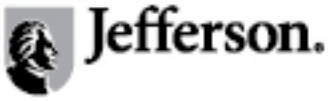


12-1-2011

An evaluation of neuroplasticity and behavior after deep brain stimulation of the nucleus accumbens in an animal model of depression.

Steven M Falowski
Thomas Jefferson University, sfalowski@gmail.com

Ashwini Sharan
Thomas Jefferson University, Ashwini.Sharan@jefferson.edu

Beverly A S Reyes
Thomas Jefferson University, beverly.reyes@jefferson.edu

Carl Sikkema
MIRECC, VA Puget Sound Health Care System

Patricia Szot
MIRECC, VA Puget Sound Health Care System

See next page for additional authors

Authors
Steven M Falowski, Ashwini Sharan, Beverly A S Reyes, Carl Sikkema, Patricia Szot, and Elisabeth J Van Bockstaele

Editorial Manager(tm) for Neurosurgery
Manuscript Draft

Manuscript Number: NEU-D-10-01544

Title: An Evaluation of Neuroplasticity and Behavior Following Deep Brain Stimulation of the Nucleus Accumbens in an Animal Model of Depression

Short Title: Neuroplasticity and Behavior following DBS

Article Type: Research - Animal

Section/Category: Stereotactic + Functional

Keywords: deep brain stimulation, DBS, depression, dendritic plasticity ,neuroplasticity, nucleus accumbens,

Corresponding Author: Steven M Falowski, M.D.

Corresponding Author's Institution: Thomas Jefferson University Hospital- Dept of Neurosurgery

First Author: Steven M Falowski, M.D.

Order of Authors: Steven M Falowski, M.D.;Ashwini Sharan, MD;Beverly A.S. Reyes, DVM, PhD;Carl Sikkema, BS;Patricia Szot, PhD;Elisabeth J Van Bockstaele, PhD

Manuscript Region of Origin: UNITED STATES

Abstract: Background:Recent interest has demonstrated the nucleus accumbens(NAcc) as a potential target for the treatment of depression with DBS.

Objective:This study sought to demonstrate that DBS of the NAcc is an effective treatment modality for depression and that there is chemical and structural changes associated with these behavioral changes that are markers of neuroplasticity.

Methods:A deep brain stimulator was placed in the NAcc of male Wistar-Kyoto rats. Groups were divided into sham(no stimulation), intermittent(3 hrs/day for two weeks) or chronic(constant stimulation for two weeks). Exploratory and anxiety-like behaviors were evaluated using the open field test before and after stimulation. Tissue samples of the prefrontal cortex(PFC) were processed using Western blot analysis of markers of noradrenergic activity that included the noradrenergic synthesizing enzyme tyrosine hydroxylase(TH). Analysis of tissue levels for catecholamines was achieved using high performance liquid chromatography. Finally, morphological properties of cortical pyramidal neurons were assessed using Golgi-cox staining.

Results:Subjects undergoing intermittent and chronic stimulation of the NAcc exhibited an increase in exploratory behavior and reduced anxiety-like behaviors.TH expression levels were decreased in the PFC following intermittent and continuous DBS, and dopamine and norepinephrine levels were decreased following chronic stimulation. Golgi-Cox staining indicated that DBS increased the length of apical and basilar dendrites in pyramidal neurons of the PFC.

Conclusion:DBS induces behavioral improvement as well as

neurochemical and morphological alterations in the PFC that demonstrate changes within the circuitry of the brain different from the target area of stimulation. This observed dendritic plasticity may underlie the therapeutic efficacy of this treatment.

Suggested Reviewers: Ali Rezai MD
Neurosurgeon, Neurosurgery, Ohio State University
ali.rezai@osumc.edu
Leader in functional neurosurgery

Andre Machado MD
Neurosurgery, Cleveland Clinic
machada@ccf.org
Leader in RAT research pertaining to functional neurosurgery

Brian Kopell MD
Neurosurgery, Medical College of Wisconsin
bkopell@neuroscience.mcw.edu

Richard Byrne MD
Neurosurgery, Rush University
richard_byrne@rush.edu

Opposed Reviewers:

Cover Letter

Please find attached the manuscript entitled “An evaluation of neuroplasticity and behavior following deep brain stimulation of the nucleus accumbens in an animal model of depression” by Steven M. Falowski, Ashwini Sharan, Beverly A.S. Reyes, Carl Sikkema, Patricia Szot and Elisabeth J. Van Bockstaele. The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this manuscript. This manuscript is not currently submitted to other journals.

Thank you for taking the time to consider this article.

Steven M. Falowski
Department of Neurosurgery
Thomas Jefferson University
Philadelphia, PA 19107

An Evaluation of Neuroplasticity and Behavior Following Deep Brain Stimulation of the Nucleus Accumbens in an Animal Model of Depression

Steven M. Falowski, MD[1], Ashwini Sharan, MD[1], Beverly A.S. Reyes, DVM, PhD[2], Carl Sikkema, BS[3], Patricia Szot, PhD[3], and Elisabeth J. Van Bockstaele PhD[2*],

[1]Department of Neurosurgery
Thomas Jefferson University
Philadelphia, PA 19107

[2]Department of Neuroscience
Farber Institute for Neurosciences
Thomas Jefferson University
Philadelphia, PA 19107

[3]MIRECC, VA Puget Sound Health Care System,
1660 S Columbian Way
Seattle, WA 98108

Running Title: Neuroplasticity and Behavior following DBS

Corresponding Author: Steven M. Falowski, M.D.
Department of Neurosurgery
Thomas Jefferson University
909 Walnut Street
Philadelphia, PA 19107
Voice: (215) 955- 7000
e-mail: sfalowski@gmail.com

Abstract

Background: Recent interest has demonstrated the nucleus accumbens(NAcc) as a potential target for the treatment of depression with DBS.

Introduction: This study sought to demonstrate that DBS of the NAcc is an effective treatment modality for depression and that there is chemical and structural changes associated with these behavioral changes that are markers of neuroplasticity.

Methods A deep brain stimulator was placed in the NAcc of male Wistar-Kyoto rats. Groups were divided into sham(no stimulation), intermittent(3 hrs/day for two weeks) or chronic(constant stimulation for two weeks). Exploratory and anxiety-like behaviors were evaluated using the open field test before and after stimulation. Tissue samples of the prefrontal cortex(PFC) were processed using Western blot analysis of markers of noradrenergic activity that included the noradrenergic synthesizing enzyme tyrosine hydroxylase(TH). Analysis of tissue levels for catecholamines was achieved using high performance liquid chromatography. Finally, morphological properties of cortical pyramidal neurons were assessed using Golgi-cox staining.

Results: Subjects undergoing intermittent and chronic stimulation of the NAcc exhibited an increase in exploratory behavior and reduced anxiety-like behaviors.TH expression levels were decreased in the PFC following intermittent and continuous DBS, and dopamine and norepinephrine levels were decreased following chronic stimulation. Golgi-Cox staining indicated that DBS increased the length of apical and basilar dendrites in pyramidal neurons of the PFC.

Conclusion: DBS induces behavioral improvement as well as neurochemical and morphological alterations in the PFC that demonstrate changes within the circuitry of the brain different from the target area of stimulation. This observed dendritic plasticity may underlie the therapeutic efficacy of this treatment.

INTRODUCTION

Deep brain stimulation (DBS) is a neurosurgical method that involves a lead or electrode being placed in a selected region of the brain [1]**.** It is a reversible procedure that allows an adjustable treatment tailored to the patient. It has become a safe and effective treatment in various neurological disorders including Parkinson's disease and essential tremor [2 3; 4]. More recently, this approach has been applied for the treatment of obsessive-compulsive disorder, epilepsy, and depression [4].

Depression is a major cause of disability worldwide [9] and accounts for more than $83 billion in domestic costs in the US alone [10]. Lifetime prevalence varies widely, from 3% in Japan to 17% in the US; however, in most countries, the number of people who suffer from depression at one time in their lives falls within an 8–12% range [11; 12]. Most patients are treated with antidepressant medications and some with psychotherapy or counseling. A minority is treated with electroconvulsive therapy [13].

The role of the prefrontal cortex is important in understanding the nucleus accumbens and its circuitry. It has been shown that the nucleus accumbens core is involved in addiction and drug behavior and the nucleus accumbens shell (NAccS) is involved in pleasure, fear behavior, and food intake. [27; 28; 29] The nucleus accumbens has intricate connections with the prefrontal cortex and limbic system. It is these connections that are thought to be involved in the underlying symptomatology seen in depression. Therefore, DBS to the nucleus accumbens is a potential target in the treatment of depression and its effects on the prefrontal cortex become of interest.

Animal models of epilepsy have benefited from stimulation of the subthalamic nucleus, substantia nigra and anterior thalamus [5; 6; 7]. Rats maintained on a high fat diet, as a model of obesity, experienced significant and sustained weight loss following continuous stimulation of

the lateral hypothalamic nucleus [8]. However, despite potential advances and therapeutic benefits of DBS, the cellular basis for the efficacy of this treatment remains elusive. Thus, studying the impact of DBS in pre-clinical models is important in establishing mechanisms underlying potential therapeutic improvements.

The Wistar-Kyoto rat strain has been shown to be a useful preclinical model exhibiting a depressive and anxious-like phenotype [14; 15; 16]. This strain exhibits hormonal [14; 17] and behavioral [15; 16] abnormalities consistent with a depressive and anxious phenotype. Several studies have demonstrated differing responses to antidepressants, as well as deficits in reward behavior and changes in hormonal levels in this strain [18; 19; 20].

While DBS has been theorized to work by various mechanisms [21; 22] neuroplasticity is a potential mechanism by which DBS results in therapeutic improvements [23]. Neuroplasticity, also known as brain plasticity can be defined as changes that occur in organization of the brain as a result of experience [24]. Areas related to memory formation, such as the hippocampus and dentate gyrus are highly plastic and produce new neurons in a continuous fashion into adulthood [24]. It has been shown that changes in long-term potentiation of cells induced by high-frequency stimulation of the subthalamic nucleus is a mechanism that determines the effectiveness of DBS in the treatment of Parkinson's disease [25]. Thus, this study aimed to show a relationship between DBS-induced changes in behavior with potential neurochemical and morphological changes.

Owing to the importance of the NAcc in regulating motivated behaviors and mood control [26; 27; 28; 29], the hypothesis that DBS of this region may be relevant in the treatment of depression was tested in the present study. Behavioral studies were conducted following stimulation of the NAcc and subsequently neurochemical and morphological changes were assessed in the prefrontal cortex (PFC).

MATERIALS AND METHODS

Animals

The animal procedures used were approved by the Institutional Animal Care and Use Committee at Thomas Jefferson University and conform with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. Seventy nine adult male Wistar-Kyoto strain (250-275g; Charles River Laboratories International, Inc., Wilmington, MA) were used in the present study. Rats were caged individually on a 12-h light schedule (lights on at 0700) in a temperature-controlled (20 ºC) colony room. Food and water was made freely available. Rats were allowed to acclimate to the animal housing facility for several days prior to the onset of the study. All efforts were made to utilize only the minimum number of animals necessary to produce reliable scientific data, and experiments were designed to minimize any animal distress.

Study Design

All rats were randomly divided into three experimental groups (15 rats per group). Each rat underwent behavorial testing via an open-field prior to implantation. For the sham stimulation group, animals were sedated and underwent surgery with implantation of the electrode with no stimulation. For the intermittent group, animals were administered stimulation for the same 3hrs/day for two weeks (14 days). The stimulation was turned on daily from 8am until 11am each day of the 14 day period. For the chronic group, animals were administered constant stimulation for two weeks (14 days). Chronic stimulation occurred 24 hours per day for the full 14 days. They underwent behavioral evaluation post stimulation period, and were then exposed to isoflurane and euthanized by decapitation. Brains were removed and used for protein extraction and analysis.

DBS Implantation

Each rat underwent stereotactic placement of a 0.25mm bipolar stimulating electrode (Plastics One Products) on the right side. Rats were initially anesthetized with a combination of ketamine hydrochloride (100 mg/kg) and xylazine (2mg/kg) in saline and placed in a stereotaxic apparatus for surgery. Anesthesia was supplemented with isoflurane (Abbott Laboratories; 0.5-1.0%, in air) via a specialized nose cone affixed to the sterotaxic frame (Stoelting Corp.). The electrodes were placed via the coordinates determined by the Paxinos and Watson rat brain atlas (1.5mm anterior from bregma, 0.8mm medial/lateral, -7.4mm ventral from the top of the skull). The electrode was secured using dental cement and the incision was closed using staples.

Each rat was allowed a 7-day recovery period after implantation. After this period they underwent stimulation via a continuous stimulus generator (Medtronic Screener Model 3638). The stimulation parameters were frequency 120Hz, pulse width 200msec, and strength 2 volts. The rats in the control group were connected to the generators which were not turned on.

Open field test

The open field was a square arena consisting of plexiglass walls and floor, measuring 43.2cm x 43.2 cm x 30.5 cm. Rats were placed in the testing room 1 hour prior to behavioral tests. The behavioral analysis pre- and post-stimulation was kept constant by ensuring that the same testing room, same lighting and analysis was performed during the same time period of the day, by the same observer who was blinded to the experimental groups. The behavioral test post-stimulation was done three hours after the last stimulation for both intermittent and chronic DBS.

The open field test was utilized to evaluate the exploratory, anxiety, and depression-like behavior of the rats. Rats were placed in the open field test and recorded by the observer. They were observed for a 5-minute period in which their activity was measured based on the amount of quadrant changes and time spent on their hind legs as a marker of exploratory behavior or

locomotor activity. Crossing of midline was also analyzed and used as a marker of anxiety based on the difference of the rat spending time in either the periphery or center of the field test.

Histology

Following the stimulation period and final behavioral analysis the rats were immediately euthanized. The brain tissue was rapidly harvested for protein extraction and analysis. The electrode track was stained with Cresyl violet to confirm accurate placement of the electrode. The placement of the electrode targeted the NAcc at antero-posterior level that corresponded to Figure 12 of the brain atlas of Paxinos and Watson [30] (Figure 1)**.** Rats that did not show optimal placement of the electrode were not included in the analysis.

Brain Harvesting

The PFC extends from the rostrocaudal segment of the cerebral cortex and is located at a level 5.2 mm anterior to bregma and extends 3.3 posterior to bregma [30]. Anatomically forming the anterior part of the forebrain, the PFC is bounded medially by the median plane dividing the cerebral lobes, ventrally by the medial orbital cortex (5.2 mm anterior to bregma - 3.2 mm anterior to bregma), dorsal peduncular cortex (3.2 mm anterior to bregma - 2.2 mm anterior to bregma) or corpus callosum (1.7 mm anterior to bregma - 3.3 mm posterior from bregma) and caudally by the occipital area [30]. In the present study, the PFC was microdissected (approximately covering the PFC area at a level 3.7 mm anterior to bregma extending at a level 1.7 posterior to bregma).

Protein extraction

Brain tissue was rapidly removed from each animal and put on ice following the final behavioral analysis. Using a trephine, the prefrontal brain region was microdissected from each animal. The PFC was homogenized with a pestle and extracted in radioimmunoprecipitation assay lysis buffer (Santa Cruz Biotechnology) on ice for 20 min. Lysates were cleared by centrifugation at 13,000 rpm for 12 min at 41°C. Supernatants or protein extracts were diluted with an equal volume of Novex 2® tris glycine sodium dodecyl sulfate sample buffer (Invitrogen) containing dithiothreitol (Sigma-Aldrich Inc.). Protein concentrations of the undiluted supernatants were quantified using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL).

Western blot analysis

Cell lysates containing equal amounts of protein were separated on 4–12% tris-glycine polyacrylamide gels and then electrophoretically transferred to Immobilon-P polyvinylidene fluoride membranes (Millipore). Membranes were incubated in mouse anti-TH (Immunostar Inc.) primary antibody overnight and then in alkaline phosphatase-conjugated secondary antibodies for 30 min to probe for the presence of proteins using a Western blotting detection system (Western Breeze Chemiluminescent Kit; Invitrogen). Following incubation in a chemiluminescent substrate (Western Breeze Chemiluminescent Kit), blots were exposed to X-OMAT AR film (Kodak) for different lengths of time to optimize exposures. TH was readily detected by immunoblotting in rat PFC extracts. TH immunoreactivity was visualized as a single band that migrates at approximately 60kDA. Blots were incubated in stripping buffer (Restore Stripping Buffer, Pierce) to disrupt previous antibody-antigen interactions and then re-probed with β-actin (1:5,000, Sigma-Aldrich Inc.) with 1-hour incubation to ensure proper protein

loading. The density of each band was quantified using Un-Scan-It blot analysis software (Silk Scientific Inc.). TH was normalized to β-actin immunoreactivity on each respective blot.

High Pressure Liquid Chromatography

Following the final behavioral analysis, PFC was sonicated in 1 ml of 0.1 M perchloric acid. A 100 µl aliquot of the sonicated material was stored at 80°C for protein determination using Pierce BCATM Protein Assay Kit (Thermo Scientific). The supernate was collected from centrifugation of the sonicated material at 13,000 g for15 min and stored at 70 °C until catecholamine extraction was performed. Catecholamines were extracted by alumina extraction from 100 µl of the sonicated supernate as previously described [31]. The eluted catechols were filtered through 0.22 Millex®GV syringe driven filter and detection was performed with the ESACoulochem II electrochemical detector (conditioning cell set at_350 mV, electrode 1 of analytical cell set at 90 mV, electrode2 of analytical cell set at _300 mV) (ESA). Phenomonex reverse phase c18 Gemini column (150_4.6 mm, 3_C, 110 A) (Phenomonex) and ScientificSoftware Inc. were used for data collection and analysis. The catecholamines, norepinephrine and dopamine were measured in the PFC. Catecholamine values were expressed as ng catecholamine/mg protein. Data were adjusted to percent control, and values were expressed as the average percent change from control ± SEM. Experimental data were analyzed by using the computer program GraphPad Prism (v. 5.0, GraphPad Software Inc.).

Golgi impregnation and morphological analysis

The brains were prepared using the FD Rapid Golgi Stain kit (FD Technologies (FD Neurotechnologies). Brains were placed in 20 ml Golgi-Cox solution (potassium dichromate, mercuric chloride and potassium chromate), where they were stored in the dark for 14 days. After this incubation, sections containing PFC were obtained using a freezing microtome (100

μm; Micron HM550 cryostat; Richard-Allan Scientific) mounted onto gelatinized slides and coverslipped. Individual neurons and their processes were visualized at x100 using camera lucida. Dendritic length was measured in triplicate using National Institutes of Health Image J (National Institutes of Health).

Data Analysis

Statistical analyses were performed using Graph Pad In Stat software (Graph Pad Software Inc.). For comparison of two groups in experiments, unpaired Student's *t*-test was used to analyze data. One way-analysis of variance followed by post-hoc Newman-Keuls multiple comparisons test was used to analyze differences among three independent groups. Averages are expressed as means ± SEM. Differences were considered to be statistically significant when the probability value was < 0.05

RESULTS

DBS increases exploratory behavior and reduces anxiety-like behavior in an open field paradigm

To investigate whether DBS of the NAcc exerts an effect on depression and anxiety-like behaviors in rats, an assessment of behavior in the open field test was carried out. Open-field is a traditional paradigm used to assess locomotor or exploratory activity and anxiety-like behaviors wherein the inherent drive to explore a novel environment is opposed by the tendency to stay in protective areas. The rodent that exhibits anxiety-like behaviors avoids the unsafe and aversive space of the inner fields and thus spends more time in the outer perimeter of the field [32]. Data obtained was based on the amount of quadrant changes and time spent on their hind legs as a marker of exploratory behavior or locomotor activity. Crossing of midline was also analyzed and used as a marker of anxiety based on the difference of the rat spending time in either the periphery or center of the field test.

Shown in Table 1 is the average value (n = 15 rats per group) of each criterion analyzed in the open field test. This data is used to determine the exploratory nature of the rat as well as the level of anxiety of the rat during the test as a marker of depressive and anxiety-like behaviors. Assessment was conducted for 5-minute intervals pre and post stimulation.

The behavioral data obtained from the control rats that received sham stimulation of the NAcc showed a significant decrease ($P < 0.001$) in all parameters analyzed when compared to pre-stimulation time point. Specifically, these subjects exhibited an increase in depression and anxiety-like behaviors that were manifested as a decrease in locomotor and exploratory behaviors in the open field test, as well as decrease crossing of midline (Table 1). Wistar-Kyoto rats that received DBS of the NAcc showed significant increases ($P < 0.05$) in exploratory behavior and crossing of midline indicative of a reduction in depression and anxiety-like behavior.

When comparing the three treatment groups to each other, baseline behavior during pre-implantation showed no significant difference across the treatments. Post-implantation data showed a significant difference across all groups ($P < 0.01$) with the largest difference seen when comparing either the intermittent or chronic stimulation group to the control group. Control rats continued to exhibit depression-like and anxiety- like behaviors compared to either the intermittent or chronic stimulated groups ($P < 0.01$). There was a significant decrease ($P < 0.01$) in quadrant changes, as well as less time spent on hind legs ($P < 0.001$). In addition, the sham-stimulated control group demonstrated almost no tendency to cross the midline and there was a significant decline ($P < 0.001$) in exploratory behavior and an increase in anxiety. Intermittent and chronic stimulation groups showed a significant increase ($P < 0.01$) in quadrant changes and time spent on hind legs, demonstrating increased exploratory behavior when compared to pre-implantation and sham-stimulated controls. There was also a significant increase ($P < 0.001$) in crossing midline indicating less anxiety. There was a larger change observed in the chronic stimulation group than with the intermittent stimulation group when comparing to all pre-implant values. These data demonstrate that there was a more significant behavioral response to treatment with chronic stimulation as opposed to intermittent stimulation.

DBS alters levels TH expression in the prefrontal cortex

In the present study, the PFC was microdissected (approximately covering the PFC area at a level 3.7 mm anterior to bregma extending at a level 1.7 posterior to bregma). Tyrosine hydroxylase protein expression following DBS was assessed using Western blot analysis. Prefrontal cortex extracts from rats (n = 4 rats per group) that were euthanized following chronic stimulation group showed a significant decrease ($P < 0.05$) in TH expression levels compared to intermittent stimulation group and sham-stimulated control (Figur e 2). There was also a

decrease in TH expression level following intermittent stimulation. However, it has not reached a statistically significant difference.

DBS alters levels NE and DA expression in the prefrontal cortex

DA and NE content in the PFC following chronic stimulation were assessed using HPLC. DA and NE were chosen for testing secondary to being downstream in the biosynthetic pathway for catecholamine's and should be directly correlated with the effects observed with TH **(Figure 3).** This aimed to determine if the decrease levels of the converting enzyme TH following chronic stimulation observed on Western blot analysis (Figure 2) correlated with a decrease in the production of its neurotransmitters in the biosynthetic pathway for catecholamines. Statistical analysis showed that PFC extracts from rats (n = 4-5 rats per group) that were euthanized following chronic stimulation demonstrated a significant decrease in the content of NE ($P < 0.05$) and DA ($P < 0.05$) compared with sham-stimulated control (Figure 4).

DBS increases dendritic length in layer V pyramidal cells in the prefrontal cortex

Statistical analysis showed that intermittent and chronic stimulation for 14 days in the NAcc significantly increased apical ($P < 0.01$) and basilar ($P < 0.01$) dendritic length in the layer V pyramidal cells in the PFC (Figure 5). However, there was no significant difference observed in the number of basilar dendritic branches in the three groups studied. Apical dendritic length did not significantly differ between intermittent stimulation group and chronic stimulation group. Figure 6 presents representative camera lucida drawings of Golgi-cox stained neurons found in PFC in sham-stimulated, intermittent and chronic stimulation groups. These drawings clearly illustrate that the length of the dendrites and the length of the branches have increased with both intermittent and chronic stimulation as compared to the sham-stimulated control group. Deep

brain stimulation of the nucleus accumbens therefore influences dendritic plasticity in the prefrontal cortex of Wistar-Kyoto rats.

DISCUSSION

The present study provides the first in vivo evidence that DBS of the NAcc reduces anxiety and depression in an animal model via an open field test. These results are consistent with a recent report demonstrating that DBS in the NAcc can reduce anhedonia in patients with treatment-resistant depression [33]. Moreover, DBS of the NAcc induces neurochemical and morphological modifications in the PFC that may partly explain the neural mechanism by which DBS exerts its effects and that the locations of changes in the brain circuitry are yet to be defined. This study set out to correlate behavioral changes directly with structural changes within this circuit. By establishing changes in the circuit it can be demonstrated that the effects of DBS may be upstream or downstream from its target and cause neuroplasticity and not be directly at the area of stimulation. DBS may have either excitatory or inhibitory effects which may be leading to changes in a different location of the brain.

Methodological considerations

There are inherent caveats that exist with respect to Western blot analysis experiments. These caveats include the accuracy of sampling of the region of interest and the comparison of equal protein quantities across treatment groups. In order to circumvent the variability in tissue excision, a single investigator obtained the brain samples for each experiment. Moreover, to ensure equivalent loading of protein, blots were reprobed with β-actin and results were normalized to this internal standard. β-actin expression was comparable across treatment groups examined.

Some effects may not have been visualized, such as the absence of change in TH expression levels with intermittent stimulation that could be attributed to the short stimulation

and observation in the conduct of DBS. The stimulation period only lasted for two weeks and this may not have been long enough to demonstrate all the changes that were occurring. This could partly explain the similar dendritic branching among groups studied. It would also be interesting to examine how long these effects on behavior, neurochemical and structural changes would last after a period of stimulation. Shi et al. suggested that there may be highly plastic neural networks that may undergo lasting effects with DBS [27]

The NAcc as target for DBS-treatment

The NAcc is known to integrate limbic and cortical inputs which then send projections to the basal forebrain and in turn sends back projections into the PFC [34; 35]. Neurochemical manipulation of the NAcc has been linked to changes in pleasure [28; 36]. Anhedonia which is the inability to experience pleasure from previously pleasurable activities, is a prominent clinical feature of depression [37; 38]. DBS via bilateral electrodes placed in the NAcc in patients suffering from extremely resistant forms of depression that did not respond to pharmacotherapy, psychotherapy and electroconvulsive therapy had significantly reduced anhedonia [33]. While effectively reducing anhedonia, DBS of NAcc did not produce any neurological and psychological side effects [33]. Therefore, DBS of the NAcc can be a potential target for the treatment of depression to alleviate the symptoms of anhedonia.

DBS in the NAcc reduces anxiety-like behavior and alters catecholamine levels in the prefrontal cortex.

Our present behavioral data are consistent with previous reports showing that Wistar-Kyoto rats exhibit depressive-like behaviors [14; 15; 16]. An increase in depression-like behaviors in control subjects most likely results from rats being individually housed throughout the experimental conditions. Previous data have shown that single rat housing is considered a mild

stress [39] indicating that sham stimulated rats may have experienced this mild stress that resulted in decreases in locomotor and exploratory behaviors. More importantly, our behavioral data support previous clinical reports on bilateral DBS of the NAcc which showed immediate effects on ratings of depression on patients with treatment-resistant depression [33].

We specifically demonstrated in an open-field test that while sham-stimulated control rats continued to manifest signs of depression and anxiety, rats that received either intermittent or chronic DBS showed a significant reduction of depression and anxiety-like behaviors as evidenced by quadrant changes, time spent on hind legs, midline crossing, exploratory behavior and reduced anxiety when compared to pre-implantation and controls. There was a more significant behavioral response to treatment with chronic stimulation as opposed to intermittent stimulation. Control rats continued to be more depressed with age. The open field test is usually used to measure locomotor behavior and although useful in our experiment its interpretation of the behavior change is somewhat limited secondary to correlating behavioral change with depression and anxiety. That being said, the true significance of the behavioral data lies in that the control group worsened in all areas tested for behavioral analysis while the stimulation groups improved. This demonstrates that DBS of the NAcc did not only halt the progression of the disease process, but improved it, consistent with evidence showing that neuronal adaptation is disrupted in mood disorders and that treatments for depression would need to enhance neuroplasticity [40]. The present study also determines whether the behavioral changes following stimulation of the NAcc lead to changes in neural circuits, specifically the PFC.

It is anatomically established that NAcc receives afferent projections from PFC [41; 42; 43]. The PFC is known for its vital role in a cognitive and behavioral processes including arousal, attention, cognition, motivation, working memory and vigilance [44]. The release of

catecholamines, NE and dopamine, is related to arousal states that consequently have profound effects on cognitive and behavioral processes involving PFC functions [45; 46]. The PFC is very sensitive to changes to neurochemical environment so that any minute changes in catecholamine generate profound effects on the activity of PFC [47]. Indeed, the role of the medial PFC is important in understanding the NAcc and its circuitry therefore making it also important to understand the neuroadaptations in the PFC following DBS of NAcc.

Using Western blotting and HPLC, the levels of TH, norepinephrine (NE) and dopamine (DA), were determined, respectively, following DBS of the NAcc. Western blot analysis demonstrated a decrease in expression of TH in the PFC after both intermittent and chronic stimulation, although statistical significance was only observed in chronic stimulation. To determine if the decreased levels of the converting enzyme TH correlated with a decrease in the production of its neurotransmitters in the biosynthetic pathway for catecholamines, the levels of DA and NE were evaluated. The levels of DA and NE were significantly decreased in the PFC with chronic stimulation as compared to controls demonstrating a correlation with the decreased levels of TH. This was not observed in the intermittent stimulation group, as the levels of DA and NE mimicked that of the control group. This may be secondary to the less pronounced response in TH levels seen on the western blot analysis for intermittent stimulation. There may be a threshold in TH levels which needs to be breached before there are changes of levels in these neurotransmitters. It can also be hypothesized that this is why there was not as large a response in behavior changes in the intermittent stimulation group as compared to the chronic stimulation group.

DBS in the NAcc induces neuroplasticity in the prefrontal cortex.

The results of the present study showed that neurochemical alterations were evident on the expression levels of TH, DA and NE. Whether these neurochemical changes lead to morphological changes in neuronal structure, dendritic growth and branching were examined as markers for neuroplasticity. As a fundamental mechanism of neuronal adaptation, neuroplasticity is altered in stress paradigm in animals. For instance, chronic restraint stress significantly reduced the length and number of branches of apical dendrites but not of basilar dendrites of the pyramidal neurons of lamina II-III of medial PFC [48; 49]. Stress also caused atrophy of the hippocampal neurons [50]. An evaluation with golgi- cox staining demonstrated that the length of the apical and basilar dendrites of the pyramidal neurons of layer V of medial PFC increased with both intermittent and chronic stimulation as compared to the control group. This data confirms the behavioral data in open-field where rats that received intermittent and continuous stimulation exhibited reduced depressive and anxiety-like behavior. Although, the neurochemical data were consistent with significant reduction in TH, NE and DA in PFC only in chronic but not in intermittent stimulation, it is likely that the reduction of TH, NE and DA, though not statistically significant may have efficiently induced behavioral changes and morphological changes in apical and basilar dendrites. Further investigation is needed to elucidate this data. Nevertheless, the present morphological data indicate that DBS of the NAcc influences dendritic plasticity in the PFC.

CONCLUSION

Our present results highlight DBS of the NAcc as a potential treatment modality for depression. In addition, our results present DBS of the NAcc as a mechanism that induces defined neurochemical changes and structural dendritic changes in the PFC that demonstrates neuroplasticity in the circuitry of the brain. It was shown that the effects of DBS may not only be

at the area of stimulation or placement of the electrode, but can lead to changes either upstream or downstream in the circuits of the brain. This further solidifies the thought process that there are long term structural changes that are occurring with DBS. Understanding DBS, as well as the brain as a circuit may be a link to unlocking the brain's ability to undergo neuroplasticity. The neurochemical alterations in the expression levels of TH, dopamine and norepinephrine, as well as the dendritic neuroplasticity observed in the cortical areas may underlie the behavioral changes following DBS of the nucleus accumbens shell.

REFERENCES

1. **Machado A, Rezai AR, Kopell BH, Gross RE, Sharan AD, Benabid AL. Deep brain stimulation for Parkinson's disease: surgical technique and perioperative management. *Mov Disord.* 2006;21(Suppl 14)S247-258.**
2. **Gildenberg PL. Evolution of neuromodulation. *Stereotact Funct Neurosurg.* 2005;83(2-3)71-79.**
3. **Perlmutter JS, Mink JW. Deep brain stimulation. *Annu Rev Neurosci.* 2006;29 229-257.**
4. **Wichmann T, Delong MR. Deep brain stimulation for neurologic and neuropsychiatric disorders. *Neuron.* 2006;52(1)197-204.**
5. **Hamani C, Hodaie M, Chiang J, et al. Deep brain stimulation of the anterior nucleus of the thalamus: effects of electrical stimulation on pilocarpine-induced seizures and status epilepticus. *Epilepsy Res.* 2008;78(2-3)117-123.**
6. **Mirski MA, Ziai WC, Chiang J, Hinich M, Sherman D. Anticonvulsant serotonergic and deep brain stimulation in anterior thalamus. *Seizure.* 2009;18(1)64-70.**
7. **Shi LH, Luo F, Woodward D, Chang JY. Deep brain stimulation of the substantia nigra pars reticulata exerts long lasting suppression of amygdala-kindled seizures. *Brain Res.* 2006;1090(1)202-207.**
8. **Sani S, Jobe K, Smith A, Kordower JH, Bakay RA. Deep brain stimulation for treatment of obesity in rats. J Neurosurg. 2007;107(4)809-813.**
9. **World Health Organization. The world health report 2001 - Mental Health: New Understanding, New Hope. 2001.**
10. **Gelenberg AJ. The prevalence and impact of depression. *J Clin Psychiatry.* 2010;71(3)e06.**
11. **Andrade L, Caraveo-Anduaga JJ, Berglund P, et al. The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys. *Int J Methods Psychiatr Res.* 2003;12(1)3-21.**
12. **Kessler RC, Berglund P, Demler O, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMA.* 2003;289(23)3095-3105.**
13. **Ciapparelli A, Dell'Osso L, Tundo A, et al. Electroconvulsive therapy in medication-nonresponsive patients with mixed mania and bipolar depression. *J Clin Psychiatry.* 2001;62(7)552-555.**
14. **Armario A, Gavalda A, Marti J. Comparison of the behavioural and endocrine response to forced swimming stress in five inbred strains of rats. *Psychoneuroendocrinology.* 1995;20(8)879-890.**
15. **Lopez-Rubalcava C, Lucki I. Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. *Neuropsychopharmacology.* 2000; 22(2)191-199.**
16. **Ramos A, Berton O, Mormede P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behav Brain Res.* 1997;85(1)57-69.**
17. **Rittenhouse PA, Lopez-Rubalcava C, Stanwood GD, Lucki I. Amplified behavioral and endocrine responses to forced swim stress in the Wistar-Kyoto rat. *Psychoneuroendocrinology.* 2002;27(3)303-318.**

18. Malkesman O, Braw Y, Weller A. Assessment of antidepressant and anxiolytic properties of NK1 antagonists and substance P in Wistar Kyoto rats. *Physiol Behav.* 2007;90(4)619-625.

19. Rauhut AS, Zentner IJ, Mardekian SK, Tanenbaum JB. Wistar Kyoto and Wistar rats differ in the affective and locomotor effects of nicotine. *Physiol Behav.* 2008;93(1-2)177-188.

20. Will CC, Aird F, Redei EE. Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. *Mol Psychiatry.* 2003;8(11)925-932.

21. Hemm S, Wardell K. Stereotactic implantation of deep brain stimulation electrodes: a review of technical systems, methods and emerging tools. *Med Biol Eng Comput.* 2010; 48(7)611-624.

22. Leiphart JW, Valone FH. Stereotactic lesions for the treatment of psychiatric disorders. *J Neurosurg.*

23. Hammond C, Ammari R, Bioulac B, Garcia L. Latest view on the mechanism of action of deep brain stimulation. *Mov Disord.* 2008;23(15)2111-2121.

24. Urakubo H, Honda M, Tanaka K, Kuroda S. Experimental and computational aspects of signaling mechanisms of spike-timing-dependent plasticity. *Hfsp J.* 2009; 3(4)240-254.

25. Shen KZ, Zhu ZT, Munhall A, Johnson SW. Synaptic plasticity in rat subthalamic nucleus induced by high-frequency stimulation. *Synapse.* 2003 50(4)314-319.

26. Zahm DS. Functional-anatomical implications of the nucleus accumbens core and shell subterritories. *Ann N Y Acad Sci.* 1999;877 113-128.

27. Liu HY, Jin J, Tang JS, et al. Chronic deep brain stimulation in the rat nucleus accumbens and its effect on morphine reinforcement. *Addict Biol.* 2008;13(1)40-46.

28. Pecina S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? *J Neurosci.* 2005;25(50)11777-11786.

29. Reynolds SM, Berridge KC. Glutamate motivational ensembles in nucleus accumbens: rostrocaudal shell gradients of fear and feeding. *Eur J Neurosci.* 2003; 17(10)2187-2200.

30. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates,* Academic Press, New York. 1986

31. Eisenhofer G, Goldstein DS, Stull R, et al. Simultaneous liquid-chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma, and their responses to inhibition of monoamine oxidase. *Clin Chem.* 1986;32(11)2030-2033.

32. Crawley JN. Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev.* 1985;9(1)37-44.

33. Schlaepfer TE, Cohen MX, Frick C, et al. Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology.* 2008;33(2)368-377.

34. Groenewegen HJ, Wright CI, Beijer AV, Voorn P. Convergence and segregation of ventral striatal inputs and outputs. Ann N Y Acad Sci 1999;877 49-63.

35. O'Donnell P, Lavin A, Enquist LW, Grace AA, Card JP. Interconnected parallel circuits between rat nucleus accumbens and thalamus revealed by retrograde transynaptic transport of pseudorabies virus. *J Neurosci.* 1997 17(6)2143-2167.

36. Pecina S, Smith KS, Berridge KC. Hedonic hot spots in the brain. *Neuroscientist* 2006;12(6)500-511.

37. Argyropoulos SV, Nutt DJ. Anhedonia and chronic mild stress model in depression. *Psychopharmacology (Berl).* 1997;134(4)333-336.

38. Rush AJ, Weissenburger JE. Melancholic symptom features and DSM-IV. *Am J Psychiatry.* 1994; 151(4)489-498.

39. Wu HH, Wang S. Strain differences in the chronic mild stress animal model of depression. *Behav Brain Res.* 213(1)94-102.

40. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology.* 2008; 33(1)88-109.

41. Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol.* 1993;338 (2) :255-278.

42. Hurley KM, Herbert H, Moga MM, Saper CB. Efferent projections of the infralimbic cortex of the rat. J Comp Neurol. 1991;308(2)249-276.

43. Sesack SR, Pickel VM. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol.* 1992;320(2)145-160.

44. Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci.* 2001;24 167-202.

45. Arnsten AF. Catecholamine and second messenger influences on prefrontal cortical networks of "representational knowledge": a rational bridge between genetics and the symptoms of mental illness. *Cereb Cortex.* 2007;17(Supp 1)16-15.

46. Lapiz MD, Morilak DA. Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. *Neuroscience.* 2006;137(3)1039-1049.

47. Arnsten AF, Li BM. Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions. *Biol Psychiatry.* 2005;57(11)1377-1384.

48. Cook SC, Wellman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol.* 2004;60(2)236-248.

49. Radley JJ, Sisti HM, Hao J, et al. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience.* 2004;125(1)1-6.

50. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry.* 2000;57(10)925-935.

Figure legends

FIGURE 1. Brightfield photomicrograph of a representative coronal section showing the site of deep brain stimulation into the NAcc used for various experiments. Inset shows a schematic diagram adapted from the rat brain atlas of Paxinos and Watson (1988) showing the region targeted for the deep brain stimulation. Big arrow indicates the location where the electrode was placed. Asterisks indicate lateral ventricle. Small arrows indicate dorsal (D) and medial (M) orientation of the tissue sections. Scale bars = 100 μm.

FIGURE 2. Western blot analysis of tyrosine hydroxylase (TH) expression in the prefrontal cortex (PFC) following deep brain stimulation/sham stimulation of the NAcc of Wistar-Kyoto rats.TH expression in the PFC of the animals is expressed as a fold change from the control mean when the control equals 1.0 ± SEM. β-actin immunoblotting was used as a control to verify equal protein loading.TH was significantly decreased ($P < 0.05$) following continuous stimulation compared to the sham stimulation group. *$P < 0.05$ vs sham stimulation.

FIGURE 3. Biosynthetic pathway for catecholamine

FIGURE 4. Dopamine (DA) and norepinephrine (NE) levels in the prefrontal cortex of Wistar-Kyoto rats are altered following deep brain stimulation/sham stimulation of the NAcc. Values are means ± SEM of four rats per group. * $P < 0.05$ vs sham stimulation.

FIGURE 5. Mean dendritic length (μm) and number of dendritic branches in the NAcc of rats following brain stimulation/sham stimulation of the NAcc of Wistar-Kyoto rats. Values are means ± SEM of four rats per group. * $P < 0.05$ vs sham stimulation

FIGURE 6. Representative camera lucida drawings of the NAcc following deep brain stimulation/sham stimulation of the NAcc of Wistar-Kyoto rats.

Figure 1

Click here to download high resolution image

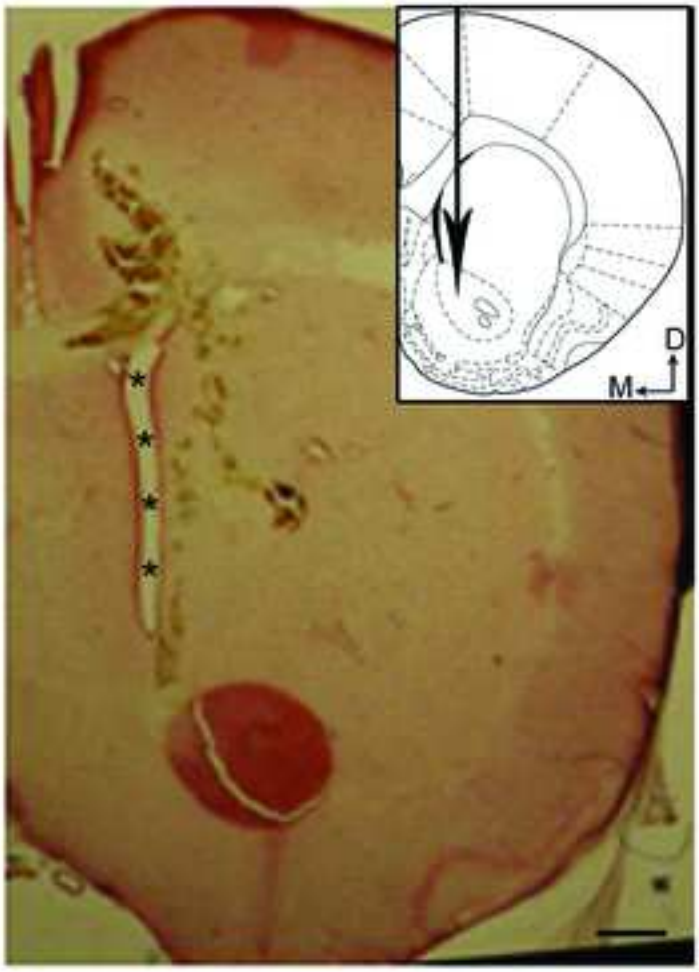


Figure 2
Click here to download high resolution image

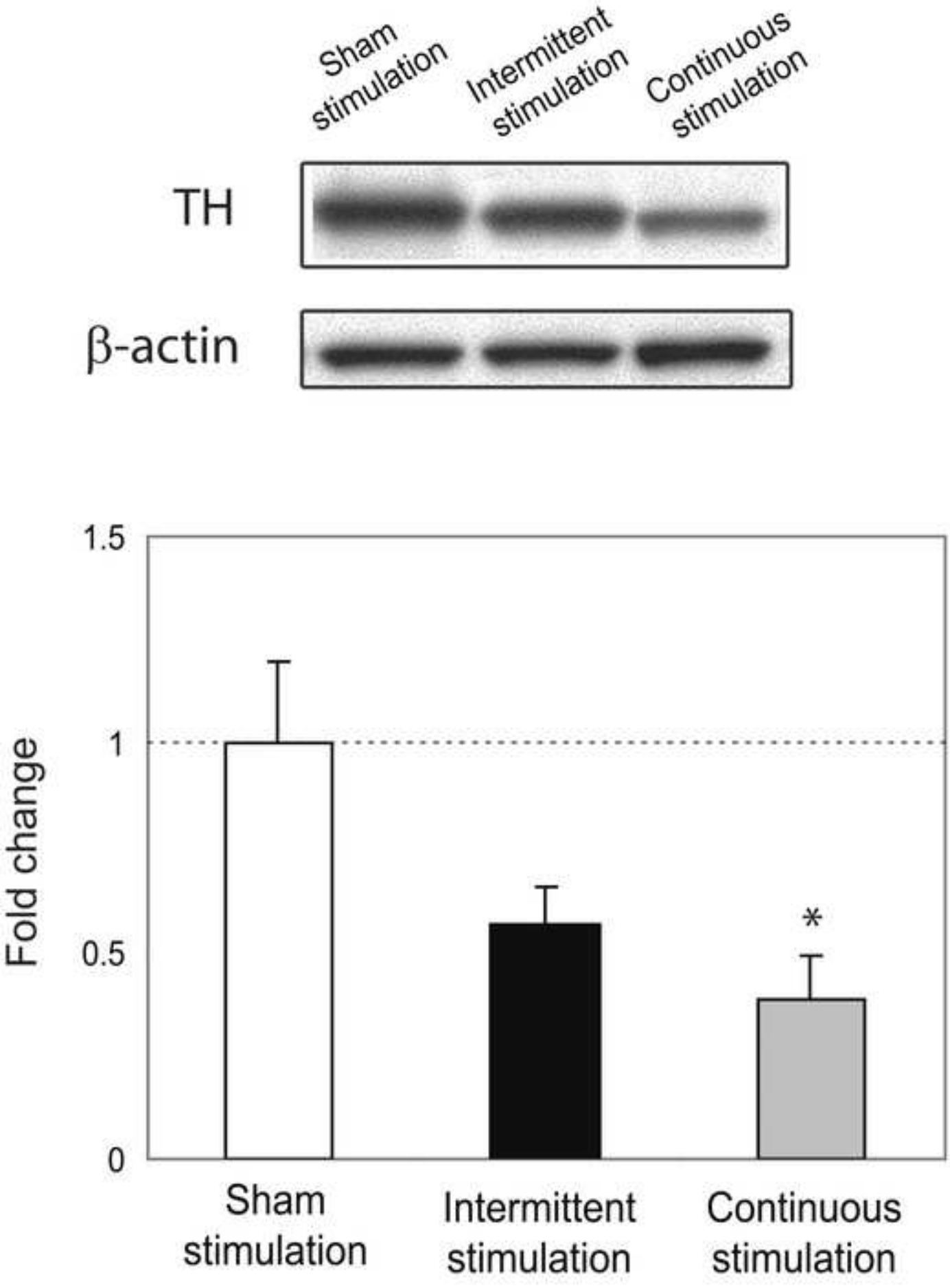


Figure 3

Click here to download high resolution image

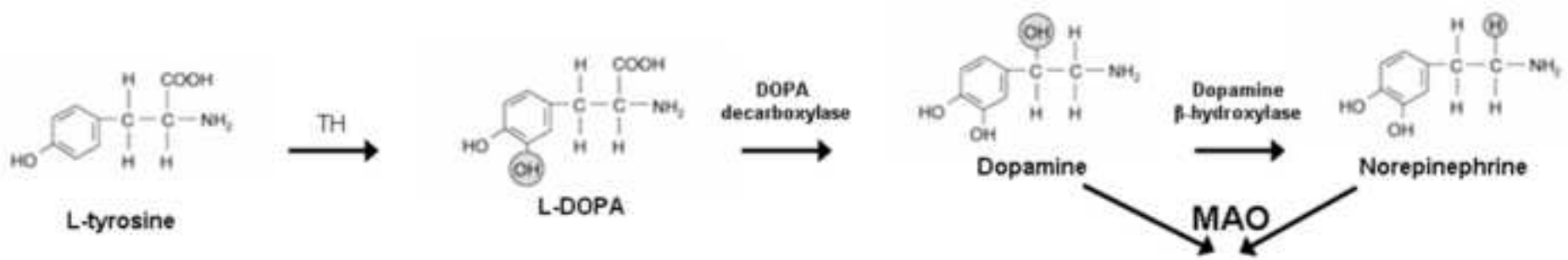


Figure 4

Click here to download high resolution image

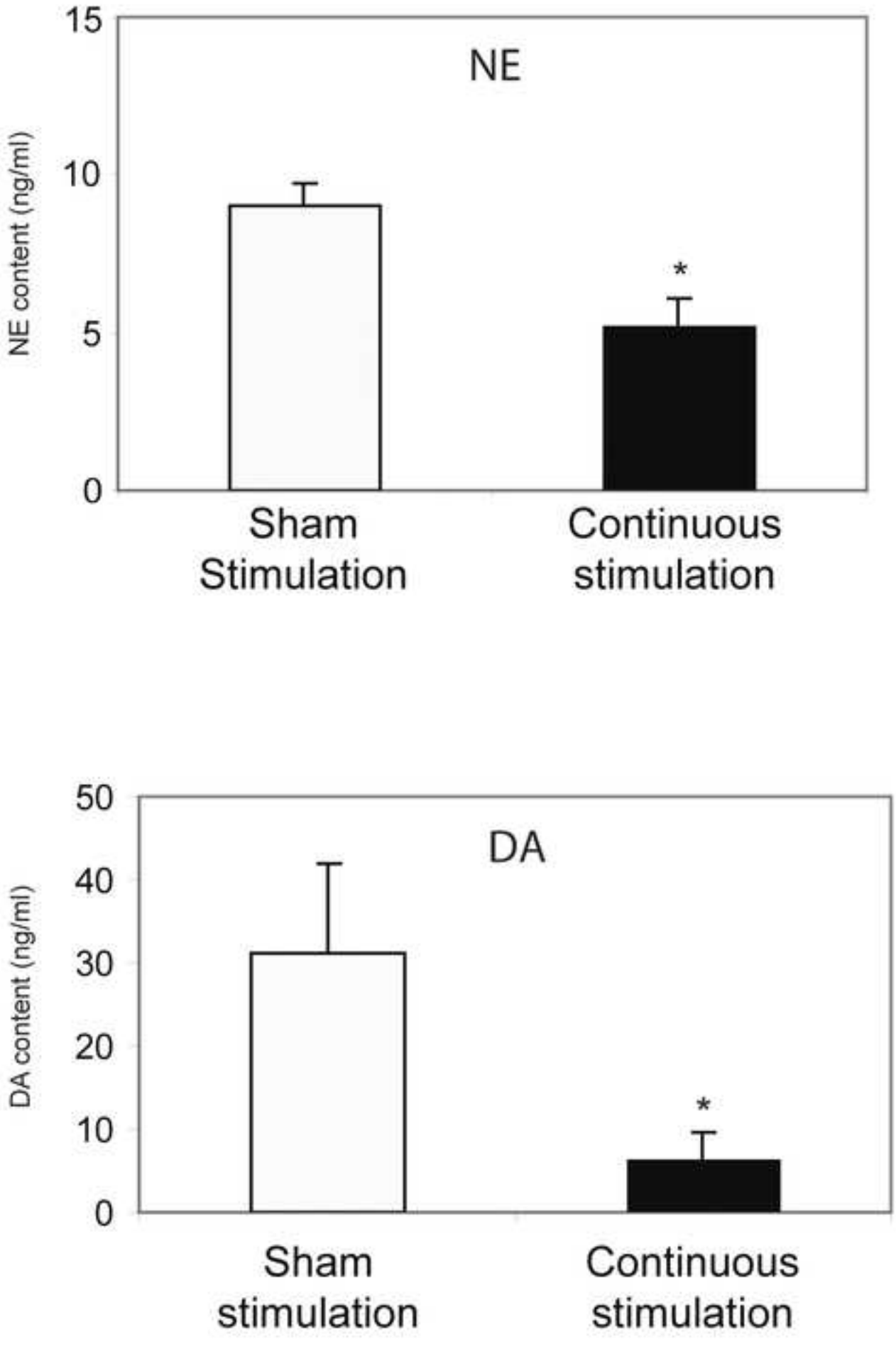
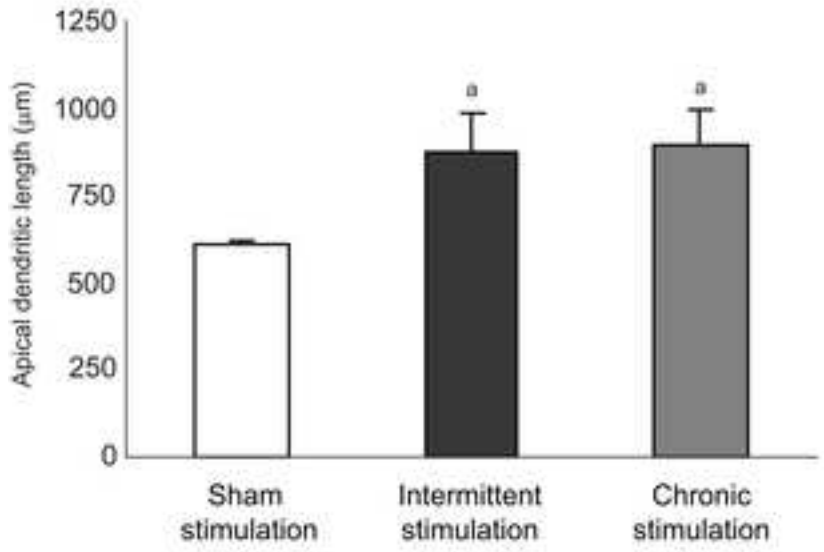
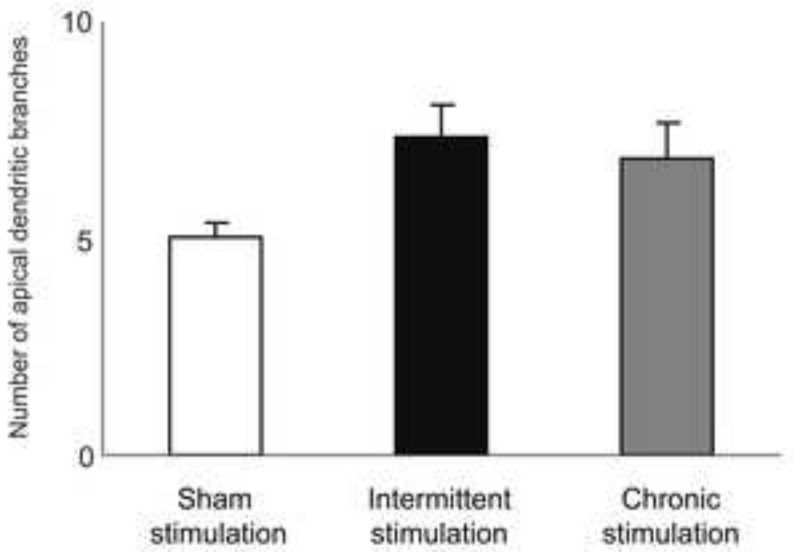
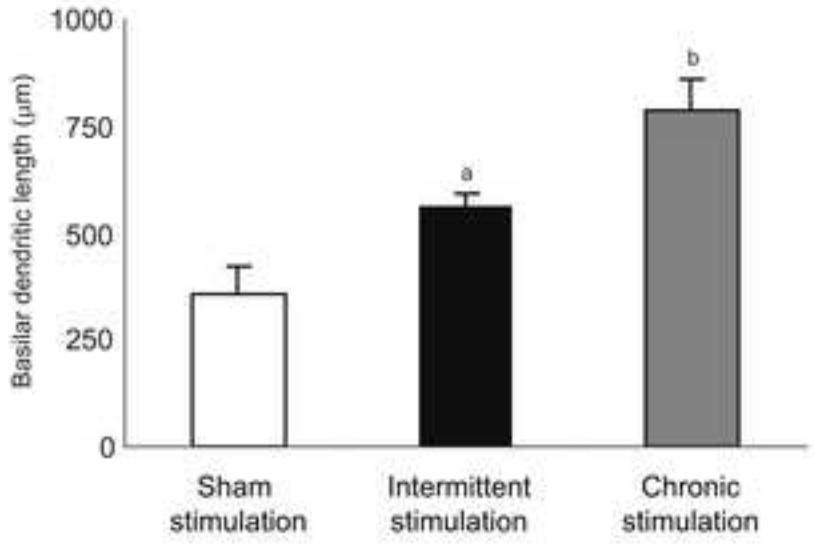


Figure 5

Click here to download high resolution image







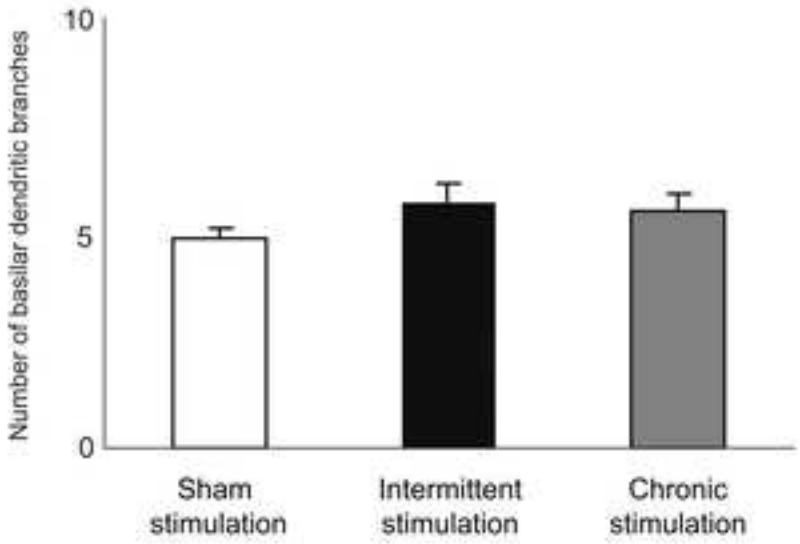


Figure 6

Click here to download high resolution image

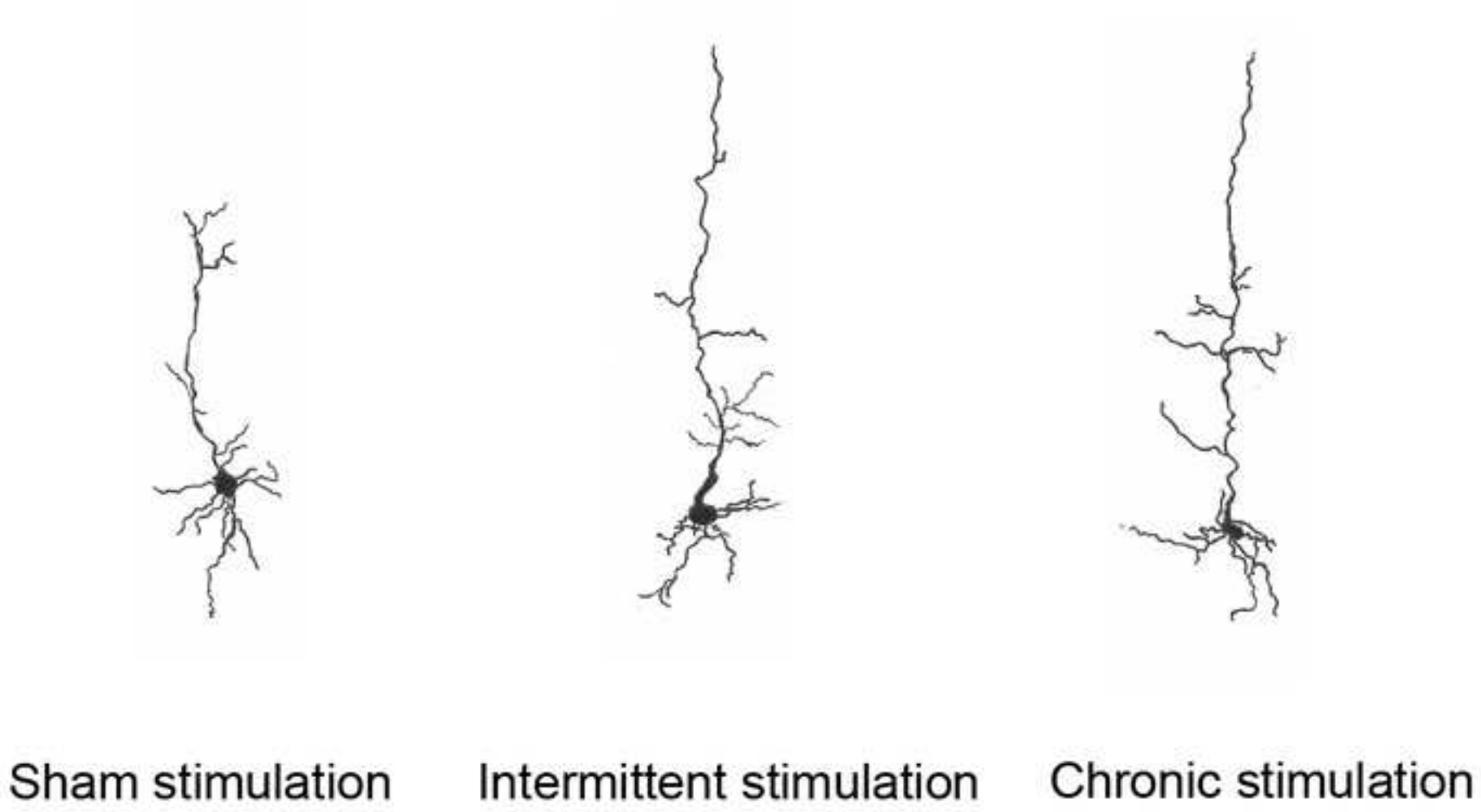


Table 1

Table 1: Behavioral Analysis with an Open- Field Test

	Quadrant Changes	***Time on Hind Legs(seconds)***	***Cross Midline***
Control	24.3 ± 1.38	35.1 ± 2.35	1.1 ± 0.32
Intermittant	19.9 ± 2.19	25.9 ± 4.20	1.3 ± 0.41
Chronic	22.1 ± 2.0	31.9 ± 2.90	1.1 ± 0.30
Post- Implantation			
Control	15.88 ± 1.37	21.56 ± 1.77	0.1 ± 0.09
Intermittant	23.9 ± 3.06*	36.6 ± 6.61**	1.79 ± 0.53**
Chronic	28.3 ± 1.91**	45.5 ± 4.89**	2.3 ± 0.51**

*$P < 0.01$ **$P < 0.001$ compared with control

Difference			
Control	-8.42	-13.54	-1
Intermittant	4	10.7	0.5
Chronic	6.2	13.6	1.2