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
Deep brain stimulation increases hippocampal neurogenesis and xylazine (10 mg/kg) administered intraperitoneally. The animals’ 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 Zif/268 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 at the respective working concentrations: 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 (1:300, 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).

**Cell Proliferation in the Subgranular Layer of the Hippocampus**

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 sec), or vehicle plus sham surgery (controls). Three days after the last HFS 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.

### Results

We administered BrdU 1, 3, 5, or 7 days after stimulation (4 animals per group) to label dividing cells, and the animals were killed the day after BrdU injection (Fig. 1C). 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 (Fig. 1D–H). The maximum number of labeled cells was seen with BrdU injection 3 and 5 days after stimulation (p < 0.01, p = 0.02, respectively) (Fig. 1I).

Because therapeutic benefits of DBS are critically dependent on the stimulation rate, we studied whether neural cell birth also depends on stimulation frequency. Rats were exposed to stimulation at 10 Hz, 50 Hz, or 130 Hz (4 animals per group; 2.5 V, 80 μs sec). The animals were injected with BrdU on the 3rd day poststimulation and killed the following day. Stimulation at 50 Hz and 130 Hz significantly increased the number of BrdU-positive cells in the granule cell layer and subgranular zone of the DG (by ~2.2-fold compared with controls, p < 0.01). In contrast, low frequency stimulation at 10 Hz did not (Fig. 1J) produce this effect. Interestingly, this finding mirrors clinical situations in which DBS at high frequency produces clinical benefits while stimulation at 5 Hz or 10 Hz is ineffective. The increase in the number of novel cells during the first week after stimulation does not appear to occur in response to increased apoptotic cell death because in animals killed 1–5 days after HFS the number of TUNEL-positive cells in the DG was similar to that in controls (2.2 ± 0.6 cells per section in the stimulated group and 1.9 ± 0.5 cells in nonstimulated controls; p = 0.25; 3 animals per group).

To examine the long-term fate of stimulation-induced BrdU-positive cells, we administered BrdU to 4 AN HFS-treated animals on the 3rd day post stimulation and allowed them to survive for 28 days. At this later time point, we found a 2.2-fold increase in the number of BrdU-positive DG cells in the HFS group (4 animals) compared with sham-treated controls (3 animals, p = 0.02; Fig. 1D, G, and H). This finding shows that not only does stimulation increase the number of newborn DG cells, but that these cells are capable of long-term survival.
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. 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...
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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 Hes1 and Id2, and increase their expression of NeuroD, a positive regulator of neuronal differentiation.8

The behavioral consequences of an increase in hippocampal neurogenesis are still unclear. Experimental manipulations that impair hippocampal neurogenesis, including stress1 or radiation,14 are associated with deterioration in the behavioral performance of rodents. In contrast, interventions that increase hippocampal neurogenesis, including exposure to enriched environments12,19 or the administration of antidepressants and certain trophic factors enhance behavioral performance in animals with pathological conditions and in normal animals.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.3,6 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.

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