Characterization of Parkinsonian Neuropathophysiology and its Modulation by Deep Brain Stimulation in the Behaving, Nonhuman Primate Model

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ABSTRACT

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by debilitating motor disturbances. It is believed that the signs and symptoms of PD are caused by idiopathic cell death in the basal ganglia (BG), a network of subcortical nuclei with a well-established connection to motor control. Although there is no cure for PD, deep brain stimulation (DBS) of the internal Globus pallidus, a constituent nucleus of the BG, offers hope for patients who don’t respond well to conventional medications. However, the mechanisms underlying the therapeutic effects of DBS remains unclear, a fact largely attributed to a poorly characterized pathophysiology. Here we identify characteristic, electrophysiological biomarkers of PD that preempt the emergence of its behavioral signs and show that DBS works to shift cortical activity back towards a more “normal” state. Using a behaving non-human primate model of PD, we observed a disruption in the normal firing patterns and frequencies of both single motor units and neuronal populations in the primary motor cortex (M1) and supplementary motor areas (SMA) following the induction of parkinsonism. During DBS, we observed an increase in task-related neuromodulation. Taken together, our results hint at a therapeutic mechanism for DBS whereby signaling in M1 and SMA is made more salient and shifted towards “normal” activity. We anticipate that our findings will serve to guide future research and instruct the development of more effective, adaptive DBS technologies.
INTRODUCTION

Introduction to the Problem

Parkinson’s disease (PD) is a debilitating, chronic, and progressive disorder of the motor system. Affecting approximately 1% of the global population over the age of 60, it is the second most prevalent neurodegenerative disorder behind only Alzheimer’s disease (de Lau and Breteler, 2006). Clinical manifestations include motor disturbances, autonomic dysfunction, psychiatric abnormalities, and cognitive impairment. Although genetics may play a limited role in determining an individual’s susceptibility for PD, extensive research has yielded limited results and potential causative factors remain poorly characterized (Bergman and Deuschl, 2002; Greenamyre, 2004). There is currently no cure for PD, and its progressive nature makes the few medications available to physicians ultimately ineffective in long-term care. To improve the quality of life of those individuals suffering from this devastating disease, it is imperative that we develop therapeutic interventions to be employed as more effective treatments for the symptoms of PD.

The following summarizes my efforts to elucidate the therapeutic mechanisms of deep brain stimulation in the context of Parkinson’s disease. It will first address our current understanding regarding the etiology, symptomatology, and pathophysiology of Parkinson’s disease, describe present treatment options, and discuss the models used to study PD. This material will lay the foundation for the presentation of my own work, using a nonhuman primate (NHP) model of PD to characterize changes in behavior and the electrophysiology of two cortical areas tightly linked to motor control, the primary motor cortex (M1) and the supplementary motor area (SMA), following the induction of parkinsonism and during deep brain stimulation.
Parkinson’s Disease

Pathophysiology

The motor disturbances characteristic of PD are thought to be produced by dysfunctional activity of the basal ganglia, a network of subcortical nuclei (See Figure 1.) with a well-established, but poorly understood role in motor control (Mink, 1996). This aberrant activity is thought to be caused by the degeneration of dopamine-producing neurons in the substantia nigra pars compacta (SNc), a darkly pigmented nucleus of the basal ganglia (Bergman and Deuschl, 2002; Jankovic, 2008; Turner et al., 2003; Watts and Mandir, 1992). As depicted in Figure 2, the SNc is thought to play a key role in regulating the basal ganglia-thalamocortical circuit, a web of cortical and subcortical structures that work to coordinate movement. In PD the loss of dopaminergic signaling to the striatum, the major input area of the basal ganglia, is believed to significantly affect the activity of other structures within the basal ganglia as well as the cortical structures with which it interfaces (Bergman and Deuschl, 2002; Mink, 1996).

The predominant cell type in the striatum (~95% of total cells) is the medium spiny neuron (MSN), which is characterized by a large, roughly spherical distribution of its dendrites about the soma (Wilson and Groves, 1980). Compared to its soma, which is only 15 microns in diameter, the MSN’s relatively enormous 500-micron dendritic arbor permits a single cell to simultaneously receive input from multiple cortical and subcortical areas (Gerfen, 1988). The axons of MSNs project to deeper regions of the basal ganglia, namely the globus pallidus. These properties make the striatum exceptionally well suited to serve the functions of both signal integration and relay (Mink, 1996).

In a healthy individual, the action of dopamine signaling in the basal ganglia may be either excitatory or inhibitory. The valence of this action is determined by the relative abundance of two families of dopamine receptors, D1 and D2. Research suggests that both receptors
modulate the activity of adenylyl cyclase, an enzyme which can precipitate significant cellular changes, with D1 receptors stimulating adenylyl cyclase and D2 receptors inhibiting it (Mink, 1996; Sibley and Monsma, 1992). When the substantia nigra pars compacta (SNc) begins to degenerate, so does the delicate chemical balance maintained within the basal ganglia (Bergman and Deuschl, 2002). As illustrated in Figure 2, this is thought to cause a substantial shift in the relative levels of excitatory and inhibitory activity or “tone” in the basal ganglia-thalamocortical circuit. Key structures affected include the internal globus pallidus (GPi), the subthalamic nucleus (STN), the primary motor cortex (M1), and the supplementary motor area (SMA).

The GPi and STN are the regions of the basal ganglia most proximal to the SNc and striatum. The GPi and STN are thought to serve somewhat complementary roles in that the STN, like the striatum, is believed to act as an input and integration center whereas the GPi is primarily considered to be the major output component of the basal ganglia (Bergman and Deuschl, 2002; DeLong, 1990; DeLong and Wichmann, 2007; Mink, 1996). Interestingly, the GPi regulates motor activity not through stimulation of the thalamus and its downstream effectors, but rather through its inhibition or disinhibition. Increases in GPi activity precipitate muscle relaxation whereas decreases are associated with contraction (Mink, 1996; Wichmann and DeLong, 1996). The inhibitory tone of the GPi, thus works in concert with excitatory input on opposing muscle groups to generate fluid movements (Anderson and Horak, 1985; Georgopoulos et al., 1988; Mitchell et al., 1987).

While there have been substantial attempts to characterize the neuronal activity of the STN and GPi in the parkinsonian state (Anderson and Horak, 1985; Hashimoto et al., 2003; Montgomery Jr, 2006), relatively few have attempted to assess the concurrent, aberrant cortical activity seen in PD (Doudet et al., 1990; Watts and Mandir, 1992). Existing research is limited,
contradictory, and inconclusive with regard to how PD and its treatments might modulate cortical activity (Doudet et al., 1990; Goldberg et al., 2002; Watts and Mandir, 1992). However, the primary motor cortex (M1) is a major destination for the neurons of the GPi, therefore it is suggested that M1 may play a role in transforming abnormal neuronal activity in the basal ganglia into the motor signs of PD (Hoover and Strick, 1993). Furthermore, the SMA is believed to be both functionally and anatomically connected to the STN (Litvak et al., 2012). Therefore, in PD, the cortical areas of greatest interest are the primary motor cortex (M1) and the supplementary motor area (SMA), which are thought to be responsible for the execution and planning of movement, respectively (Greenamyre, 2004; Watts and Mandir, 1992).

Presently, there exist two competing models that seek to explain the mechanisms driving the motor signs of PD. In the theoretical rate model, the dysfunctional signaling in the striatum results in hyperactivity of the GPi and STN, which ultimately results in decreased levels of excitatory signaling back to M1 and SMA (DeLong and Wichmann, 2007; Wichmann and DeLong, 1996). Most research suggests that it is the abnormal neural activity in these two regions that ultimately precipitates the motor signs characteristic of PD, making this model an attractive candidate (Bronstein et al., 2012; Deuschl et al., 1996; Greenamyre, 2004; Jankovic, 2008).

A newer model however that is steadily increasing in favor points to changes in the firing patterns and frequencies of cortical and sub-cortical neurons (Hashimoto et al., 2003; Johnson et al., 2009). This model proposes that not only are the motor signs due to disproportionate excitatory and inhibitory tone in the basal ganglia but rather, are emergent based on other factors such as burst frequency and coherence. Of particular interest is the widely reported linkage of movement and changes in synchronized bursting of the so-called beta frequency band of 14-35
Hz in the basal ganglia (Kühn et al., 2004; Levy et al., 2002; Priori et al., 2004). Most notably, these studies have successfully identified event-related decreases in beta power before and during movement. Furthermore, additional studies have managed to link a decrease in beta synchronicity with clinical improvements (Brown, 2003; Kühn et al., 2006). However, the prevalence and degree of beta modulation in patients with PD is inconsistent in both human and animal studies (Bronte-Stewart et al., 2009; Devergnas et al., 2014; Rosa et al., 2011). In short, we have failed to conclusively identify “archetypical” electrical activity that is consistently present in parkinsonian subjects.

**Symptomatology**

The clinical manifestations of PD can be divided into four cardinal features: bradykinesia, tremor at rest, rigidity, and postural instability. The presence or absence of these symptoms and their severity varies widely between patients but as a general rule, most tend to get worse as the disease progresses (Bergman and Deuschl, 2002; Jankovic, 2008). To assist physicians and scientists with their analysis of the disease, the Unified Parkinson’s Disease Rating Scale (UPDRS) was developed to quantify the extent of disability and impairment (Ramaker et al., 2002). Interestingly, studies making use of the UPDRS indicate that the progression of the disease is not linear; rather, the rate of progression appears to be variable and more rapid in the earlier stages of the disease (Jankovic and Kapadia, 2001; Post et al., 2007).

Bradykinesia is perhaps the most extensively researched feature of PD and poses one of the greatest impacts on quality of life. It is characterized by a pronounced slowness of movement (Berardelli, 2001; Jankovic, 2008). Several studies making use of neuroimaging technologies provide evidence for a strong correlation between the degree of bradykinesia and the neuronal density of the substantia nigra (SNc) (Bergman and Deuschl, 2002; Turner et al., 2003). This
data suggests that the severity of bradykinesia is directly proportional to the deficit of dopamine in the striatum. In addition to the classic slowness of movement, bradykinesia may be punctuated with dangerous episodes of “freezing” or akinesia in which a patient briefly loses conscious control of a muscle group. The sudden loss of motor control can cause harm to the patient if he or she happens to fall during one of these episodes. Freezing seems to be at least in part, affected by perception as freezing episodes are relatively common when patients are made to cross a threshold like a doorway (Barbe et al., 2014; Nieuwboer and Giladi, 2013; Schaafsma et al., 2003; Vercruysse et al., 2012).

Tremor at rest is perhaps the most well-known and readily observable feature of PD. The tremor is typically most pronounced in the distal regions of limbs such as the hands or feet although it may present in the lips, chin, and jaw (Deuschl et al., 1996; Rajput et al., 1991). As indicated by its name, this tremor disappears with action and during sleep. Unlike bradykinesia, tremor severity is independent of dopaminergic deficit in the striatum (Antonini et al., 1998; Ceballos-Baumann and Brooks, 1997). Therefore, it is suggested that tremor at rest is relatively binary in that it is either present or absent and only appears when the dopamine levels of the basal ganglia fall below a given level.

Rigidity is characterized by increased resistance of the musculature to both passive and active manipulation. Rigidity is often associated with pain in the shoulder and is thus, often misdiagnosed as arthritis or a joint injury (Riley et al., 1989). It is believed that rigidity arises from an inability of the CNS to properly react to signal from stretch receptors, resulting in long-latency stretch reflexes (Berardelli et al., 1983). This feature commonly affects distal joints such as the wrists and ankles in addition to the more proximal joints of the neck, shoulders, and hips.
Rigidity in the proximal joints can cause postural deformities such as scoliosis and extreme flexion of the neck and trunk (Ashour and Jankovic, 2006; Askmark et al., 2001).

Postural instability is a feature of late-stage PD attributed to a loss of the postural reflexes and characterized by a reduced ability to maintain or regain balance following upset (Jankovic, 2008). Thus, postural instability, along with freezing, presents the most significant risk for falls and hip fractures (Williams, 2006). Not surprisingly, the frequency of falls has been correlated with increased scores on UPDRS (Koller et al., 1989).

**Treatment of Parkinson’s Disease**

**Medicinal Intervention**

The base cause of the symptoms of PD is a deficit in dopaminergic input to the striatum following idiopathic cell death in the SNc (Bergman and Deuschl, 2002; Jankovic, 2008). Because regeneration of the SNc is not yet possible, the gold standard of treatment is the administration of levodopa, a precursor of dopamine capable of crossing the highly selective blood brain barrier after which it is metabolized into dopamine (Jankovic, 2008; Marsden and Parkes, 1976). This method of treatment for PD, most aptly called chronic replacement therapy, supplements the deficit of endogenous dopamine and is a relatively old, effective, and well-tested tool (Marsden and Parkes, 1976; Moro et al., 2002). Unfortunately, chronic replacement therapy has its limitations, the most notable being the diminished response to levodopa over time and its tendency to induce abnormalities in voluntary movement (dyskinesia) at high doses (Contin et al., 1990; Muenter and Tyce, 1971). The former requires that the dose of levodopa gradually be increased to maintain a therapeutic effect which ultimately makes the latter relevant when the therapeutic dose exceeds the threshold at which dyskinesia occur, a phenomenon
known as medically refractive PD. Therefore, chronic replacement therapy is inherently limited in that its effectivity will always diminish; it is simply a question of when.

**Deep Brain Stimulation**

The progressive nature of PD and the side effects to levodopa make chronic replacement therapy only a temporary treatment for the disease. In order to continue treatment of patients with medically refractive PD, it is necessary that we develop novel therapies or new medications that work when conventional medicines fail. Fortunately, work on the deep brain stimulation (DBS) of the internal globus pallidus (GPi) and the subthalamic nucleus (STN) discussed earlier offers a potential solution to the limitations of chronic replacement therapy.

The advent of therapeutic DBS is a very recent development in the field of medicine. For example, the significance of the GPi with regard to the treatment of PD was not fully realized until 1952 when its unilateral chemical ablation, termed “pallidotomy”, resolved both the symptoms of PD and levodopa-induced dyskinesia on one side of the body (Bronstein et al., 2012; Narabayashi et al., 1956). Unfortunately, PD is a bilateral disease and it was necessary to lesion both sides of the brain to fully realize bilateral motor benefits. However, performing bilateral pallidotomies posed a significant risk of complications and therefore, most surgeons were only willing to ablate the globus pallidus of just one of the brain’s hemispheres (Laitinen et al., 1992). This provided a relief from symptoms on only one side of the body and thus, patients were largely unsatisfied.

A change in thinking was required for the quantum leap recently experienced in the treatment of medically refractive PD: stimulate rather than ablate. In deep brain stimulation (DBS), electrodes are stereotaxically implanted into either the subthalamic nucleus (STN) or internal globus pallidus (GPi) where their frequency and amplitude of stimulation are modified
to achieve a relief in symptoms. DBS is therefore particularly advantageous in that unlike the “all-or-none” nature of ablation, its effects are reversible and the stimulation can be tailored to suit an individual’s needs. For these reasons, DBS made physicians much more comfortable with bilateral therapy and the procedure was approved by the FDA in 2002 (Bronstein et al., 2012). It is important to note that DBS is not a cure-all and is contraindicated for patients with certain cognitive or psychiatric problems (Mentzel et al., 2012).

Despite its success, very little is known as to how deep brain stimulation (DBS) induces the observed changes in behavior. In fact, even the ideal target of intervention is still up for debate. The STN and GPI are targeted in DBS because their primary roles as the input and output structures of the basal ganglia, respectively make them particularly well situated for the modulation of aberrant neural activity (DeLong and Wichmann, 2007). Both targets seem to be similarly effective in ameliorating the motor signs of PD and patients commonly report a reduction in the amount of levodopa medication they take (Bronstein et al., 2012; Deuschl et al., 1996; Johnson et al., 2009). However, recent studies have linked DBS of the STN to an increased risk for depression, an effect not observed in DBS of the GPi (Follett et al., 2010). This may suggest that the GPi may be the ideal target for DBS due to the less often considered non-motor effects of DBS.

**Modeling Parkinson’s Disease**

Historically the methods employed by many studies have involved electrophysiological recordings of parkinsonian patients in a resting state, inside a less than experimentally-hospitable operating room. This methodology, which utilizes very fine microelectrodes to record the activity of only one or two neurons at a time, is useful in that it reduces the number of movement-related artifacts found in the data and minimizes the chance of stress or injury to the
patient. Unfortunately, this type of experiment is self-limiting in that it fails to appropriately replicate the everyday conditions in which the cardinal features of PD are most apparent (Connolly et al., 2015). Furthermore, as discussed earlier, the prevalence and degree of neuromodulation in humans varies considerably between studies (Bronte-Stewart et al., 2009; Devergnas et al., 2014). Although it is improbable that there is a singular explanation to this observed variation, it has been postulated that the symptoms of PD are characterized by transient, episodic events which may not be apparent in the resting-state conditions utilized by these earlier studies (Connolly et al., 2015). Furthermore, multiple studies have demonstrated that the symptoms of PD are most easily observed during repetitive, sequential tasks that require processing by the dysfunctional basal ganglia (Vercruysse et al., 2014). Taken together, these findings suggest that PD pathophysiology and DBS mechanisms are best studied in a behaving model.

It is presently impossible to reliably predict if an individual will develop PD and it is therefore, unethical to study asymptomatic patients using anything other than non-invasive, minimal risk techniques such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG). Although it may be possible to detect decreased neuronal density in the SNc via a positron emission tomography (PET) scan prior to the appearance of symptoms, it makes use of ionizing radiation which could prove harmful to an otherwise healthy subject (Jankovic, 2008). There is thus, an absence of behavioral and electrophysiological data for pre-PD humans, which imposes a significant limitation to PD research. Data from PD patients can only be compared within the disease state, either within or between affected subjects, or with healthy controls. In other words, it is impossible to quantify how exactly PD altered the original healthy state of the individual. Furthermore, the studies involving humans have required
experimental paradigms that are limited in their length, sample size, and scope of their task, which is often performed in a seated or reclined position in an operating room (Kühn et al., 2005).

A powerful animal model for the study of PD is the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) nonhuman primate (NHP). MPTP is a neurotoxin that selectively ablates the dopaminergic neurons of the SNc in a dose-dependent manner, simulating the motor signs of PD. This type of chemical ablation is particularly useful in that it provides a controlled, non-progressive model of the disease. This property is unique to the animal model as PD in humans is a neurodegenerative disorder and thus, may only be observed on a continuous spectrum of severity (Doudet et al., 1990; Fox and Brotchie, 2010; Pasquereau and Turner, 2010). Therefore, the MPTP model permits the controlled observation and analysis of the disease at discrete stages in its development, which may be broadly classified as naïve, mild, moderate, and severe.

**Present Research**

Deep brain stimulation offers significant hope for patients living with medically refractive PD. However, the mechanisms underlying its therapeutic effects remain to be seen, a fact that is largely due to our general lack in understanding of the disease’s pathophysiology (McIntyre et al., 2004). Furthermore, there exists little research regarding the effects of DBS in the context of behavior (Connolly et al., 2015; Leventhal et al., 2012). Therefore, it is the goal of this study is to elucidate the therapeutic mechanisms of DBS by first characterizing electrophysiological and behavioral differences between the naïve and parkinsonian states, and then correlating those findings with observed changes during DBS. More specifically, we examine how DBS of the internal globus pallidus (GPi) affects cortico-cortical and cortico-
subcortical communication during a motion-tracked, reach behavior in the MPTP nonhuman primate (NHP) model.

While many tests are well suited to study motor function in humans, relatively simple behavioral tasks in animal models have been effective in preclinical studies (Courtemanche et al., 2003; Leventhal et al., 2012). The use of an actively behaving NHP model of PD is necessary to identify the exact way in which DBS modifies behavior. To consistently analyze the same parameters of reaching behavior, we employed a cued, center-out reaching task (Connolly et al., 2015; Georgopoulos, 1993; Georgopoulos et al., 1988). Although it may appear exceedingly simple to most, a “reach” is a relatively complex physiological process by which a sequence of multi-joint muscle activations guide the hand appropriately through space. An experimental protocol utilizing a reaching task is therefore well-suited to elucidate changes in a number of parameters such as reach speed and reaction time due to its extensive recruitment of motor neurons through the dysfunctional basal ganglia.

With respect to DBS-induced changes in electrophysiology, neural activity within and between the primary motor cortex (M1) and supplementary motor area (SMA) are of particular interest. As discussed earlier, these structures are instrumental in the planning and execution of movement and it is believed that their aberrant activity directly causes the cardinal features of PD (Agnesi et al., 2013; Georgopoulos, 1993; Hashimoto et al., 2003; Pearce and Moran, 2012; Samuel et al., 1997). The consensus of research points to the conclusion that in general terms, DBS manages to restore normal neuronal signaling and cognitive functioning despite the dopaminergic deficit in the striatum (Cleary et al., 2013; Johnson et al., 2009; Silberstein, 2005). The present research aims to verify these claims and evaluate their meaning in the context of behavior.
MATERIALS & METHODS

The study was performed in compliance with The National Institutes of Health Guide for Care and Use of Laboratory Animals and with the University of Minnesota – Twin Cities guidelines for the use and care of laboratory animals in research (National Research Council of the National Academies, 2011). All protocols received approval from the Institutional Animal Care & Use Committee at the University of Minnesota – Twin Cities.

Subjects

Two female rhesus monkeys (Macaca mulatta) – “L” & “T” – were used in this study. They were housed in a light- and temperature-regulated vivarium maintained by the University’s Research Animal Resources (RAR) organization. Each monkey was fitted with a commercial non-human primate collar, which permitted safe transport between its home cage and a specialized primate, restraint chair that restricted movement to maximize animal and investigator safety.

Behavioral Assessments and Analysis

Adaptation & Training

Upon their arrival to the lab, the NHPs were acclimatized to the primate chair and the collar/pole system used for restraining as well as guiding the animal. Acclimation to the chair is determined by the animal's ability to transfer to chair while using a pole. Signs of animal acclimation included presenting collar for transfer, assisting with pole-collar attachment, transferring to chair with minimal pole redirection, and minimized vocalizations.

Following the adaptation period, the NHPs were trained to do a motor task (typically on a daily basis until proficiency is reached, then up to 5 times/week thereafter) to be later performed as a quantitative assessment of motor behavior at different times and conditions. Additionally,
they were trained to permit passive manipulation of their limbs by the experimenter in order to assess their parkinsonian motor signs.

Clinical Rating of Motor Signs

The modified Unified Parkinson’s Disease Rating Scale (mUPDRS) is used to individually rate joint rigidity, akinesia, bradykinesia, tremor, and other parkinsonian motor signs on a scale of 0-3 (Vitek et al., 2012). The mUPDRS composite score defines the severity of the parkinsonian state (mild: 3-13, moderate: 18-28, and severe: 32-42). Motor signs were assessed frequently to verify the stability of parkinsonian motor signs through time.

Center-Out Reaching Task

Motor behavior was assessed by training the animal to perform a visually-cued, center-out reach task. As shown in Figure 3, the primate chair was placed in front of a touchscreen monitor and start-pad with the arm ipsilateral to the DBS lead lightly restrained. The animal was trained to use its free arm to hold the start pad for a variable period of 2-2.5 seconds, after which one of eight targets was presented randomly for each trial. The monkey then had to reach and touch the target within 1.8 seconds of its presentation to receive a juice reward.

The task was performed during one of two broad conditions: naïve and diseased-state. The data collected during the naïve condition were obtained prior to induction of parkinsonism and served as the animal’s healthy baseline. Data collected from monkey “T” following MPTP administration serves as the animal’s diseased-state baseline which can be compared with naïve data. Data collected from monkey “L”, which has a DBS lead, following MPTP administration may be further subdivided into two categories: DBS “ON” and DBS “OFF”. Data from the OFF condition serve as the animal’s disease-state baseline whereas data from the ON condition serve as the model of therapeutic intervention and can be compared with both the Naïve and OFF
conditions.

Each condition “block” required a minimum of 80 trial attempts to be considered valid. Data collected during condition blocks with less than 80 trials were excluded from further analysis. Because it has been observed that the effects of DBS persist for up to an hour following arrest of stimulation, data collected during the “wash-out” following DBS ON periods was excluded from further analysis.

Our system recorded the precise location and timing of each screen touch and divided each reach behavior into three distinct and contiguous epochs: reaction, reach, and return time. The events dividing these epochs were reach onset, target-touch, and return to start-pad, respectively (See Fig 3C).

**Reach Kinematics**

During the experimental reach paradigm, the movement of the NHPs’ arm was recorded by three IR cameras that monitored the relative location of IR-reflective spheres placed on the animal’s arm as shown in Figure 3 (Motion Analysis Corp., Santa Rosa, CA). Off-line analysis of marker position was performed using Cortex Motion Analysis software (Motion Analysis Corp.) and evaluated to identify motor features such as freezing episodes and peak reach and return velocity.

**Surgical Procedures**

**MPTP Treatment**

Once data collection was complete for the naïve (non-parkinsonian) state, both monkeys were made mildly hemi-parkinsonian via unilateral administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In a quarantined, aseptic environment and under isoflurane sedation, MPTP (0.2-0.8 mg/kg, 1 mg/ml solution) was infused over 10-30 minutes
into the left carotid artery. Following a 72-hour quarantine period, parkinsonian motor signs were evaluated via the mUPDRS.

Curiously, the attempt to induce a moderate hemi-parkinsonian state in monkey “L” failed and instead caused the monkey to stop freezing and improved its mUPDRS score. Therefore, to transition it from a mild to moderate parkinsonian state, MPTP was administered systemically under light ketamine sedation via repeated intramuscular injections of approximately 0.4 mg/kg. As with intracarotid infusions, intramuscular injections were performed in a quarantined room and the monkey was not returned to its home cage until at least 72 hours after the most recent injection. Injections were halted when its score on the mUPDRS reached the “moderate” level.

**MRI/ CT Imaging**

Cranial chamber locations and orientations were planned prior to surgery by visualizing each animal’s brain noninvasively with MRI and CT. Because these imaging protocols are susceptible to motion artifacts and would otherwise be extremely distressing for the animals, anesthesia was induced with continuous isoflurane (1-3%) during imaging. An intravenous propofol bolus was supplemented as needed during the imaging session to maintain sedation. The approximate time of each CT scan was 5 minutes whereas the MRI scan took anywhere from 1-3 hrs. If possible, both sets of images were acquired on the same day to limit the number of times the animals were anesthetized. Following chamber surgery and DBS lead implantation, a repeat CT scan was performed to estimate the anatomical location of the DBS electrode.

**Implantation of Cephalic Chambers**

Pre-operative MRI and CT scans were co-registered using the computer program Monkey Cicerone for the stereotactic planning of cephalic chamber placement (Miocinovic et al., 2007).
In an aseptic environment and under isoflurane anesthesia, the animal’s head was secured in a stereotaxic frame, the scalp and temporalis muscle was partially dissected away from the cranium, and two craniotomies were made, leaving the underlying dura intact. Titanium cephalic recording chambers (16 mm, Gray Matter Research, Bozeman, MT) were aligned over each cranial opening and secured with a combination of surgical bone screws and dental acrylic. A stainless steel head post for head fixation was mounted along the midline and towards the rear of the cranium with surgical bone screws. In both animals, the two recording chambers were placed so as to target the right primary motor cortex (M1) and the supplementary motor areas (SMA) of both hemispheres, a feat made possible due to their location along the midline (See Figure 4). Furthermore, the M1 chambers were situated such that they were also aligned with the sensorimotor segment of the GPi.

Following implantation of the cephalic recording chambers, the exact location of the sensorimotor region of GPi was determined via microelectrode mapping techniques as described in Vitek et al. 1998. Briefly, glass-coated platinum-iridium microelectrodes (0.5-1.0 MΩ at 1kHz) were advanced into the brain using a chamber-mounted multichannel hydraulic microdrive system (AlphaEPS, AlphaOmega). The GPi was identified by cross-referencing the location of characteristic firing rates patterns (DeLong, 1971) with stereotactic information from Cicerone (Miocinovic et al., 2007). The dorsal surface of the GPi was defined as the location at which isolated cells exhibit high frequency (82 ± 24 Hz) tonic discharge, a firing pattern characteristic of GPi neurons (Vitek et al., 1998). The ventral surface was defined as the approximate location at which the high frequency discharge signal was lost and replaced by “border cells” with slower rates of discharge (34 ± 19 Hz).
The DBS lead, a chronic stimulation electrode used in humans (Model 3387, Medtronic Inc.) was then implanted into the right GPi of monkey “L” (monkey “T” has not yet received its DBS lead). The stimulating electrode consisted of eight metal contacts (impedances of 100-150 MΩ) designated “C0-7”, each with a diameter of 0.76 mm, width of 0.50 mm, and separated from one another by 0.50 mm (See Figure 5). The distal tip of the lead was targeted to the ventral aspect of the GPi. The remainder of the lead extends out of the brain into the recording chamber where it is held in place by a specially designed ring that fits into the chamber. As noted earlier, proper placement of the lead contacts was confirmed by merging a post-implant CT with the pre-operative MRI using the Cicerone software package as well as through an assessment of behavioral responses to therapeutic stimulation at contacts C3 & C4 (2µA biphasic pulse, 135 Hz).

**Electrophysiology**

**Neuronal Recording**

Recording sessions were generally 2-3 hours long. All of the data were collected either during the reaching task or in the awake, but resting state.

Single motor units in the primary motor cortex (M1) and supplementary motor area (SMA) were identified using extracellular microelectrode techniques. Three glass-coated platinum-iridium microelectrodes (0.5-1.0 MΩ at 1kHz) were independently advanced via a chamber-mounted multichannel hydraulic Microdrive system into M1 (Alpha EPS, Alpha Omega for “L” and SC32, Gray Matter Research for “T”) and SMA (SC32, Gray Matter Research). Neuronal activity was transduced acoustically, allowing for real-time (on-line) evaluation of isolated neurons for motor responsiveness to passive limb manipulation and microstimulation. Neuronal activity was amplified (x10k), band-pass filtered (300Hz-6kHz),
and digitized (25 kHz) for off-line analysis. The use of multiple electrodes permitted the collection of cortical local field potentials (LFPs), which describe the activity of local neuronal populations, as well as paired, synchronized recordings of neurons within and across M1 and SMA.

**Data Analysis**

Single motor unit (SMU) neuronal activity was analyzed off-line with custom software in MATLAB (Mathworks) and Offline Sorter (Plexon). Perievent time histograms of neuronal activity were analyzed to verify if the cells were related to the reaching movement. SMUs isolated from both SMA and M1 were determined to be reach related if they exhibited modulation in their firing frequency and/or pattern during the reaching task. Reach-related neurons were further analyzed in Neuroexplorer (Nex Technologies) by determining the average magnitude and timing of peak firing frequency as well as the behavioral epochs (reaction, reach, or return times) during which they preferentially fired.

To assess the effects of DBS on neuronal activity, perievent time histograms of reach-related neurons were compared across the two conditions of DBS On and DBS Off. To determine if an individual neuron’s firing was modulated significantly by DBS, its average perievent histograms from the two conditions were overlaid on one another and compared with the 95% confidence bands calculated by Neuroexplorer (Nex Technologies). Furthermore, the average peak firing frequencies were compared across these conditions. The paired, synchronized recordings within and across M1 and SMA were also analyzed in Neuroexplorer to detect changes in coherence.

**RESULTS**

The motor behavior and electrophysiology of two NHPs was assessed to determine the effect of PD and DBS on the firing patterns and frequencies of cortical neurons. Data was
collected from NHP “T” in the naïve and mild hemi-parkinsonian states to study the effects of PD on cortical activity and motor behavior. A DBS lead was implanted into the posterolateral GPi of Monkey “L” and data was collected in the naïve, mild hemi-parkinsonian, and moderate parkinsonian states to further study the pathophysiology of PD and its modulation by DBS.

**Verification of DBS Targeting & Action**

Post-operative CTs of NHP “L” were performed to assess the quality of GPi targeting. As these scans were co-registered with pre-operative MRI scans to verify the location of electrode placement. As shown in Figure 5, the DBS lead’s distal tip is located at the ventral surface of the GPi, which permitted the majority of the electrode’s contacts to lie within the GPi itself. Critically, the NHP exhibited no adverse side-effects to sub-therapeutic stimulation (1V, 135Hz) and even more importantly, therapeutic DBS (3V, 135 Hz) decreased the severity of all measured parkinsonian signs as determined by the mUPDRS (See Figure 6). Together, these findings confirm that the DBS leads were appropriately implanted.

**Behavioral Assessment of Mild & Moderate PD**

**Task Performance Declined with Increasing Disease Severity**

Five key measures of motor performance were calculated during the visually-cued center-out behavioral task: reaction time (sec), reach time (sec), return time (sec), success rate (%), and trial rate (trials/ min). As shown in Figure 7, most of these measures significantly worsened between the mild and moderate PD states in the unstimulated NHP. Interestingly, the same observation was not made in the transition between the naïve state and mild parkinsonism.

Mean success rates, as defined by the percentage of targets touched in time for the juice reward, decreased with increasing disease severity (t=169, p<0.01). Mean reaction times, as defined by the average time between go cue presentation and reach onset, and mean reach times,
as defined by the average time between reach onset and target touch, both increased with disease severity (t=3.54, p<0.02 and t=4, p<.048, respectively). Trial rate, as defined by the number of trial completed each minute, decreased with increasing disease severity (t=2.73, p=0.05).

Interestingly, as shown in Figure 8, motor performance was more inhibited during reaches towards contralateral targets appearing across the monkey’s midline. This was most pronounced in the moderate Parkinsonian state and correlates with relatively increased reach and reaction times as shown in Figure 9.

**DBS Improved the Rate of Task Performance in Moderate PD**

As shown in Figure 10, of the objective measures of motor function described in Figure 9, all but one was unchanged with therapeutic DBS. Mean success rates, reaction times, reach times, and return times did not significantly change with DBS in either the mild or moderate states (t=0.016, p>0.05 and t=0.017, p>0.05, respectively). The notable exception was trial rate, which did significantly increase with DBS in the moderate state (p <0.05).

**Effects of DBS Varied with Reach Direction**

To further analyze the effects of DBS, the data for behavioral measures of no significant difference discussed above were separated based on whether the target appeared on either the left (ipsilateral) or right (contralateral) halves of the screen. Interestingly, the only variable affected by DBS and reach direction was success rate (See Figure 11). DBS improved the percentage of successful trials when reach was directed across the midline towards contralateral targets.

**DBS Reduced the Incidence and Duration of Freezing**

Perhaps the most obvious effect of DBS on motor behavior was its effect on episodes of freezing. Using data gathered from motion analysis of the infrared tracker on the wrist, freezing episodes were operationally defined as periods after target touch during which wrist velocity fell
below 50 cm/sec for greater than 200ms. Freezing period were further categorized base on their relative onset after target touch with “short-latency” freezes occurring within 700ms of target touch and “long-latency” freezes occurring 700ms or after target touch. As shown in Figure 12, DBS reduced both the incidence (percentage of trials) and duration (seconds) of freezing. Notably, DBS seemed to be most effective at treating long-latency-type freezes.

**DBS Reduced Variability in Objective Measures of Motor Behavior**

Of significant note was DBS’s reduction in behavioral variance in reaction, reach, and return times. Although none of these measures exhibited any notable difference in their means between conditions of DBS ON and OFF, a plot of their values across time reveals a relatively large variance in the DBS OFF condition as compared to periods of pallidal stimulation. As shown in Figure 13, there was a markedly tighter grouping of the values for these measures during the DBS ON condition.

**Electrophysiological Results**

**PD Disrupted Normal Cortical Single Motor Unit Functioning**

To better understand how PD affects normal cortical functioning, the activity of single motor units (SMUs) from both M1 and SMA were recorded during the behavioral reach task across both the naïve and parkinsonian conditions. These data were analyzed and compared with one another to identify characteristic changes following the induction of parkinsonism.

As shown in Figure 14, we observed a striking difference between the functioning of reach-related, cortical single motor units (SMUs) isolated from M1 and SMA in the naïve and parkinsonian states. The most notable differences include a reduction in peak firing frequency, an elevation in baseline activity, and increased variability of event-related neuromodulation in the parkinsonian state. Although this figure shows data from only two recording sessions, the
results are representative of population data (data not shown).

Of particular note are the findings of a cross-correlation analysis of the data presented in Figure 14. As shown in Figure 15, we observed a relatively high correlation in the firing of SMUs from M1 and SMA in the naïve condition. More specifically, the SMU in SMA consistently fired about 200ms prior to a discharge by the SMU in M1. This is a stark contrast to the same analysis of cortical SMUs from the parkinsonian state also shown in Figure 15. An entire order of magnitude separates the peak correlation of cortical SMU activity between these two conditions.

**PD Disrupted Population Functioning in SMA**

In an effort to look beyond the activity of single neurons, we next used spectral analysis to identify changes in the local field potentials (LFPs) generated by populations of neurons in SMA. As shown in Figure 16, Parkinsonian LFPs in SMA are characterized by an increased breadth and decreased spectral power of movement-related desynchronization (decrease in spectral power) by the beta frequency domain (14-35 Hz) as compared to the naïve condition. Also observed in PD was a decreased duration of beta desynchronization and suppression of movement-related synchronization (increase in spectral power) in the gamma frequency domain (>60 Hz).

**DBS Increased the Depth of Neuromodulation During Cells’ Preferred Task Epoch**

Cortical SMUs were recorded during performance of the behavioral reach task across all experimental conditions. To better understand the therapeutic mechanism of DBS, the activity of individual SMUs was compared between the DBS ON and OFF conditions. Perievent histograms of cortical single unit activity from both M1 and SMA illustrate an increased depth of modulation during the cells’ “preferred” task epoch. As shown in Figure 17A, M1 neurons
exhibited a spike in their firing frequency above baseline shortly after the onset of reach whereas SMA cells exhibited a similar spike in their firing frequency shortly before the onset of reach.

Peak modulation by the SMU in M1 exceeds at least one of the limits in a 95% confidence interval with or without DBS (See Figure 17A). Any data that surpasses one of these limits is considered to a significant change from baseline (p<0.05). Furthermore, as noted in Figure 17B, the magnitude of relative peak modulation was significantly greater during pallidal stimulation.

Relative peak modulation by the SMU in SMA was not significantly changed during DBS (See Figure 17B). However, a closer look at Figure 17A reveals that it was only during conditions of pallidal stimulation that the SMU’s maximal firing frequency exceeded the upper limit of a 95% confidence interval.

**DISCUSSION**

**Model and Therapy Verification**

As illustrated by Figure 6, MPTP administration succeeded in inducing both a mild and later, a moderate parkinsonian state. Scores of ~1-2 in nearly every category coincide with the UPDRS classification for moderate parkinsonism. This validates the efficacy of our model in approximating the signs of PD.

Figure 6 also serves to illustrate the relative efficacy of pallidal DBS in treating the signs and symptoms of PD in that despite stimulating only one hemisphere of the brain, we observed a profound decrease in the severity of parkinsonism across both sides of the body. Taken together with the imaging findings depicted in Figure 5, the significant improvement of UPDRS scores during DBS, indicates that the DBS lead was indeed localized to the appropriate region of the brain. Furthermore, these finding validate the therapeutic efficacy of pallidal stimulation. At the
Conclusion of the present study of which this report is only a part, DBS lead placement will be confirmed via perfusion and histology of the animal.

**Behavioral Analysis**

Figures 7-9 make clear the impact of parkinsonian symptoms on motor behavior. In both mild and moderate parkinsonism, task performance as measured by success rate, reaction time, reach time, and trial rate, declined across every variable. Taken together, these findings validate our use of a center-out reaching task to measure behavioral changes induced by PD and serve as the standard to which we can compare data obtained during pallidal DBS.

Figure 10 illustrates the difficulty inherent in teasing out how exactly DBS exerts its therapeutic benefit. Consistent with the literature, DBS did not improve average behavioral measures taken from the epochs externally driven by the “Go-cue” (i.e. success rate, reaction time, and reach time) (Jahanshahi et al., 1995; Majsak et al., 1998; Siegert et al., 2002). The only significant DBS-induced change observed across the analyzed days was an increase in the trial rate of the moderately parkinsonian animal. When regarded in the context of our findings regarding freezing as described in Figure 12, we concur with previous suggestions that the basal ganglia is less involved in externally paced movements and that therefore, the benefit of DBS may partially lie in restoring internally regulated, non-cued movements (Georgiou et al., 1993; Kuhn, 2004).

Further analysis by subdividing the mean measures of motor behavior into ipsilateral and contralateral reaches revealed only that DBS seemed to exert a greater effect on contralateral reaches (See Figure 11). Although the explanation for this phenomenon remains unclear, we propose that a contralateral reach requires a greater degree of motor coordination than an ipsilateral one. Therefore, we believe these findings to be supportive of the suggestion that DBS
of the basal ganglia might restore the functioning of motor circuits involved in the planning and coordination of movement (Fasano et al., 2011; Schneider et al., 1992).

While the above results are certainly interesting, the most important behavioral finding of this project are those shown in Figure 13. The variability in reaction, reach, and return times was markedly larger during conditions of DBS OFF as compared to DBS ON, which suggests that parkinsonism might be degrading stereotypy in movement. This is a plausible explanation when one considers that one of PD’s fundamental features is “arrhythmokinesis”, a variability in repetitive movements (Trager et al., 2015). These findings are consistent with related studies, which suggest that DBS is the major therapeutic contributor in reducing variability in gait of parkinsonian patients (Faist, 2001; Hausdorff et al., 2009). How exactly behavioral variability is improved however, requires consideration of electrophysiological data.

**Electrophysiological Analysis**

As shown in Figures 14-16, we succeeded in identifying characteristic and abnormal cortical activity at both the single motor unit and population levels in the parkinsonian monkey. At the level of individual neurons, we observed a decrease in peak firing frequency, an increased degree of baseline activity, and increased variability as compared to the naïve state. In the most simple of terms, these changes all work to decrease the resolution of the “message” encoded in event-related neuromodulation (See Figure 14). These findings are interesting in that they serve as mixed evidence both for and evidence against what might be considered the canonical model of PD pathophysiology: the “rate” model. As illustrated in Figure 2, the rate model predicts that dopamine depletion in PD leads to an increase in the activity of the GPi which should in turn, reduce the activity of cortical neurons (Albin et al., 1989; DeLong, 1990; DeLong and Wichmann, 2007). Although a decrease in peak firing frequency certainly supports this
hypothesis, the observed increase in baseline activity tells quite the opposite story. In short, our data is inconclusive with respect to the rate model.

Furthermore, we identified what appeared to be almost a complete breakdown in communication between SMA and M1 in the parkinsonian state (See Figure 15). Notably in monkey “T,” these changes in the activity of M1 and SMA were observed despite the lack of any profound behavioral changes (e.g. reach time). These findings support the working hypothesis that there exists a pathophysiological threshold prior to which there is no overt manifestation of the behavioral signs of PD (Jankovic, 2008; Siderowf and Stern, 2006; Wu et al., 2011).

As for population-level activity, we observed characteristic abnormal activity across multiple frequency domains – most notably beta and gamma – in cortical LFPs (See Figure 16). Briefly, our notable findings include a suppression of movement-related synchronization in the gamma frequency domain and a broader, deeper, and abbreviated beta desynchronization. These findings lend support to the “firing pattern” model of PD pathophysiology, which proposes that disordered oscillatory activity by the basal ganglia disturbs informational processing and in turn, motor behavior (Hutchison, 2004; Nambu et al., 2015). Although it is unclear what exactly these disturbances in LFPs means for individuals with PD, they may at the very least be regarded as biomarkers of PD.

As evidenced by Figure 17, DBS profoundly modulated the peak firing rates of cells in both M1 and SMA during their “preferred” epoch. Pallidal stimulation significantly increased the relative peak of modulation by a M1 neuron that was already significantly modulated beyond baseline in the DBS OFF condition. More importantly however was its effect on the SMU from SMA. Although the relative peak modulation DBS ON was not significantly different from the DBS OFF baseline, pallidal stimulation succeeded in inducing preparatory activity by SMA that
was significantly above baseline. This finding is particularly exciting because it supports in part our earlier claim that coordination was the source of the differential effect of DBS on ipsilateral and contralateral reaches (Fasano et al., 2011; Schneider et al., 1992). If the normal functioning of SMA is indeed all but knocked out in the parkinsonian state, it seems reasonable that a more complex motor task would be more significantly impaired than a simple one. The “reactivation” of this area by DBS is therefore, likely responsible for enhanced coordination and motor planning. Furthermore, these findings are consistent with the suggestion that pallidal lesioning improves motor behavior via SMA-induced improvements in motor planning and although DBS doesn’t actually lesion the GPi, its action has been likened to an “informational lesion” (Agnesi et al., 2013; Samuel et al., 1997).

When evaluated in the context of the results presented in Figure 13, these findings hint at a therapeutic mechanism whereby DBS shifts cortical activity towards a more “normal” state. More specifically, we propose that pallidal stimulation acts to somehow “clarify” or resolve the activity of neurons in M1 and SMA and in doing so, make their encoded messages more salient for downstream components of the motor pathway.

**Future Work**

The above research has the potential to have significantly impact our understanding of PD pathophysiology and the development of more effective therapeutic technologies. Although the data and conclusions are diverse, they can all be applied to the same target: adaptive deep brain stimulation (aDBS). Presently, DBS therapies are considered “open-loop” in that the stimulation is chronic and neither monitors nor responds to activity in the brain. aDBS represents the next step by DBS therapies in that it is a “closed-loop” technology that monitors neural activity and adjusts its own output accordingly (Beudel and Brown, 2016). Although the effects
of conventional open-loop DBS technologies are nothing short of magical, they are limited in
terms of effectivity, side effects, and battery life. Therefore, the need for aDBS devices is great.

In identifying individual- and population-level biomarkers of PD, our research both
informs and instructs future research necessary for the development of aDBS technologies. The
data presented here identifies parameters to which an aDBS device could be programmed to
recognize and respond. Just one example of the many avenues future research might pursue
would be the use of experimental aDBS devices to explore these biomarkers to verify their
validity as therapeutic “targets” in humans. Although our findings are encouraging, there remains
a substantial amount of work before aDBS devices receive approval for widespread use in human
patients with PD. The wait however, will most certainly be worth it.
Figure 1. An anatomically correct illustration of the basal ganglia, its constituent structures, and their relative location within the brain. Image adapted from https://syntaptogaming.files.wordpress.com/2012/01/basalganglia_image1.jpg.
Figure 2. Adapted schematic illustration of the basal ganglia–thalamocortical circuits of healthy (A) and parkinsonian individuals (B) proposed in the “rate” model of parkinsonian pathophysiology (DeLong, 1990). The relative strength of excitatory and inhibitory innervation is indicated by arrow thickness. As depicted in B, the loss of dopaminergic input to the striatum from the SNC significantly alters the excitatory and inhibitory tone within this pathway. The lightning bolts indicate the target of deep brain stimulation in the present study. M1 = Primary Motor Cortex; SMA = Supplementary Motor Area; GPe = External Globus Pallidus; GPi = Internal Globus Pallidus; SNC = Substantia Nigra pars compacta; SNr = Substantia Nigra pars reticulate; D1 = D1-like dopamine receptor; D2 = D2-like dopamine receptor; FEF = Frontal Eye Fields; STN = Subthalamic Nucleus; TH = Thalamus; SC = Superior Colliculus
Figure 3. A. The visually-cued center-out reach behavioral task used to assess behavior. The animal was seated in front of a touchscreen monitor and a capacitive start-pad. Reach kinematics were collected using an infrared video-based tracking system. B. One of eight possible targets, each centered 15 cm from the middle of the screen, was presented randomly for each trial. This sample shows the location of all screen touches, both successful and failed, for a single experimental block in the naïve state. C. Epochs were determined by the presentation of the go cue, reach onset, target touch, and return to start pad.
Figure 4. Monkey cicerone was used in the stereotaxic planning of cephalic chamber placement. A illustrates the positional identity of the different recording electrodes in M1 (top) and SMA (bottom) by overlaying the recording chambers and microelectrode map with a 3D reconstruction of the monkey’s brain. Local identifying sulci are labeled and highlighted in red. B illustrates the location of the M1 & SMA chambers relative to both the cortex and the CT scan. M1: Primary motor cortex; SMA: Supplementary motor area; CS: Central sulcus; SPS: Superior prefrontal sulcus; AS: arcuate sulcus; sp: spur of the arcuate sulcus.
Figure 5. Co-registered pre-operative MRI and post-operative CT scans from NHP “L” overlaid with coronal section from a brain atlas illustrates proper DBS lead placement. The DBS lead has been outlined and identified for clarity’s sake. The lead with 8 cylindrical contacts (C0:7, 0.5 mm wide with 0.5 mm spacing) was implanted within the posterolateral (sensorimotor) segment of the GPi (highlighted in red) and targeted to its ventral surface. This placement permitted the complete and chronic immersion of 5 contacts in the GPi. During periods of DBS ON, stimulation was delivered to contacts 3 and 4.
Figure 6. DBS improved clinical scores of parkinsonian motor signs as measured by the mUPDRS. This particular example is taken from the average of monkey “L”’s mUPDRS scores in the moderate parkinsonian state. Of particular note is the ability with which DBS reduced the scores for every parameter measured by the mUPDRS. Furthermore, although stimulation was only unilateral, it succeeded in ameliorating the signs and symptoms of PD on both sides of the body. Although not pictured here, data from the mild hemi-parkinsonian state was very similar, the two differences being that only the left side was affected and a reduced magnitude of the scores for both DBS OFF and DBS On.
Figure 7. Objective measures of task performance by monkey “L” in the naïve (black) and parkinsonian (blue) conditions. A. Mean success rates (% of targets touched) decrease with disease severity (t=169, p<0.01). B. Mean reaction times increased with PD state (t=3.54, p<0.02). C. Average cued-reach times changed with disease severity (t=4, p<0.05). D. Rate of task performance (average trials per minute) decreased with increased disease severity (t=2.73, p=0.05).
Figure 8. Sample recording sessions indicate that the effect of PD on motor performance varied with reach direction. In the above comparison of successful trials in the naïve (left), mild PD (middle), and moderate PD (right) conditions, mean success rates (percentage of targets touched) decreased with disease severity (p<0.01) and were largely correlated to targets presented on the side contralateral to the reaching arm. This effect was not observed when comparing any other directions (e.g., top and bottom).
Figure 9. A directionally variable effect of PD on reach and reaction time was only observable in moderate PD. In the mild state (left), there were no marked differences in either reaction or reach times between ipsilateral (positions 5-7) or contralateral (positions 1-3) targets. However, while not significant, there was an observed increase in both measures for contralateral targets as compared to ipsilateral targets. The overall average reach times were significantly increased in both monkeys as compared to their respective naïve data (p<0.05). The overall average reaction time was only significantly greater in the moderately parkinsonian monkey (p<0.05).
Figure 10. DBS (red) only significantly altered the “rate” measure of behavioral performance.

DBS failed to significantly change success rate (A), reaction time (B), reach time (C), and return time (D) in both the mild (left) and the moderate (right) parkinsonian states (t=0.016, p>0.05 and t=0.017, p>0.05, respectively). However, the rate of trials (E) did improve significantly (p<0.05) with DBS in the moderate state.
Figure 11. Sample recording sessions in A show the effect of pallidal DBS on task performance as a function of reach direction. As indicated in B, success rates improved during DBS On (red) when reach was across the midline towards a contralateral target. Reaction time and reach time did not vary with reach direction or with DBS (data not shown).
Figure 12. Pallidal DBS reduced both the incidence (A) and duration (B) of “freezing” in the moderately parkinsonian monkey (Subject “L”). Freezing incidents (A) identified by motion analysis data from the wrist marker (v < 50cm/sec for t > 200ms after target touch and before return to start pad) were significantly reduced during DBS (red; p<0.05). The duration of freezing (B) was also significantly reduced by DBS (red; p<0.05). DBS was most effective at reducing the duration and incidence of freezes with a relatively late onset following target touch (short < 700 ms ≤ long).
Figure 13. DBS decreased variability in reaction time, reach time, and return time. This figure illustrates each component of an experimental block with data from a typical recording session for monkey “L”: the DBS OFF “baseline”, DBS ON, and the return to baseline following a 30-minute “washout” period during which behavior and neural activity were allowed to return to baseline. Although the mean reaction, reach, and return time are relatively unchanged between DBS OFF and ON, the standard deviation is significantly reduced during DBS. This suggests that DBS may improve repetitive behavior by making the reaching movement markedly more stereotypic in nature.
Figure 14. Sample perievent histograms of reach-related, cortical single motor unit (SMU) activity in monkey “T” across two recording sessions of 80 trials each: Naïve (top) and Mild PD (bottom). SMUs in M1 (left, aligned on reach) fired preferentially during the reach epoch (See Figure 3C) whereas SMUs in SMA (right, alight on go-cue) fired during the reaction time epoch. Raster plot markers (“Go-Cue” and “Reach”) represent the trial-by-trial onset of adjoining epochs/events relative to the reference event (t=0). Notable findings include reduced peak firing frequencies, increased baseline activity, and less salient event-related neuromodulation in the mild parkinsonian state as compared to naïve data.
Figure 15. Induction of parkinsonism significantly reduced the correlation of cortical SMUs in M1 and SMA. The proportion and timing of coincidental firing of the paired SMUs shown in Figure 14 was calculated to generate the above cross-correlogram. The reference event (t=0) represents activity by the SMUs in M1 whereas the graphed lines represent coincidental activity by the SMUs from SMA in the naïve (blue) and parkinsonian (orange) conditions. In the naïve condition, nearly 20% of all SMA activity preceded activity in M1 by ~200 ms. In contrast, less than 2% of SMA activity is correlated with activity by M1 in the parkinsonian state.
Figure 16. An example of spectral analysis of local field potentials (LFPs) recorded in SMA in both the naïve (top) and parkinsonian (bottom) conditions. Warmer colors indicate points of greater spectral power whereas cooler colors indicate points of decreased spectral power relative to target touch (t=0). From left to right, the dotted lines identify go-cue, reach, target touch, and return (if present). Notable findings from the numerous spectral analyses include an increased breadth and decreased spectral power of movement-related desynchronization (decrease in spectral power) by the beta frequency domain (14-35 Hz) in the parkinsonian condition. Also observed were an increased degree of transience by the aforementioned beta desynchronization and an apparent suppression of movement-related synchronization (increase in spectral power) in the gamma frequency domain (>60 Hz).
Figure 17. DBS increased the depth of neuromodulation during cells’ preferred task epochs. As shown by perievent histograms aligned on reach in A, DBS markedly increased the “depth”, or deviation from the mean, of event-related neuromodulation by SMUs in both M1 (left) and SMA (right). B presents a numerical summary of the relative modulation by both SMUs across the DBS On (red) and DBS (off) conditions. The relative peak modulation by the SMU from M1 was significantly increased during pallidal stimulation (p<.001). Although the relative peak modulation by the SMU in SMA was significantly different between conditions, it was only during pallidal stimulation that cell’s maximum discharge rate exceeded the limit of the 95% confidence interval.
**SOURCES CITED**


