Antidepressant-like Effects of Medial Forebrain Bundle Deep Brain Stimulation in Rats are not Associated With Accumbens Dopamine Release

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Abstract

Background: Medial forebrain bundle (MFB) deep brain stimulation (DBS) is currently being investigated in patients with treatment-resistant depression. Striking features of this therapy are the large number of patients who respond to treatment and the rapid nature of the antidepressant response.

Objective: To study antidepressant-like behavioral responses, changes in regional brain activity, and monoamine release in rats receiving MFB DBS.

Methods: Antidepressant-like effects of MFB stimulation at 100 $\mu$A, 90 $\mu$s and either 130 Hz or 20 Hz were characterized in the forced swim test (FST). Changes in the expression of the immediate early gene (IEG) zif268 were measured with \textit{in situ} hybridization and used as an index of regional brain activity. Microdialysis was used to measure DBS-induced dopamine and serotonin release in the nucleus accumbens.

Results: Stimulation at parameters that approximated those used in clinical practice, but not at lower frequencies, induced a significant antidepressant-like response in the FST. In animals receiving MFB DBS at high frequency, increases in zif268 expression were observed in the piriform cortex, prelimbic cortex, nucleus accumbens shell, anterior regions of the caudate/putamen and the ventral tegmental area. These structures are involved in the neurocircuitry of reward and are also connected to other brain areas via the MFB. At settings used during behavioral tests, stimulation did not induce either dopamine or serotonin release in the nucleus accumbens.

Conclusions: These results suggest that MFB DBS induces an antidepressant-like effect in rats and recruits structures involved in the neurocircuitry of reward without affecting dopamine release in the nucleus accumbens.

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tract departing the midbrain that bifurcated into inferomedial and superolateral branches [5]. The former approximated the one described as the MFB in rodents.

Experiments involving stimulation of the MFB were first conducted in the 50s by Olds and Milner and highlighted the hedonic-like effects that stimulation in this area could produce in rodents [7–9]. Over the years, protocols have been perfected so that rats and mice would reliably self-administer electrical current into this target [10]. With a strong hedonic component, self-stimulation has been a commonly used model to investigate mechanisms of reward and drug addiction [10–12].

A major difference between protocols used during DBS and self-stimulation is the continuous and prolonged administration of current (weeks/months) in the former [13,14] and the use of short bursts lasting less than a second over periods of minutes/hours in the latter [10]. In addition, self-stimulation has been used in preclinical research to mimic reward/hedonic states and not depressive-like behavior.

In the present study, we delivered MFB DBS at settings that parallel those used in the clinic to rats undergoing the forced swim test (FST), a paradigm that has been shown to have good predictive validity to screen antidepressant therapies [13–15]. Thereafter, we examined neurocircuitry changes and neurotransmitter release following MFB DBS.

Materials and methods

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

Surgical procedures

Adult male Sprague–Dawley rats (250–300 g; Charles River) were anesthetized with isoflurane and had their heads fixed to a stereotaxic frame (David Kopf Instruments). Insulated stainless steel electrodes (250 μm diameter with 0.75 mm of exposed surface) were bilaterally implanted into the MFB and used as cathodes (anteroposterior −2.6, lateral ± 2.2, and depth 8.0 mm) [16]. Electrodes with similar characteristics attached to epideral screws were used as anodes. After being connected to a plastic pedestal (Plastics One), electrodes were fixed to the skull with dental acrylic cement. Controls had holes drilled to the skull but were not implanted with electrodes.

Forced swim test and electrical stimulation

Behavioral experiments were conducted seven days after surgery. On the first day of testing, rats were individually placed in a Plexiglas® cylinder filled with 25 ± 1 °C water. After 15 min of swimming, they received either continuous electrical stimulation or sham treatment for 4 h. On the second day, the same stimulation regimen was given to the animals for 2 h, followed by a second 5 min swimming session. During this session, immobility, swimming and climbing movements were scored by a blinded investigator, as previously described [17–19].

Stimulation was conducted with a handheld device (St Jude Medical model 3510, Plano, TX), connected to the animals through extension cables and a multi-channel commutator (Plastics One, Roanoke, VA). The following settings were tested: 100 μA, 90 μs of pulse width, and either 130 Hz (high frequency stimulation; HFS) or 20 Hz (low frequency stimulation; LFS). These settings were chosen based on our previous DBS studies in other targets [17–19]. In this protocol we did not use higher settings during behavioral studies, as MFB stimulation at currents above 300 μA was associated with stereotypic movements.

Open field test

Two days after the FST, animals received either stimulation or sham-treatment for 4 h. On the next day, the same treatment was provided for 2 h. Thereafter, locomotor activity was assessed for 30 min in a square 0.49 m² Plexiglas® open field apparatus (Med Associates) with infrared photo beams placed every 15 cm along the walls of the equipment. Crossing of the beams provided counts of motor activity.

Microdialysis

In a batch of animals not undergoing behavioral studies (n = 5), a microdialysis cannula was implanted into the right nucleus accumbens (AP + 1.8 mm, ML ± 2.4 mm, and DV −8 mm) along with bilateral MFB electrodes. Seven days later, animals were anesthetized with isoflurane. A microdialysis probe (MAB4.15.4, Scientific Products) was inserted into the target and perfused with Ringer’s solution at a constant flow rate of 0.7 μL/ min. Following an equilibration period (3 h), dialysate samples were collected every 30 min. Four baseline samples were collected over 2 h. The average of these measures was used as a single baseline value during analyses. Thereafter, animals received MFB stimulation at 100 μA 90 μs, 130 Hz for 1 h. Current was then increased to 500 μA (1 h collection). One hour after DBS offset, animals were given a single injection of amphetamine (3 mg/kg i.p.) as a positive control for the DBS experiment. One week later (n = 4), dialysis experiments were repeated with animals being injected with fenfluramine (10 mg/kg i.p.). Details on the monoamine assay and analysis of the samples have been previously described [20].

In situ hybridization and histology

One week after surgery, a batch of animals that did not undergo behavioral testing received stimulation for 4 h on day 1 and 2 h on day 2. Immediately after stimulation offset, animals were sedated using ketamine/xylazine anesthesia and sacrificed by decapitation. Hybridization was performed using 35S-UTP labeled riboprobes complementary to zif268, as previously described [17,21]. After hybridization, slides were exposed to Kodak BioMax film for 6 days at 4 °C along with calibrated radioactivity standards. Film analyses were conducted with an MCID system (Interfocus, UK). In this study, the expression of zif268 was measured in regions implicated in psychiatric disorders (Table 1). 3D modeling of structures expressing zif268 was conducted as previously described [17]. To assess electrode placement, brains were stained with cresyl violet (Fig. 1).

Statistical analysis

One-way ANOVA (Tukey post-hoc), repeated one-way ANOVA or Student’s t test were used to compare behavioral, microdialysis and zif268 data across groups.

Results

Behavioral tests

MFB DBS induced a significant antidepressant-like effect in the FST (F(2,31) = 5.72, P = 0.008 for immobility; F(2,31) = 5.67, P = 0.008 for swimming). Animals treated with 100 μA, 90 μs and
Immediate early gene expression

In animals receiving DBS, significant increases in zif268 expression were detected in the piriform cortex, prelimbic cortex, shell of the nucleus accumbens, anterior striatum and ventral tegmental area (Fig. 3, Table 1). In contrast, zif268 mRNA levels in stimulated animals were reduced in the dentate gyrus of the dorsal hippocampus.

Table 1

<table>
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Values represent average measures in microCuries/gram (SE). Pir—piriform cortex; PL— prelimbic cortex; AcbS—nucleus accumbens shell; CPU ant —caudate putamen anterior; Dgd—dentate gyrus dorsal; VTA—ventral tegmental area; IL—infrahypothalamic cortex; Cg1—Cingulate gyrus, area 1; Cg2—Cingulate gyrus, area 2; AcbC—nucleus accumbens core; CPU DM—caudate putamen dorsomedial; CPU DL—caudate putamen dorsolateral; LS—lateral septum; VP—ventral pallidum; Amg Me—amygdala medial nucleus; Amg BL—amygdala basolateral nucleus; Ang BM—amygdala basomedial nucleus; LH—lateral hypothalamus; MD—mediodorsal nucleus of the thalamus; DGv—dentate gyrus ventral; CA1d—CA1 dorsal; CA1v—CA1 ventral; CA3d—CA3 dorsal; CA3v—CA3 ventral; LHb—lateral habenula; SNc—substantia nigra compacta; DMPAG—dorsomedial periaqueductal grey; LPAG—lateral periaqueductal grey; DRnv—dorsal raphe nucleus dorsal; DRsol—dorsal raphe nucleus ventral; MnR—medial raphe nucleus; LC—locus ceruleus.

130 Hz (n = 10) had a 30% decrease in immobility scores as compared to controls (n = 14; P = 0.008; Fig. 2A). In animals receiving DBS at 20 Hz (n = 10), reduction in immobility was in the order of 20% (P = 0.1).

To assess whether the observed effects of DBS were due to simple locomotor changes, MFB stimulation was delivered to animals during open field testing. No differences were found between animals treated with DBS at 130 Hz (n = 8) or 20 Hz (n = 7) and non-stimulated controls (n = 6; F(2,18) = 0.32, P = 0.7; Fig. 2B).

Microdialysis

MFB DBS induced no changes in dopamine (F(6,24) = 2.3; P = 0.20) or serotonin release (F(6,18) = 1.1; P = 0.38) at either 100 μA or 500 μA (Fig. 4; comparison of baseline, DBS and post stimulation periods). As a positive control, animals were given single injections of amphetamine or fenfluramine. After the administration of the former, levels of dopamine in the nucleus
DBS MFB

A

DBS MFB

B

Structures with an increase in zif 268 expression

Structures with a decrease in zif 268 expression

Anterior Caudate/Putamen

VTA

Accumbens Shell

Pir

Dentate Gyrus

Figure 3. Differences in zif268 expression between controls and animals receiving medial forebrain bundle DBS. (A) In the upper panel, 3D reconstructions give an overview of structures with an increase (red for cortical and orange for subcortical) or a decrease (blue) in zif268 expression after DBS. (B) In the lower panel, individually labeled structures are represented in different colors. Pir- Piriform cortex; Ptl- Prelimbic cortex; VTA- Ventral tegmental area.

Monoamine release during medial forebrain bundle DBS. No significant changes in dopamine (A) or serotonin (B) levels were observed when samples obtained during stimulation were compared to those at baseline. In contrast, significant increases in dopamine or serotonin have been recorded after single doses of amphetamine 3 mg/kg (A) or fenfluramine 10 mg/kg (B), respectively. Samples were collected every 30 min. Bsl-average of four baseline samples collected 2 h before the experiments. Horizontal bar represents the interval during which DBS was administered. * Significantly different from Bsl at P < 0.05.

Figure 4.

Discussion

Our findings suggest that MFB DBS induces frequency-dependent antidepressant-like effects in the FST and modulates activity in structures that project to or receive projections from the MFB. In contrast to self-stimulation studies [22–25], no significant dopamine or serotonin release was detected during stimulation at the settings used in our experiments.

Over 50 structures within the brainstem, hypothalamus, thalamus, basal ganglia, basal forebrain and cortex send their axons through the MFB [26,27]. Perhaps the most thoroughly characterized of these connections is the pathway that projects from the VTA to the nucleus accumbens. These fibers are crucial for prediction of reward and for maintenance of a normal hedonic state [28–31]. In addition, fibers in the VTA-Acb pathway are thought to play a role in mechanisms of addiction and schizophrenia [32].

Since the original work by Olds and Milner, electrical stimulation has been applied to various brain sites in order to map reward-processing areas [7–9]. Robust behavioral responses have been described with current applied to the septal area, cingulate cortex, MFB, among others [7–9]. At present, self-stimulation of the MFB is a well-characterized paradigm to study hedonic states and mechanisms of reward. Typical anatomical target, current and frequency are similar to those applied in our DBS study, except for the fact that our stimulation was continuous and self stimulation is applied in a cyclic on/off mode [10]. In one of the few studies in which DBS was co-administered with self-stimulation, the administration of current to the ventromedial prefrontal cortex of Flinders rats was shown not to have an anti-anhedonic-like effect [33]. We have made similar observations in Sprague–Dawley rats (data not shown).

Since self-stimulation of the MFB induces hedonic-like responses and dopaminergic fibers run through this pathway, stimulation-induced release in dopamine has been postulated as a potential mechanism for the hedonic-like effects of self-stimulation. Whether this is actually the case, however, is still debatable. While some studies have indeed reported that self-stimulation induces dopamine release [22–25,34], others have suggested that a high dopamine turnover was equally important for the behavioral effects observed in rats [23].

It is quite conceivable that mechanisms other than a simple increase in dopamine release may be responsible for the behavioral responses of self-stimulation. The vast majority of axons running through the MFB are non-myelinated. Of those, only 0.2% are dopaminergic [35]. Both self-stimulation and DBS are often delivered at high frequencies (e.g. 100–140 Hz) [10,14]. The only neural elements capable of following stimulation at such settings are myelinated axons. In addition, when the chronaxie of fibers is considered, DA pathways are more easily recruited with pulse widths in the order of 500 µs (i.e. much longer than the 100 µs used in our study) [36]. Even when optimal settings for stimulating non-myelinated fibers are used, it is 3–6 times harder to recruit non-myelinated DA axons than non-DA myelinated ones [35]. In this context, it is not surprising that MFB DBS did not induce dopamine release in our study. That said, it is possible that different results might have been attained with the use of smaller electrode tips, a
higher charge density, the delivery of stimulation bursts, or DBS conducted in other regions of the MFB [34,35].

We chose to implant MFB electrodes near the lateral hypothalamus since this is the most commonly used target for self-stimulation and the region where the bundle is most prominent. In the clinic, the target used for DBS is more posterior, closer to the VTA. Our rationale for not selecting this target was threefold: 1) Near the brainstem, the MFB is small and difficult to isolate (i.e. stimulation would have certainly spilled over to other structures that might have influenced results). 2) The likelihood of animals developing side effects at relatively low settings with electrodes implanted in the brainstem would have been higher (as observed in humans). 3) Dopaminergic pathways of the mesolimbic and mesostriatal systems run through the MFB at the level of the lateral hypothalamus (e.g. injections of toxins into the MFB to induce parkinsonism in rats are conducted at the level of the lateral hypothalamus) [37]. Though the spread of current to the lateral hypothalamus has likely occurred in our study, similarities between effects recorded in animals and humans suggest that a common element—the MFB—may have likely been important for an antidepressant-like response.

Structures recruited in the region of the MFB during self-stimulation have been previously examined using functional markers such as cytochrome oxidase and 2-deoxyglucose [38,39]. Stimulation have been previously examined using functional

IEGs in various structures involved in the neurocircuitry of reward frequency-dependent antidepressant-like effects in the FST. In depression.

One of the common features between MFB stimulation and DBS applied to other targets (e.g. vmPFC, nucleus accumbens and white matter fibers of forceps minor) is an increased expression of IEGs in the piriform cortex [17]. This structure is part of the olfactory system and also seems to be involved in medication-induced antidepressant-like responses observed in the FST [40,41]. Future studies are still needed to better ascertain the role of the piriform cortex in depression.

In summary, our results suggest that MFB stimulation induces frequency-dependent antidepressant-like effects in the FST. In addition, we found that this therapy increases the expression of IEGs in various structures involved in the neurocircuity of reward that project to, or receive projections from, the MFB. As no changes in monoamine levels were detected after DBS, additional mechanisms need to be explored to ascertain potential substrates involved in the acute antidepressant-like effects of MFB DBS.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.brs.2015.02.007.

References


