels. In contrast, DBS did not improve stress-induced anhedonic-like behavior in animals bearing serotonin-depleting raphe lesions with associated normal hippocampal BDNF levels.

Conclusions: vmPFC stimulation was effective in a chronic model of depression. Our results suggest that the integrity of the serotonergic system is important for the anti-anhedonic-like effects of DBS but question a direct role of hippocampal BDNF.

Key Words: BDNF, cingulate gyrus, deep brain stimulation, depression, prefrontal cortex, psychiatry, serotonin

Subcallosal cingulate gyrus (SCG) deep brain stimulation (DBS) is currently being investigated as a potential therapy for treatment-resistant depression. Despite the promising results of initial trials (1–4), mechanisms underlying an antidepressant DBS response remain largely unknown (4). We have recently shown that DBS applied to the ventromedial prefrontal cortex (vmPFC), the rodent homologue of the human SCG, induces an antidepressant-like effect in rats undergoing the forced swim test (FST) (5,6). Although the FST is a well-established screener to assess the antidepressant activity of clinical therapies, a common criticism to its face validity is the short timeframe for an antidepressant-like response (e.g., 1–2 days). In this context, models such as chronic unpredictable mild stress (CUS) would be more suitable for the investigation of chronic effects of DBS. As commonly applied, CUS involves subjecting rodents to a series of unpredictable stressors over a period of weeks. A decline in the preference for sucrose solutions over water has been suggested to reflect an anhedonic-like behavior (7–12).

In the present study, we report on a significant anti-anhedonic-like effect of vmPFC DBS in rodents undergoing CUS. The kinetics of a DBS response and mechanisms for the effects of vmPFC stimula-

tion are investigated and discussed from a translational perspective.

Methods and Materials

Protocols were approved by the Animal Care Committees of the Centre for Addiction and Mental Health and the Universidade Federal de São Paulo (UNIFESP), Brazil. Forty male 250–275-g Wistar rats (UNIFESP, Sao Paulo, Brazil) were housed in individual cages and initially trained to drink a 1% sucrose solution with a two-bottle choice procedure. Animals were paired according to a sucrose preference index (SPI), to balance the groups to undergo stress or no-stress. This is defined as (sucrose)/(sucrose + water) consumed during 1 hour. In a second set of experiments to study mechanisms of DBS, 45 animals were used. Of every three animals, two were randomly assigned to receive stress and one was randomly assigned to become a control.

CUS

Rats were subjected to 12 different stressors on a weekly schedule. These included four periods of water deprivation (16 hours, 19 hours, 20 hours, and 30 hours), two periods of food deprivation (16 hours and 30 hours), two periods in a soiled cage (wet bedding, 17 hours and 18 hours), two periods of cage tilt (45°–60°, 21 hours and 41 hours), two periods of paired housing (18 hours and 19 hours), two 5-hour and one 4-hour periods of continuous noise (85 dB), three periods of exposure to a cloth with cat smell (1 hour), one period of continuous illumination in the dark phase (12 hours), two periods of darkness in the light phase (12 hours), two 16-hour periods of partial food deprivation (30 g of food), two 1-hour periods with an empty water bottle, and two 3-hour periods of stroboscopic illumination. The SPI measurements were obtained once/week.

Four weeks after the beginning of stress, all rats (including non-stressed control subjects) were once again matched for SPI scores. Members of each pair were randomly assigned to receive DBS or sham surgery.

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Surgical Procedures

Rats were anesthetized with ketamine/xylazine (100/7.5 mg/kg IP) and had monopolar vmPFC stainless steel electrode implanted (anteroposterior + 3.0; medial-lateral \pm .4; dorsal-ventral 5.2 mm) (13). These had 250 μ m in diameter and .75 mm of exposed surface and were used as cathodes. Electrodes connected to an epidural screw were used as anodes. Control subjects (sham surgery) were anesthetized and had holes drilled into the skull but were not implanted with electrodes.

Serotonin (5-HT) depletion was achieved by injections of 5,7-dihydroxytryptamine (5,7-DHT) into the dorsal and median raphe nuclei (5). A 30-gauge needle was first lowered into the median raphe (AP -7.8 , L \pm 0, D8.8 mm) and .2 μ L of a solution containing 4 μ g of 5,7-DHT creatinine sulphate (Sigma-Aldrich, Oakville, ON, Canada) in .1% ascorbic acid was injected over 4 min. After an additional 4 min, the needle was raised by 2.2 mm, and the process was repeated in the dorsal raphe. Control subjects received .1% ascorbic acid (5). During the same surgical procedure, animals were either implanted with vmPFC electrodes or had holes drilled into their skull, as described in the preceding text.

DBS and Postoperative Assessment

Animals were allowed 1 week to recover from surgery before stress was restarted. The DBS was only commenced 1 week later (on the third postoperative week), when SPI measurements were similar to preoperative levels. Stimulation was conducted with a handheld device (St. Jude Medical 3510, St. Jude Medical, St. Paul, Minnesota) at 200 μ A, 130 Hz, and 90 μ sec of pulse width. These settings were chosen because they were effective in our previous FST studies (5,6) and have been suggested to approximate those used in clinical practice (5,6,14). Stimulation was applied daily from 9:00 AM to 5:00 PM for 2 weeks. Rats were not stimulated during SPI measurements or when they were paired-housed. Animals continued to receive stress for an additional 3 weeks, to study the recurrence of anhedonic-like behavior after DBS offset.

Histology

In the first set of experiments, rats were sacrificed 3 weeks after stimulation was discontinued and had their brains stained with cresyl violet (5,6). One animal lost the stimulation cap, and one died during CUS. Both were excluded from the analysis. The location of the electrodes in these animals is presented in Figure 1. Because the exposed tip of our electrodes covered .75 mm, most of the current was delivered to the ventral prelimbic cortex (vPL). This region was chosen as a target on the basis of our previous studies showing that stimulation of the vPL induces significant antidepressant-like effects in the FST (6,15,16).

In experiments designed to investigate mechanisms of DBS, animals were decapitated immediately after stimulation was discontinued (2 weeks after DBS onset). Two animals lost the stimulation cap and was excluded from the study. Two animals died during CUS. After removal from the skull, brains were quickly frozen in dry ice. Hippocampi were bilaterally dissected for the analysis of brain derived neurotrophic factor (BDNF) and 5-HT levels (one hemisphere each). For BDNF, tissue was homogenized in lysis buffer and centrifuged. Protein levels in the supernatant were detected with enzyme-linked immunosorbent assay (Promega Corporation, Madison, Wisconsin) (17). The extent of 5-HT depletion in animals undergoing 5,7-DHT lesions was assessed by capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) (18,19). The 5-HT levels in these animals may be found in Figure S1 in Supplement 1. Three animals that did not have a significant decrease in 5-HT levels after 5,7-DHT were excluded from behavioral and neurochemical analyses (two in the stress alone group, and one in the DBS stress group).

Statistical Analysis

One-way analysis of variance was used to compare data when three or more independent groups were considered (Fisher post hoc). For experiments with only 2 samples, independent or paired Student *t* tests were used. Statistical significance was

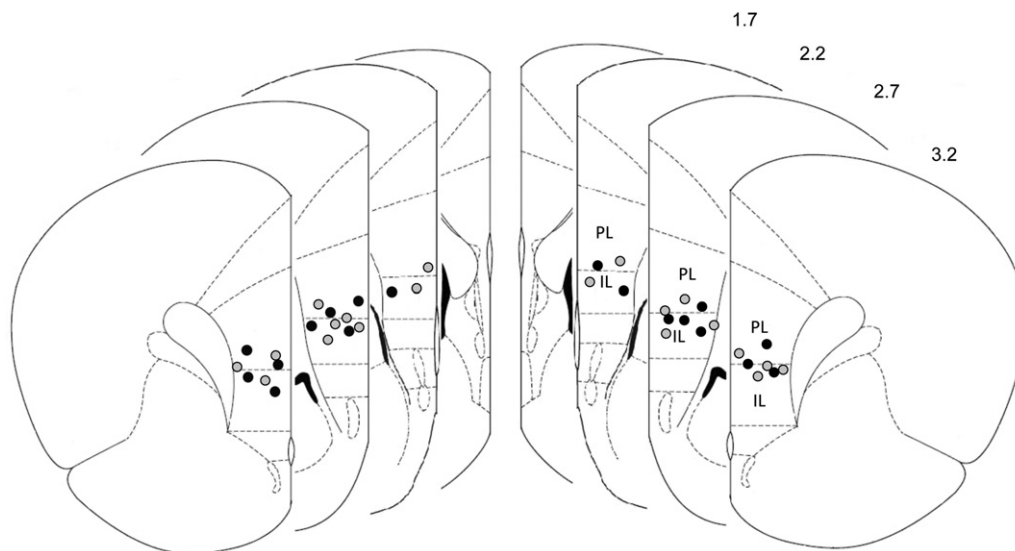


Figure 1. Location of electrodes in the ventromedial prefrontal cortex in animals undergoing deep brain stimulation. Schematic representation of coronal brain sections showing the location of the tip of the electrodes implanted in stressed (black circles) and nonstressed (gray circles) animals. Because the exposed tip of our electrodes covered .75 mm, most of the current was delivered to ventral regions of the prelimbic cortex (PL). Numbers to the right denote distance from bregma. IL, infralimbic cortex. Reprinted from Paxinos and Watson (13), with permission from Elsevier, Copyright 1998.

set at $p = .05$. Results in the text and figures are expressed as mean \pm SEM.

Results

Effects of vmPFC DBS in the CUS Model: Kinetics and Timeframe of Response

As shown in Figure 2, SPI at baseline was approximately 66%. One week after CUS, differences between stressed and nonstressed rats were already noticeable. These became particularly pronounced after 2 weeks of stress. During weeks 3 and 4, SPI stabilized at approximately 30%–40% in the stressed group. Animals were then implanted with electrodes or had sham surgery. One week later, the CUS protocol was restarted.

On the first postoperative assessment, stressed animals with implanted electrodes had a slight increase in SPI scores (54% vs. 40% before surgery; $p > .05$). Return to near baseline levels was only observed on the second postoperative week. A 2-week regimen of DBS (8 hours/day) was then commenced. On the first post-DBS measurement, a significant increase in SPI was observed when stressed animals that had or did not have DBS were compared (60% vs. 23%, respectively, $p < .002$). Results became even more pronounced on the second week of stimulation (64% vs. 15%, $p = .0001$), with SPI in DBS-treated stressed animals reaching levels similar to those in nonstressed control subjects. After discontinuing DBS, the recurrence of an anhedonic-like behavior in stressed animals was gradual. Three weeks after stimulation was ceased, SPI

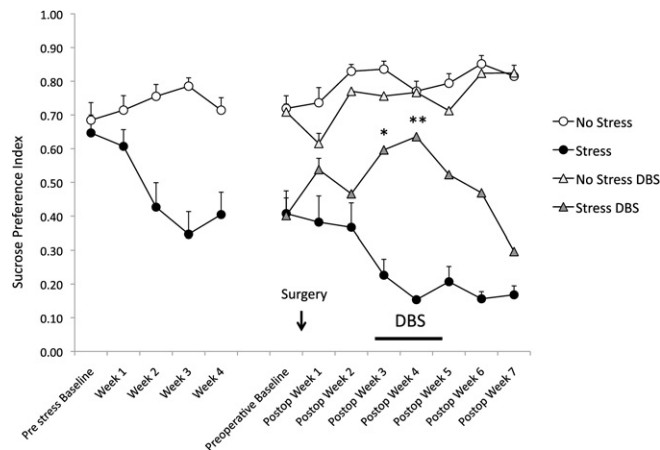


Figure 2. Changes in sucrose preference index (SPI) as a function of stress and deep brain stimulation (DBS). The figure is divided in two parts, before and after surgery (arrow). Before surgery, rats were exposed to chronic unpredictable mild stress or remained in their home cages for 4 weeks. Except for baseline, all points were statistically different between stressed and nonstressed animals ($p < .03$). Indicators of statistical significance were omitted for clarity. In the preoperative baseline assessments immediately before surgery, one may notice that SPI in stressed and nonstressed animals to undergo DBS or sham surgery were similar. Stress was reintiated 1 week after the procedure. DBS (8 hours/day) was commenced on the third postoperative (postop) week and continued for 2 weeks (horizontal bar). On the first week after stimulation onset, SPI in stress DBS animals ($n = 9$) was significantly different from those recorded in the stress alone group ($n = 9$, $p < .002^*$). Differences were even more accentuated on the second week of stimulation ($p < .0001$ vs. stress alone**) with SPI in DBS-treated stressed animals, reaching levels comparable to those of nonstressed control subjects. Thereafter, stimulation was discontinued for the assessment of changes in hedonic-like behavior. Decrease in sucrose preference was gradual, with values approximating preoperative baseline levels only 3 weeks later. No significant differences were observed between nonstressed animals that did ($n = 10$) or did not receive DBS ($n = 10$).

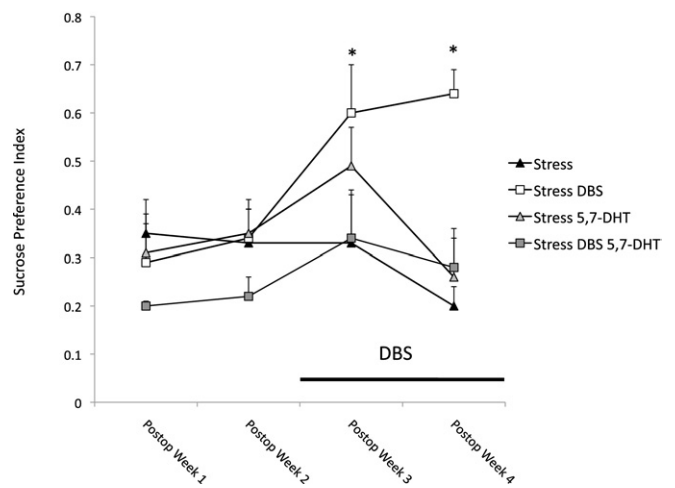


Figure 3. Changes in SPI as a function of stress and DBS in animals undergoing 5,7-dihydroxytryptamine (5,7-DHT) raphe lesions. As in our previous experiment, DBS ($n = 5$) induced a significant increase in SPI as compared with the nonstimulated stressed group ($n = 6$; $p = .05$ and $p < .001$ for the first and second weeks of stimulation, respectively). The effects of stimulation were blocked in stressed animals given raphe microinjections of 5,7-DHT, a toxin for serotonergic neurons ($n = 6$). Differences in SPI were not significant when stressed ($n = 6$) and nonstressed ($n = 5$) serotonin-depleted animals were compared with their respective control subjects (results of nonstressed animals were not depicted in the figure for the sake of clarity). *Statistically significant differences. Abbreviations as in Figure 2.

scores declined by 30% but were still significantly higher than those recorded in sham-treated stressed rats (17%). Of note, DBS had no effect on SPI in nonstressed control subjects.

Changes in sucrose consumption were similar to the ones described in the preceding text for the SPI (Figure S2 in Supplement 1). Stressed rats treated with DBS had a significant increase in sucrose consumption ($p < .001$) and a significant decrease in water intake ($p < .001$) as compared with nonstimulated stressed control subjects. After 4 weeks of CUS, body weight in stressed animals was 12% lower than in nonstressed control subjects ($p < .01$). Two weeks after DBS onset, body weight in stressed animals that did and did not receive DBS were 18% and 13% lower than in nonstressed control subjects, respectively ($p < .01$ for both comparisons). Differences in body weight between groups receiving stress alone or DBS + stress were not significant.

Mechanisms for the Effects of vmPFC DBS: 5-HT Depletion and BDNF Levels

We found that the anti-anhedonic-like effects of vmPFC stimulation were abolished in rats bearing 5-HT depleting raphe lesions (Figure 3). In contrast, 5-HT depletion did not affect sucrose preference in either sham stressed animals or nonstressed control subjects.

As expected, hippocampal BDNF levels in animals undergoing CUS were significantly lower than in nonstressed control subjects ($p = .0007$) (Figure 4A). DBS partially reversed this deficit with a trend toward an increase in BDNF levels ($p = .055$) (Figure 4A). In contrast, stimulation did not exert a significant effect on BDNF levels in nonstressed animals.

Stressed animals injected with 5,7-DHT showed a small but significant increase in BDNF levels relative to 5-HT depleted control subjects ($p = .02$) (Figure 4B). In contrast, BDNF levels in 5,7-DHT stressed animals with or without DBS were similar to those recorded in nonstressed, nonlesioned control subjects. This is of im-

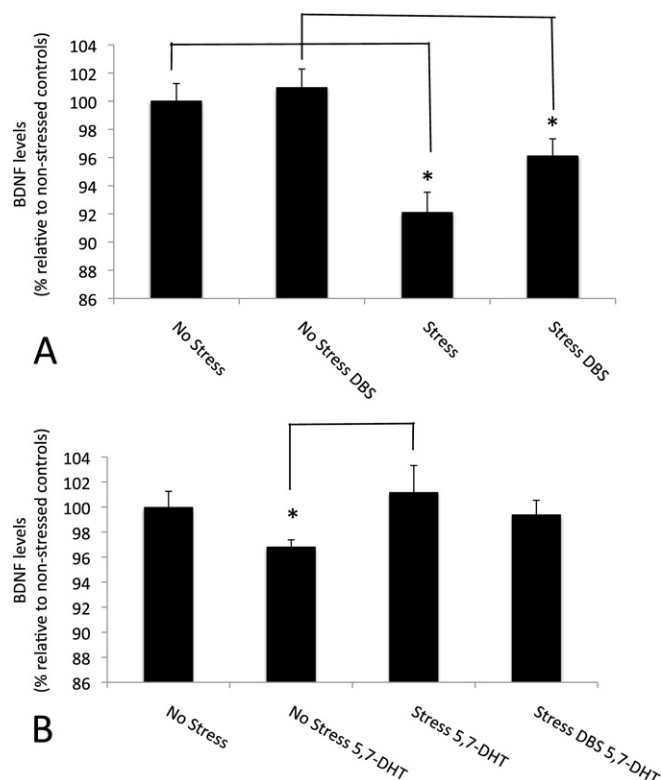


Figure 4. Brain derived neurotrophic factor (BDNF) levels in the hippocampus of animals receiving ventromedial prefrontal cortex stimulation during chronic unpredictable mild stress. **(A)** Hippocampal BDNF levels were significantly lower in stressed animals as compared with nonstressed control subjects ($p = .0007$). DBS partially reversed this deficit with a trend toward an increase in BDNF levels ($p = .055$). **(B)** In stressed animals given 5,7-DHT raphe injections, BDNF levels were higher than in corresponding serotonin-depleted control subjects ($p = .02$). In contrast, BDNF levels in 5,7-DHT stressed animals with or without DBS were similar to those recorded in nonstressed, nonlesioned control subjects. Values in the graph represent percentages relative to animals that did not undergo chronic unpredictable mild stress or 5,7-DHT injections. *Statistically significant. Abbreviations as in Figure 3.

portance, because DBS did not exert an anti-anhedonic-like response in 5-HT-depleted animals, despite normal BDNF levels.

Discussion

In the present study, chronic vmPFC DBS improved anhedonic-like behavior in animals undergoing CUS. This shows that DBS is effective in ameliorating this cardinal symptom of depression in rodents, in addition to resulting in a general antidepressant-like response. As in our previous report (5), rats tolerated DBS very well and behaved normally during stimulation. In addition, chronic DBS was found to have no effect on SPI in nonstressed control subjects, thus ruling out the possibility of a stimulation-induced nonspecific increase in sucrose preference.

Translation of data from animal models to humans must always be considered with caution. That being said, we believe our findings might yield interesting parallels and possibly raise issues to be considered in clinical trials. One observation was that rats implanted with electrodes showed a slight increase in SPI right after the surgery in the absence of stimulation. In a parallel scenario, a so-called “insertional effect” has been characterized as an improvement in clinical symptoms after the implantation of electrodes in the absence of stimulation (20,21). This effect should be taken into

account when data from clinical trials is interpreted and when experiments with DBS in animal models are conducted.

In our study, a significant improvement in sucrose preference after DBS was already apparent after 1 week of treatment. On the second week, anhedonic-like behavior was remarkably improved, with SPI levels approximating those recorded in nonstressed control subjects. This short timeframe for a DBS response suggests that the mechanisms involved in the effects of stimulation might be different from the ones reported for medications, which might require weeks for a positive outcome (8–11).

Animals continued to receive CUS for 3 additional weeks, so that recurrence of anhedonia after termination of DBS could be studied. We found that SPI began to decline a few weeks after stimulation was ceased, suggesting that vmPFC stimulation might be associated with a carry-over effect (i.e., persistence of a response after treatment is discontinued). In the clinical scenario, Holtzheimer *et al.* (22) have recently conducted a study in which a few depression patients receiving DBS had stimulation discontinued. Symptom recurrence in these subjects did not occur immediately but in a few weeks. Crossover studies are commonly used in the DBS literature (23). Trials with such design involve the administration of either active or sham stimulation for a period of time, after which patients receive the inverse treatment. Our findings suggest that a washout period without stimulation in between arms might be of importance and crossover only be carried out when symptom recurrence is recorded.

Neural substrates commonly thought to be involved in mechanisms of antidepressant therapies include monoaminergic systems, neurotrophins, and neurogenesis (24–27). The relatively short timeframe required for a behavioral DBS response in the CUS model suggests that neurogenesis might not be involved (e.g., this interval would be too short for newly born cells to differentiate and become incorporated into the hippocampal circuitry). We therefore decided to focus our initial investigation on the serotonergic system and BDNF.

We have previously shown that vmPFC stimulation induces a fourfold increase in hippocampal 5-HT release and that the antidepressant-like effects of DBS in the FST are dependent on the integrity of the serotonergic system (5). Our current results are in line with those findings, because the anti-anhedonic-like effects of stimulation in stressed animals were abolished after 5-HT-depleting raphe lesions. However, although in our initial report naive animals received DBS for only a few hours, in the present study stressed rats were stimulated for 2 weeks. These results suggest that the serotonergic system might be involved in both acute and chronic effects of vmPFC DBS in rats. Mechanisms underlying the antidepressant-like effects of vmPFC DBS remain unclear but might involve the modulation of prefrontal projections to the raphe, a key structure in 5-HT synthesis and release (6,15,16,28). Future studies are still required to characterize the effects of chronic stimulation on the serotonergic system and how DBS modulates activity of raphe nuclei.

BDNF levels are significantly reduced in rodents undergoing stress and in patients with depression (29–31). Commonly used antidepressant treatments such as medications and electroconvulsive therapy upregulate BDNF in various brain regions (31,32). In our study, vmPFC DBS partially reversed the stress-induced reduction in hippocampal BDNF levels observed in animals undergoing CUS. This is in line with previous reports showing an increase in BDNF after electrical stimulation of the ventral tegmental area, nucleus accumbens, and vPL in rodents (33,34). In one of these studies, increased BDNF levels after vPL stimulation were positively

correlated with sucrose preference scores in animals undergoing CUS (34).

Reports on interactions between BDNF and 5-HT have mainly focused on changes in 5-HT levels in transgenic animals and preparations that increase or decrease BDNF (35–39). Studies addressing the consequences of 5-HT depletion on BDNF levels are far less common, particularly in stressed animals. In our study, 5-HT-depleting raphe lesions prevented the reduction in BDNF levels observed in animals undergoing CUS. Furthermore, BDNF levels in these animals were significantly higher than in nonstressed rats undergoing 5,7-DHT injections. These findings are in line with previous reports showing that 5-HT depletion might interfere with mechanisms of stress adaptation, leading to an increase in the expression of hippocampal glucocorticoid receptors and BDNF (40). Perhaps the most intriguing aspect of our study was that vmPFC DBS did not improve SPI scores in 5-HT-depleted stressed animals, despite the concurrent presence of normal hippocampal BDNF levels. These results question a direct role for hippocampal BDNF in the anti-anhedonic-like effects of vmPFC DBS. That being said, it is possible that an increase in BDNF might contribute to the effects of DBS in conjunction with other substrates and that this neurotrophin might play a role in animals with an intact serotonergic system.

A few methodological considerations need to be addressed in our study. The first includes the timeframe of surgery and stimulation. In an initial study, Friedman *et al.* stimulated the ventral tegmental area in naïve and Flinders rats at settings designed to mimic the neuronal firing pattern of the nucleus (33). Deep brain stimulation administered 20 min before testing induced an antidepressant-like response in different behavioral paradigms (33). In a more recent study, rats undergoing CUS were treated with vPL and nucleus accumbens stimulation at settings suggested to mimic those used for transcranial magnetic stimulation in humans (10 min/day, 5-sec pulses at 20 Hz with 20-sec pauses for 10 days) (34). Deep brain stimulation in either target but not the dorsal prefrontal cortex (PL) induced an antidepressant/anti-anhedonic-like effect (34). In the present study, our goal was to mimic the clinical scenario, in which depressed patients receive DBS continuously for a long time (3). In this context, electrodes were implanted in rats that were already stressed, and stimulation was conducted for 8 hours/day during 2 weeks. Because our DBS system is external and we could not leave the animals connected to the equipment at night, we were unable to conduct experiments with stimulation being delivered 24 hours/day. As mentioned in Methods, frequency and pulse width in our study were similar to those used in clinical practice. Current was selected on the basis of our previous experiments in the FST (6).

Another important aspect to be discussed is the stimulation target. Although the anatomy of the PFC varies considerably across species (41,42), when anatomical connections and cytoarchitectural features are considered, ventral regions of the medial prefrontal cortex (e.g., infralimbic cortex [IL] and vPL) are suggested as the anatomical correlates of the SCG (43,44). In our previous studies, DBS in PL was associated with a more prominent antidepressant-like response in the FST as compared with the IL (6). In this context, we decided to implant the tip of our electrodes in the IL/PL border so that most of the exposed surface of the electrodes would be placed within the vPL.

Finally, the CUS protocol in our study was similar to that described by others (11). Although stressors in our protocol were largely mild, some might be considered somewhat stronger (e.g., paired housing and food deprivation). We do not know whether DBS would still be effective in animals only subjected to severe forms of stress.

In summary, we report an anti-anhedonic-like effect of vmPFC DBS in a chronic model of depression. The kinetics involved in such response were somewhat similar to those described in clinical practice, including the presence of insertional and carryover effects. Our study also shows that the effects of DBS in the CUS model were abolished in animals bearing 5-HT-depleting lesions. This suggests that, as for the antidepressant-like effects of DBS in the FST, the integrity of the serotonergic system is important for the chronic effects of vmPFC stimulation in CUS. We found that, in contrast to stressed animals, rats receiving 5,7-DHT raphe injections with or without DBS had normal hippocampal BDNF levels. This is of importance, because in 5-HT-depleted animals DBS did not exert an anti-anhedonic-like response, despite normal BDNF levels. These results question a direct role for hippocampal BDNF in the anti-anhedonic-like effects of vmPFC DBS. Future studies are still required to further address this issue.

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Supplementary material cited in this article is available online.

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