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Original Articles

# 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 μA, 90 μs 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 *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.



## Introduction

One potentially promising deep brain stimulation (DBS) target for the treatment of depression is the medial forebrain bundle (MFB). In a recent open label clinical trial, over 80% of treatment-refractory patients undergoing surgery showed a significant degree of improvement [1]. In contrast to medications, the initial therapeutic response to MFB DBS was quite dramatic, occurring within days after stimulation onset [1]. The rationale for conducting MFB DBS in depression stems from imaging studies originally carried out in patients with Parkinson's disease (PD) treated with subthalamic nucleus (STN) stimulation [2]. Commonly reported side effects when STN electrodes are misplaced medially include dysphoria and mania [3,4]. While these have initially been attributed to the stimulation of medial regions of the limbic STN, Coenen and colleagues have argued that such psychiatric responses could be attributed to the stimulation of the MFB [2,5,6]. Using diffusion tensor imaging and tractography, the authors described a

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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 isofluorane 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 epidural 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 $m^2$ 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 + or −2.4 mm, and DV −8 mm) along with bilateral MFB electrodes. Seven days later, animals were anesthetized with isofluorane. 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 $^{35}$S-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