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Thalamic Stimulation in Awake Rats Induces Neurogenesis in the Hippocampal Formation

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ABSTRACT

Background: Deep brain stimulation (DBS) provides clinical benefits for a variety of movement disorders and lately emerged as a potential treatment for cognitive and mood disorders. Modulation of adult hippocampal neurogenesis may play a role in mediating its effects.
Objective: To investigate the effects of unilateral anteromedial thalamic nucleus (AMN) stimulation on adult hippocampal neurogenesis in awake and unrestrained rats.
Methods: Four groups of adult Sprague–Dawley male and female rats received unilateral stimulation (n = 6 each) or sham surgery (n = 4 each) in the right AMN; another group of males (n = 4) was stimulated in the right ventral posterolateral thalamic nucleus (VPL). A naive group of males and females (n = 4 each) was also included. Rats received 4 injections (50 mg/kg/injection) of 5′-bromo-2′-deoxyuridine (BrdU) 3 days post-surgery and were euthanized 24 h later. The fractionator method was used together with confocal microscopy to count BrdU, GFAP and NeuN positive cells in the dentate gyrus (DG) and hilar zone of the hippocampus.
Results: Focal neurogenesis was induced in the ipsilateral DG after AMN but not VPL stimulation. Stimulation-induced effects were sex-independent and translated into a 76% increase in proliferation of neural stem/progenitor cells. Increased neurogenesis was most prominent at the caudal region of the DG, while no effect was detected in the hilar and the subventricular zones.
Conclusions: The exclusive hippocampal neurogenic response to AMN stimulation suggests an involvement of the Papez circuitry in mediating DBS effects and in the treatment of cognitive and behavioral disorders.

Introduction

Deep brain stimulation (DBS) is a surgical therapeutic modality that has been used for more than 30 years, whereby high frequency stimulation is delivered to specific targets in the brain [1]. The established clinical success of DBS in the treatment of movement disorders such as Parkinson's disease, essential tremor, and primary dystonia [2,3] prompted scientists to evaluate its applications in psychiatry as a treatment for depression [4], obsessive compulsive disorder [5,6] and more recently, dementia [7]. For example, open-label DBS to ventral capsule/ventral striatum [8], the subgenual cingulate [9,10], the nucleus accumbens [4] or medial forebrain bundle [11] in patients with refractory depression was associated with antidepressant benefits. Interestingly in an investigational study, hypothalamic/fornix DBS to a patient with obesity generated prominent memory improvements, especially those dependent on hippocampal activity such as verbal recollection [12]. The findings on reversible memory effects of hypothalamic/fornix stimulation encouraged the same group to investigate fornix stimulation as a potential treatment for memory impairments associated with neuropsychiatric disorders. In a pilot trial on patients with Alzheimer disease, DBS to the same limbic targets was accompanied by amelioration of dementia-associated cognitive decline, partly by evoking neural activity in the memory circuits of the hippocampus and entorhinal cortex [7]. A recent paper introduced the first

Abbreviations: DBS, deep brain stimulation; AMN, anteromedial thalamic nucleus; VPL, ventral posterolateral thalamic nucleus; BrdU, 5′-bromo-2′-deoxyuridine; DG, dentate gyrus.

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line of evidence for cognitive improvements of bilateral DBS to another limbic target, the anterior thalamic nucleus (AMN) in patients with refractory epilepsy. Indeed, AMN stimulation not only resulted in controlled seizures, but also in improving verbal memory and oral information processing [13]. While the underlying mechanisms conferring these therapeutic outcomes remain elusive, the effects of AMN stimulation suggest a role for the activation of limbic memory pathways [13].

The hippocampus is a structural and functional component of the limbic system with extensive interconnections between the hippocampal formation and the AMN [14,15]. Among other regions of the brain, the hippocampus is distinguished by persistent generation of new neurons in the subgranular zone (SGZ) of the dentate gyrus (DG) [16]. Neural precursors residing in the SGZ undergo a process of division, differentiation and functional integration into existing hippocampal circuits [17]. Modulation of hippocampal neurogenesis is attributed to a wide number of factors including enriched environment, learning and stress [17–19]. The key role of the hippocampus in mood regulation and memory suggested that DBS might exert pro-cognitive improvements through increased adult hippocampal neurogenesis and functional lifelong integration of newly generated neurons into hippocampal networks [20]. In this context, Lozano and colleagues reported an enhanced neurogenesis in the DG, a sub region of the hippocampus, upon bilateral DBS applied to the AMN of anesthetized adult rats [21].

This study aimed to reexamine the specificity of unilateral AMN stimulation on adult hippocampal neurogenesis in awake, freely moving adult rodents. We also compared it to the stimulation of the ventral posterolateral thalamic nucleus (VPL), a region not known to have direct connection to the hippocampus.

Materials and methods

Animals

Experiments were performed on adult male and female Sprague–Dawley rats (250–300 g) in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals) and approved by the Institutional Animal Care and Use Committee (IACUC) at the American University of Beirut (AUB) [22]. Animals were maintained in a controlled environment, temperature (20–22 °C), 12 h light/dark cycle and provided with water ad libitum. Surgical procedures were performed under deep anesthesia. Postoperative behavioral and body weight monitoring was conducted during the light phase of the cycle by a researcher blind to the treatment conditions.

Stereotactic surgery

Rats were deeply anesthetized with a solution of atropine (0.05 mg/kg, i.p.) and ketamine (50 mg/kg, i.p.), after which the head was rigidly fixed on a stereotaxic frame (DKI) and an incision was made in the scalp. For bipolar electrical stimulation, two insulated copper electrodes (200 μm diameter with 0.3 mm of exposed tip) inserted into a stainless steel guide cannula were implanted unilaterally into the right anteromedial thalamic nucleus according to the following stereotaxic coordinates: –1.4 mm from bregma, +0.8 mm lateral and 6 mm vertical from the surface of the brain [23]. In another control group for nucleus specificity, electrodes were implanted in the right thalamic VPL nucleus at the coordinates: –2.7 mm from bregma, –3 mm lateral and 6 mm vertical from the surface of the brain [23]. At the end of surgery, a topical antibiotic was applied on the sutured wound and the rats were returned to their home cages. Rats were given 3–5 days of post-surgical recovery before instituting the stimulation or sham procedure, as illustrated in Fig. 1A.

The correct localization of electrodes in the right AMN (Fig. 1B and 1C) or right VPL (Fig. 1D and 1E) was confirmed postmortem by histological observation on free floating sections (50 μm) stained with cresyl violet. Data collection and analysis are based on animals with correct electrode placement.

Deep brain stimulation and BrdU administration

We designed a stimulator capable of delivering balanced biphasic charge with constant current at 100 μA. High-frequency electrical stimulation was delivered using the stimulator for 1 h at the following parameters (100 μA, 125 μs, 130 Hz), which approximates the settings used in clinical practice [20,21]. The output cables of the stimulator were connected to the implanted electrodes and rats were allowed to roam freely in their setting during stimulation. Sham rats underwent the same stereotactic surgery and wires were connected to implanted electrodes without current delivery.

Three days after stimulation, all rats were injected intraperitoneally four times at 3 h interval with 5-bromo-2-deoxyuridine (BrdU, 50 mg/kg/ injection) dissolved in 0.9% warm saline. Rats were killed and electrode placement was verified for all animals.

Perfusion and tissue preparation for stereology

Twenty-four hours after the last BrdU injection, rats were deeply anesthetized and perfused transcardially with 0.9% saline and 4% formalin. Brains were removed, fixed overnight in 4% paraformaldehyde and then cryoprotected with 30% sucrose solution for 3 days. Systemic-random sampling of brain sections was achieved following the Fractionator principle [24]. In brief, 50 μm coronal frozen sections were cut serially using a freezing microtome, from the caudal to the rostral extent of the DG at the following caudo-rostral coordinates: –6.3 to –3.3 mm relative to bregma, for use in immunostaining. Sections were collected in six sets, each set containing 12 slices, where each slice is 300 μm apart from the next. All sections were collected in 0.1 M phosphate-buffered saline (PBS) and additional sets were stored in a PBS solution containing 15 mM sodium azide for future processing.

Immunofluorescence

For BrdU detection, DNA was denatured by incubating the sections in 2N HCl for 2 h at 37 °C. Sections were then rinsed with 0.1 M PBS and washed with 0.1 M sodium borate (pH 8.5) for 10 min at room temperature (RT) to neutralize acidic effect. Tissues were then washed with 0.1 M PBS and transferred to the blocking and permeabilization solution (10% NGS, 3% BSA, 0.1% Triton-X diluted in PBS) for 1 h at 4 °C. In order to minimize non-specific cross labeling between different primary antibodies, we opted to sequentially stain the sections. Therefore, sections were incubated overnight at 4 °C with mouse monoclonal anti-NeuN (1:1000; Millipore) and/or rabbit monoclonal anti-GFAP (1:1000; Millipore) diluted in PBS with 10% NGS, 0.1% Triton-X. The following day, sections were washed and incubated in the dark with fluorochrome-conjugated secondary antibodies: Alexa Fluor-488 anti-mouse (1:250; Invitrogen) and Alexa Fluor-660 anti-rabbit (1:250; Invitrogen) diluted in PBS with 10% NGS and 0.1% Triton-X for 2 h at RT on a rotator. Sections were then washed and incubated with rat monoclonal anti-BrdU at 4 °C overnight and the next day the secondary antibody Alexa Fluor-568 anti-rat (1:100; Invitrogen) was applied as before. Finally, sections were mounted onto slides with Fluoro-Gel containing DAPI (Electron Microscopy Sciences, USA) and covered with a thin glass coverslip.