Video Article

Analysis of Gene Expression Changes in the Rat Hippocampus After Deep Brain Stimulation of the Anterior Thalamic Nucleus

Tharakeswari Selvakumar[1], Kambiz N. Alavian[2], Travis Tierney[1]

[1]Department of Neurosurgery, Brigham & Women's Hospital, Harvard Medical School

[2]Division of Brain Sciences, Department of Medicine, Imperial College London

Correspondence to: Travis Tierney at tstierney@partners.org

URL: https://www.jove.com/video/52457
DOI: doi:10.3791/52457

Keywords: Neuroscience, Issue 97, anterior thalamic nucleus, deep brain stimulation, dentate gyrus, hippocampus, epilepsy, gene expression, high-frequency stimulation, quantitative RT-PCR

Date Published: 3/8/2015

Citation: Selvakumar, T., Alavian, K.N., Tierney, T. Analysis of Gene Expression Changes in the Rat Hippocampus After Deep Brain Stimulation of the Anterior Thalamic Nucleus. *J. Vis. Exp.* (97), e52457, doi:10.3791/52457 (2015).

Abstract

Deep brain stimulation (DBS) surgery, targeting various regions of the brain such as the basal ganglia, thalamus, and subthalamic regions, is an effective treatment for several movement disorders that have failed to respond to medication. Recent progress in the field of DBS surgery has begun to extend the application of this surgical technique to other conditions as diverse as morbid obesity, depression and obsessive compulsive disorder. Despite these expanding indications, little is known about the underlying physiological mechanisms that facilitate the beneficial effects of DBS surgery. One approach to this question is to perform gene expression analysis in neurons that receive the electrical stimulation. Previous studies have shown that neurogenesis in the rat dentate gyrus is elicited in DBS targeting of the anterior nucleus of the thalamus[1]. DBS surgery targeting the ATN is used widely for treatment refractory epilepsy. It is thus of much interest for us to explore the transcriptional changes induced by electrically stimulating the ATN. In this manuscript, we describe our methodologies for stereotactically-guided DBS surgery targeting the ATN in adult male Wistar rats. We also discuss the subsequent steps for tissue dissection, RNA isolation, cDNA preparation and quantitative RT-PCR for measuring gene expression changes. This method could be applied and modified for stimulating the basal ganglia and other regions of the brain commonly clinically targeted. The gene expression study described here assumes a candidate target gene approach for discovering molecular players that could be directing the mechanism for DBS.

Video Link

The video component of this article can be found at https://www.jove.com/video/52457/

Introduction

The history behind the development of Deep Brain Stimulation as a neurosurgical technique dates back to the 1870s when the possibility of electrically stimulating the brain circuitry was explored[2]. The use of chronic high-frequency stimulation as treatment for neuronal disorders started in the 1960s[3]. Later in the 1990s with the advent of chronic implantation DBS electrodes[4-6], the number of neuronal disorders that were treated by DBS continued to increase. Deep Brain Stimulation was first used in the United States as a treatment for essential tremor[6]. Today the surgery is used widely to treat neuronal disorders that are currently untreatable by pharmacological intervention. DBS is currently used to treat movement disorders of Parkinson's disease and dystonia[7-9]. Alzheimer's type dementia, Huntington's disease, epilepsy, pain and neuropsychiatric diseases such depression, OCD, Tourette's syndrome and addiction are some of the conditions amenable to treatment by DBS[10-12]. While DBS surgery is FDA approved for treating Parkinson's disease, dystonia and essential tremor, the use of DBS for treating other conditions mentioned above are in various stages of lab and clinical studies offering much promise to patients[13,14].

Clinically, DBS surgery is performed in two stages. The first stage involves surgically positioning the DBS electrodes at the targeted anatomical location using a combination of radiological positioning, CT, MRI as well as microelectrode readings for enhanced precision. The second stage involves implanting a pulse generator in the patient's upper chest and installing extension leads from the scalp to the pulse generator. Based on the neurological condition, several programming schemes for the pulse generator have been standardized and will be used to deliver the desired voltage. The final voltage is reached in a stepwise fashion so as to receive the best clinical response with minimal voltage[15]. However, in our studies, unlike the chronic DBS implants used clinically, for the sake of simplicity, we have resorted to studying a one-time high frequency stimulation (for 1 hr) in our animal models.

Part of our group's research focuses on investigating the use of DBS surgery for treatment-refractory epilepsy. Stereotactic surgical approaches using high frequency stimulation has been explored by many others as an effective option to treat medically-refractory epilepsy which constitutes about 30% of all incidences of epilepsy[10,16,17]. Cerebellar stimulation targeting the cortical surface as well as the deep cerebellar nuclei have been used in the past as targets to treat epilepsy[10,18,19]. In addition, hippocampus stimulation has also been tried but with mixed results[20,21]. Some of the other investigated DBS targets for epilepsy include the cerebral cortex, thalamus, subthalamic nucleus and vagus nerve[8]. However, following results from several studies in the past few years, the anterior thalamic nucleus (ATN) has emerged as the most common DBS target for epilepsy treatment[10,22]. Based on knowledge about neuroanatomical circuitry and findings from animal models, several studies have

focused on the therapeutic effect of deep brain stimulation of the ATN in treating epilepsy [23-26]. The ATN is part of the limbic circuit and is located in the region of the brain that affects seizure frequency. Studies by Hamani *et al*., have tested the efficacy of ATN-DBS in a pilocarpine induced epilepsy model and found that bilateral ATN stimulation prolonged latencies for pilocarpine-induced seizures and status epilepticus[24]. Furthermore, high frequency stimulation of the ATN was found to reduce seizure frequency in a pentylenetetrazol (PTZ) model of epilepsy[25,27-29]. Lee *et al*., have reported a mean reduction in seizure frequency by about 75% upon chronic deep brain stimulation of the ATN in treating refractory partial epilepsy[30].

A recent clinical study on treatment-refractory epilepsy has shown promising results after DBS surgery targeting the anterior thalamic nucleus (ATN)[22]. A multicenter randomized clinical trial with 110 patients undergoing bilateral DBS of the ATN for treatment refractory epilepsy (SANTE trial) indicated a drop in seizure frequency by approximately 40%[31]. The results from this study also hinted on a delayed optimal anti-epileptic effect observed at 2-3 months post surgery. Further studies by Toda *et al*., corroborated with these findings where they demonstrated neurogenesis happening at a later time post DBS (days 3-5) in animal models[1]. In addition, Encinas *et al.,* have reported hippocampal neurogenesis in the adult mouse dentate gyrus after high frequency stimulation of the ATN[32]. Previous studies[33-35] have reported declining hippocampal neurogenesis in certain epileptic cases such as chronic temporal lobe epilepsy and an association with learning deficits, memory impairment and spontaneous recurrent motor seizures. Furthermore, there was a reduction in neural stem cell progenitor factors such as FGF2 and IGF-1 in the chronically epileptic hippocampus in animal models [33]. Considering this, interventional strategies such as DBS that show an augmentation of neurogenesis in the dentate gyrus are exciting avenues for research. These findings have encouraged us to explore further deeply into the mechanism underlying neurogenesis post-DBS treatment for epilepsy. We have targeted the ATN both unilaterally (data not reported) as well as bilaterally (in representative results) and seen elevated neurotrophin (BDNF) expression in the rat dentate gyrus. Our current hypothesis is that BDNF expression initiates a gene expression cascade that culminates in neurogenesis that translates to the anti-epileptic effect of DBS surgery. In this paper, we present our methods for DBS surgery targeting the ATN in rats followed by gene expression analysis as an attractive approach to study the mechanism underlying the benefits of DBS.

Protocol

NOTE: Ethics Statement: All procedures discussed in this manuscript are in accordance with the NIH guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals) and are approved by the Harvard Medical School IACUC Committee.

1. Pre-surgical Preparation

1. Make sure that all surgical instruments are sterilized by either autoclaving or by cleansing with antiseptic solution and/or ethanol as necessary. Where possible, use sterile disposable equipment such as scalpels, needle and syringes.
2. Cover the workbench with surgical drapes and ensure that there is a biohazard waste disposal available.
3. Weigh the rat and calculate anesthesia dose. Use a Ketamine/Xylazine mix (Ketamine 75 mg/kg and Xylazine 10 mg/kg) to anesthetize the rats.
   NOTE: Between 200-250 g is the optimal weight for proper fixing of the animal in the stereotactic frame as well as for accurate targeting of the ATN. Isoflurane can also be used as the anesthetizing agent.
4. Mount and secure two sterile electrodes (sterilized by autoclaving or with ethylene oxide) on the electrode holder (**Figure 2**) of a stereotactic surgical frame and with the help of a microscope (10-40X magnification), inspect the tips of the electrode for proper alignment. Secure the two electrodes 3.0 mm apart.
   NOTE: Take care to avoid damaging the electrode tip by touching on hard surfaces.

2. DBS Surgery

1. Inject the Ketamine/Xylazine mix intraperitoneally to the animal and confirm that the animal has reached a surgical plane of anesthesia (by checking for the toe-pinch reflex, respiratory rate and depth and regularity of breathing). Remove hair from the region of the scalp that will be incised and disinfect the scalp with 3 alternating scrubs each of betadine and either alcohol or sterile saline. Secure and position the animal on the stereotactic frame.
2. Use circulating warm water pads to maintain the animal's body temperature at an optimal level. Apply eye lubricant to the animal's eyes to protect from overdrying.
3. Use the following stereotactic coordinates for targeting the ATN (Anterior thalamic nucleus): anterioposterior -1.6 mm, mediolateral 1.5 mm and dorsoventral 5.2 mm[1].
   NOTE: The stereotactic coordinates are based on the Paxinos and Watson (6th edition) rat brain atlas[36].
4. Make an incision in the scalp sagittally to reveal the skull. Using a pair of retractors secure the incised scalp to expose the skull. Use sterile saline to flush the incision. If incision is too wet, use sterile cotton swabs to dry. Locate the bregma and mark with a black marker. To guide the position of the burr holes, make two more marks at approximately 1.5 mm mediolaterally on both sides from the sagittal suture and 1.6 mm posterior to the coronal suture.
5. Use a hand-held drill to make the burr holes. Make sure the tip of the burr hole is sterile by sterilizing it with ethanol. Hold the drill at about a 45° angle to the skull surface when drilling. Frequently switch between the two burr holes to avoid excessive heat at the location of any burr hole.
6. Continue drilling until the dura is exposed. Using a needle with its tip bent resembling the shape of an 'L', remove any broken pieces of bone that would obstruct the insertion of the electrode. Take care to avoid damaging the underlying dura and/or brain tissue while removing bone fragments using the bent needle.
   NOTE: Using a blunted needle or fine blunt forceps is also an option.
7. Fix the dual electrode assembly to the rotating handle of the stereotactic frame and fix the handle at a 90° angle. Using the adjustments in the stereotactic frame, position the left electrode exactly above the bregma.
8. Using the stereotactic adjustments for mediolateral positioning, precisely move the left electrode 1.5 mm to the left side of bregma such that now there are two electrodes perfectly aligned along the coronal suture but spaced apart at 1.5 mm mediolaterally from the bregma.

9. Using the anterioposterior stereotactic adjustments, move the electrodes 1.6 mm posterior to the coronal suture.
10. Use the dorsoventral adjustments to lower the electrodes to first check if the burr holes have been made at the right location such that the electrodes can be inserted with ease, without touching the rough edges of the burr holes. If so, insert the electrodes to a depth of 5.2 mm from the surface of the skull.
11. Connect the electrodes via leads to a stimulator set at 130 Hz, 2.5 V and 90 µsec pulsewidth[1].
12. Deliver high frequency stimulation for an hour (or for a desired period of time as per experimental setup). During the course of the stimulation, remember to assure proper anesthetic depth regularly by checking for the absence of foot withdrawal in response to a toe pinch. Supplement anesthesia with approximately half the initial dose used to induce anesthesia. Avoid contamination of your sterile gloves when checking anesthetic depth and change them if necessary. Perform unilateral or bilateral stimulation based on one's experimental needs. Include controls such as low frequency stimulation (for *e.g.*, 10 Hz) and unstimulated animals (inserting electrodes with no subsequent stimulation).
13. After stimulation is done, remove the electrodes carefully and suture the incision with 3-0 sutures or with sterile surgical staples.
14. Administer buprenorphine (0.05 mg/kg) subcutaneously as analgesia. Monitor the animal until it returns to normal activity and then return it to the housing facility.
15. After a set period of time based on the experimental design (for *e.g.*, 0, 3, 6 or 12 hr post DBS surgery), euthanize the animal with anesthesia overdose. After confirming the absence of vital signs, decapitate the animal.
16. Dissect out the brain by first removing the skin using scissors. Cut through the bone along the sagittal suture using dissection scissors. Make two more incisions (about an inch each) through the bone on both the lateral sides. Using forceps, lift the partially severed piece of bone from the top of the skull to expose the brain.
17. Using fine scissors or forceps, dislodge the brain from the skull and transfer to a Petri dish with cold PBS on ice.
    NOTE: Take care to avoid damage to the brain while making the incisions through the bone.

3. Hippocampus Isolation

NOTE: Perform all the subsequent steps in this section on ice.

1. Place the brain on a pre-cooled acrylic brain matrix on ice. Using a razor blade, cut the brain coronally at approximately 7-8 mm from the anterior-most edge of the brain. Make a second cut coronally and posterior to the first cut such that an approximately 5 mm thick brain slice could be removed.
2. Transfer the brain slice to a Petri dish with ice-cold PBS. Using razor blade, sever the two hemispherical sections and take care to note which hemisphere corresponds to the left and right sides respectively. This is especially important while performing unilateral stimulations.
3. Using fine forceps and scissors remove the hippocampus carefully.
4. Flash freeze the hippocampal tissue on dry ice and store in -20 °C freezer until ready for the subsequent RNA extraction steps.
   NOTE: For long term storage, it is advisable to store tissue in a -80 °C freezer.

4. RNA Extraction and Quantitative PCR

1. Make sure the tissue stays frozen on dry ice until ready for homogenization.
2. NOTE: Perform this step in the hood.
    1. Add 1 ml of Tri reagent to hippocampal tissue in a 1.5 ml centrifuge tube and homogenize it by first pipetting multiple times until the tissue is broken in smaller pieces. Further homogenize the tissue by passing through a syringe with a 25 G needle until there is no unbroken tissue visible. Perform the homogenization steps on ice.
    2. After homogenizing the tissue, allow the cell suspension to stand at room temperature for 5 min to allow cell lysis.
       NOTE: After this point the cell suspension can be quick frozen and stored in -70 °C until further processing.
3. Add 0.2 ml chloroform and mix by vortexing for 20 sec and let the solution rest at room temperature for 15 min.
4. Centrifuge the samples at 12,000 x g for 15 min at 4 °C. After centrifugation, remove the tubes carefully without disturbing the three separate layers that will be visible.
   NOTE: The bottom (red) phase contains proteins, middle cloudy phase contains DNA and the top clear phase contains RNA.
5. Transfer the top clear phase (RNA) into a fresh 1.5 ml centrifuge tube (typically 500 µl of the RNA containing phase is obtained). To this add 0.5 ml isopropanol and mix by vortexing. To this add 2-3 µl glycogen (20 mg/ml) as the carrier for the RNA. Allow the sample to rest on the table at room temperature for 10 min.
6. Centrifuge the sample at 12,000 x g for 1 hour at 4 °C. Make sure that the RNA pellet is visible at the bottom of the tube.
7. Discard the supernatant and wash the RNA pellet by adding 1 ml of 75% ethanol (made with nuclease free water). Invert the sample multiple times until the pellet dislodges from the bottom of the tube and floats in the solution. Centrifuge the solution at 12,000 x g for 10 min at 4 °C.
   NOTE: The pellet in 75% ethanol can be stored at -20 °C until further steps.
8. Allow the RNA pellet to air dry by leaving the tubes open for 5 min at room temperature. Take precaution to avoid over drying the pellet as this might affect its dissolution in water in the subsequent step.

5. Removing DNA from the RNA Preparation

1. Add 9 µl of nuclease free water to the RNA pellet and make sure the pellet is dissolved before proceeding by gently vortexing the tube. To this add 1 µl of 10x Dnase I Buffer and 1 µl recombinant DNase (from DNase I kit), mix by gently flicking the tubes, briefly spin the tubes and incubate at 37 °C in a water bath for 30 min.
2. Add 2 µl DNase inactivation reagent (from DNase I kit) to the sample and incubate at room temperature for 2 min and mix often by gently flicking the tube. Centrifuge at 12,000 x g for 10 min and transfer approximately 10 µl of the clear supernatant to a fresh 1.5 ml centrifuge tube.
3. Measure RNA concentration using a nanodrop or spectrophotometer.

6. Making cDNA from RNA

1. Add requisite volume that contains 1 µg of RNA and bring the total volume to 8 µl by adding nuclease free water. To this add 1 µl 10 mM dNTP mix and 1 µl of random hexamers from the Superscript First Strand Synthesis Kit. Gently mix and incubate at 65 °C for 5 min in either a water bath or on a pre-programmed thermocycler.
2. Make a premix containing the following reagents: 2 µl of 10x RT Buffer, 4 µl of 25 M $MgCl_2$, 2 µl of 0.1 M DTT and 1 µl of RNAse OUT (40 U/µl).
   NOTE: Volumes given are per reaction, user would need to scale up according to one's experimental needs.
3. After removing the RNA containing sample from 65 °C, set the tube at room temperature for a minute. Add 9 µl premix solution and incubate at room temperature for 2 min. Add 1 µl of Superscript II reverse transcriptase enzyme (50 U/µl) and then incubate at room temperature for 10 min.
4. Incubate the samples at 42 °C for 50 min for the reverse transcription to occur.
5. Incubate the samples at 70 °C for 15 min to terminate the reaction.
6. Place the samples on ice briefly and add 1 µl RNAseH (2 U/µl) and incubate at 37 °C for 20 min.
7. Briefly centrifuge the tubes at 3,000 x g to spin down the condensate liquid.
   NOTE: This is the cDNA that will be used for quantitative PCR. cDNA can be stored in -20 °C freezer until PCR setup. cDNA can also be diluted with nuclease free water before proceeding to PCR.

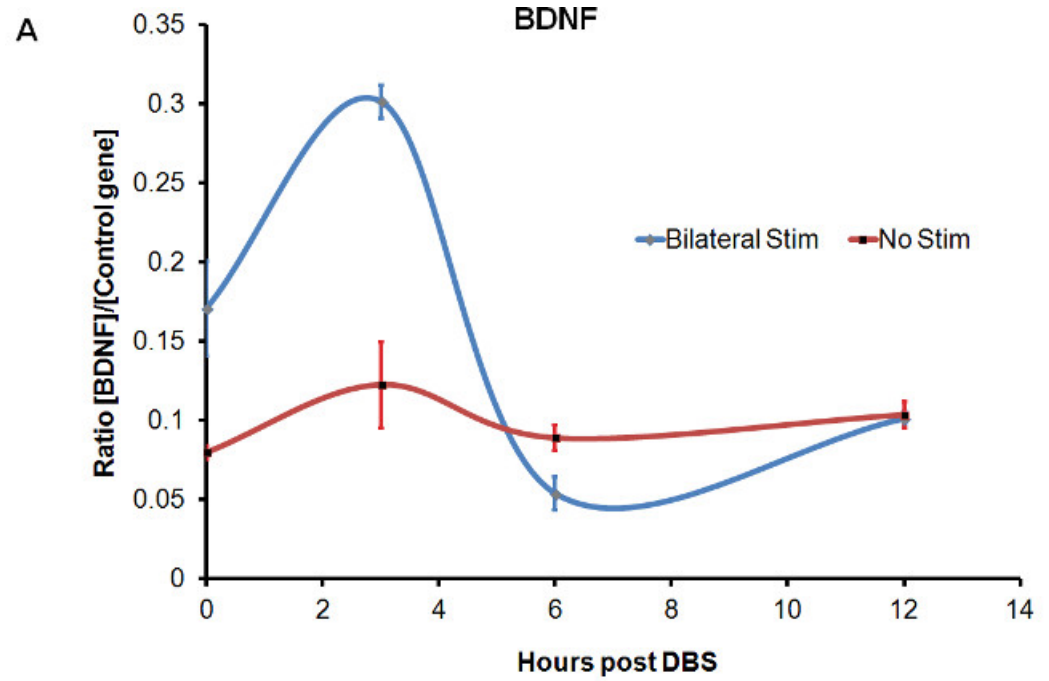
7. Quantitative PCR

1. Make a master mix containing the following reagents (volumes given are per reaction):6.3 µl of 2x Sybr Green Mix, 0.6 µl of Forward Primer (10 µM), 0.6 µl of Reverse Primer (10 µM) and 0.5 µl of Nuclease free water.
   NOTE: Primer design was done using the 'Pick Primers' option in NCBI's nucleotide sequence page for the gene of interest.
2. Add 10 µl mastermix to each well of a 96 well- PCR Plate. Add 2.5 µl of cDNA made earlier to the well containing the mastermix. Make sure to set up triplicate PCR reactions for each sample.
3. After completing the addition of mastermix and cDNA, cover the PCR plate using an optical adhesive sheet to seal the wells. Spin the plate briefly for 5 min at 500 x g in a tabletop centrifuge to settle all the liquid to the bottom of the well.
4. Load the PCR plate onto a pre-programmed RT-PCR machine. Use the PCR parameters provided in **Table 1**.
5. Data analysis: Use the C_t values from the qPCR output for further calculations. Along with the test gene, set up PCR reactions for internal control genes, 18S rRNA (to ensure equal input) and β-actin (negative control). Calculate fold changes based on ΔΔCt method [37].

Representative Results

Figures 1A and **1B** show the relative expression of BDNF and GABRD relative to the control gene β-actin. BDNF, a neurotrophin is often associated with neuroprotective effects in many neuronal diseases[38-41]. It is therefore interesting to analyze the expression profile of BDNF in response to stimulation of the ATN which yields therapeutic benefits to epileptic patients. In **Figure 1A** which shows the gene expression profile of BDNF across the indicated time-points post DBS stimulation, BDNF up-regulation is observed immediately (0 hr) after DBS surgery along with the peak expression (3 fold greater than unstimulated) at 3 hr post stimulation. This observation suggests that enhanced BDNF expression and the resulting neuroprotection could contribute to the therapeutic benefit of DBS. Another gene GABRD (**Figure 1B**) was also investigated using the qPCR method. GABRD is a GABA receptor which is one of the potential targets for designing anti-epilepsy drugs[42]. The expression profile of GABRD also shows enhanced expression in the stimulated animals compared to the unstimulated control animals at 3 hr post DBS. Considering that GABA agonists are used as effective seizure suppressors, it is interesting to observe enhanced GABRD expression post DBS, implicating a possible role for GABA in the anti-epileptic effect of DBS.

The RT-PCR protocol described here yields reproducible and quantitative results that reveal gene expression patterns and the relative fold differences compared to the control animals. The data analysis is performed in the following manner: The qPCR output gives the threshold C_t value for the test gene for each sample analyzed. C_t values are also obtained for the control gene β-actin and 18S rRNA (input control). The $\Delta\Delta C_t$ method will then be used to calculate the gene expression profile using these C_t values[37]. For example, to calculate the gene expression changes for BDNF, for a given sample, the difference between the C_t value for BDNF and 18S rRNA is calculated and is the first ΔC_t. For the same sample, the difference between the C_t value for β-actin and 18S rRNA is calculated to give the second ΔC_t. The difference between the two ΔC_t values is calculated to give $\Delta\Delta C_t$. This $\Delta\Delta C_t$ value is used to calculate 2^ (-$\Delta\Delta C_t$) which gives the relative template abundance for BDNF compared to β-actin. By plotting this value across the different time-points alongside the unstimulated control, the gene expression changes induced by DBS across time-points can be visualized. The above described method could be used effectively to investigate changes in expression for other genes which are potential candidates that are responsive to DBS stimulation and to investigate some of the downstream effects of modulating the expression of these genes.



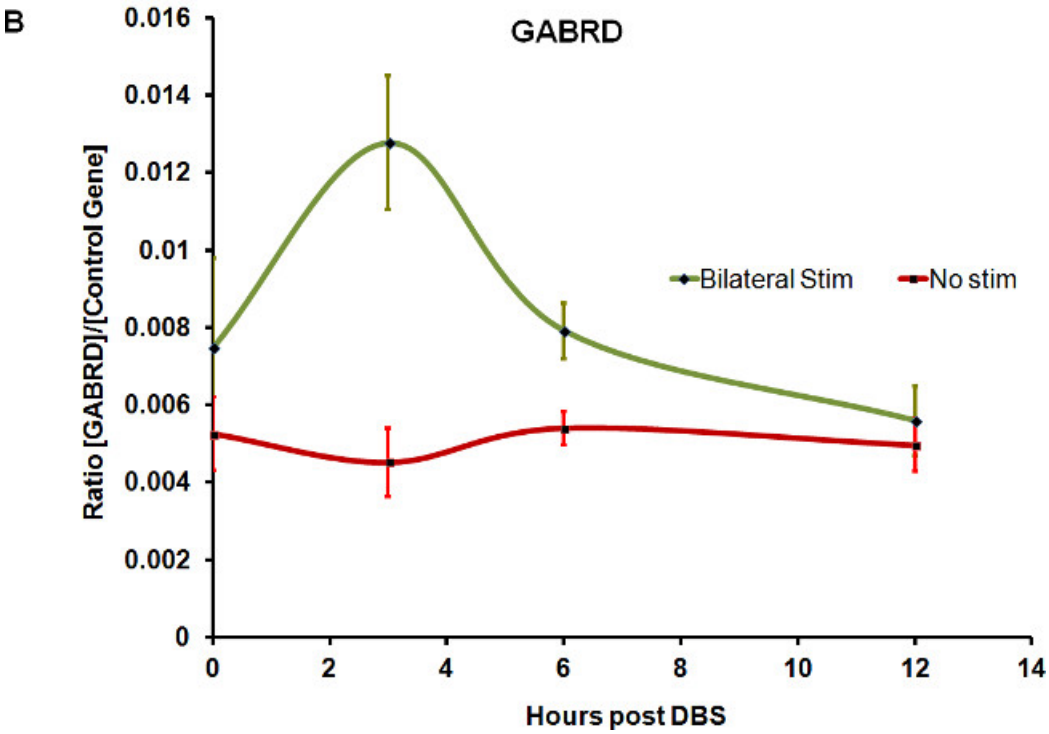


Figure 1: (A) Time course analysis of BDNF expression in response to high frequency stimulation of the ATN. Tissue harvesting, RNA extraction, cDNA preparation and q-PCR were performed as explained in the protocol. Relative changes in gene expression are calculated after normalizing for input (by amplifying 18S rRNA) as well as a control gene (β-actin). C_t values obtained from the real-time PCR were used to calculate expression levels by the $\Delta\Delta C_t$ method [37]. The time-points analyzed are 0, 3, 6 and 12 hr post DBS stimulation. Note: The timepoints selected here are with respect to a particular study and is subject to change according to the hypothesis and experimental plan. **(B)** Time course analysis of GABA A receptor delta subunit (GABRD) levels in response to DBS at 130 Hz targeting the ATN. Methods and calculations were done as similar to the BDNF data.

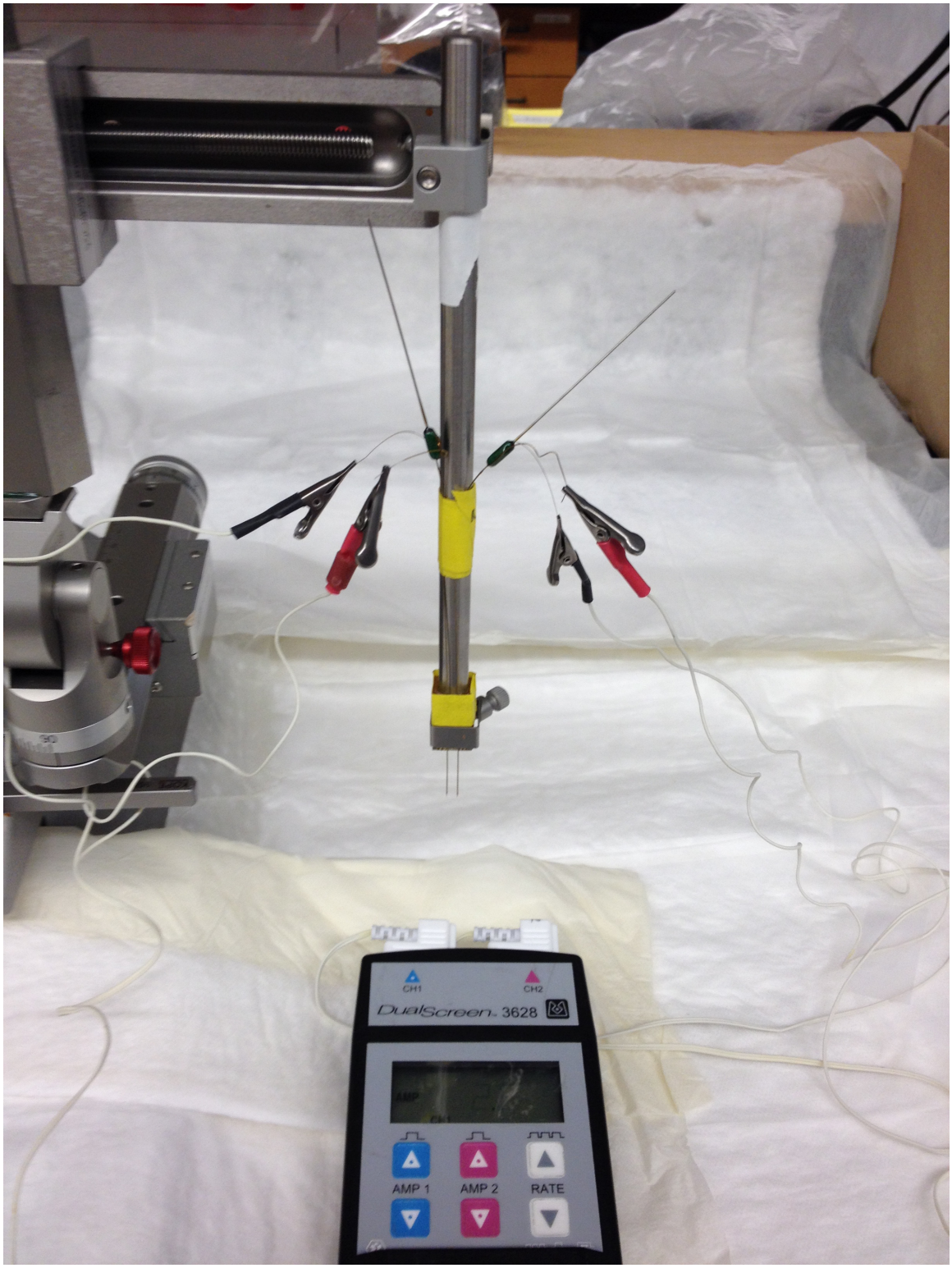


Figure 2: DBS electrodes and stimulator set up.

	PCR Cycles		
Stage 1:	Initial Denaturation	95 °C	15 min
Stage 2:	Denaturation	95 °C	15 sec
	Annealing	60 °C	30 sec
	Extension	72 °C	30 sec

	40 cycles of Step 2		
Note: The annealing temperature varies according to the			
primer melting temperature. Primers are typically designed			
to have an optimal annealing temperature of 60 °C			

Table 1: PCR Parameters.

Discussion

Following the landmark work by Benabid *et al.* in using deep brain stimulation to treat Parkinson's disease and essential tremor, the DBS surgical technique has been investigated with much interest over the past decade to treat many neurological disorders[6,10,43]. DBS studies targeting various neuro-anatomical regions of the brain circuitry are currently performed by many groups to address major neuronal diseases and are in various stages of clinical trials. Stimulation of the subthalamic nucleus (STN) or the internal segment of the globus pallidus (GPi) is FDA approved and used in treating movement disorders in Parkinson's disease[10]. The SANTE clinical trial has shown promising trends for epileptic patients receiving high frequency stimulation of the anterior thalamic nucleus[31]. Results from a phase I clinical trial of bilateral forniceal stimulation have shown a delay in the rate of cognitive decline and a reversal of the glucose hypometabolic uptake seen in Alzheimer's disease as well as activation of the memory circuitry[8,44]. Furthermore, in recent years neurosurgeons have conducted DBS trials for treating neuropsychiatric disorders such as OCD (Obsessive Compulsive Disorder), treatment-resistant depression, Tourette syndrome and addiction[10,45-55].

In addition to the clinical trials, over the past few years, animal surgery has offered us great opportunities to study the physiological changes induced by the surgical technique in a live animal, in a manner unparalleled by any *in vitro* technique. In this manuscript, we have discussed the methods involved in performing deep brain stimulation surgery in rodents. Stereotactic surgery in rodents as described here could also be used for potential DBS target searches and to test out the efficacy of the surgery using disease model animals. One of the challenges for the experimenter here is to be able to target the correct anatomical locus in a reproducible manner. There is especially a need for a skilled technician for the surgery because checking for correct targeting is possible only after the stimulation is done and the animal euthanized. Also occasionally, one might accidentally injure a key blood vessel which could lead to significant blood loss and sometimes even death of the animal. In addition, the need to dissect out the hippocampus for further biochemical analysis limits the possibility of immunohistological verification for proper electrode targeting in the same animal which requires an intact brain specimen for tissue sectioning. Proper electrode targeting could possibly be checked on a different animal stimulated in an identical manner. However, this does not provide evidence for proper targeting in the test animal and is a limitation of this approach. Recent publications have tried to circumvent this problem by conducting DBS surgery with simultaneous fMRI[56]. One possible improvement of the technique described here could be a study on the effects of chronic stimulation via an implanted stimulator in the animal. However, we have limited our analysis for a single dose (1 hr) of high-frequency stimulation as the first step to understanding the changes induced by DBS at a cellular level.

Considering the use of DBS surgery for a variety of neuronal disorders, it is essential that we know the mechanism underlying the beneficial effects of DBS. This information is critical for developing future improvements in the surgery and also to explore the utility of DBS as a treatment for other conditions which haven't been investigated by experts in DBS. In addition, modifications to the surgical technique can be implemented to avoid certain deleterious side-effects of the procedure and to effectively deal with recurrence of the disease condition.

An in-depth mechanistic analysis of DBS is possible by examining the gene expression changes induced by DBS. Either a candidate gene approach based on existing knowledge about neuronal pathways that respond to depolarizing stimuli such as high frequency stimulation, or a global transcriptome analysis can give important insights into the molecular events triggered by DBS. The gene expression analysis techniques (candidate gene approach as well as high-throughput method) are powerful tools that are key to exploring the molecular mechanisms and cellular changes associated with DBS surgery. Recent developments in this area have made it possible for us to get a wealth of information about several aspects of cellular physiology in a very short time, which was not possible a few years ago. With the advent of high throughput sequencing technology such as ChIPseq, it is possible to characterize the genome-wide location of important transcription factors which respond to DBS[57,58]. Recent discoveries linking non-coding RNA such as microRNA with neurodegenerative diseases, enable us to analyze possible changes in miRNA levels in neurons post-DBS using miRNA sequencing technology[59,60]. Possible changes in epigenetic signatures such DNA methylation and histone modifications in response to DBS could also be explored. However, despite the advantages, it is important to acknowledge some of the limitations of these techniques as well as potential problems that might arise during analyses. An important concern with gene expression analyses has been reproducibility and technical errors. It is important that the experimenter takes note of this and plans on having adequate number of repetitions to ensure reliability. A common problem with some of the high throughput screening studies has been the difficulty in interpretation of the tremendous amount of data that is generated. Sometimes it becomes important to determine whether the gene expression changes observed are due to the direct effect of the experimental treatment or is a downstream effect. This usually requires additional studies that are designed to address this issue.

In addition to the genomic studies, an extensive immunohistochemical analysis of the spatio-temporal localization of the key players that respond to DBS and a quantitation of the changes in their levels in various regions of the brain will be a great asset to future developments of the DBS surgical procedure. The cumulative findings from the gene expression analyses as well as the immunohistochemical studies can reveal novel interactions between key factors as well as key molecular events that regulate cellular processes such as neurogenesis or neurodegeneration. Identification of such critical molecular markers may also enable future drug discoveries. Such findings could shed light on the general functioning of the brain circuitry, which is valuable information from the perspective of scientists working to understand many neuronal diseases. Directing our future efforts on integrating latest technological advances made in the clinic as well as the laboratory is likely to offer us substantial advantages in our fight against disease.

Disclosures

The authors have no disclosures.

Acknowledgements

We are grateful for the support of the NREF foundation.

References

1. Toda, H., Hamani, C., Fawcett, A. P., Hutchison, W. D., Lozano, A. M. The regulation of adult rodent hippocampal neurogenesis by deep brain stimulation. *Journal of.* **108**, 132-138 (2008).
2. Perlmutter, J. S., Mink, J. W. Deep brain stimulation. *Annual review of neuroscience.* **29**, 229-257 (2006).
3. Bergstrom, M. R., Johansson, G. G., Laitinen, L. V., Sipponen, P. Electrical stimulation of the thalamic and subthalamic area in cerebral palsy. *Acta physiologica Scandinavica.* **67**, 208-213 (1966).
4. Benabid, A. L., *et al.* Chronic electrical stimulation of the ventralis intermedius nucleus of the thalamus as a treatment of movement disorders. *Journal of neurosurgery.* **84**, 203-214 (1996).
5. Benabid, A. L., *et al.* Long-term electrical inhibition of deep brain targets in movement disorders. *Movement disorders : official journal of the Movement Disorder Society.* **13**, Suppl 3. 119-125 (1998).
6. Benabid, A. L., *et al.* Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. *Lancet.* **337**, 403-406 (1991).
7. Tierney, T. S., Lozano, A. M. Surgical treatment for secondary dystonia. *Movement disorders : official journal of the Movement Disorder Society.* **27**, 1598-1605 (2012).
8. Tierney, T. S., Sankar, T., Lozano, A. M. Some recent trends and further promising directions in functional neurosurgery. *Acta neurochirurgica. Supplement.* **117**, 87-92 (2013).
9. Tierney, T. S., Sankar, T., Lozano, A. M. Deep brain stimulation emerging indications. *Progress in brain research.* **194**, 83-95 (2011).
10. Sankar, T., Tierney, T. S., Hamani, C. Novel applications of deep brain stimulation. *Surgical neurology international.* **3**, S26-S33 (2012).
11. Tierney, T. S., Vasudeva, V. S., Weir, S., Hayes, M. T. Neuromodulation for neurodegenerative conditions. *Frontiers in bioscience.* **5**, 490-499 (2013).
12. Mayberg, H. S., *et al.* Deep brain stimulation for treatment-resistant depression. *Neuron.* **45**, 651-660 (2005).
13. Lyons, M. K. Deep brain stimulation: current and future clinical applications. *Mayo Clinic proceedings.* **86**, 662-672 (2011).
14. Ch. 4. *Rehabilitation in movement disorders.* Lansek, R., Morris, M. E. 36-43 Cambridge University Press (2013).
15. Goodwin, R., Tierney, T., Lenz, F., Anderson, W. Ch. 15. *Single Neuron Studies of the Human Brain: Probing Cognition.* Fried, I., Rutishauser, U., Cerf, M., Kreiman, G. **15**, 275-293 MIT Press (2014).
16. Hauser, W. A. Epidemiology of epilepsy in children. *Neurosurgery clinics of North America.* **6**, 419-429 (1995).
17. Hauser, W. A. Recent developments in the epidemiology of epilepsy. *Acta neurologica Scandinavica. Supplementum.* **162**, 17-21 (1995).
18. Davis, R., Emmonds, S. E. Cerebellar stimulation for seizure control: 17-year study. *Stereotactic and functional neurosurgery.* **58**, 200-208 (1992).
19. Levy, L. F., Auchterlonie, W. C. Chronic cerebellar stimulation in the treatment of epilepsy. *Epilepsia.* **20**, 235-245 (1979).
20. Velasco, A. L., *et al.* Electrical stimulation of the hippocampal epileptic foci for seizure control: a double-blind, long-term follow-up study. *Epilepsia.* **48**, 1895-1903 (2007).
21. Tellez-Zenteno, J. F., McLachlan, R. S., Parrent, A., Kubu, C. S., Wiebe, S. Hippocampal electrical stimulation in mesial temporal lobe epilepsy. *Neurology.* **66**, 1490-1494 (2006).
22. Kerrigan, J. F., *et al.* Electrical stimulation of the anterior nucleus of the thalamus for the treatment of intractable epilepsy. *Epilepsia.* **45**, 346-354 (2004).
23. Hamani, C., *et al.* Deep brain stimulation of the anterior nucleus of the thalamus: effects of electrical stimulation on pilocarpine-induced seizures and status epilepticus. *Epilepsy research.* **78**, 117-123 (2008).
24. Hamani, C., *et al.* Bilateral anterior thalamic nucleus lesions and high-frequency stimulation are protective against pilocarpine-induced seizures and status epilepticus. *Neurosurgery.* **54**, 191-195 (2004).
25. Mirski, M. A., Rossell, L. A., Terry, J. B., Fisher, R. S. Anticonvulsant effect of anterior thalamic high frequency electrical stimulation in the rat. *Epilepsy research.* **28**, 89-100 (1997).
26. Mirski, M. A., Ferrendelli, J. A. Interruption of the mammillothalamic tract prevents seizures in guinea pigs. *Science.* **226**, 72-74 (1984).
27. Mirski, M. A., Ferrendelli, J. A. Anterior thalamic mediation of generalized pentylenetetrazol seizures. *Brain research.* **399**, 212-223 (1986).
28. Mirski, M. A., Ferrendelli, J. A. Interruption of the connections of the mammillary bodies protects against generalized pentylenetetrazol seizures in guinea pigs. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* **7**, 662-670 (1987).
29. Mirski, M. A., McKeon, A. C., Ferrendelli, J. A. Anterior thalamus and substantia nigra: two distinct structures mediating experimental generalized seizures. *Brain research.* **397**, 377-380 (1986).
30. Lee, K. J., Jang, K. S., Shon, Y. M. Chronic deep brain stimulation of subthalamic and anterior thalamic nuclei for controlling refractory partial epilepsy. *Acta neurochirurgica. Supplement.* **99**, 87-91 (2006).
31. Fisher, R., *et al.* Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. *Epilepsia.* **51**, 899-908 (2010).
32. Encinas, J. M., Hamani, C., Lozano, A. M., Enikolopov, G. Neurogenic hippocampal targets of deep brain stimulation. *The Journal of comparative neurology.* **519**, 6-20 (2011).
33. Hattiangady, B., Rao, M. S., Shetty, A. K. Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. *Neurobiology of disease.* **17**, 473-490 (2004).
34. Kuruba, R., Hattiangady, B., Shetty, A. K. Hippocampal neurogenesis and neural stem cells in temporal lobe epilepsy. *Epilepsy & behavior : E&B.* **14**, Suppl 1. 65-73 (2009).

35. Hattiangady, B., Shetty, A. K. Implications of decreased hippocampal neurogenesis in chronic temporal lobe epilepsy. *Epilepsia.* **49**, Suppl 5. 26-41 (2008).
36. Paxinos, G., Watson, C. *The Rat Brain in Stereotaxic Coordinates.* Elsevier Inc (2007).
37. Livak, K. J., Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 22DDCT Method. *METHODS.* **25**, 402-408 (2001).
38. Kells, A. P., *et al.* AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. *Molecular therapy : the journal of the American Society of Gene Therapy.* **9**, 682-688 (2004).
39. Han, B. H., Holtzman, D. M. BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* **20**, 5775-5781 (2000).
40. Hetman, M., Kanning, K., Cavanaugh, J. E., Xia, Z. Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. *The Journal of biological chemistry.* **274**, 22569-22580 (1999).
41. Stadelmann, C., *et al.* BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells. *Brain : a journal of neurology.* **125**, 75-85 (2002).
42. Treiman, D. M. GABAergic mechanisms in epilepsy. *Epilepsia.* **42**, Suppl 3. 8-12 (2001).
43. Benabid, A. L., Pollak, P., Louveau, A., Henry, S., de Rougemont, J. Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for bilateral Parkinson disease. *Applied neurophysiology.* **50**, 344-346 (1987).
44. Laxton, A. W., *et al.* A phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. *Annals of.* **68**, 521-534 (2010).
45. Tierney, T. S., Abd-El-Barr, M. M., Stanford, A. D., Foote, K. D., Okun, M. S. Deep brain stimulation and ablation for obsessive compulsive disorder: evolution of contemporary indications, targets and techniques. *The International journal of neuroscience.* **124**, 394-402 (2014).
46. Kennedy, S. H., *et al.* Deep brain stimulation for treatment-resistant depression: follow-up after 3 to 6 years. *The American journal of psychiatry.* **168**, 502-510 (2011).
47. Lozano, A. M., *et al.* Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. *Biological psychiatry.* **64**, 461-467 (2008).
48. Ackermans, L., *et al.* Double-blind clinical trial of thalamic stimulation in patients with Tourette syndrome. *Brain : a journal of neurology.* **134**, 832-844 (2011).
49. Houeto, J. L., *et al.* Tourette's syndrome and deep brain stimulation. *Journal of neurology, neurosurgery, and psychiatry.* **76**, 992-995 (2005).
50. Maciunas, R. J., *et al.* Prospective randomized double-blind trial of bilateral thalamic deep brain stimulation in adults with Tourette syndrome. *Journal of neurosurgery.* **107**, 1004-1014 (2007).
51. Koning, P. P., Figee, M., van den Munckhof, P., Schuurman, P. R., Denys, D. Current status of deep brain stimulation for obsessive-compulsive disorder: a clinical review of different targets. *Current psychiatry reports.* **13**, 274-282 (2011).
52. Greenberg, B. D., *et al.* Deep brain stimulation of the ventral internal capsule/ventral striatum for obsessive-compulsive disorder: worldwide experience. *Molecular psychiatry.* **15**, 64-79 (2010).
53. Muller, U. J., *et al.* Successful treatment of chronic resistant alcoholism by deep brain stimulation of nucleus accumbens: first experience with three cases. *Pharmacopsychiatry.* **42**, 288-291 (2009).
54. Zhou, H., Xu, J., Jiang, J. Deep brain stimulation of nucleus accumbens on heroin-seeking behaviors: a case report. *Biological psychiatry.* **69**, e41-e42 (2011).
55. Porta, M., *et al.* Thalamic deep brain stimulation for treatment-refractory Tourette syndrome: two-year outcome. *Neurology.* **73**, 1375-1380 (2009).
56. Younce, J. R., Albaugh, D. L., Shih, Y. Y. Deep brain stimulation with simultaneous FMRI in rodents. *Journal of visualized experiments : JoVE.* e51271 (2014).
57. Park, P. J. ChIP-seq: advantages and challenges of a maturing technology. *Nature reviews. Genetics.* **10**, 669-680 (2009).
58. Valouev, A., *et al.* Genome-wide analysis of transcription factor binding sites based on ChIP-Seq data. *Nature methods.* **5**, 829-834 (2008).
59. Fiore, R., Siegel, G., Schratt, G. MicroRNA function in neuronal development, plasticity and disease. *Biochimica et biophysica acta.* **1779**, 471-478 (2008).
60. Hebert, S. S., De Strooper, B. Alterations of the microRNA network cause neurodegenerative disease. *Trends in neurosciences.* **32**, 199-206 (2009).

