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DEEP BRAIN STIMULATION

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A functionally relevant and long-term model of deep brain stimulation of the rat subthalamic nucleus: advantages and considerations

A. L. Spieles-Engemann,^{1,2} T. J. Collier³ and C. E. Sortwell³

¹Department of Neurology, University of Cincinnati, Cincinnati, OH 45267, USA

²The Neuroscience Graduate Program, University of Cincinnati, Cincinnati, OH, USA

³Translational Science and Molecular Medicine, Michigan State University, Grand Rapids, MI 49503, USA

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Abstract

In this review we outline some relevant considerations with regards to the rat model of deep brain stimulation of the subthalamic nucleus (STN DBS). In order to optimize the rat STN DBS model in terms of predictive validity for the clinical situation we propose that the STN stimulation experimental design parameters in rodents should incorporate the following features: (i) stimulation parameters that demonstrate functional alleviation of symptoms induced by nigrostriatal dopamine (DA) denervation; (ii) stimulation duration that is relatively long-term and continuous; (iii) stimulation that is initiated at a time when the denervation status of the nigrostriatal system is known to be partial and progressing; (iv) stimulation current spread that is minimized and optimized to closely approximate the clinical situation; (v) the appropriate control conditions are included; and (vi) implantation to the STN target is verified post-mortem. Further research that examines the effect of long-term STN DBS on the neurophysiology and neurochemistry of STN circuitry is warranted. The rat model of functionally relevant long-term STN DBS provides a most favorable preclinical experimental platform in which to conduct these studies.

Introduction

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has become the most often practiced neurosurgery for treatment of the cardinal motor features of Parkinson's disease (PD), specifically bradykinesia, rigidity and resting tremor. More than 40,000 patients have received this treatment as of 2007 (Pereira et al., 2007). Highfrequency DBS was successfully developed to replace ablative procedures due to the advantage of it being reversible and adjustable. Initially, bilateral stimulation of the STN or the pars interna of the globus pallidus (GPi) were both utilized with similar frequency to treat PD. However, in recent years STN DBS has gained greater popularity and is now practiced more often by most centers performing DBS due to greater improvements in motor performance, which allows for a greater reduction in dopaminergic medication (Vitek, 2002; Benabid et al., 2005; Moro et al., 2010). Bilateral high-frequency STN DBS is a safe, stable and reliable therapy that leads to dramatic long-term improvements in the quality of life for PD patients (Benabid et al., 2005; Hamani et al., 2005; Lyons & Pahwa, 2005). Recent clinical studies indicate that bilateral STN DBS may be more effective in treating PD than medication alone (Deuschl et al., 2006). Additionally, STN DBS is economically beneficial as it is less expensive than medications for PD, with the advantage in cost realized within the second year (Charles *et al.*, 2004; Meissner *et al.*, 2005). More recently, STN DBS has also been investigated as a treatment strategy for other disorders such as obsessive–compulsive disorder and seizures (Vercueil *et al.*, 1998; Lado *et al.*, 2003; Usui *et al.*, 2005; Winter *et al.*, 2008; Klavir *et al.*, 2009).

The clinical use of STN DBS has proceeded without a complete understanding of its mechanism of action. This circumstance has spurred intense investigation into the impact of STN DBS in preclinical animal models, with the overwhelming majority of preclinical research utilizing rats as experimental subjects. We, and others, have used the 6-hydroxydopamine (6-OHDA)-lesioned rat as a model of PD to study the effects of STN DBS. This model offers many advantages, not the least of which are the lack of financial and experimental design constraints that are associated with basic research in non-human primates. Further, rats are better suited for STN DBS studies than their mouse counterparts on a number of levels. However, the rat model of STN DBS is not without its limitations. In this review we outline some relevant considerations with regards to the rat model of STN DBS.

Rat STN neuroanatomy

Rats represent good candidates for preclinical investigation as the organization of structures and circuitry adjacent to the STN compares

Correspondence: Caryl E. Sortwell, as above. E-mail: caryl.sortwell@hc.msu.edu

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favorably between rats and humans. The STN is a small, densely populated structure located between the zona incerta and the cerebral peduncle. It is primarily comprised of projection neurons with axons that extend for $> 750 \ \mu m$. The GPi (in rodents, entopeduncular nucleus) and the substantia nigra pars reticulata (SNr) are the principal target structures of the glutamatergic neurons of the STN. These same structures also receive inhibitory input from the striatal GABAergic direct pathway, making the GPi and the SNr the major output structures of the basal ganglia (Whittier & Mettler, 1949; Carpenter & Strominger, 1967). The STN exerts powerful glutamatemediated excitatory effects on the GPi and the SNr as well as the frontal cortex, the pars externa of the globus pallidus (GPe) and the striatum (Jackson & Crossman, 1981; Kita et al., 1983; Kita & Kitai, 1987; Parent & Hazrati, 1995; Kitai & Kita, 2006; Degos et al., 2008) whereas it receives inhibitory input from GABAergic neurons of the GPe via the indirect pathway. As a result, the STN is in the unique position of being the main relay nucleus of the indirect pathway that can act in concert with the direct pathway to modulate the activity of the GPi and SNr. Therefore, the STN is in a strategic position to influence the output of motor information from the basal ganglia.

Targeting the small STN deep within the brain can be difficult. From a neuroanatomical standpoint, rats represent much more favorable candidates than mice to serve as a preclinical model of STN DBS as the size of the rat STN is approximately twice that of the mouse STN $[1.2 \times 1.2 \text{ mm} \text{ in rats}$ (Hardman *et al.*, 2002) vs. 0.6×0.65 mm in mice (Franklin & Paxinos, 2001)], making targeting of the STN easier in rats. That being said, the STN of rats is approximately 300 × smaller than the human STN (rat STN $\approx 0.8 \text{ mm}^3$; human STN $\approx 240 \text{ mm}^3$, Hardman *et al.*, 2002) whereas the size of the stimulating electrode used in rodent studies is only $6 \times$ smaller than electrodes used clinically. The large size of the stimulating electrode relative to the size of the rat brain increases the likelihood that current aimed at the rat STN will spread to structures outside of the STN. Previous studies in our laboratory have directly examined this issue (fig. 1A; also Spieles-Engemann et al., 2010). We estimated the distance of current spread associated with STN stimulation by utilizing simultaneous STN stimulation and extracellular recordings. Concentric bipolar stimulating electrodes were placed within the STN of anesthetized rats using extracellular guided recording techniques. A tungsten recording electrode was then used to record from sites at varying distances from the STN. We estimated that under our experimental stimulation parameters current spread impacts not only the STN but also a sphere immediately surrounding the STN with an approximate radius of at least 250 μ M. These findings indicate that the interpretation of results generated in rat STN DBS studies should always consider that observed effects may be attributable to stimulation of fibers and structures outside of the STN.

The question in terms of clinical relevancy is: does the mismatch in the scale of the rat brain relative to the size of the stimulating electrode, as compared to stimulators placed in the STN of PD patients, introduce the probability of current spread outside the STN that may not normally occur in the clinical situation? In PD patients stimulation parameters are meticulously refined over multiple postsurgical sessions in order to optimize functional improvements (Deuschl *et al.*, 2006). The area of current spread, which has been termed the volume of tissue activation (Butson & McIntyre, 2005), is not a factor in this programming. Functional efficacy of the stimulation is the ultimate goal. The stimulation parameters routinely used in PD patients have been estimated to result in stimulation of a large volume of neural tissue that is not limited to the STN proper, resulting in activation of neighboring large-diameter myelinated axons (McIntyre *et al.*, 2004a; Butson *et al.*, 2007). A schematic representation of structures and fiber pathways that can potentially be impacted by STN stimulation is illustrated in Fig. 1B. Nonetheless, it is reasonable to assume that the relative stimulation area in the rodent model of STN DBS will be larger. Under these conditions careful positioning of the stimulating electrode confirmed by post-mortem placement analysis becomes critical. Further, the stimulation parameters, particularly stimulation amplitude, must be carefully considered, as large-amplitude current will impact a larger volume of tissue. Lastly, and in our opinion most importantly, the STN stimulation parameters utilized in rat studies must demonstrate functional alleviation of symptoms induced by nigrostriatal dopamine (DA) denervation.

STN DBS in the rat

Stimulation parameters

In PD patients with STN stimulation, the precise stimulation parameters are determined after implantation surgery for each patient in extensive consultation with their neurologist. Nonetheless, there are ranges within which most stimulus parameters fall: stimulus frequency between 130 and 185 Hz, pulse duration between 60 and 210 μ s and stimulus amplitude between 1 and 3.5 V (Kuncel & Grill, 2004). With the exception of stimulus amplitude (intensity), preclinical studies in the rodent model of STN DBS are likely to be most informative when STN stimulus parameters are set within these same ranges. Further, while therapeutic STN stimulation frequencies have been reported to be > 100 Hz, frequencies < 50 Hz are non-therapeutic (Garcia et al., 2005) and stimulation at 5-10 Hz has been demonstrated to worsen parkinsonism (Rizzone et al., 2001; Moro et al., 2002). With regards to stimulus amplitude, due to the large differences in STN volume between rodents and humans, the intensity used in rats cannot replicate clinical parameters and every attempt should be made to minimize current spread to neuronal structures not normally impacted in the clinical setting. Estimates of the volume of tissue activated in the human brain range from 30 to 114 mm³ with approximately 70 mm³ generating the most therapeutic benefit (Maks et al., 2009). This volume of activation would probably include the lenticular fasciculus and ansa lenticularis (see Fig. 1B) but not the substantia nigra (SN). Based on our current-spread studies in anesthetized rats (Fig. 1A), stimulation of the STN at amplitudes > 100 μ A should therefore be interpreted with caution as stimulation of the SN cannot be excluded. Further, stimulus amplitudes utilized in anesthetized rats may not directly translate to awake rats as anesthesia can impact the firing properties of neurons (Chen et al., 2001; Erchova et al., 2002). When possible, refinements in stimulus parameters should be made in awake rats in order to minimize the effects of STN stimulation on SN and the medial forebrain bundle. For example, the behavioral responses of differing stimulus intensities can be immediately observed after stimulation is turned on in awake rats (Maesawa et al., 2004; Spieles-Engemann et al., 2010). These responses range from minor to major involuntary movements. For example, at 30-50 µA contralateral dyskinetic orofacial movements are typically apparent, increasing the stimulation intensity to 120 μ A leads to dyskinetic movements of the contralateral forepaw, and stimulation > 200 μ A results in severe tonic twisting or rotational behavior. These rotational responses are indicative of direct SN stimulation (Arbuthnott & Ungerstedt, 1975; Watanabe et al., 1983). Therefore, we contend that stimulation intensities set to just below the level of apparent contralateral orofacial dyskinetic movements (approximately 30-50 μ A) may be optimal in terms of restricting current spread.

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FIG. 1. What exactly is stimulated by STN DBS? (A) Schematic of area of current spread in rat STN DBS experiments. Short-term dual stimulation-recording experiments were conducted using concentric bipolar stimulating electrodes (black line) identical to the electrodes we use for chronic STN stimulation. A tungsten recording electrode was then placed at varying distances from the stimulating electrode; at each site we recorded from individual cells before and after STN stimulation (AP -3.8 mm, L +2.5 mm, DV -7.6-8.0 mm; frequency 130 Hz, pulse duration 60 µs, amplitude 100 µA). Locations of the recording sites were verified histologically at the conclusion of the experiment utilizing Kluver Barrera staining. At each site a single neuron was isolated and the response of the cell to STN stimulation (frequency 130 Hz; pulse duration 60 µs, amplitude 100 µA) was recorded by constructing a peristimulus time histogram (PSTH) using 100 sweeps and bin width of 1 ms, prestimulation of 40 ms and poststimulation of 200 ms. At this current intensity the stimulus artifact was between 2 and 3 ms. During the recording, the occurrence of antidromic activation of the cell was tested by measuring the response jitter. If the PSTH showed a distinct peak with latency between 5 and 8 ms, it was concluded that the cell was activated orthodromically. It was assumed that the cell was activated by spread of current if (i) no distinct peak in PSTH was observed during the first 10 ms after the stimulus artifact, (ii) the cell was not antidromically activated, and (iii) the number of spikes in bins within the 10 ms following the onset of the stimulation current was $> 3 \times$ the number of spikes during the prestimulation period. No response was detected when the cells recorded from were located approximately 300 µM or further rostral to the STN (green lines). However, when recording electrodes were placed in the rostral SN, approximately 250 μ M from the stimulating electrode, we did detect a current spread-induced response (red line). Based on these experiments we estimate that under our stimulation parameters, in anesthetized rats, current spread impacts not only the STN but also a sphere immediately surrounding the STN with an approximate radius of at least 250 µM (red sphere). Therefore, in order to avoid direct stimulation of the SN we position our stimulating electrode in the anterior portion of the STN (Bregma - 3.5), a full 900 µM rostral to the rostral border of the SN. Sagittal section of the rat brain is reprinted with permission from Paxinos & Watson (2005). (B) Schematic of the human STN and surrounding structures. The STN is a small, densely populated structure located between the zona incerta and the cerebral peduncle, and is primarily comprised of glutamatergic projection neurons. Its main targets are the substantia nigra pars reticulata (SNpr) and the internal globus pallidus (GPi); however, it also has projections to the SN pars compacta (SNpc), external GP (GPe), striatum and cortex (red arrows). The STN receives glutamatergic input from the cortex and GABAergic input from the GPe (blue dashed arrows). Immediately dorsal and ventral to the STN are the lenticular fasciculus and ansa lenticularis (green arrows), which are comprised of GABAergic projections from the GPi to the thalamus. Also dorsal to the STN is the zona incerta, which contains both glutamatergic and gamma-aminobutyric acid (GABA) ergic projections to areas of the cortex, diencephalon, basal ganglia, brainstem and spinal cord. During STN DBS, STN efferents, afferent inputs to the STN and fibers of passage all have the potential to be stimulated as a result of current spread. Further, STN DBS can also increase the activity of target structures of the STN efferents.



Chronic vs. acute stimulation

An additional consideration to keep in mind when establishing a clinically relevant model of rat STN DBS is an appreciation for the duration of stimulation. The overwhelming majority of studies published to date examine the acute effects of STN DBS lasting from minutes to hours (Benazzouz *et al.*, 2000; Windels *et al.*, 2000, 2003, 2005; Salin *et al.*, 2002; Bruet *et al.*, 2003; Boulet *et al.*, 2006; Schulte *et al.*, 2006; Shi *et al.*, 2006). However, patients that receive STN DBS experience stimulation 24 h a day, 7 days a week, often for many years. The brain is a remarkably plastic environment and the biology associated with acute effects of DBS may not be representative of the biology generated by chronic stimulation. Therefore, an important part of studying the impact of STN DBS in the clinical

FIG. 2. Time course, magnitude and behavioral impact of nigrostriatal degeneration following intrastriatal 6-OHDA. Schematic illustrating the time course of degeneration of nigral tyrosine hydroxylase (TH)-immunoreactive (ir) neurons (red squares), nigral Neuronal Nuclei (NeuN)ir neurons (red triangles), striatal THir neurites (white circles) and impairments in contralateral forepaw use (black circles) following intrastriatal 6-OHDA. In contrast to the progressive loss of nigral neurons that we observe over the course of 4 weeks after 6-OHDA, the loss of striatal THir and the behavioral manifestations of this loss occurs over a much more condensed time frame and is essentially complete within 2 weeks post-6-OHDA.

situation is examining in the rat model the long-term effects STN DBS, which has been the subject of only a very limited number of reports (Maesawa *et al.*, 2004; Temel *et al.*, 2006; Harnack *et al.*, 2008; Spieles-Engemann *et al.*, 2010).

Verification of stimulator placement and damage to the STN

The rat STN is a relatively small structure, which spans only 200 μ M in the dorsal-ventral axis. Targeting the STN can therefore be extremely difficult. While extracellular-guided recordings can lower STN placement failure rates they cannot ensure correct placement. Correct placement must be verified post-mortem and data analyzed accordingly. Such a post-mortem analysis has often yielded important findings, indicating that placement of STN stimulating electrodes outside of the STN can actually have detrimental effects on the nigrostriatal system (Chang et al., 2003; Shi et al., 2004; Spieles-Engemann et al., 2010). Additionally, examination of the STN itself is critical as lesions of the STN can directly counteract the effects of parkinsonian insults (Delfs et al., 1995; Guridi et al., 1996; Piallat et al., 1996, 1999; Blandini et al., 1997; Carvalho & Nikkhah, 2001). Many previous studies have carefully determined the status of the STN following stimulator implantation to eliminate the confounding effects of STN lesions on the interpretation of results (Aziz et al., 1991; Dostrovsky & Lozano, 2002; Maesawa et al., 2004; Temel et al., 2006; Wallace et al., 2007; Spieles-Engemann et al., 2010). Finally, implantation of the stimulator can have unknown consequences on brain neurochemistry and excitability. Rats in which stimulators are implanted but in which stimulation is never initiated serve as the best control condition to determine the specific effects of stimulation. Our laboratory and others consistently incorporate these critical quality control measures into our experimental procedures (Chang et al., 2003; Maesawa et al., 2004; Shi et al., 2004; Spieles-Engemann et al., 2010).

STN DBS in the parkinsonian rat

Neurotoxin models of PD

Established neurotoxin models of PD [6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)] offer the distinct advantage of allowing investigators to study the effects of STN DBS in a severely DA-depleted environment (Langston et al., 1984; Sauer & Oertel, 1994; Lee et al., 1996; Meredith et al., 2008). The nigrostriatal degeneration induced by the toxins produces measurable motor impairments and triggers a succession of events within the basal ganglia that parallels many of those characteristic of PD. Following dopamine depletion in PD, STN activity becomes extremely disorganized (Bergman et al., 1994; Hassani et al., 1996; Hutchison et al., 1998; Wichmann et al., 1999; Magnin et al., 2000). Additionally, significant alterations occur within the GPi and the SNr, which become hyperactive due to enhanced glutamatergic inputs from the STN. Similarly, experiments utilizing the 6-OHDA lesion model of PD in rodents reveal that after DA depletion the firing properties of the STN are altered, specifically leading to an increase in both the rate and the percentage of STN neuron burst firing (Hassani et al., 1996; Ni et al., 2001; Breit et al., 2005; Gajendiran et al., 2005). Additionally, 6-OHDA and MPTP lesions of the nigrostriatal tract display increases in markers of neuronal activity (Crossman et al., 1985; Schwartzman & Alexander, 1985; Blandini et al., 2007) and a downregulation of glutamate receptors in the SNr and GPi (Porter et al., 1994; Wullner et al., 1994; Kaneda et al., 2005). These findings are consistent with the findings in humans that DA depletion leads to hyperactivity in SNr and GPi and a compensatory downregulation of receptors in those structures due to enhanced glutamatergic input from the STN (Difazio *et al.*, 1992; Vila *et al.*, 1997; Rodriguez *et al.*, 1998).

Another feature of the neurotoxin models is the ability to perform precise analysis of motor performance, which can detect impairments due to DA depletion and improvements in response to STN DBS. In this regard the 6-OHDA rat model appears to hold a slight advantage over the MPTP-lesioned mouse as the lesion and detection of motor deficits is more stable and reliable in 6-OHDA-treated rats as compared to MPTP-treated mice (Meredith & Kang, 2006). Specifically, MPTP treatment in mice may have no effect on motor performance, or it may cause a transient deficit followed by full recovery, or it can even produce hyperactivity (Colotla *et al.*, 1990; Sedelis *et al.*, 2000, 2001; Meredith & Kang, 2006) whereas 6-OHDA treatment produces a reliable lesion and motor deficits (Meredith & Kang, 2006; Spieles-Engemann *et al.*, 2010).

Previously in our laboratory we characterized an intrastriatal 6-OHDA lesion protocol in rats that results in relatively protracted nigral DA neuron degeneration that is first expressed as a loss of DA phenotype and ultimately leads to overt cell loss of nigral DA neurons. In contrast, the loss of striatal DA innervation and the behavioral manifestations of this loss occur over a much more condensed time frame, specifically within 2 weeks after 6-OHDA lesioning (Spieles-Engemann et al., 2010) (Fig. 2). The time course of degeneration produced by our lesion parameters in the rat allows for STN DBS to be initiated at specific intervals that can model either early or late PD. For example, the changes in motor function symptomatic of PD are not usually apparent until DA levels in the striatum have dropped to < 20% of normal accompanied by a 60% loss of nigral DA neurons (Bernheimer et al., 1973). Further, more recent evidence indicates that loss of terminals in the putamen of early PD patients precedes nigral neuron loss (Dodiya et al., 2009). This early-stage pattern of nigrostriatal degeneration is most accurately modeled at the 2-week post-lesion time point in our rat paradigm, with later-stage PD more accurately modeled at 4 or 6 weeks following 6-OHDA. Initiation of STN DBS at various points after 6-OHDA may reveal differences in the impact of STN DBS during early vs. late stages of PD. Up to recently, the typical PD patient receiving STN DBS is in late-stage PD, having a mean disease duration of 14 years (Volkmann, 2004). However, an ongoing clinical trial seeks to determine the safety and tolerability of STN-DBS in early PD (Charles, 2010). Our intrastriatal lesion protocol allows us to evaluate the effects of STN DBS within a partially DA-depleted or a near-complete DA-depleted environment. In conclusion, although the causes of PD are unknown and the neurotoxin models of PD (6-OHDA, MPTP) are unlikely to completely model PD etiology, these models recapitulate the major relevant characteristics of the disease within the structures of the basal ganglia and provide an appropriate background on which to test the impact of STN DBS.

Functionally relevant STN DBS in the rat model

The nigrostriatal DA denervation in the rat that results from intrastriatal 6-OHDA yields numerous motor impairments that can be objectively quantified. As stated previously, one of the considerations of the rat model of STN DBS is the fact that the structures and pathways within the area of current spread may not accurately model the clinical situation. However, current spread can be minimized by reducing stimulus amplitude and observing stimulus-induced behaviors in awake rats. Further, as stimulation parameters in PD patients are programmed to optimize symptomatic relief a case can be made for similarly 'programming' rat stimulation parameters at current intensities that alleviate 6-OHDA-induced motor impairments. In this regard the ability to demonstrate functional efficacy of stimulation in the rat model becomes essential and, ideally, examinations of the impact of STN DBS on neurochemical and morphological outcome measures should utilize functionally effective stimulation parameters.

Previous work has demonstrated that short-term STN DBS can positively impact deficits in motor performance induced by 6-OHDA by its ability to reverse or attenuate reaction time deficits and amphetamine-induced contralateral rotations, and improve treadmill locomotion, walking speed, forelimb akinesia and rearing activity (Meissner et al., 2002; Chang et al., 2003; Darbaky et al., 2003; Shi et al., 2004, 2006; Fang et al., 2006; Vlamings et al., 2007). The main focus of these studies was to determine the impact of acute STN DBS on these behavioral outcomes with the stimulation lasting from minutes to hours. Only two studies have evaluated the ability of longterm STN DBS to alleviate 6-OHDA-induced deficits in motor performance. The first study demonstrated that 2 weeks of continuous STN DBS can ameliorate amphetamine-induced contralateral rotations (Maesawa et al., 2004). In our laboratory we recently evaluated the impact of long-term STN DBS on 6-OHDA-induced deficits in rat rearing activity and forepaw akinesia in the cylinder task (Spieles-Engemann et al., 2009). Rats that received 2 weeks of continuous active STN stimulation exhibited significant increases in both rearing activity and contralateral forepaw use, demonstrating that long-term STN DBS is functionally effective in alleviating parkinsonian symptomology. Using identical stimulation parameters we have previously demonstrated that long-term STN DBS can provide neuroprotection in the face of previous significant nigral DA neuron loss (Spieles-Engemann et al., 2010).

Optogenetics provide insight into circuitry involved in functionally relevant STN DBS

In a recent study by Gradinaru *et al.* (2009) the power of optogenetics was harnessed to specifically uncover which distinct component of STN circuitry may be involved in alleviating 6-OHDA-induced motor deficits in rats and mice. Unlike electrical DBS, in which the volume of tissue activated can include local STN circuitry, afferents, efferents and neighboring fibers of passage (See Fig. 1B), the elegant technique of optogenetic stimulation can be tailored to drive or inhibit specific cell populations or circuits. Using this approach it was revealed that antidromic activation of primary motor cortex (M1)-STN afferents was sufficient to impact 6-OHDA-induced deficits whereas inhibition of local STN neurons or astroglia was not. In this study motor performance was assessed by measuring rotational asymmetry in response to amphetamine, correction of head position bias, and distance and speed of locomotion. Further, only high-frequency

stimulation (> 100 Hz), not stimulation at lower frequencies (20 Hz), elicited the therapeutic effects. These optogenetic results functionally confirm the findings from previous studies in which antidromic M1 activation was revealed to be a consequence of STN DBS in anesthetized rats (Li. *et al.*, 2007). Collectively these results highlight the potential importance of activation of M1-STN circuitry in the therapeutic efficacy of STN DBS in rodent models and may aid in optimizing clinical stimulation parameters in the patient population.

Predictive ability of the 6-OHDA rat model of STN DBS

Beyond the advantages and limitations of the rat model of STN DBS that have been raised, a final advantage of the 6-OHDA rodent model of STN DBS for preclinical research is that the cost of utilizing rodents compared to non-human primates allows for greater flexibility in experimental design, including treatment and control groups and types of in vivo and post-mortem analyses. Clearly the ultimate utility of the rat model of STN DBS can be determined by evaluating its predictive ability for results in non-human primate studies and clinical trials. Although fewer studies have been conducted in this regard, evidence exists which demonstrates that results in monkey and human studies are consistent with previous findings in rat studies. Specifically, studies in rats, primates and humans have all shown that STN DBS inhibits neural activity in the STN itself (Hashimoto et al., 2003; Tai et al., 2003; Meissner et al., 2005; Shi et al., 2006) while exciting STN output structures (Windels et al., 2000, 2003; Hashimoto et al., 2003; Stefani et al., 2005, 2006). Additionally, examinations of the impact of STN DBS on levels of striatal DA suggest that the level of nigrostriatal degeneration will predict whether STN DBS will increase (Bruet et al., 2001; Zhao et al., 2009) or not affect (Meissner et al., 2001, 2002; Hilker et al., 2003; Strafella et al., 2003) striatal DA. Lastly, STN DBS has been shown to be neuroprotective for the DA neurons of the SN in both rats and monkeys (Maesawa et al., 2004; Temel et al., 2006; Wallace et al., 2007; Spieles-Engemann et al., 2010). These findings illustrating the predictive validity of STN DBS are detailed in Table 1. In conclusion, while the 6-OHDA rat model of STN DBS is not without limitations, it as an important experimental platform in which findings can aid in understanding the mechanism of STN DBS. This understanding can inform the experimental design of necessary follow-up studies in non-human primates and PD patients and ultimately provide insights into the mechanisms of STN DBS.

Conclusions

Numerous *in vitro* and *in vivo* studies have been conducted to determine the effect of stimulation on the rat STN and its circuitry (for review see (Chang *et al.*, 2008; Gubellini *et al.*, 2009; McIntyre *et al.*, 2004b). To date there have been over 45 manuscripts published that utilize the *in vivo* rat model of STN DBS. These published reports

TABLE 1. Predictive validity of the rat model of STN DBS

Major finding	Rat studies	Monkey/human studies
STN DBS inhibits the STN	To $i d (2003)$. Shi $d d (2006)$	Maissner at al. (2005): Hashimoto at al. (2003)
STN DDS millions lie STN	$W_{in} d_{i} l_{i} = (2000), Sin et ut. (2000)$	$H_{2} = \frac{1}{2} \left(\frac{1}{2} \left(\frac{1}{2} \right) \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \left(\frac{1}{2} \right) \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \left(\frac{1}{2} \right) \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac{1}{2} \right), H_{2} = \frac{1}{2} \left(\frac{1}{2} \right) \left(\frac$
SIN DBS excites SIN output structures	windels et al., (2000) ; windels et al., (2003)	Hashimoto et al. (2003); Stefani et al. (2005, 2006)
STN DBS increases striatal DA (<i>partial lesions</i>)	Bruet <i>et al.</i> (2001)	Zhao et al. (2009)
STN DBS does not increase striatal DA (complete lesions)	Meissner et al. (2001, 2002)	Strafella et al. (2003); Hilker et al. (2003)
STN DBS protects against dopaminergic toxins	Maesawa <i>et al.</i> (2004); Temel <i>et al.</i> (2006); Harnack <i>et al.</i> (2008); Spieles-Engemann <i>et al.</i> (2010)	Wallace et al. (2007)

vary in experimental design features including stimulus intensity, frequency and duration as well as in quality control measures including verification of stimulator placement within the STN and lack of STN damage. The features and outcome measures utilized in any particular study provide criteria with which to evaluate how findings in rodent studies can be extrapolated to the clinical situation. Most notably, only five studies have evaluated the impact of continuous STN stimulation for a period of > 4 days (Bacci *et al.*, 2004; Maesawa et al., 2004; Oueslati et al., 2007; Harnack et al., 2008; Spieles-Engemann et al., 2010) with the majority of these examining the issue of whether STN DBS can provide neuroprotection from 6-OHDA (Maesawa et al., 2004; Harnack et al., 2008; Spieles-Engemann et al., 2010). Further research that examines the effect of long-term STN DBS on the neurophysiology and neurochemistry of STN circuitry is warranted. The rat model of functionally relevant long-term STN DBS provides an optimal preclinical experimental platform in which to conduct these studies.

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Abbreviations

6-OHDA, 6-hydroxydopamine; DA, dopamine; DBS, deep brain stimulation; GABA, gamma-aminobutyric acid; GPe, pars externa of the globus palidus; GPi, pars interna of the globus pallidus; ir, immunoreactive; M1, primary motor cortex; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NeuN, Neuronal Nuclei; PD, Parkinson's disease; SN, substantia nigra; SNr, SN pars reticulata; STN, subthalamic nucleus; TH, tyrosine hydroxylase.

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