The regulation of adult rodent hippocampal neurogenesis by deep brain stimulation

Laboratory investigation

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OBJECT. To examine the influence of deep brain stimulation on hippocampal neurogenesis in an adult rodent model.

METHODS. Rats were anesthetized and treated for 1 hour with electrical stimulation of the anterior nucleus of the thalamus (AN) or sham surgery. The animals were injected with 5′-bromo-2′-deoxyuridine (BrdU) 1–7 days after surgery and killed 24 hours or 28 days later. The authors counted the BrdU-positive cells in the dentate gyrus (DG) of the hippocampus. To investigate the fate of these cells, they also stained sections for doublecortin, NeuN, and GFAP and analyzed the results with confocal microscopy. In a second set of experiments they assessed the number of DG BrdU-positive cells in animals treated with corticosterone (a known suppressor of hippocampal neurogenesis) and sham surgery, corticosterone and AN stimulation, or vehicle and sham surgery.

RESULTS. Animals receiving AN high-frequency stimulation (2.5 V, 90 μsec, 130 Hz) had a 2- to 3-fold increase in the number of DG BrdU-positive cells compared with nonstimulated controls. This increase was not seen with stimulation at 10 Hz. Most BrdU-positive cells assumed a neuronal cell fate. As expected, treatment with corticosterone significantly reduced the number of DG BrdU-positive cells. This steroid-induced reduction of neurogenesis was reversed by AN stimulation.

CONCLUSIONS. High-frequency stimulation of the AN increases the hippocampal neurogenesis and restores experimentally suppressed neurogenesis. Interventions that increase hippocampal neurogenesis have been associated with enhanced behavioral performance. In this context, it may be possible to use electrical stimulation to treat conditions associated with impairment of hippocampal function. (DOI: 10.3171/JNS/2008/108/01/0132)

KEY WORDS • deep brain stimulation • dentate gyrus • hippocampus • neurogenesis • thalamus

The production of neurons from neural stem/progenitor cells in the mammalian brain is an active process that continues throughout life.10,11,17 In adults, neurogenesis occurs in two main sites: the subventricular zone and the hippocampal subgranular zone.17 Several physiological and pathological conditions affect neuronal proliferation and differentiation in the subgranular zone.9,16 Electroconvulsive therapy,22 seizures,7,27,25,31 glutamatergic agonists,8 and ionotropic depolarizing currents8 enhance the proliferation of neural precursors suggesting that electrophysiological activity may be an important modulator of neurogenesis.

Chronic electrical stimulation through implanted electrodes, commonly referred to as deep brain stimulation or DBS, has now been used to treat > 30,000 patients with various neurological and psychiatric disorders. Although the mechanism of action is incompletely understood, it is believed that DBS exerts its therapeutic effects by altering neural activity leading to the disruption of pathological outputs in dysfunctional brain circuits.20 The effects of DBS in regulating neurogenesis are unknown.

We examined the influence of DBS on hippocampal neurogenesis in an adult rodent model.

Materials and Methods

Experimental Animals

This study was approved by the Toronto Western Research Institute Animal Care Committee and is in accordance with the guidelines of the Canadian Council on Animal Care. Adult male Sprague–Dawley rats (180–200g) were housed with ad libitum access to food and water in a room maintained at a constant temperature (20–22°C) on a 12 hour:12 hour light–dark cycle.

Electrical Stimulation of the Anterior Thalamic Nucleus and BrdU Administration

Anesthesia was induced with a solution of ketamine (75 mg/kg)
and xylazine (10 mg/kg) administered intraperitoneally. The animal's heads were then fixed to a stereotactic instrument (Model 900, David Kopf Instruments). To stimulate the AN, platinum concentric bipolar electrodes (model SNEX-100; Rhodes Medical Instruments) were bilaterally implanted at the following coordinates (relative to the bregma): anteroposterior -1.6 mm, medial-lateral 1.5 mm, dorsoventral 2.2 mm, according to Paxinos and Watson. Stimulation was conducted for 1 hour with a Medtronic 3628 screener at the following parameters: 2.5 V, 90 μs of pulse width, and variable frequencies (10, 50, 130 Hz). Control animals were also anesthetized, had electrodes implanted in the AN but did not receive stimulation. After the procedures, the electrodes were removed, the surgical planes were closed and the animals were allowed to recover.

The BrdU (Sigma) was dissolved in saline and steriley filtered. Animals received a total of 200 mg/kg of BrdU (50 mg/kg administered intraperitoneally every 3 hours) 1, 3, 5, or 7 days after stimulation. One day or 28 days after the last BrdU injections, animals were deeply anesthetized with pentobarbital (50 mg/Kg administered intraperitoneally) and subsequently perfused with saline 0.9%, followed by 4% paraformaldehyde. Brains were then removed from the skull and postfixed overnight in a phosphate buffered 30% sucrose solution.

The expression of the immediate early gene Zif268 was examined in animals killed 3 hours after 1 hour of stimulation.

**Immunohistochemistry, Immunofluorescence, and TUNEL Assay**

Free-floating 40-μm sections were cut on a cryostat, collected in cryoprotectant, and immunostained with the following primary antibodies: rabbit anti-Zif/268 (1:300, Santa Cruz Biotechnology); mouse anti-NeuN (1:500, Zymed); guinea pig anti-GFAP (1:500, Harlan); goat anti-DCX (1:500, Santa Cruz Biotechnology), and rat anti-BrdU (1:500, Harlan). Secondary antibodies were used at a 1:500 concentration (Jackson ImmunoResearch Laboratories). TUNEL histochemistry was performed according to the manufacturer’s protocol (Promega).

**Stereology and Confocal Microscopy**

Cell counting was limited to the DG granule cell layer and the region comprising the 50-μm border along the hilar margin (subgranular zone). Stained BrdU-positive nuclei were scored in every sixth section (that is, 240 μm apart) throughout the rostrocaudal extent of the hippocampal DG using the optical fractionator method (100 objective, Nikon Eclipse light microscope). The TUNEL-positive cells were counted in every sixth section of the rostrocaudal extent of the hippocampal DG. Overestimation was corrected using the Abercrombie method for nuclei with an empirically determined average diameter of 13 μm within a 40-μm section.

For confocal microscopy we used a Zeiss 510 microscope (Carl Zeiss). Appropriate gain and black-level settings were determined on control tissues stained only with the secondary antibody. The number of BrdU-positive cells displaying specific cell markers (DCX, NeuN, and GFAP) was determined in every 12th section throughout the rostrocaudal extent of the DG. Counts were performed using a multichannel configuration, an ×63 objective, and an electronic zoom of 1. Each cell was manually examined in its full Z dimension. Only cells whose nuclei were unambiguously associated with lineage-specific markers were scored.

**Corticosterone Administration**

Animals received daily subcutaneous injections of 40 mg/kg of corticosterone (Sigma) or vehicle during the 5 days that preceded and the 3 days that followed AN stimulation or sham surgery (a total of 8 days). Three groups were considered for the experiment: corticosterone plus sham surgery, corticosterone plus AN HFS (130 Hz, 2.5 V, 80 μs), or vehicle plus sham surgery (controls). Three days after the last FFS or sham surgery (last day of corticosterone or vehicle administration) animals received BrdU 200 mg/kg (50 mg/kg intraperitoneally every 3 hours); they were killed 1 or 28 days later. Brains were processed as described above.

**Statistical Analysis**

Unpaired, two-tailed Student t-tests were used in all comparisons.
Fig. 1. High-frequency stimulation of the AN increases hippocampal neurogenesis. A and B: Photomicrographs of DG sections from animals that underwent sham surgery (A) or AN HFS (B), showing increased expression of the immediate early gene Zif/268 at 3 hours after stimulation. C: Graphic illustration of the timing of BrdU administration and death. Injections of BrdU were administered at variable time points from postoperative Day 1 to Day 7 (indicated in the figure by ×) and the animals were killed 24 hours or 4 weeks later (indicated by solid black circles). D: Graph showing the relationship between the number of BrdU-positive cells in DG sections and time after last BrdU injection in the AN HFS (dark bars) and sham surgery (light bars) groups. Animals that received AN HFS had a 2- to 3-fold increase in the number of BrdU-positive cells in the granule cell layer and subgranular zone of the DG compared with nonstimulated controls. E–H: Photomicrographs of DG sections from animals that underwent sham surgery (E and G) or AN HFS (F and H) and were killed 24 hours (E and F) or 28 days (G and H) after BrdU injections (BrdU-positive cells are stained red). I: Graph showing the relationship between the number of BrdU-positive cells and timing of BrdU injection. The maximum number of labeled cells was seen in sections from animals that received BrdU 3 and 5 days after stimulation in the AN HFS (dark bars) and the sham surgery (light bars) groups. J: Graph showing that the effect of AN stimulation was frequency dependent. While rats treated with stimulation at 50 Hz and 130 Hz had a significant increase in the number of BrdU-positive cells, stimulation at 10 Hz did not have such an effect. Scale bars = 200 μm (B and H). Values in the graphs represent the mean number of BrdU-positive cells (± standard error of the mean) per group of animals. *Statistically significant difference in comparison with controls.
Increase in Number of Neural Progenitor Cells With AN HFS

We used cell-specific markers to identify the phenotype of stimulation-induced BrdU-positive cells. In animals that received BrdU 3 days after AN HFS and were killed 1 day later (Fig. 2A), 85% of the BrdU-positive cells in the DG expressed DCX, a marker of immature neuron lineage, while only 2% of the BrdU-positive cells expressed the astrocytic marker GFAP (Fig. 2B). In animals treated with AN HFS that were killed 28 days after the BrdU injections (Fig. 2C), 46% of the BrdU-positive cells expressed NeuN, a marker of mature neurons, while 7% expressed GFAP (Fig. 2D). The proportion of cells that expressed these markers did not differ between animals treated with AN HFS and controls (Fig. 2B, D). These findings show that a large fraction of new cells in the granule cell layer and subgranular zone of the DG after stimulation develop into mature neurons and suggests that HFS, as applied here, does not significantly alter the proportion of cells assigned to the various intrinsic differentiation paths.

Reversal of Cortisone Suppression of Hippocampal Neurogenesis

We were interested in whether HFS could not only enhance baseline neurogenesis but also ameliorate disruptions of neurogenesis that may occur as a consequence of pathological conditions. Stress and one of its chemical mediators, corticosterone, are known powerful suppressors of hippocampal neurogenesis.\textsuperscript{5,24} We tested whether AN HFS could overcome this suppression. Animals received daily injections of vehicle or corticosterone for 8 days and were assigned to 3 groups: corticosterone plus sham surgery, vehicle plus sham surgery, or corticosterone plus HFS (4 animals per group). Sham or stimulation surgery took place...
5 days after the initial dose of corticosterone or vehicle. Animals received BrdU on Day 8 and were killed on Day 9. As expected, administration of corticosterone reduced the number of BrdU-positive DG cells by 68.3 ± 11.3% compared with findings in vehicle-treated controls (p = 0.001, Fig. 3A and C). In contrast, in corticosterone-treated animals that also received AN HFS for 1 hour, the number of BrdU-positive cells was restored to near control levels (reduced by 23.2 ± 15.1% compared with vehicle treated controls, p = 0.12, Fig. 3B and C). The mitigation of the steroid effect was also observed with longer survival times. Animals that received corticosteroid medication for 8 days and were killed 28 days later had a 68.9 ± 1.9% reduction in the number of BrdU-positive DG cells compared with vehicle-treated controls (p = 0.002). Animals similarly treated with corticosterone but also receiving AN HFS for 1 hour on Day 5 had only a 32.2 ± 8.1% reduction in BrdU-positive cells (p = 0.013) 28 days later (Fig. 3D).

**Discussion**

Our study shows that high-frequency electrical stimulation at parameters similar to that in current clinical use not only drives hippocampal neurogenesis but also reverses the suppression of neurogenesis that occurs with corticosterone administration. These observations support the concept of an excitation–neurogenesis coupling.

A variety of neurotransmitters and receptors including excitatory GABAergic stimulation\(^2\) can drive adult hippocampal neurogenesis. However, the mechanism through
which neural stimulation drives neurogenesis is not fully understood. There is evidence that in response to excitatory stimuli, adult hippocampal neural progenitor cells alter their cell differentiation programs away from glial cell fate genes, such as \textit{Hes} and \textit{Idz}, and increase their expression of NeuroD, a positive regulator of neuronal differentiation.\textsuperscript{8}

The behavioral consequences of an increase in hippocampal neurogenesis are still unclear. Experimental manipulations that impair hippocampal neurogenesis, including stress\textsuperscript{3} or radiation,\textsuperscript{14} are associated with deterioration in the behavioral performance of rodents. In contrast, interventions that increase hippocampal neurogenesis, including exposure to enriched environments\textsuperscript{12,19} or the administration of antidepressant drugs and certain trophic factors enhance behavioral performance in animals with pathological conditions and in normal animals.\textsuperscript{21,23,30} This suggests that there may be beneficial functional consequences to the enhancement of hippocampal neurogenesis. With this in mind, it may be possible to apply electrical stimulation to enhance hippocampal function in various disorders characterized by pathological conditions of the hippocampus.\textsuperscript{5,6} Whether electrical stimulation–induced neurogenesis occurs in humans and whether this has functional consequences remains to be determined. We have the opportunity to study these questions given the increasing use of DBS in patients with various neurological and psychiatric disorders.

**Conclusions**

High-frequency stimulation of the AN induces a 2- to 3-fold increase in hippocampal neurogenesis in an adult rodent model. The neurogenic effect can restore experimentally suppressed neurogenesis. Interventions that increase hippocampal neurogenesis have been associated with enhanced behavioral performance. In this context, it may be possible to apply electrical stimulation to treat conditions associated with impairment of hippocampal function.

**Disclosure**

Dr. Lozano is a consultant for Medtronic, Inc. and Functional Neuroscience, Inc.

**References**


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