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# The Role of Beta Activity in Blink Abnormalities in a Rat Model of Parkinson's Disease

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by

**Jaime Kaminer**

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**Jaime Kaminer**

We, the dissertation committee for the above candidate for the
Doctor of Philosophy degree, hereby recommend
acceptance of this dissertation.

**L. Craig Evinger, PhD, Professor**
**Departments of Neurobiology and Behavior and Opthalmology**
**(Advisor)**

**John Robinson, PhD, Professor**
**Department of Psychology**
**(Defense Chairperson)**

**Brenda Anderson, PhD, Associate Professor**
**Department of Psychology**

**Aprajita Mohanty, PhD, Assistant Professor**
**Department of Psychology**

**Alfredo Fontanini, PhD, MD, Associate Professor**
**Department of Neurobiology and Behavior**

This dissertation is accepted by the Graduate School

Charles Taber
Dean of the Graduate School

Abstract of the Dissertation

**The Role of Beta Activity in Blink Abnormalities in a Rat Model of Parkinson's Disease**

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Parkinson's disease (PD) is a devastating neurodegenerative disease that causes difficulty initiating voluntary movements among other symptoms. Electrophysiological work highlights the presence of exaggerated synchronized beta band (10-30 Hz) oscillations throughout the cortico-basal ganglia network in PD. Although a causal role of beta oscillations in PD symptoms has not been established, many hypothesize that beta activity is "antikinetic" and the source of Parkinsonian motor symptoms. This relationship remains unclear because the strength of beta oscillations changes dynamically with cues that initiate movement and because the voluntary motor symptoms of PD are often not present in animal models of PD. Therefore, studying an involuntary movement shared by all mammals is critical to understanding the role of beta oscillations in creating Parkinsonian symptoms. The blink system provides this opportunity as dopamine depletion induces identical symptoms in PD patients and the 6-hydroxydopamine (6-OHDA) rat model of PD. My investigations utilized the blink system to examine the role of beta oscillations in Parkinsonian motor symptoms.

My first study examined the effect of dopamine on spontaneous blinking in rats. Dopamine depletion decreased the spontaneous blink rate as occurs in PD patients and reduced the regularity of spontaneous blinking. Disrupting beta oscillations with 130 Hz Subthalamic Nucleus Deep Brain Stimulation (STN DBS) did not attenuate these symptoms. Second, I examined the role of beta oscillations in PD blink reflex abnormalities, impaired blink plasticity and increased trigeminal reflex blink excitability. Disrupting beta oscillations with 130 Hz STN DBS restored normal plasticity and attenuated increased blink reflex excitability displayed by 6-OHDA lesioned rats. In the third study, I directly tested the role of beta oscillations in blink reflex abnormalities in PD by determining whether beta (16 Hz) stimulation was sufficient to induce Parkinsonian-like abnormalities in normal rats. Only 16 Hz STN DBS impaired reflex blink plasticity and produced reflex blink hyperexcitability in normal rats. The role of beta oscillations in blink reflex abnormalities but not spontaneous blink reduction (Study 1), suggests separate circuits for basal ganglia modulation of these behaviors. Overall, these data provide strong support for the importance of beta oscillations in the generation of Parkinsonian symptoms.

I dedicate this thesis to my amazingly generous, thoughtful, supportive, hardworking, and fun parents and to the memory of Avron Grossman who asked me every time I saw him for the past ten years if he could call me “doc” yet.

## TABLE OF CONTENTS

**LIST OF FIGURES** ..... **vii**

**CHAPTER 1: GENERAL INTRODUCTION** ..... **1**

*Organization and Function* ..... *1*

*Introduction to Parkinson's Disease* ..... *4*

*Rate Model of PD* ..... *6*

*Pattern Abnormalities in PD* ..... *7*

*Beta Band Activity* ..... *9*

*6-OHDA Lesioned Rat Model of PD* ..... *10*

*Blinking in PD* ..... *12*

*Treatment for PD* ..... *15*

*Mechanisms of Deep Brain Stimulation* ..... *17*

*Conclusions* ..... *19*

**FIGURES** ..... 20

**CHAPTER II: DOPAMINERGIC MODULATION OF THE SPONTANEOUS BLINK GENERATOR** ..... **26**

*Introduction* ..... *26*

*Materials and Methods* ..... *28*

*Results* ..... *33*

*Discussion* ..... *42*

**FIGURES** ..... 46

**CHAPTER III: DISRUPTING BETA OSCULLATIONS RESTORES NORMAL BLINK BEHAVIOR IN 6-OHDA LESIONED RATS** ..... **58**

*Introduction* ..... *58*

*Materials and Methods* ..... *60*

*Results* ..... *65*

*Discussion* ..... *69*

**FIGURES** ..... 73

**CHAPTER IV: BETA STIMULATION INDUCES PARKINSONIAN LIKE BLINK ABNORMALITIES IN RATS** ..... **77**

*Introduction* ..... *77*

*Materials and Methods* ..... *79*

*Results* ..... *83*

*Discussion* ..... *86*

**FIGURES** ..... 90

**CHAPTER V: GENERAL DISCUSSION** ..... **92**

*General Summary* ..... *92*

*Implications* ..... *95*

**REFERENCES** ..... **99**

**LIST OF FIGURES**

FIGURE 1 ..... 20
FIGURE 2 ..... 21
FIGURE 3 ..... 22
FIGURE 4 ..... 23
FIGURE 5 ..... 24
FIGURE 6 ..... 25
FIGURE 7 ..... 46
FIGURE 8 ..... 47
FIGURE 9 ..... 48
FIGURE 10 ..... 49
FIGURE 11 ..... 50
FIGURE 12 ..... 51
FIGURE 13 ..... 52
FIGURE 14 ..... 53
FIGURE 15 ..... 54
FIGURE 16 ..... 55
FIGURE 17 ..... 56
FIGURE 18 ..... 57
FIGURE 19 ..... 73
FIGURE 20 ..... 74
FIGURE 21 ..... 75
FIGURE 22 ..... 76
FIGURE 23 ..... 90
FIGURE 24 ..... 91

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# I. GENERAL INTRODUCTION

## General Basal Ganglia Function and Organization

The basal ganglia are composed of several interconnected sub cortical nuclei including the caudate and the putamen (the striatum), the globus pallidum (internal (GPi) and external (GPe) components), the substantia nigra (pars reticulata (SNr) and pars compacta (SNc)), and the subthalamic nucleus (STN). Although the basal ganglia were traditionally thought of as purely a motor structure, recent evidence reveals its critical role in learning, action selection, motivation, and a variety of other higher order cognitive and emotional functions. The now well established, far reaching influence of the basal ganglia is not surprising considering its widespread connections with cortical, limbic, and brainstem structures. An informative way to understand how the basal ganglia modulate these higher order processes is to examine how the basal ganglia regulate movement.

As outlined in classical models, cortical inputs synapse directly onto the dendritic spines of medium spiny neurons (MSNs) that comprise the vast majority of neurons in the striatum. The striatum also receives dopaminergic input from the SNc, one of the main sites of dopamine containing cells in the brain. MSNs project to the GPi or SNr, the output structures of the basal ganglia, though either a "direct" or "indirect" pathway (Delong *et al.*, 1984; Albin *et al.*, 1989). The GPi/SNr exerts a tonic inhibitory control over target structures that include the thalamus (which relays information back to the cortex), the superior colliculus (SC), and the pedunculopontine nucleus (PPT). A brief pause of this tonic inhibition allows a movement to occur.

Early models postulated that the direct and indirect pathway are initiated with MSNs that express either dopamine type 1 ($D_1$) or dopamine type 2 ($D_2$) receptors, respectively. MSNs that

express $D_1$ receptors make a monosynaptic projection to the SNr/GPi. When activated, these MSNs inhibit SNr/GPi neurons. Phasic inhibition of these tonically active neurons transiently disinhibits basal ganglia target structures that allow a planned movement to go forward. MSNs that express $D_2$ receptors relay information through the GPe and the STN before projecting to the GPi/SNr. Activation of these MSNs leads to an increased inhibition of the basal ganglia target structures that prevents the occurrence of unwanted movements (Penney & Young, 1986; Albin *et al.*, 1989). Additionally, the classical model held that nigrostriatal dopamine differentially effected $D_1$ and $D_2$ expressing MSNs. Under normal conditions, nigrostriatal dopamine was thought to facilitate $D_1$ expressing MSNs, but inhibit $D_2$ expressing MSNs (*Figure 1A*) (Albin *et al.*, 1989; Gerfen *et al.*, 1990).

Additional early anatomical and physiological work in monkeys demonstrated the presence of similarly organized, but distinct cortico-basal ganglia-thalamic-cortical loops that regulated different functions depending upon the origin of the cortical input. These parallel loops were termed the skeletal motor loop, the associative loop, the oculomotor loop, and the limbic loop (Alexander *et al.*, 1986). For example, the motor loop relayed information through the putamen, lateral regions of the GPi, and the ventral lateral and ventral anterior nuclei of the thalamus before it was sent back to motor related cortical regions. In contrast, the limbic loop, relayed information through the ventral striatum (the nucleus accumbens), the ventral pallidum, and the medial dorsal thalamic nuclei before it was sent back to limbic regions. Initially based on anatomical studies in non-human primates, functional magnetic resonance imaging (fMRI) studies in humans demonstrated the presence of these loops in humans and supports their proposed functional roles (Lehericy *et al.*, 2005; Draganski *et al.*, 2008).

Investigators elucidated the direct and indirect pathway organization in the late 1980s (Penney & Young, 1986; Albin *et al.*, 1989). More recent evidence, however, indicated that this classical model was over simplified (DeLong & Wichmann, 2009; Obeso & Lanciego, 2011). It is now known that the basal ganglia is not organized in the uni-directional manner hypothesized by the classical models (Graybiel, 2008). There are reciprocal connections between most nuclei including the GPe and the striatum (Sato *et al.*, 2000) and the SNc and the striatum (Haber *et al.*, 2000). Additionally, the role of the STN changed drastically in newer models of basal ganglia organization. In addition to indirect input from the striatum, the STN receives a direct input from the cortex, forming a "hyperdirect" cortico-STN-GPi/SNr pathway that plays an important role in inhibiting and changing motor plans (Nambu *et al.*, 2002). Furthermore, the STN has reciprocal connections with the SNc and the GPe, and also receives direct input from the thalamus (*Figure 1B*).

Nevertheless, the oversimplified classical model makes accurate predictions about the roles of the indirect and direct pathways. The general organization and function of the direct and indirect pathways have been recently validated using optogenetic techniques to specifically activate MSNs expressing either $D_1$ or $D_2$ receptors. Using this technique in alert mice, investigators demonstrated that bilateral stimulation of $D_2$ expressing MSNs increases freezing and decreases locomotion, whereas activation of $D_1$ expressing MSNs increases locomotion. These data clearly support the classical model's hypothesis that activation of the direct pathway facilitates movement, whereas activation of the indirect pathway inhibits movement (Kravitz *et al.*, 2011). The continued development of these novel methods and developing a deeper understanding of basal ganglia pathologies, like Parkinson's disease (PD), will provide more insight into the functional organization of the basal ganglia.

**An Introduction to Parkinson's Disease**

PD is a neurodegenerative disorder that results from progressive dopaminergic cell death in the SNc. Approximately 70-80 percent of these dopamine neurons die before the onset of most Parkinsonian symptoms (Ruberg *et al.*, 1995). Although there are a small amount of cases that are genetic (Klein & Westenberger, 2012), the vast majority of PD cases are idiopathic. The cardinal motor symptoms of PD include small and slow voluntary movements (bradykinesia), rigidity, postural instability, difficulty walking, and a resting tremor at around 4-6 Hz. Although they are extremely debilitating, these Parkinsonian motor symptoms often respond relatively well to dopamine replacement treatment early in the disease progression.

Although PD is predominantly thought of as a movement disorder, patients also suffer from a variety of non motor symptoms that include severe cognitive and emotional deficits. One prominent symptom is impaired implicit learning (Roncacci *et al.*, 1996) that occurs early in the progression of PD signs and symptoms. This learning deficit appears to result from aberrant activity in the sensorimotor cortical loop that severely impairs the performance of automatic and habitual actions (Graybiel, 2008). There are also executive functioning deficits in PD, including working memory, set shifting, and attentional impairments (Dubois & Pillon, 1997). These symptoms tend to progress with disease severity, and are therefore difficult to separate from the dementia experienced by 30% of PD patients (Aarsland *et al.*, 2005). In addition to these cognitive symptoms, PD patients commonly suffer from depression (Burn, 2002). Evidence consistently shows that depression in PD plays a critical role in patients' decreased quality of life (Karlsen *et al.*, 1999). Unfortunately, these non motor symptoms rarely respond to typical antiparkinsonian treatments. This problem has led to an increase in the attention and resources focused on the neural bases of these cognitive and emotional problems in PD.

In addition to these typical motor and more recently recognized cognitive symptoms, PD patients also suffer from a variety of symptoms that reveal abnormal sensory (Sandyk & Snider, 1985) and brainstem (Grinberg *et al.*, 2010) functioning. The most prominent sensory deficit is impaired olfactory perception. Early and severe olfactory dysfunction is extremely common in PD, and is therefore often considered a preclinical symptom of the disease (Liberini *et al.*, 2000). Although the pathophysiology of olfactory dysfunction in PD is not totally understood, there is evidence for marked dopamine denervation in the olfactory tubercles (Bogerts *et al.*, 1983). Investigators hope that closer examination of these deficits will provide insight into the pathophysiological processes occurring in other brain regions related to odor perception, and provide a useful tool in early detection and diagnosis of PD (Liberini *et al.*, 2000).

Parkinsonian brainstem related symptoms include severe autonomic (Walter *et al.*, 2006), sleep (Boeve *et al.*, 2007; Focke & Trenkwalder, 2010), pain control (Quinn *et al.*, 1986), and oculomotor (Corin *et al.*, 1972) abnormalities. Many of these symptoms affect the vast majority of PD patients, and are regularly reported before the manifestation of motor symptoms such as bradykinesia or resting tremor. These observations provide some support for the Braak model of PD that postulates that lewy body (LB) formation in several brainstem nuclei associated with these behaviors (Greenfield & Bosanquet, 1953; Grinberg *et al.*, 2010) may occur before the formation of LBs in the SNc (Braak *et al.*, 2003). Nevertheless, it is also important to note that several basal ganglia nuclei, exhibit strong anatomical connections with brainstem structures including the SC, the cuneiform, the periaqueductal grey, and the PPT and parabrachial nuclei (McHaffie *et al.*, 2005; Redgrave & Coizet, 2007). The role of the aberrant basal ganglia – brainstem interactions in the generation of these abnormal behaviors in PD, however, is not well

understood. Closer investigations of these "pre – motor" PD symptoms may provide unique insights into the pathophysiology and new treatments for PD.

**The Rate Model of Parkinson's Disease**

The significant death of dopamine containing neurons in the SNc critically alters the pattern of basal ganglia activity. Early experiments aimed at elucidating exactly how PD altered basal ganglia circuits utilized the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate model of PD (Burns *et al.*, 1983). MPTP, a neurotoxin that primarily kills dopamine containing neurons in the SNc, was discovered when a chemist accidentally synthesized the designer drug MPPP with MPTP, and induced acute Parkinsonism in seven individuals who took this drug (Langston & Ballard, 1984). Non-human primates injected with MPTP present with PD symptoms strikingly similar to PD patients, including akinesia, rigidity, and resting tremors. Therefore, MPTP treated monkeys are an especially useful animal model to characterize the changes in neural activity and behavior in response to the severe dopamine depletion that occurs with PD.

MPTP treated monkeys were critical in the development of the rate model of PD, which was the predominant theory of Parkinsonism in the late 1980s and 90s (Albin *et al.*, 1989). Investigators using electrophysiological techniques to record neural activity in different basal ganglia nuclei discovered that specific nuclei were either overactive or underactive in MPTP treated monkeys compared to controls. Specifically, it was demonstrated that firing frequency of neurons in the STN (Bergman *et al.*, 1994) and the GPi (Filion & Tremblay, 1991) were significantly increased, whereas the firing frequency of neurons in GPe significantly decreased (Filion & Tremblay, 1991) after treatment with MPTP. Metabolic studies examining 2-deoxyglucose (2DG) in MPTP treated monkeys further supported these electrophysiological data

(Schwartzman & Alexander, 1987). These data led to the interpretation that MSNs that give rise to the indirect pathway were overactive in PD, while MSNs of the direct pathway were underactive (*Figure 2*). Dopamine depletion reduces the inhibition of indirect pathway MSNs by D2 receptors. These MSNs then exert more inhibition of the GPe that leads to STN neuron disinhibition. This overactive glutamatergic projection from the STN to the GPi increases GPi inhibition of the thalamus. Conversely, dopamine depletion decreases the activity of direct pathway MSNs through the loss of excitatory drive from D1 receptors. As a result of this reduced activity, there is disinhibition of GPi neurons, further increasing the inhibition of thalamic neurons already caused by increased STN activity. Overall, the reduced activity of the direct pathway and the increased activity of the indirect pathway produced a pathological increase in basal ganglia output that blocked or slowed movement.(Albin *et al.*, 1989; Bergman *et al.*, 1990). Further support for the rate model came from experiments demonstrating that lesions of the STN and GPi in MPTP treated monkeys reduced the increased output of the GPi and ameliorated bradykinesia and akinesia symptoms (Wichmann *et al.*, 1994; Guridi *et al.*, 1996). Consistent with the pathophysiologic rate model, animal models of hyperkinetic disorders such as hemiballism, chorea, and dyskinesias consistently exhibited reduced basal ganglia output (Mitchell *et al.*, 1985; Crossman, 1990).

**Pattern Abnormalities in PD**

Soon after the formation of the rate model, investigators began to notice inconsistencies suggesting that rate changes could not solely explain PD pathophysiology. The most obvious challenge to the rate hypothesis is that GPi lesions do not cause dyskinesias, or unwanted involuntary movements, as the rate model predicts. Based on the rate model, lesioning the GPi would remove the inhibition from the basal ganglia to the thalamus and allow unwanted

movements to occur. In fact, a pallidotomy, a GPi lesion, does not cause dyskinesias as the rate model would predict. An additional challenge to the rate hypothesis is that lesions of the motor thalamus do not result in akinesia, or aggravate Parkinsonian motor symptoms (See *Figure 2*) (Marsden & Obeso, 1994). These observations led investigators to shift their attention to the role of other changes in neural activity being responsible for PD pathophysiology.

Several of the early electrophysiological studies in MPTP treated monkeys reported that dopamine depletion not only changed the spontaneous firing rate of basal ganglia neurons, but also altered their firing pattern. Recordings from the GPi and the STN in MPTP treated monkeys showed that neurons developed a bursting pattern instead of the irregular tonic activity common to normal basal ganglia neurons (Filion & Tremblay, 1991; Bergman *et al.*, 1994). Single cell recordings clearly demonstrated the increased bursting activity after MPTP treatment (*Figure 3*). Although the physiological mechanisms underlying these bursting patterns are not well understood, it is hypothesized that the bursting pattern results into abnormal information processing that likely occurs in PD (Wichmann & Soares, 2006).

Another prominent characteristic of neurons in the Parkinsonian state is that they fire more synchronously than basal ganglia neurons in a normal state (*Figure 3*). Evidence from MPTP treated monkeys (Bergman *et al.*, 1994) and more recently from PD patients (Levy *et al.*, 2000) demonstrates that dopamine depletion synchronizes individual neurons and basal ganglia nuclei. Additionally, enhanced synchrony has also been reported in the motor cortex of monkeys after MPTP treatment (Goldberg *et al.*, 2002). Therefore, it seems likely that dopamine plays a critical role in regulating neuronal discharges throughout cortico-basal ganglia networks; a critical function that is lost with PD.

**Beta Band Activity**

Careful analysis of this aberrant synchronized bursting activity revealed that basal ganglia neurons spontaneously oscillate between 10-30 Hz, in the "broad beta band." Electrophysiological recordings of individual neurons allow investigators to perform autocorrelations on individual neurons, cross correlations on pairs of neurons, and power spectrum analyses to determine the frequency at which these individual or pairs of neurons oscillate (*Figure 4*). These single cell recording techniques revealed the presence of spontaneous beta band oscillatory activity of neurons in the STN, GPe, and GPi of MPTP treated monkeys (Wichmann & Soares, 2006). The most prominent synchronous oscillations have been reported to be between 12-20 Hz in MPTP treated monkeys (Heimer *et al.*, 2006). Importantly STN inactivation (Wichmann *et al.*, 1994) and dopamine replacement therapies (Heimer *et al.*, 2006) that attenuate the motor symptoms in MPTP treated monkeys also suppress the beta band oscillatory activity in the GPi.

Although it is more difficult to record individual neuron activity in PD patients, neurosurgeons are often able to record local field potentials (LFP) prior to performing neurosurgical treatments or from implanted deep brain stimulating electrodes. The LFP recordings provide valuable information about the neural activity of large pools of neurons. LFP recordings from patients off of their dopamine replacement consistently revealed powerful oscillations in the broad beta band in the basal ganglia (Brown & Williams, 2005; Kuhn *et al.*, 2005). Oscillations within this beta band have also been reported in the cerebral cortex of PD patients (Brown & Williams, 2005). Moreover, there is an impressive correlation with the levodopa (L-dopa) induced reduction of beta band LFP activity and improvement in akinesia score in PD patients (Hammond *et al.*, 2007). These data and those from MPTP treated monkeys

indicate that synchronous oscillatory activity is an important feature of the basal ganglia cortical network with dopamine depletion.

The strong correlations between synchronized beta oscillations and slowed movement in PD led to the theory that beta band activity is anti-kinetic and potentially the source of Parkinsonian symptoms (Hammond *et al.*, 2007; Jenkinson & Brown, 2011). This theory is further supported by evidence that STN stimulation within the beta band can slow movements (Chen *et al.*, 2007) and worsen akinesia in PD (Timmermann *et al.*, 2004). However, the role of beta band activity in the generation of PD symptoms like bradykinesia and rigidity is difficult to assess as the strength of beta band activity changes with cues that are used to initiate voluntary movements (Leventhal *et al.*, 2012). The exact function of beta band activity is further complicated by its complementary relationship with gamma activity that is thought to enable voluntary movements (Lopez-Azcarate *et al.*, 2010; Tan *et al.*, 2013). Furthermore, there is evidence with MPTP monkeys that bradykinesia can develop prior to synchronized beta oscillatory activity (Leblois *et al.*, 2007). The study of involuntary movements may provide the opportunity to clarify the role of exaggerated beta band oscillatory activity on Parkinsonian movement abnormalities.

**6-OHDA Lesioned Rat Model of PD**

Although critical advances in our understanding of basal ganglia organization and the pathophysiology of PD were made using the MPTP treated monkey model, rodent models of PD have also proved extremely useful. The most commonly used rodent model of PD is the 6-hydroxydopamine (6-OHDA) lesioned rat model (Ungerstedt, 1968), as rats are insensitive to MPTP. Similar to MPTP, 6-OHDA is a neurotoxin that selectively kills catecholamine containing neurons. Unlike MPTP that is most commonly injected systemically, 6-OHDA does

not cross the blood brain barrier and must be injected into the brain. It is most commonly injected into the SNc, medial forebrain bundle, and the striatum to restrict its damage to dopamine producing neurons (Ungerstedt, 1968).

Although there are reports that rats with severe bilateral 6-OHDA lesions display postural deficits and reduced spontaneous movements analogous to PD patients (Wolfarth *et al.*, 1996), these rats also require intensive nursing care, and are therefore not commonly used as animal models of PD (Cenci *et al.*, 2002). The more frequently used model is a unilateral 6-OHDA lesioned rat that displays a hemiparkinsonian phenotype with sensorimotor deficits contralateral to the lesioned dopamine neurons, but does not require nursing care to survive. Another advantage to this hemiparkinsonian model is that it can provide a within animal control. A commonly employed behavioral measure of dopamine cell loss is rotation induced by systemic apomorphine or amphetamine induced rotation. Animals with unilateral lesions will turn away from the side with the strongest striatal dopamine receptor activation. A dopamine releasing drug, like amphetamine, will cause the rat to turn toward the lesion because only the intact side has dopamine terminals that can be activated by amphetamine. Because the denervated dopamine receptors in the lesioned striatum are more sensitive than those on the innervated side, a dopamine agonist, like apomorphine causes the rat to turn away from the lesion. The validity of rotation as a model for PD motor impairment, however, is in question (Cenci *et al.*, 2002). Additional analyses of sensorimotor behaviors in 6-OHDA lesioned rats reveal subtle impairments in reaction time to sensory stimuli (Carli *et al.*, 1985) and reach to grasp movements (Metz *et al.*, 2001) of the affected limbs. Nevertheless, the general, the motor symptoms displayed by a unilaterally lesioned 6-OHDA rat are mild and difficult to identify, unlike those

present in PD patients. These discrepancies highlight the importance of evaluating appropriate behavioral deficits in rodent models of PD (Cenci *et al.*, 2002).

Unlike the subtle behavioral symptoms, changes in the neural activity of basal ganglia neurons are analogous to the aberrant neural characteristics found in PD patients. Similar to PD patients and MPTP treated monkeys, the most consistent finding with 6-OHDA lesioned rats is an increased bursting pattern of neuronal activity in the SNr (Tseng *et al.*, 2000; Jubault *et al.*, 2009) and STN (Beurrier *et al.*, 2001). Most importantly, LFP recordings throughout cortico-basal ganglia networks after chronic and acute dopamine denervation in rodents also demonstrate excessive synchronized oscillatory activity (Sharott *et al.*, 2005; Costa *et al.*, 2006) in a broad beta frequency range similar to that occurring in PD patients (Hammond *et al.*, 2007). Some investigators even report the dominant frequency of oscillatory activity in 6-OHDA lesioned rats is more similar to PD patients than that of MPTP treated monkeys (Hammond *et al.*, 2007). Although nearly identical to the oscillatory behavior of neurons present in PD, single cell recordings of SNr neurons in unilateral 6-OHDA lesioned rats fail to support the classical rate model of PD. There are conflicting reports of increased (Burbaud *et al.*, 1995), lowered (Rohlfs *et al.*, 1997) and unchanged (Weick & Walters, 1987) average firing rates in 6-OHDA lesioned rats. These striking electrophysiological similarities between PD patients and 6-OHDA lesioned rats highlight its value as an animal model for investigating PD.

**Blinking in Parkinson's Disease**

Animal models have been very useful in advancing our understanding of the pathophysiology and treatment options for Parkinson's disease. Nevertheless, these models have also exposed the difficulty of examining the role of this aberrant activity in the development and treatment of Parkinsonian motor symptoms, especially in the most commonly used rodent

models of PD. This is partially due to the fact that vast majority of behavioral deficits studied in rodent models of PD do not accurately model deficits observed in PD patients. Therefore, the study of a set of symptoms that presents the same in both animal models of PD and Parkinsonian patients may be very useful in further advancing our understanding of the pathophysiology of the disease.

The trigeminal blink circuit provides an ideal model system to study movement disorders because all mammals blink in the same way. Furthermore, nigrostriatal dopamine depletion in PD patients and in animal models of PD causes the same blink disturbances. One such disturbance is that unmedicated PD patients typically have a low spontaneous blink rate (Bologna *et al.*; Karson *et al.*, 1982b). This low spontaneous blink rate contributes to one of the hallmark features of PD, masked faces. Importantly, the low spontaneous blink rate is recovered when patients are on L-dopa (Bologna *et al.*; Agostino *et al.*, 1987). Dopamine depleted animal models of PD also exhibit a decreased spontaneous blink rate (Lawrence & Redmond, 1991; Taylor *et al.*, 1999).

In addition to spontaneous blink rate disturbances, PD patients (Agostino *et al.*, 2008) and the 6-OHDA lesioned rat model of PD (Basso *et al.*, 1993) exhibit strikingly similar trigeminal blink reflex hyperexcitability. Stimulation of the supraoribital branch of the trigeminal nerve (SO) evokes an EMG response in the lid closing orbicularis oculi muscle (OOemg)(Shahani & Young, 1972; Pellegrini & Evinger, 1995) in all mammals. Pairs of identical SO stimuli with a short interstimulus interval have been employed to assess this brainstem reflex in both humans and animal models under normal and pathological conditions. Normally, the response to the 2nd of the two identical SO stimuli is smaller than the response to the 1st stimulus (*Figure 5*, Control). Nigrostriatal dopamine depletion, however, enhances the

response to the 2nd stimulus (*Figure 5*, 6OHDA). This enhanced excitability is also exhibited by a failure of blink reflex habituation with repeated blink evoking stimuli (Basso *et al.*, 1993).

The simplicity of this brainstem behavior enabled investigators to identify the neural circuits through which the basal ganglia alter the blink reflex. The SNr inhibits neurons in the intermediate layers of the SC (Basso & Evinger, 1996). These neurons excite neurons in the nucleus raphe magnus (NRM) that inhibit neurons in the spinal trigeminal complex (Vc). In PD, the altered output of SNr neurons leads to a reduced inhibitory input from the NRM onto Vc neurons (Basso & Evinger, 1996), resulting in an increased excitability of the Vc. Although it is clear that aberrant nigra-collicular interactions are responsible for this hyperexcitability, the altered neural activity that produces this Parkinsonian behavior is unknown. This well established circuitry provides a unique opportunity to examine the role of aberrant firing patterns of basal ganglia output in the generation of PD motor symptoms.

Additionally, the blink circuit provides an ideal system to assess brainstem plasticity under normal and pathological conditions. Experiments have shown the presence of long-term potentiation (LTP) and long-term depression (LTD) -like effects on the trigeminal reflex blink circuit under normal conditions by modifying lid feedback through the presentation of high-frequency stimulation (HFS) to the SO branch of the trigeminal nerve at different times during the blink (Mao & Evinger, 2001). This form of blink reflex plasticity is impaired in several basal ganglia pathologies (Crupi *et al.*, 2008; Suppa *et al.*, 2011), including PD (Battaglia *et al.*, 2006). While we know this type of blink reflex plasticity is cerebellar dependent (Ryan *et al.*, 2014), the neural mechanisms underlying this aberrant plasticity in PD are unknown. It is likely that altered basal ganglia activity in PD perturbs normal basal ganglia-cerebellar interactions (Bostan & Strick, 2010).

**Treatments for PD**

As the cause of the dopaminergic cell death in the SNc is not well understood, the vast majority of PD treatments focus on treating PD symptoms rather than preventing the loss of dopamine neurons. The main goal of pharmacological PD treatments is to restore dopamine levels in the brain or to recover the balance between dopamine and cholinergic levels in the striatum. Since L-dopa, the dopamine precursor, was first demonstrated to improve the motor symptoms in PD patients by Dr. George Cotzias in 1968 (Cotzias, 1968), it has remained the "gold standard" of antiparkinsonian treatment. The surviving dopamine terminals convert L-dopa to dopamine. Combining L-Dopa with other dopamine agonists and dopamine breakdown inhibitors creates an effective "cocktail" of dopamine replacement therapy established individually for each patient. It was recently discovered that L-dopa's ability to attenuate motor symptoms correlates highly with suppression of beta band activity, providing significant support to the role of this synchronized activity in the pathophysiology of the disease (Hammond *et al.*, 2007).

Initially, L-dopa is highly effective at treating motor symptoms in PD, especially bradykinesias, akinesia, tremor, and rigidity (Mercuri & Bernardi, 2005). Although extremely successful at treating motor symptoms for the first couple years of the disease progression, the efficacy of dopamine replacement therapy drastically decreases as the disease progresses. Additionally, after several years of L-dopa treatment, the vast majority of patients fail to experience a stable clinical response to L-dopa and begin to develop frequent, debilitating dyskinesias (Mercuri & Bernardi, 2005). The mechanism of these L-dopa induced dyskinesias is not well understood, but the most convincing evidence points towards an alteration in dopamine turnover and the development of aberrant corticostriatal plasticity after chronic treatment with

the drug (Picconi *et al.*, 2003). Importantly, L-dopa treatment does not consistently attenuate the non-motor symptoms of PD.

Although the initial effectiveness of L-dopa supports its position as the primary pharmacological treatment for PD, L-dopa's severe side effects ignited investigations into neurosurgical treatment options for PD. Since the 1950s, neurosurgical treatments primarily targeted at lesioning the thalamus have been reported to attenuate the motor impairments in PD dramatically (Obeso *et al.*, 1997). The field of neurosurgery for PD really took off, however, after Laitinen described dramatic symptom relief in all but one of 38 PD patients that he treated with a pallidotomy, a lesion of GPi (Laitinen, 1985). The field was further aided by advances in electrophysiological techniques that allowed for intraoperative recordings to increase the accuracy of lesion sites (Obeso *et al.*, 1997). Further support and understanding of the mechanisms of lesions of the GPi and STN came from several experiments that demonstrated pharmacological inactivations of these basal ganglia nuclei in MPTP treated monkeys (Bergman *et al.*, 1990; Wichmann *et al.*, 1994; Guridi *et al.*, 1996) and 6-OHDA lesioned rats (Burbaud *et al.*, 1995; Wichmann *et al.*, 2001) ameliorated motor impairments and restored a normal firing pattern in the basal ganglia nuclei and associated neural networks.

The side effects and irreversibility associated with brain lesions persuaded neurosurgeons to try high frequency electrical stimulation in these regions to create a functional blockade of neural activity. This high frequency electric stimulation, or Deep Brain Stimulation (DBS), was first introduced in the late 1980s, and targeted the ventrointermediate nucleus of the thalamus to treat essential tremor. The DBS system involves implanting a battery operated pulse generator that can continuously send electrical pulses to an electrode implanted in the targeted brain region. In addition to thalamic stimulation, DBS of the STN and GPi dramatically and

immediately improved PD motor symptoms including bradykinesia, tremor, and rigidity. The FDA approved DBS to treat PD in 2002. Typically, the procedure is performed bilaterally on PD patients that are either unresponsive to or exhibit severe side effects to standard pharmacologic therapies. DBS allows patients to reduce their dopamine replacement therapies by up to 50%. Since its approval for PD, DBS has been used on other brain regions and successfully treated dystonia, chronic pain, epilepsy, and neuropsychological disorders (Mayberg *et al.*, 2005; Perlmutter & Mink, 2006; Gubellini *et al.*, 2009; Kahane & Depaulis, 2010).

**Mechanisms of Deep Brain Stimulation (DBS)**

Although STN DBS has become the most commonly practiced neurosurgical option for treating PD motor symptoms, its therapeutic mechanism is not fully understood. Because the clinical effects of DBS were similar to those seen with STN lesions, early hypotheses regarding the mechanism by which DBS achieved its clinical effect assumed that DBS created a functional inhibition of the stimulated region through either a local depolarization block, inactivation of voltage dependent channels, or functional deafferentation. Such functional inhibition was thought to reduce the excitatory drive onto the GPi/SNr. Consistent with the classical model of basal ganglia function and the rate model of PD *(Figures 1 & 2)*, this inactivation was assumed to allow reactivation of thalamo-cortical networks (Benazzouz *et al.*, 1993; Benazzouz *et al.*, 1995; Benazzouz *et al.*, 2000). Patch clamp recordings *in vitro* (Beurrier *et al.*, 2001), and LFP recordings in human PD patients (Filali *et al.*, 2004) supported the functional inhibition hypothesis by demonstrating reduced activity in the STN during STN DBS. More recent experiments, however, indicated that STN DBS created more complicated local effects. There were reports of increased STN neural firing rate in response to STN DBS (Picconi *et al.*, 2003) and other reports of spike discharges at the frequency of stimulation *in vitro* (Garcia *et al.*, 2003).

Therefore, the local effects of STN DBS did not consistently support the functional inhibition hypothesis.

When considering the mechanisms of STN DBS, it is important to consider that the classical uni-directional model of the basal ganglia organization is drastically oversimplified, and that the STN has important reciprocal connections with the cortex, thalamus, and other basal ganglia nuclei (*Figure 1*). Therefore, it seems likely that STN DBS generates widespread effects throughout cortico-basal ganglia networks through both orthodromic and antidromic activations. (Deniau *et al.*, 2010). Consistent with the hypothesis that DBS generates widespread effects, some studies report that STN DBS normalizes the pathological synchronized oscillatory firing activity in the SNr of 6-OHDA lesioned rodents (Degos *et al.*, 2005) and the GPe, GPi, and STN of MPTP treated monkeys (Hashimoto *et al.*, 2003; Meissner *et al.*, 2005). Recordings from PD patients also demonstrated a suppression of beta band activity in the STN (Wingeier *et al.*, 2006; Kuhn *et al.*, 2008) and motor cortex (Silberstein *et al.*, 2005) with STN DBS.

Further studies showed that only high frequency STN DBS disrupts beta band activity throughout the basal ganglia cortical network (Dorval *et al.*, 2010; McConnell *et al.*, 2012). The ability to disrupt beta oscillations was critical in the effectiveness of STN DBS as Kuhn and colleagues demonstrated that around 60 seconds after the cessation of STN DBS beta band activity returned in the STN along with the bradykinetic symptoms (*Figure 6*) (Kuhn *et al.*, 2008). Additionally, low frequency STN DBS not only fails to attenuate beta oscillations (McConnell *et al.*, 2012), but can also worsen PD symptoms (Timmermann *et al.*, 2004; Chen *et al.*, 2007). Overall, it has become increasingly clear that 130 Hz STN DBS achieves its therapeutic effect by disrupting beta band activity in PD.

## Conclusions

Since James Parkinson's first discription of PD in 1817, our understanding of the pathophysiology of PD has significantly advanced. Animal models, particularly the MPTP treated monkey and the 6-OHDA lesioned rodent model of PD, have played critical roles in these developments. These animal models along with LFP recordings from human PD patients have highlighted the presense of synchronized beta oscillations across the basal ganglia-cortical networks in PD. Although we know that effective treatments, such as STN DBS, disrupt beta activity, we do not yet understand the role of this aberrant activity in the genertation of Parkinsonian symptoms. This is largely due to the complex relationship between beta activity and voluntary movements as well as the differences in the symptoms displayed between animal models and PD patients. Therefore, the study of simpler, involuntary Parkinsonain symptoms that present the same across all models of PD is critical to advancing our understanding of beta oscillations in PD. The goal of these studies is to utilize the blink system to elucidate the role of exaggerated beta oscillations in Parkinsonian symptoms.

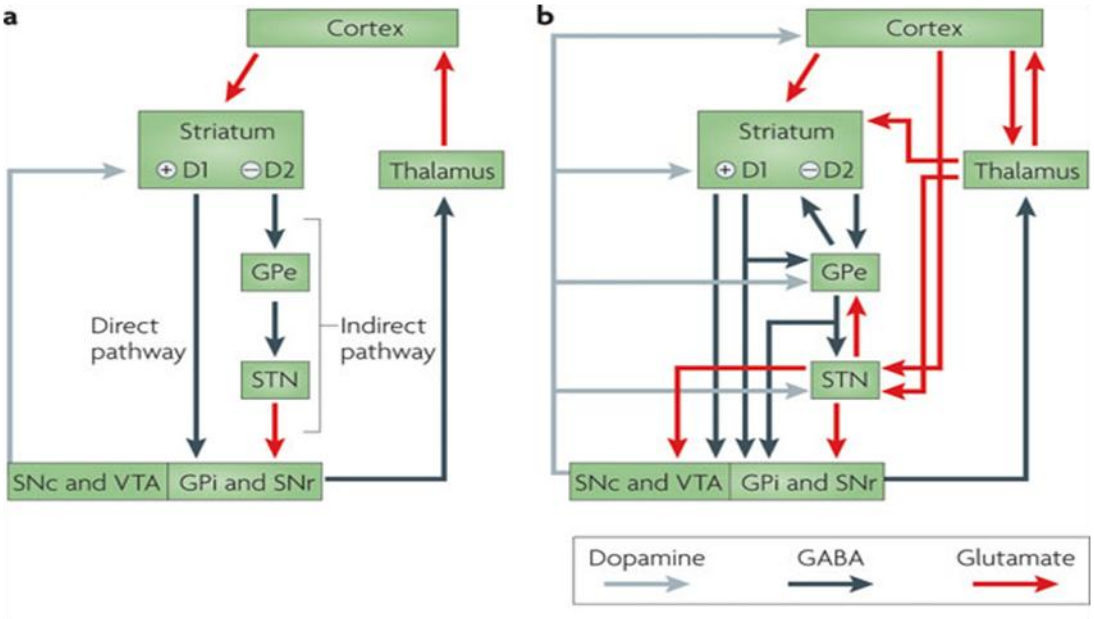


**Figure 1**. (A) Classical model of the basal ganglia. (B) New anatomical findings suggest a much more complex basal ganglia organization.

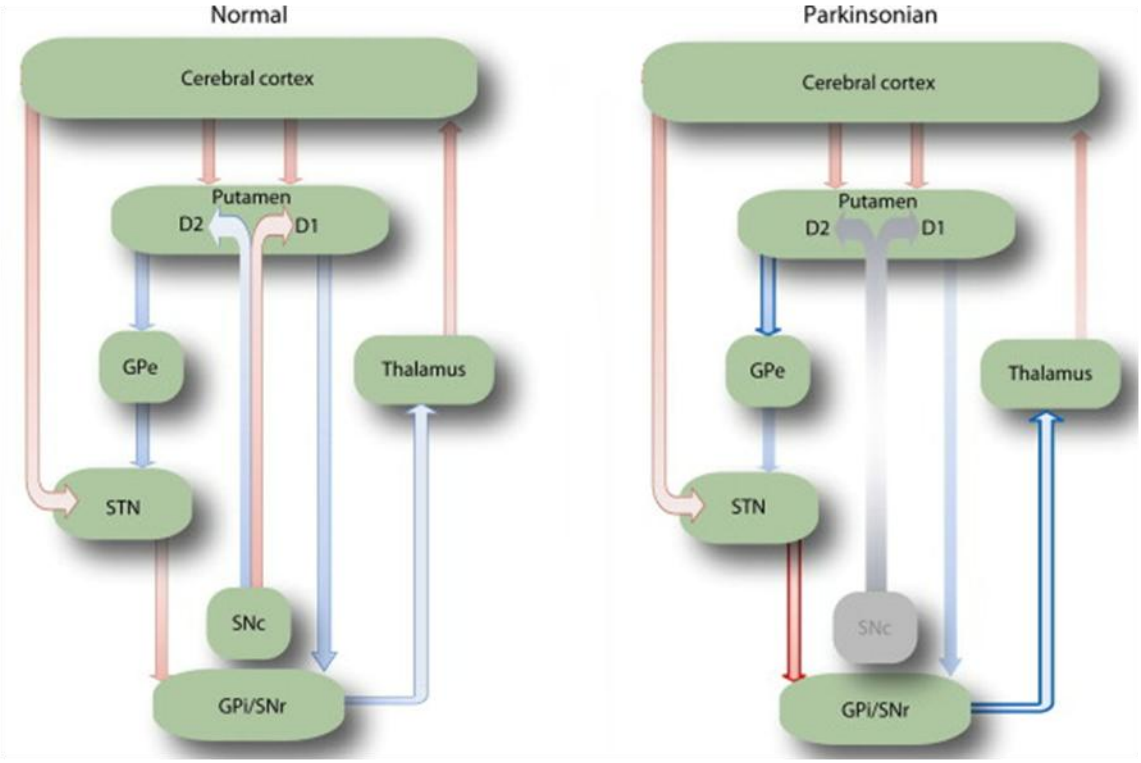


**Figure 2**. Changes in basal ganglia organization in a Parkinsonian state as proposed by the rate model.

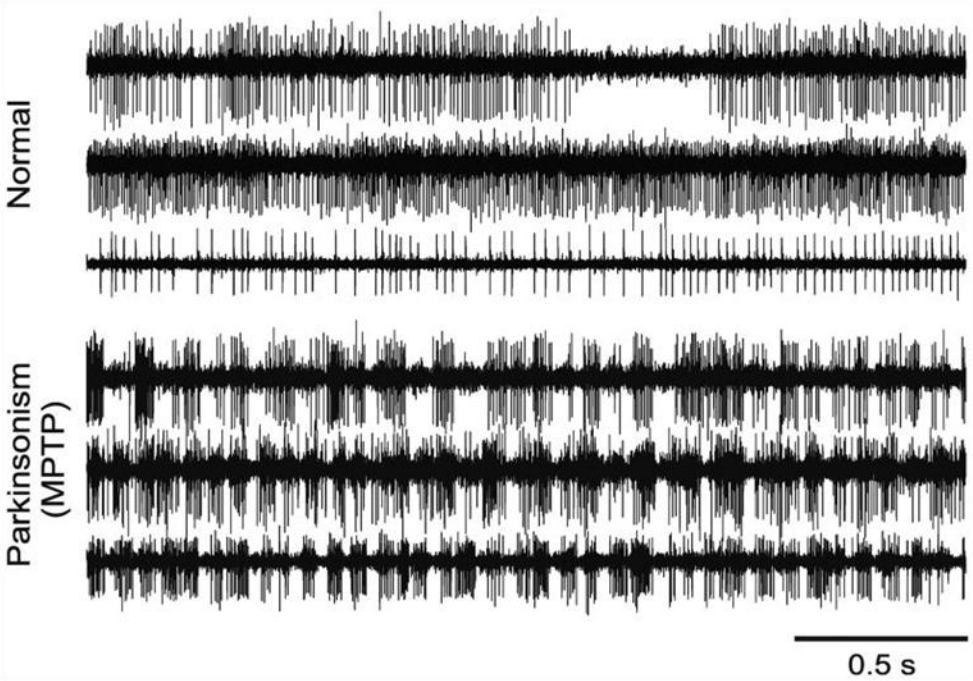


**Figure 3 (Wichmann & DeLong, 2006).** Individual cell recordings of three individual neurons in the GPi of a normal and MPTP treated monkey. Increased bursting and synchronization is clearly observed after MPTP treatment.

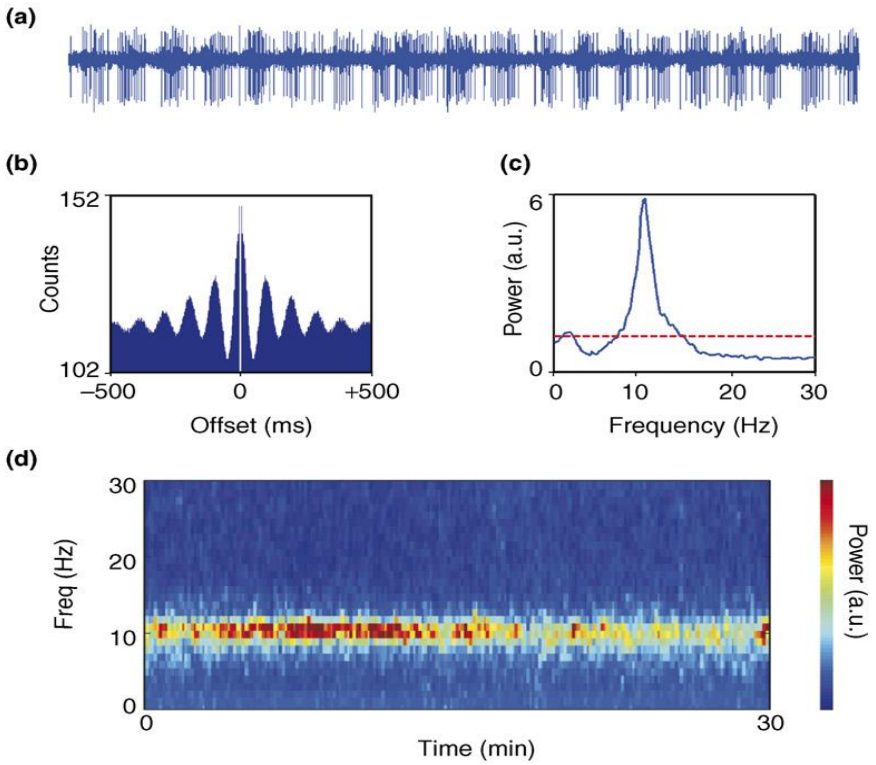


**Figure 4**. (A) Two seconds of a GPi neuron recording in the GPi of an MPTP treated monkey (B) Autocorrelation of the spike trains of this neuron (C) Power spectrum analysis and (D) Spectrogram of the discharge of this individual neuron reveals highly oscillatory activity with a frequency around 10 Hz

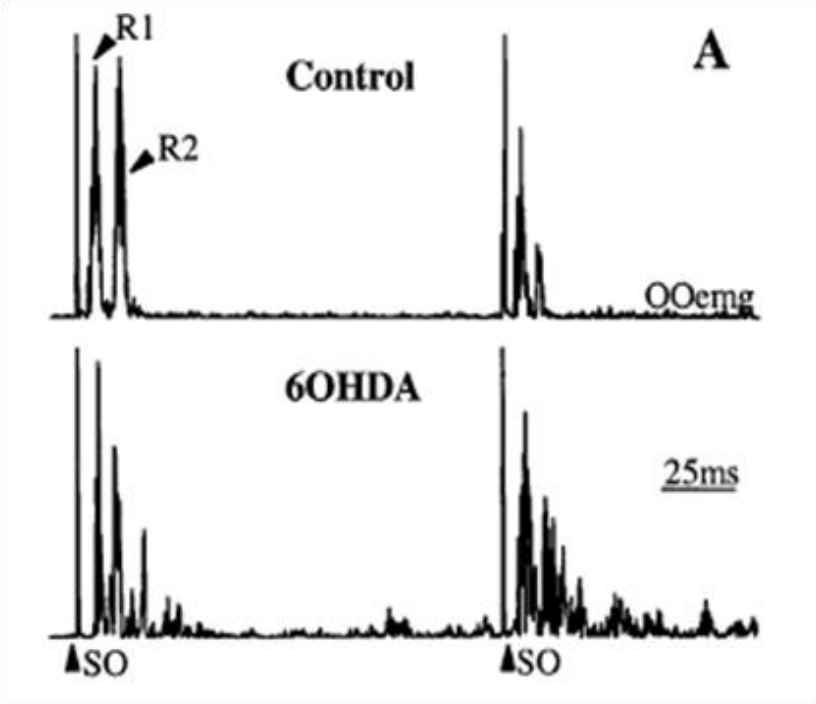


**Figure 5 (Basso *et al.*, 1993)**. Normal (Control) and hyperexcitable (6OHDA) responses to paired stimulation of the supraorbital branch of the trigeminal nerve (▲SO).

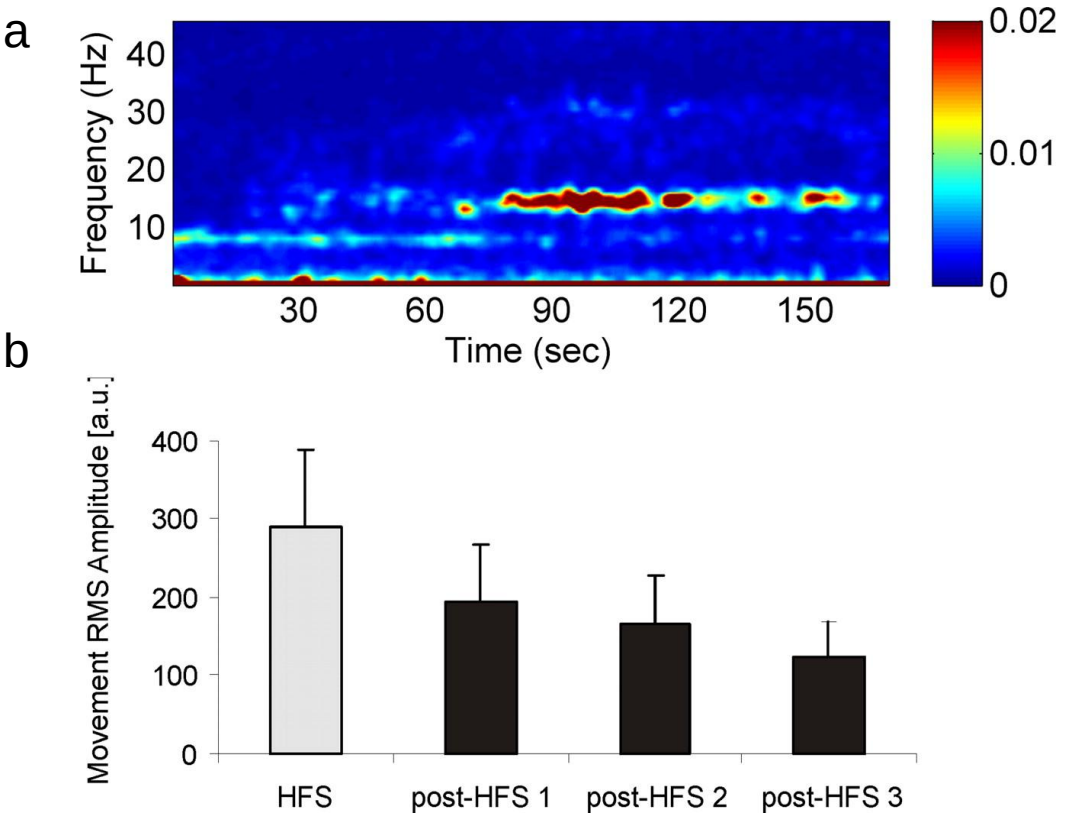


**Figure 6 (Kuhn *et al.*, 2008)**. (A) Time–frequency plot of power changes in the STN after HFS STN DBS was shut off. (B) Mean movement amplitude in the same patient during HFS STN DBS and at three separate sessions (60, 90, and 120 seconds) after HFS STN DBS was stopped.

## II. DOPAMINERGIC MODULATION OF THE SPONTANEOUS BLINK GENERATOR

### INTRODUCTION

Spontaneous blinking is one of the most frequent human movements. At an average rate of approximately 14 blinks per minute when looking straight ahead (Doughty, 2001), people make about 14,000 spontaneous blinks during a waking day. Because maintaining corneal tear film requires only three to four blinks per minute (Al-Abdulmunem, 1999), many more blinks are made than necessary to maintain corneal moisture. Given that the main purpose of the eyelids and blinking is to maintain the corneal tear film (Sibony & Evinger, 1998; Evinger, 2010), it is not surprising that corneal afferent inputs modify spontaneous blinking. The blink rate increases with ocular irritation and decreases with corneal anesthesia (Ponder & Kennedy, 1927; Tsubota & Nakamori, 1995; Tsubota *et al.*, 1996; Nakamori *et al.*, 1997; Zaman *et al.*, 1998; Schlote *et al.*, 2004; Naase *et al.*, 2005; Borges *et al.*, 2010). Nevertheless, spontaneous blinking is not simply a reflection of corneal afferent inputs (Acosta *et al.*, 1999; Al-Abdulmunem, 1999) because the kinematics of trigeminal reflex blinks are significantly different than those of spontaneous blinking (Evinger *et al.*, 1991) and corneal and conjuntival anesthesia does not eliminate spontaneous blinking (Naase *et al.*, 2005).

Non-ophthalmic processes also modify spontaneous blinking. Brain dopamine levels modify spontaneous blinking such that reduced dopamine levels decrease blink rate and elevated dopamine levels increase the blink rate in both humans and non human primates (Karson *et al.*, 1981b; Karson *et al.*, 1982a; Karson, 1983; Karson *et al.*, 1983; Kleven & Koek, 1996; Korosec *et al.*, 2006). This is exhibited most clearly in PD patients (Karson *et al.*, 1982b; Bologna *et al.*, 2012) and MPTP lesioned monkeys (Lawrence & Redmond, 1991; Taylor *et al.*, 1999) that

demonstrate drastically reduced spontaneous blink rates that can be restored with L-Dopa treatment (Bologna *et al.*, 2012). Cognitive states also modify spontaneous blink rate. For example, the average blink rate is lowest during reading and highest during conversation (Ponder & Kennedy, 1927; Fogarty & Stern, 1989; Orchard & Stern, 1991; Doughty, 2001; Pivik & Dykman, 2004).

Thus, spontaneous blinking appears to arise from the activity of an endogenous spontaneous blink generator (Blount, 1927; Ponder & Kennedy, 1927; Stern *et al.*, 1984; Karson, 1988; Naase *et al.*, 2005) that is modulated by corneal afferents, dopamine, and cognitive states. Other than blink rate, however, the properties of the spontaneous blink circuit have not been characterized and its location is unknown. To understand how cognitive processes and disease states modify spontaneous blinking, it is critical to develop a mammalian animal model with which to identify the neural circuits responsible for generating spontaneous blinks.

Rodents provide an excellent model system to identify the neural bases for spontaneous blinking. The characteristics of rodent and human reflex blinking are qualitatively similar (Pellegrini *et al.*, 1995; Powers *et al.*, 1997; Schicatano *et al.*, 2000), and basal ganglia modulation of trigeminal reflex blinks is identical in primates and rats (Basso *et al.*, 1993; Basso & Evinger, 1996; Basso *et al.*, 1996; Gnadt *et al.*, 1997). To characterize the rat spontaneous blink generator, I monitored spontaneous blinking for periods of one hour and determined the spontaneous blink rate, blink amplitude, and temporal organization of spontaneous blinking under normal conditions and with dopaminergic challenges. I further assessed the ability of Subthalamic Nucleus Deep Brain Stimulation (STN DBS), a common treatment for PD symptoms, to attenuate Parkinsonian spontaneous blink abnormalities. Finally, I compared these data with human spontaneous blinking.

## MATERIALS AND METHODS

*Subjects*

Spontaneous blinking was monitored in fourteen male Sprague Dawley rats (200 – 600 gms) maintained on a reversed twelve hour light/dark cycle and fed *ad libitum*. All rat data were collected during the rats' subjective night. Ten human subjects (aged 21 - 61, 7 female and 3 male) also participated in the study. These subjects gave informed consent for their participation. Other than refractive errors, none of the human subjects had any history of eye abnormality or neurological disease. All experiments were performed in accordance with Federal, State, and University regulations regarding the use of humans and animals in experiments and received approval of the University Institutional Review Board and the Institutional Animal Care and Use Committee.

*Procedures*

*Rats*

Under general anesthesia (ketamine 90 mg/kg, xylazine 10 mg/kg), rats were prepared for chronic recording of the orbicularis oculi EMG (OOemg) and stimulation of the supraorbital branch of the trigeminal nerve (Dauvergne & Evinger, 2007). OOemg recordings were performed with a pair of Teflon-coated stainless steel wires (0.003 inch diameter bare, 0.0055 inch coated; A-M Systems, Everett, WA) implanted into the orbicularis oculi muscle near the lateral canthus. Rats received analgesics for twenty-four hrs after the surgery (Ketorolac, 7 mg/kg). Rats were alert and eating within 24 h of the surgery, but at least 1 week passed before the experiments began. In addition to using the OOemg to monitor the occurrence and size of spontaneous blinks, I also recorded lid movements with an infrared detector (Weiss & Disterhoft, 2008) to corroborate blinks in some rats (*Figure 7*). As there was no difference between blink

patterns determined with OOemg recordings and infrared recordings, I combined these data in group analyses. Although supraorbital nerve stimulation was not utilized in these experiments, the rats participated in subsequent experiments that involved evoking reflex blinks with supraorbital nerve stimulation.

I monitored spontaneous blinking before and after treatment with apomorphine or haloperidol in three rats. After collecting one hour of spontaneous blinking, the rats received a subcutaneous injection of 0.1 mg/kg of haloperidol (Research Biochemicals Inc) in 10% ascorbic acid in water, 1 mg/kg apomorphine hydrochloride (Sigma) in saline, or saline. Ten minutes after drug injection, I collected another hour of spontaneous blinking.

*6-OHDA lesioned rats*

Immediately before OOemg implantation, three rats received unilateral 6-hydroxydopamine (6-OHDA) lesions of the right SNc and medial forebrain bundle (Basso *et al.*, 1993). These rats were then implanted unilaterally with laboratory designed stimulating electrodes in the STN. Electrodes were implanted into the right STN stereotaxically at AP: -3.8, ML: 2.5 based on the Paxinos and Watson stereotaxic atlas (Paxinos & Watson, 1998). Final STN electrode position was determined by recording through one of the DBS leads to find an increase in background activity as the electrode moved through the zona incerta into the STN. The stimulation electrodes were 4 twisted stainless steel Teflon coated wires (0.003 inch diameter bare, 0.0055 inch coated; A-M Systems, Everett, WA). These rats were alert and eating within 24 hrs of the surgery. The experiments began 10 days after surgery. After completion of experiments all rats were deeply anesthetized and perfused intracardially. The brains were sectioned at 100 µm and stained with cresyl violet to identify DBS electrode location. Brains were also sectioned at 40 µm and immunohistochemically stained for tyrosine hydroxylase to

assess lesion magnitude (Basso *et al.*, 1993). Tryosine hydroxylase staining revealed full unilateral lesions of the SNc in all three 6-OHDA injected rats. Only rats with correct STN electrode placement were reported in this study.

I monitored spontaneous blinking in three 6-OHDA lesioned rats with and without continuous 130 Hz STN DBS. STN DBS was a continuous biphasic 100 µA 100µS stimulus delivered at 130 Hz. Using a counterbalanced design, I collected thirty five minutes of spontaneous blinking for both conditions each day. The first five minutes of data was not analyzed.

*Humans*

After acquiring informed consent, an eyelid measuring system was attached to human subjects. To use the magnetic search coil system, the subjects sat in a Helmholtz coil with their head stabilized by a chin rest. A 2 mm diameter coil was taped onto the center of the lower margin of the upper eyelid (Evinger *et al.*, 1991). Great care was taken to ensure that subjects did not find the coil uncomfortable or irritating. Particularly for subjects with an epicanthal fold, I used an infrared system for measuring eyelid movements (ASL model 210 Eye Tracking System). With sensor adjustment, the infrared monitor provided an accurate measure of upper eyelid position (*Figure 7*).

*Data Collection and Analysis*

Spontaneous blinks were monitored in rats as they moved freely in their home cage in a darkened room during their subjective night. Rat OOemg and lid position were recorded for one hour. With humans, eyelid position was recorded while the subjects watched one of several films lacking strong emotional content. The screen was 1.5 m in front of the subject at eye level. Lid position was recorded continuously for at least 27 min. Lid position signals were amplified

and filtered from DC – 300 Hz. All data were sampled at 2 kHz per channel (Data Translation, Marlboro, MA; 12-bit analog-to-digital resolution), and stored for later analysis using laboratory developed software. Although spontaneous blinking was recorded for at least 60 min in rats and 27 min in humans, I did not analyze the first 5 min of data in rats or the first 3 min of data in humans to enable subjects to habituate to the experimental situation (Ponder & Kennedy, 1927; Karson *et al.*, 1981a; Naase *et al.*, 2005). Data reported here do not include the habituation period.

I employed a variety of mathematical measures to identify and characterize temporal patterns of spontaneous blinking in addition to the typical measures of blink rate, mean and median interblink interval (IBI). For the temporal analysis, I ignored spontaneous blink amplitude and treated each spontaneous blink as a point process, a unitary event that occurred at the start of OOemg activity or lid closure. To identify temporal organization in the pattern of spontaneous blinking, I divided the period of data collection into 1, 3, 6, or 10 s bins and determined the blink rate (blinks / min) for each bin spanning the analysis period. Using MATLAB scripts, I calculated the autocorrelation functions of these binned data to reveal repeating patterns of spontaneous blinking. A fast Fourier transform (FFT) was then performed on the autocorrelation to quantify the frequency components of the spontaneous blinking. I employed the Fano factor, normalized variance, to characterize the periodicity of the spontaneous blink pattern further.

I calculated the Fano factor using counting intervals that increased in 1 s increments. For example, the analysis period was divided into 1 s bins and the number of blinks occurring in each bin determined. The Fano factor for this counting interval was the variance divided by the mean number of blinks determined over all bins at this counting interval. This calculation was

repeated for each counting interval. I plotted the Fano factor as a function of the counting intervals on a log-log plot. The Fano factor provides a measure of whether the variable occurrence of blinks *(Figure 7)* matches a Poisson distribution over different time scales (Eden & Kramer, 2010).

To test whether the temporal organization of spontaneous blinks was critical in these measurements, I randomly shuffled the IBI order and recalculated the autocorrelation, FFT, and Fano factor for these randomized blink sequences. Shuffling did not change the mean blink rate or IBI distribution. Results are presented as mean ± SEM. Statistical analyses used SPSS software.

## RESULTS

*Rat Spontaneous Blinking*

The IBI distribution suggested that the average blink rate failed to characterize spontaneous blinking adequately *(Figure 7).* The mean IBI of spontaneous blinking for the eleven rats was 12.6 ± 0.75 s calculated over all rats and days of data collection. The IBI distribution, however, did not exhibit a normal distribution as the median IBI was only 4.7 s. To evaluate the IBI distribution for each day of data collection, I determined IBI probability using 0.5 s bins for each day of data collection for all rats. For rat A, short IBIs had the highest probability, and probability decreased as IBI duration increased (*Figure 8A*, symbols). Averaging the IBI probability across days provided an estimate of this rat's IBI probability distribution (*Figure 8A*, solid line) and created a metric for comparison among rats. The average IBI probability distribution was similar for all eleven rats (*Figure 8B*, symbols). Plotting the log of the average of all of the average IBI probabilities as a function of the log IBI revealed power law scaling of IBI probability for IBIs greater than 0.25 s (*Figure 8B*, inset). For IBIs larger than 0.25 s, the equation $c*IBI^{\alpha}$ described this power law scaling where α is the scaling factor and c is a constant. As c does not affect the shape of the equation, this value was not considered in our analysis. For rat A, α ranged from -0.53 to -0.94 across days. The α value for the averaged IBI probability for each rat ranged from -0.61 to -1.07.

The increased probability of short rather than long IBIs (*Figure 8A*, B) meant that a brief period of data collection should produce a higher blink rate than a long period of data collection. To test this prediction, I calculated the average blink rate in increments of one minute for the first twenty-five minutes of data collection after the habituation period for four rats (*Figure 8C*). The average blink rate was always higher when averaging across the first ten minutes of data

collection than when averaging across all twenty five minutes. For all rats across all days, the average blink rate for ten minutes of data collection (6.6 ± 0.5 blinks/min) was significantly higher than the average blink rate with fifty five minutes of data collection (5.3 ± 0.3 blinks/min; $t_{(54)} = 5.7$, $p < 0.001$). Consistent with the property of power law distributions, which do not possess means when $\alpha > -2$, the estimate of the spontaneous blink rate was a function of the length of data collection.

The combination of frequent short IBIs with a few long IBIs suggested that spontaneous blinking exhibited periodicity. Plotting the blink rate every 10 s for 4200 s of spontaneous blinking revealed such periodicity for rat E (*Figure 9A*). To identify the temporal organization of the blinks, I performed an autocorrelation on this blink pattern (*Figure 9C*). I quantified the periodicity present in the autocorrelation by performing a FFT of the autocorrelation (*Figure 9E*). The FFT revealed a dominant frequency of 0.002 Hz, indicating that this pattern of spontaneous blinking repeated every 500 s. To determine whether these calculations resulted from actual temporal patterning, I randomly shuffled the IBI order of these data and repeated the measurements (*Figure 9B, D, F*). Shuffling did not affect the blink rate or IBI probability distribution. Nevertheless, shuffling substantially reduced the periodicity revealed by the autocorrelation (*Figure 9D*) and the FFT of the autocorrelation (*Figure 9F*). The highest power of the shuffled data (*Figure 9F; 9.2*) was less than that of the normal data (*Figure 9E; 20.2*) and occurred at a higher frequency (normal: 0.002 Hz, shuffled: 0.00625 Hz). Shuffling significantly reduced the FFT peak power determined across all rats and days (normal: 17.1 ± 1.1; shuffled: 10.3 ± 0.4; $t_{(59)} = 6.6$, $p < 0.001$). Utilizing 1 s bins, the frequency at the peak power of the autocorrelation FFT was significantly lower for the normal than for the shuffled data over all rats and days tested ($t_{(59)} = -3.1$, $p < 0.005$). Averaged across animals and days, the pattern of rat

spontaneous blinking exhibited a frequency at the peak power of .0041 Hz, a 253 s period. The median frequency at the peak power was 0.0013 Hz, a 750 s period.

For all rats, the log Fano factor for spontaneous blinking increased with the log counting interval (*Figure 10*). This result demonstrated that spontaneous blinks were not independent events. If the occurrence of spontaneous blinks followed a Poisson distribution, then the Fano factor would be 1 at all counting intervals (Snedecor & Cochran, 1967). The increasing Fano factor with counting interval was consistent with spontaneous blinking exhibiting periodicity in which blinks were not independent (*Figure 9*). Supporting this interpretation, disrupting spontaneous blink periodicity by shuffling significantly reduced the mean exponent of the increase in log Fano factor from 0.36 ± 0.02 to 0.19 ± 0.01 (*Figure 10F*; $t_{(59)} = 11.9$, $p < 0.001$) over all rats and days. Given the importance of periodicity in producing the logarithmically increasing Fano factor, I anticipated that the Fano factor exponent would increase as the regularity of the spontaneous blink periodicity increased (Middleton *et al.*, 2003). When Rat E's autocorrelation FFT revealed strong power over a narrow frequency range (*Figure 10C*), the Fano factor increase was steeper (*Figure 10A*) than on a day when the peak power of the autocorrelation FFT was much less restricted to a narrow frequency (*Figure 10D*). The Fano factor exponent increased with the peak power of the autocorrelation FFT across all days and rats (*Figure 10E*). Thus, the Fano factor exponent estimated the regularity of the spontaneous blink pattern.

To determine whether the size of bins used to measure blink rate affected the computation of autocorrelation frequency and power, I calculated the blink rate, autocorrelation, and FFT of the autocorrelation for the same data from four rats using different sized bins (*Figure 11*). Increasing bin size from 1 to 3 to 6 to 10 s did not change the pattern of the autocorrelation

(*Figure 11A-D*). The maximum power of the FFT calculated for these autocorrelations decreased with bin size, but the frequency at peak power did not change for this rat (*Figure 11E*). For all four rats, frequency at peak power was unaffected by bin size (*Figure 11F*) and peak power decreased with bin size (*Figure 11G*). To utilize data arrays that most closely approximated individual blink occurrence, all values presented are from calculations using 1 s bins.

A second potential confound is that spontaneous blink periodicity may be an artifact of the spontaneous blink rate. To resolve this issue, I plotted our measures of periodicity as a function of blink rate. The peak power of the autocorrelation FFT was not correlated with the mean blink rate (*Figure 12A*; $r = 0.032$). Likewise, the frequency at the peak power of the blink pattern was unrelated to the blink rate (*Figure 12B*, $r = 0.17$). Finally, the Fano factor exponent was uncorrelated with the blink rate across all rats and days ($r = 0.2$; not illustrated). Thus, the periodicity of spontaneous blinking was independent of the spontaneous blink rate.

*Dopaminergic Modulation of Spontaneous Blinking*

Consistent with the decreased blink rate in Parkinson's disease, systemic dopamine antagonists reduced the spontaneous blink rate, whereas dopamine agonists increased the spontaneous blink rate of primates (Karson, 1983; Lawrence & Redmond, 1991; Adamson, 1995; Deuschl & Goddemeier, 1998; Taylor *et al.*, 1999; Hallett, 2000; Esteban *et al.*, 2004; Korosec *et al.*, 2006). If rodent spontaneous blinking models human blinking, then treating rats with haloperidol, primarily a $D_2$ receptor antagonist, should decrease spontaneous blink rate, whereas treatment with apomorphine, a nonselective dopamine receptor agonist, should increase spontaneous blink rate. The data supported this prediction. Treatment with 1 mg/kg of apomorphine significantly raised the blink rate from $5.7 \pm 1.1$ to $11.0 \pm 1.2$ blinks/ min

($t_{(4)} = -5.56$, $p < 0.005$; *Figure 13A*). In contrast, treatment with 0.1 mg/kg of haloperidol significantly reduced the blink rate from 6.3 ± 0.9 to 1.9 ± 0.3 blinks/min ($t_{(6)} = 7.55$, $p < 0.001$; *Figure 13A*). Injections of saline alone did not significantly alter the spontaneous blink rate (pre 6.8 ± 1.1, post 7.3 ± 1.5; $t_{(4)} = -0.61$, $p > 0.05$; Figure 13A). Coincident with the change in blink rate, dopaminergic drugs significantly altered blink amplitude. As blink rate increased with apomorphine treatment, the mean spontaneous blink amplitude significantly decreased by 67% from its pre treatment amplitude ($t_{(4)} = 11.71$, $p < 0.001$; *Figure 13B*). A 20% increase in spontaneous blink amplitude ($t_{(5)} = -2.8$, $p < 0.05$; *Figure 13B*) accompanied the reduction in blink rate produced by haloperidol. As with the absence of changes in blink rate with saline, there was an insignificant 6% increase in spontaneous blink amplitude ($t_{(5)} = 2.27$, $p > 0.05$; *Figure 13B*)

To determine if dopaminergic treatment altered the periodicity of spontaneous blink patterns, I determined the difference between the data and its shuffle for both the pre and post drug treatment. I then compared these differences to determine whether dopaminergic drugs affected spontaneous blink periodicity. I adopted this approach because the dramatic reduction in blink rate produced by haloperidol created an artificial periodicity by inserting long periods without blinks within the 3300 s periods of data collection (*Figure 14*). Before haloperidol, the blink rate as a function of time exhibited a normal periodicity (*Figure 13A*) that random shuffling of the IBIs reduced (*Figure 14B*). This loss of periodicity produced by shuffling was clear from the reduction in the Fano factor exponents produced by shuffling (normal pre = 0.42, shuffled pre = - 0.02; *Figure 14C*). After 0.1 mg/kg haloperidol, there were episodes of multiple blinks interspersed with prolonged periods without blinks (*Figure 14E*). Shuffling the IBIs, however, created apparent periodicity because the brief intervals of blinking were interleaved

with those long IBIs without a blink (*Figure 14F*). The Fano factor exponents of the normal data (0.45; *Figure 13G*) and the shuffled IBIs (0.34; *Figure 14G*) were only slightly different, indicating that the apparent periodicity was a result of haloperidol creating multiple long IBIs rather than from changes in the periodicity of the spontaneous blink generator. Comparing the difference between the Fano factor exponent for the obtained and the shuffled IBIs for pre and post drug conditions for all animals and days indicated a significant decrease in spontaneous blink regularity produced by haloperidol ($t_{(6)} = 2.51$, $p < 0.05$; *Figure 14D*), a significant increase in regularity with apomorphine treatment ($t_{(4)} = -3.08$, $p < 0.05$; *Figure 14D*), but no change following saline injections ($t_{(4)} = -0.19$, $p > 0.05$; *Figure 14D*).

Dopaminergic drugs affected spontaneous blink regularity without altering the spontaneous blink period. There were no significant differences between the frequency at the peak power between the three groups before drug treatment ($F_{(14)} = 1.05$, $p > 0.05$) or after treatment ($F_{(14)} = 1.24$ $p > 0.05$). There were also no significant differences between the frequency at peak power before and after any drug treatment. The relationship between peak power and the Fano factor exponent remained consistent before (*Figure 14H*, open symbols) and after (*Figure 14H*, filled symbols) drug or saline treatment. Although activating or blocking dopamine receptors did not affect the periodicity of rat spontaneous blinking, the data revealed that apomorphine enhanced regularity, whereas haloperidol diminished the regularity of spontaneous blinking.

*STN DBS on spontaneous blinking in 6-OHDA lesioned rats*

To test if beta oscillations alter the spontaneous blink generator in PD, I examined the effect of 130 Hz STN on spontaneous blink behavior in 6-OHDA lesioned rats. 6-OHDA lesioned rats had a lower blink rate, 3.16 ± 0.3 blinks/min, than normal rats, 5.30 ± 0.03. 130 Hz

STN DBS did not return the blink rate of 6-OHDA lesioned rats to normal levels. The blink rate with 130 Hz STN DBS, 2.81 ± 0.3, was not significantly different from the No DBS condition ($t_{(4)} = 2.38$, $p > 0.05$; *Figure 15A*). Additionally, 130 Hz STN DBS did not affect the amplitude of the spontaneous blinks of 6-OHDA lesioned rats, as 130 Hz STN DBS increased the spontaneous blink amplitude by an insignificant 14% ($t_{(4)} = -0.89$, $p > 0.05$; *Figure 15B*).

To determine if 130 Hz STN DBS altered the regularity of blink patterns of 6-OHDA lesioned rats, I determined the difference between the data and its shuffle for both the No STN DBS and 130 Hz STN DBS conditions. I compared these differences to determine whether 130 Hz STN DBS affected the pattern of spontaneous blinking. First I confirmed that 6-OHDA lesions reduced the periodicity of the spontaneous blink generator. The Fano factor exponents of the normal 6-OHDA lesioned rat data (0.30; *Figure 15C*) and the shuffled IBIs (0.34; *Figure 15C*) were only slightly different, indicating that the apparent periodicity was a result of 6-OHDA lesions creating multiple long IBIs rather than from changes in the periodicity of the spontaneous blink generator. Likewise, the Fano factor exponents of 130 Hz STN DBS data (0.25; *Figure 15D*) and the shuffled 130 Hz STN DBS data (0.19; *Figure 15D*) were also only slightly different, revealing that 130 Hz STN DBS did not alter the regularity of the spontaneous blink generator in 6-OHDA lesioned rats. Comparing the difference between the Fano factor exponent for the obtained and the shuffled IBIs for No DBS and 130 Hz STN DBS conditions for all three rats and days indicated there was no significant change in the periodicity of spontaneous blinking with 130 Hz STN DBS ($t_{(4)} = 0.66$, $p > 0.05$; *Figures 15E, F*). Therefore, 130 Hz STN DBS did not restore a normal pattern of spontaneous blinking in 6-OHDA lesioned rats, suggesting beta oscillations were not responsible for this increased variability.

*Human Spontaneous Blinking*

Similar to results of other investigations (Al-Abdulmunem, 1999; Doughty, 2001), I found the average spontaneous blink rate of ten human subjects was 17.6 ± 2.4 blinks/min when monitored for twenty-four minutes. Across all subjects, the blink rate ranged from a high of 25.5 to a low of 6.9 blinks/min. The mean IBI was 4.3 ± 0.8 s. Humans clearly blinked spontaneously more frequently than rats (*Figure 7*). Nevertheless, given that the temporal characteristics of rat spontaneous blinking were independent of blink rate (Figs. 4E; 6), it was possible that humans and rats exhibited similar temporal patterns to their spontaneous blinking.

As occurred with rodents (*Figure 8*), the human IBI probability distribution was not normally distributed. The median human IBI (2.7 ± 0.5 s) was lower than the average IBI (Fig. 11A). Plotting the log of the average of all subjects' IBI probabilities as a function of the log IBI revealed power law scaling of IBI probability for IBIs greater than 1.025 s (*Figure 16B*). The exponent, α, for this power law scaling ranged from -1.71 to -0.89 for the 10 subjects, with α = -1.24 for the averaged IBI. The power law scaling of the IBI indicated that the mean blink rate depended upon the length of data collection in humans (*Figure 16*) as it does in rats (*Figure 8*). I plotted the average blink rate as a function of the time of data collection in one minute increments for four subjects (*Figure 16C*). As occurred with rats, shorter periods of data collection typically produced higher blink rates than longer periods of data collection. Averaged across all ten subjects, the blink rate measured over the first 10 minutes of data collection was significantly higher than that measured over the first twenty-four minutes of collection ($t_{(9)} = 3.19$, $p < 0.05$).

As in rats, human spontaneous blinking exhibited periodicity (*Figure 17*). Calculating the autocorrelation of the blink rate over a twenty-four minute period revealed clear periodicity

(*Figure 17A*) that was confirmed by performing a FFT on the autocorrelation (*Figure 17B*). For the subject illustrated in Figure 12, the frequency at peak power was 0.000833 Hz (*Figure 17B*). Across all subjects, the mean frequency at the peak power was 0.0055 ± 0.003 Hz, a period of 180 s. The median frequency was 0.0022 Hz, a 460 s period. To determine whether this periodicity represented temporal organization, I randomly shuffled the order of the IBIs and calculated the autocorrelation and the autocorrelation FFT of the resulting blink order. For the subject illustrated in Fig. 12, shuffling reduced the periodicity revealed by the autocorrelation (*Figure 17C*) and the FFT of the autocorrelation (*Figure 17D*). For all subjects, the peak power of the shuffled IBIs was significantly less than that of the real data ($t_{(9)} = 3.05$, $p < 0.05$). As occurred with rats (*Figure 9*), shuffling significantly increased the frequency at the peak power for humans ($t_{(9)} = -2.49$, $p < 0.05$). As with the rat data (*Figure 12*), the frequency at peak power of the FFT autocorrelation was independent of the bin size and the peak power decreased as bin size increased from 1 to 3 to 6 to 10 s in humans (not illustrated).

The log Fano factor typically exhibited a constant value of less than 1 between one and five s counting intervals, but steadily increased with higher counting intervals in humans (*Figure 17E*). To estimate the increase in Fano factor with the counting interval, we calculated the Fano factor exponent using counting intervals between ten and one hundred forty-four s. The Fano factor exponent correlated with the regularity of the spontaneous blink pattern as measured by the peak power of the FFT of the autocorrelation (*Figure 17F*). Shuffling the IBI order significantly reduced the Fano factor exponent for all subjects, demonstrating that the increasing Fano factor with counting interval reflected a temporal organization of human spontaneous blinking ($t_{(9)} = 4.68$, $p < 0.005$).

## DISCUSSION

Although they had significantly different mean IBIs (rats: 12.6 ± 0.75 s; humans: 4.3 ± 0.8 s; $t_{(42)} = -3.74$, $p < 0.001$), both species displayed qualitatively similar temporal organization to their spontaneous blink pattern. The IBI distribution exhibited power law scaling (*Figures 8B; 16B*). When $\alpha > -2$, which is the case for rat and human blinking, it is not possible to determine the blink rate because of power law scaling properties. This characteristic is consistent with my observation that the mean blink rate depended upon the length of data collection. The higher probability of encountering a short, rather than a long IBI caused the estimated spontaneous blink rate to be higher for shorter than for longer periods of data collection (*Figures 8C, 16C*). For rats, the mean spontaneous blink rate required at least fifteen minutes to approach a near plateau level (*Figure 8C*), but continued to decrease slightly throughout the fifty-five minute recording period. In humans, the blink rate peaked in the first one to five minutes of data collection and then decreased over the next nineteen minutes (*Figure 16C*). One possible explanation for this pattern in humans was that the lid coils were mildly irritating until subjects became habituated to their presence. This is unlikely because these data were collected after three minutes of habituation to the experimental situation and my data were nearly identical to those collected using video cameras (Zaman & Doughty, 1997). I plotted the average blink rate in one minute increments relative to the average blink rate after five minutes of data collection for the ten subjects and the fourteen subjects reported by Zaman and Doughty (1997; Table 1). Blink rates determined with a lid coil (*Figure 18*, ●) or a video camera (*Figure 18*, ▲) exhibited nearly identical increases over the first five minutes of recording. In my study, however, determining blink rates for periods longer than five minutes revealed a subsequent

steady decline in the rate of human spontaneous blinking (*Figure 16C*). Thus, the average spontaneous blink rate depended upon the length of data collection for both rats and humans.

For both species, the pattern of spontaneous blinking revealed long-term periodicity composed of frequent blinking episodes interspersed with interludes of few blinks. An autocorrelation analysis of the blink rate and a FFT of the autocorrelation demonstrated this periodicity (*Figure. 9, 17*). In rats, the median period for the blink pattern was 750 s, whereas the median blink period was 460 s for humans. The exponent characterizing the increase in log Fano factor with log counting interval correlated with blink pattern regularity (*Figures 10, 17*). The periodicity and regularity identified by the FFT and Fano factor exponent reflected the temporal organization of spontaneous blinking because shuffling the IBI order eliminated or significantly reduced the periodicity in both species (*Figures 9, 10, 17*). This temporal pattern was independent of the spontaneous blink rate (*Figure 12*). Thus, rat and human spontaneous blinking exhibited similar periodic increases and decreases in blink rate that were independent of the mean blink rate measured over periods of time from 1440 to 3300 s. These similarities indicate that the rat is an appropriate animal model for investigating the neural bases of spontaneous blinking.

It is possible that the temporal organization of spontaneous blinking came about from the linkage of blinks to ongoing behaviors rather than ensuing from the activity of a blink generator. Given the numerous influences on spontaneous blink rate involving attention and task (Karson, 1988; Orchard & Stern, 1991; Pivik & Dykman, 2004), the temporal organization determined in humans could reflect changes in attention to the movie storyline that the subjects watched (Nakano *et al.*, 2009). This is unlikely, however, because subjects exhibited similar patterns of temporal organization even though they watched different movies. Moreover, the temporal

periodicity of spontaneous blinking exhibited by rats who did not receive patterned external stimulation was not significantly different from that of humans. The simplest explanation of the temporal organization of blinking is that it reflects the action of an endogenous spontaneous blink generator.

While the location of the spontaneous blink generator remains unknown, it is obvious that the basal ganglia and dopamine modulate its activity. My data (*Figures 13,15A*) are consistent with primate work that has demonstrated that treatment with dopaminergic agonists increases the spontaneous blink rate, while dopamine depletion reduces the spontaneous blink rate (Karson *et al.*, 1982b; Lawrence & Redmond, 1991; Taylor *et al.*, 1999; Bologna *et al.*, 2012). Dopamine affects the pattern of spontaneous blinking as well as the rate. Similar to the increase in birdsong variability with dopamine depletion and its reduction with elevated dopamine levels (Leblois *et al.*, 2010), increases in dopamine levels reduce blink variability and decreases in dopamine levels enhance blink variability (*Figures 14, 15C*). The effect of dopamine depletion on the spontaneous blink generator is analogous to the enhanced stride variability displayed by PD patients (Hausdorff *et al.*, 2003).

I also observed that dopamine levels affect spontaneous blink amplitude. Treatment with apomorphine decreased spontaneous blink amplitude (*Figure 13B*), while haloperidol increased the spontaneous blink amplitude. These dopaminergic drugs had the same effect on blink reflex amplitude (Evinger *et al.*, 1993) that is modulated through a circuit from the SNr to the spinal trigeminal complex via the SC and the NRM (see Chapter I) (Basso & Evinger, 1996; Basso *et al.*, 1996). However, these haloperidol data conflict with clinical data that display a reduced spontaneous and reflex blink amplitude in PD patients (Bologna *et al.*, 2012). This is possibly due to the fact that haloperidol acts primarily on $D_2$ receptors while dopamine depletion in PD

affects $D_1$ and $D_2$ expressing neurons. Clearly, these data indicate that the basal ganglia modulate spontaneous blink rate and amplitude via separate mechanisms after dopamine depletion.

One possible mechanism for this basal ganglia modulation of spontaneous blinking in PD is through exaggerated beta oscillations. Synchronized beta oscillations are exaggerated across the cortico-basal ganglia in PD (Brown & Williams, 2005; Sharott *et al.*, 2005), correlated with the severity of motor symptoms (Kuhn *et al.*, 2006; Kuhn *et al.*, 2009), and attenuated with effective PD treatments like 130 Hz STN DBS (Kuhn *et al.*, 2008; Dorval *et al.*, 2010; McConnell *et al.*, 2012). However, 130 Hz STN DBS did not restore a normal blink rate or pattern of blinking in 6-OHDA lesioned rats (*Figure 15*). This suggests that beta band oscillations do not play a role in spontaneous blink symptoms in PD or that STN DBS does not affect beta band oscillations in the region of the brain containing the spontaneous blink generator. Although our data contradict clinical reports that found a very subtle effect of STN DBS on spontaneous blinking (Bologna *et al.*, 2012), the results are less surprising when considering that STN DBS does not consistently attenuate Parkinsonain gait symptoms (Moreau *et al.*, 2008; St George *et al.*, 2010). This suggests that beta oscillations may not affect central pattern generator behaviors in PD or that STN DBS is not able to reduce these oscillations in the required circuits. Perhaps, pedunculopontine (PPT) DBS that has better efficacy in the treatment of Parkinsonian locomotor disturbances (Stefani *et al.*, 2007; Lozano & Snyder, 2008) will affect the spontaneous blink behavior in PD as well.

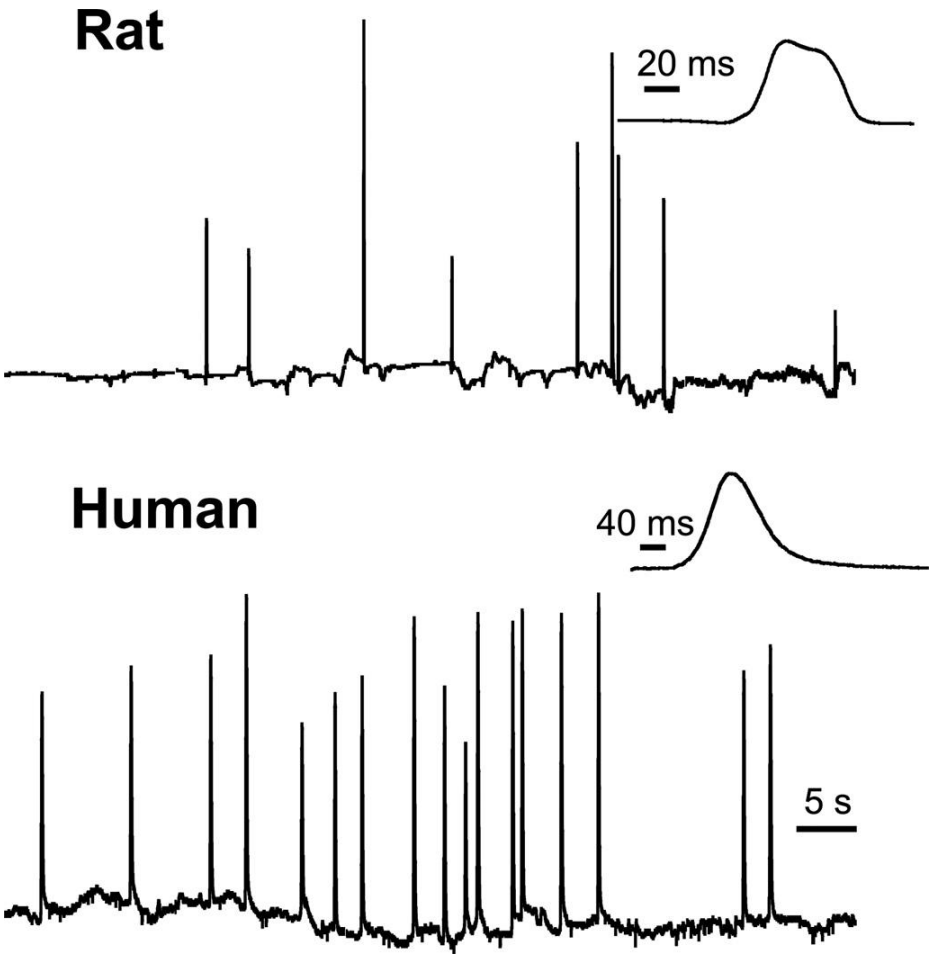


**Figure 7**. 75 s of spontaneous blinking of a rat and a human monitored with infrared eyelid detection. The insets show an individual blink for the rat and the human.

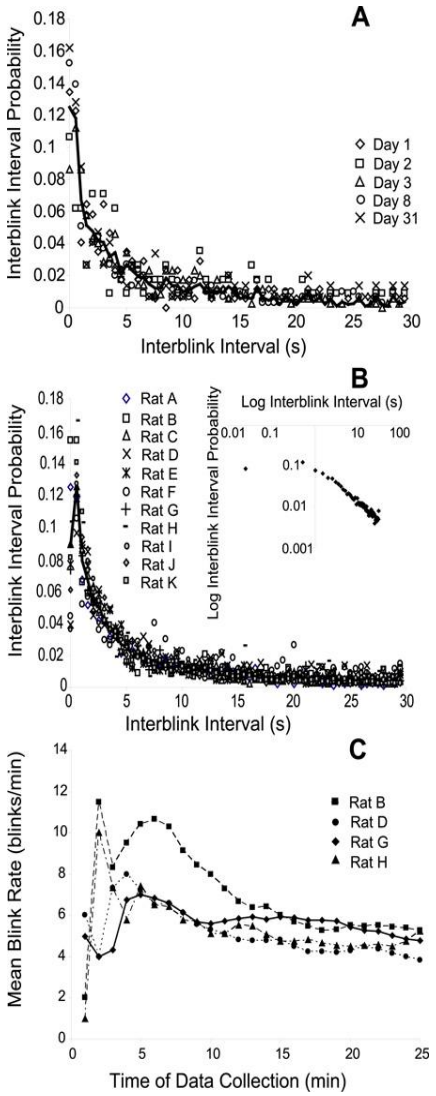


**Figure 8.** Interblink interval and spontaneous blink rate. (A) Interblink interval probability distribution calculated in 0.5 s bins for five days of data (open symbols) for rat A. The solid line is the average of the probability distribution across days. (B) Average interblink interval probability distribution across days for eleven rats (symbols) and the average of these distributions (solid line). The inset shows the average probability distribution as a function of interblink interval on a log-log scale. (C) Mean blink rate determined in increments of one minute for four rats over the first twenty-five minutes of data collection.

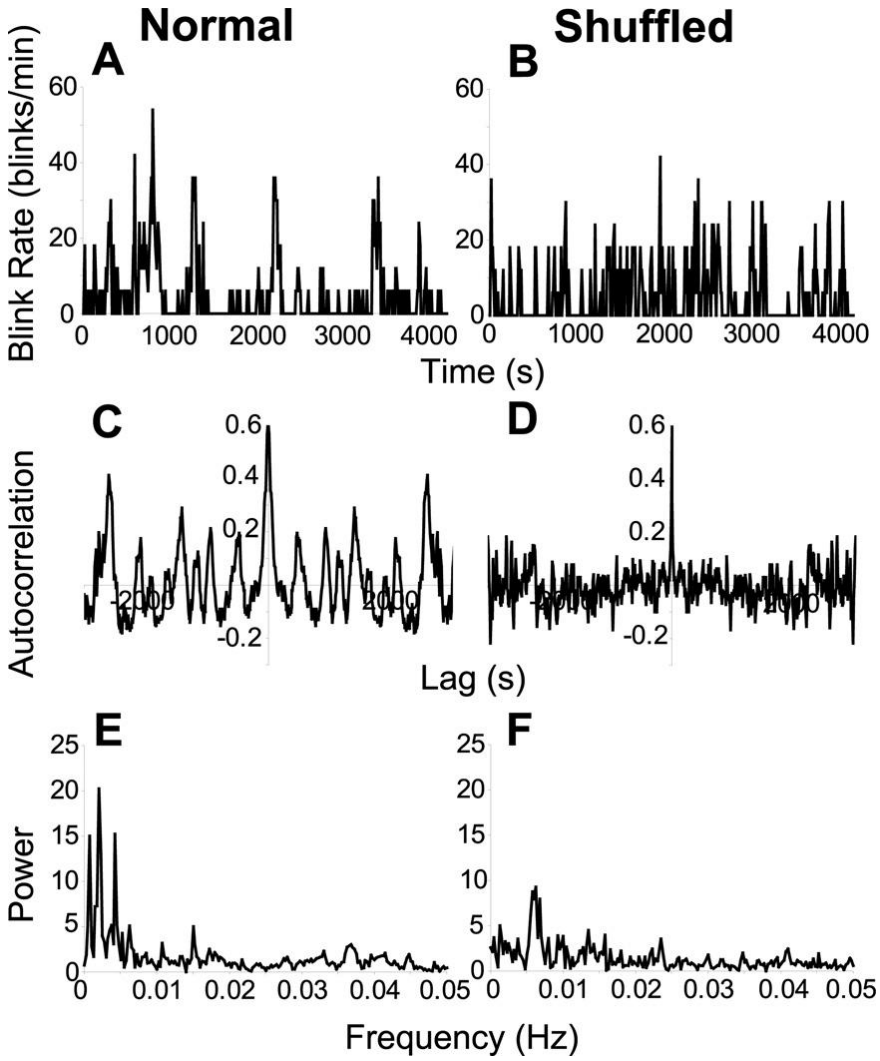


**Figure 9.** (A) The blink rate calculated from blinks in 10 s bins for 4200 s of spontaneous blinking for rat E. (C) The autocorrelation of the blink rate in A using 10 s lags. (E) The power spectrum from the FFT of the autocorrelation in C. (B, D, F) The blink rate (B), autocorrelation (D) and power spectrum from the FFT of B (F) after randomly shuffling the IBI order of the data in A.

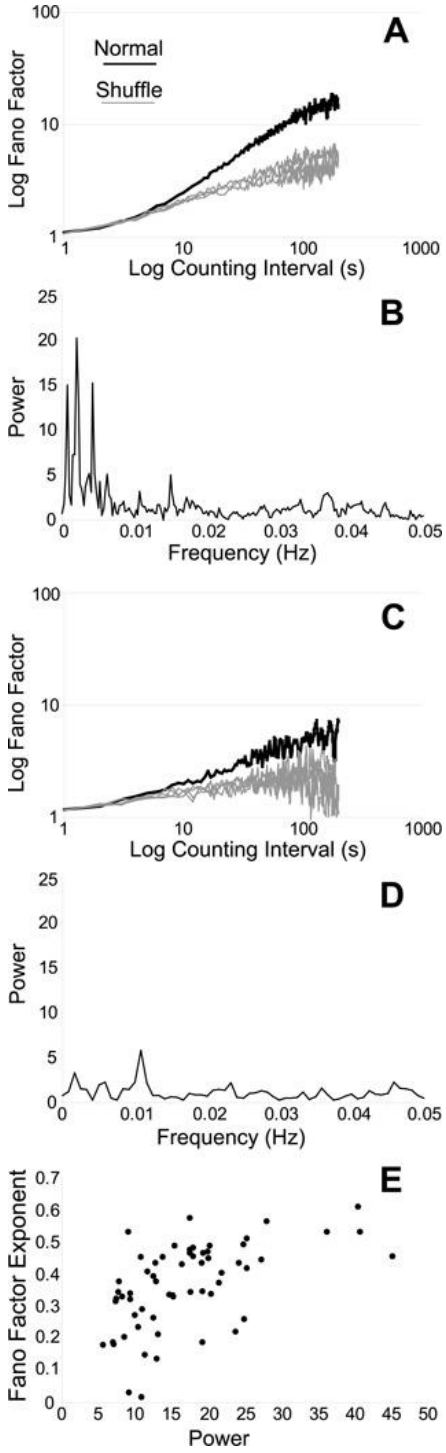


**Figure 10.** (A, B) Log Fano factor as a function of the Log Counting interval for two days data from Rat E for normal data (black line) and for four shuffles of the normal data (gray lines) one day (A) and fifteen days later (B). (C, D) FFT of the autocorrelation for the blink rate for data from A and B using 10 s bins. (E) Plot of the Fano factor exponent as a function of the peak power of the FFT for that day's data for all rats and days.

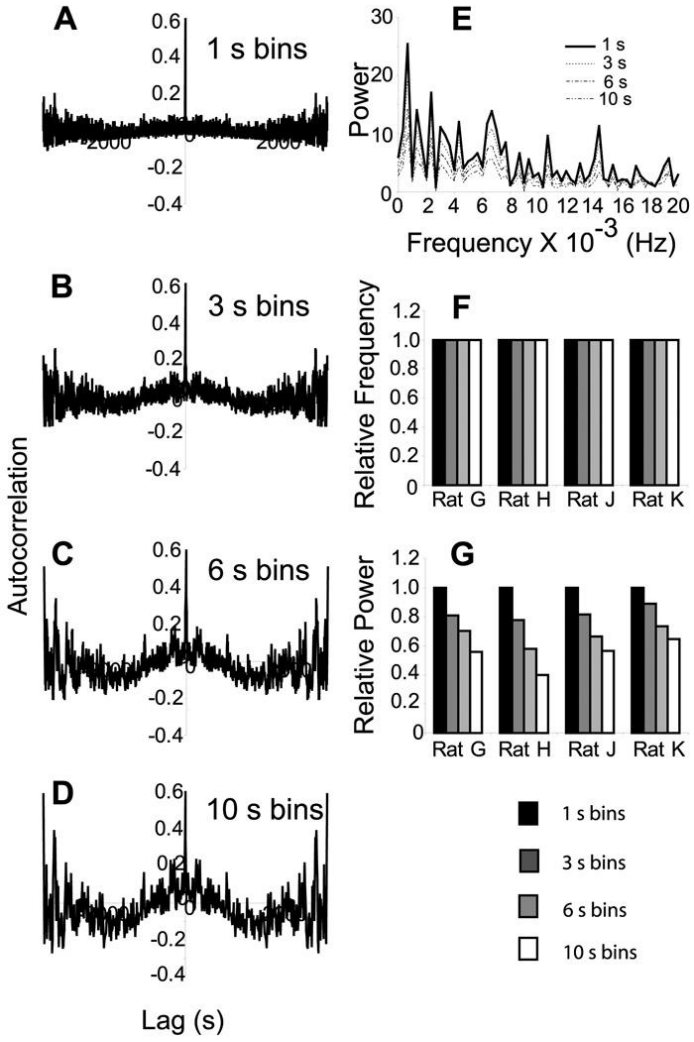


**Figure 11.** Effect of bin size on autocorrelations of blink rate (A –D), peak frequency (E, F) and power of autocorrelations (G) for four rats. Autocorrelation of blink rate determined using 1 (A), 3 (B), 6 (C) and 10 (D) s bins for one day of Rat H's spontaneous blinking. (E) The power spectrum from FFTs of the autocorrelations illustrated in A – D. (F) The relative frequency at the peak power for FFTs calculated using data binned with 1, 3, 6 and 10 s bins for four rats. (G) The relative peak power for FFTs calculated using data binned with 1, 3, 6 and 10 s bins for four rats.

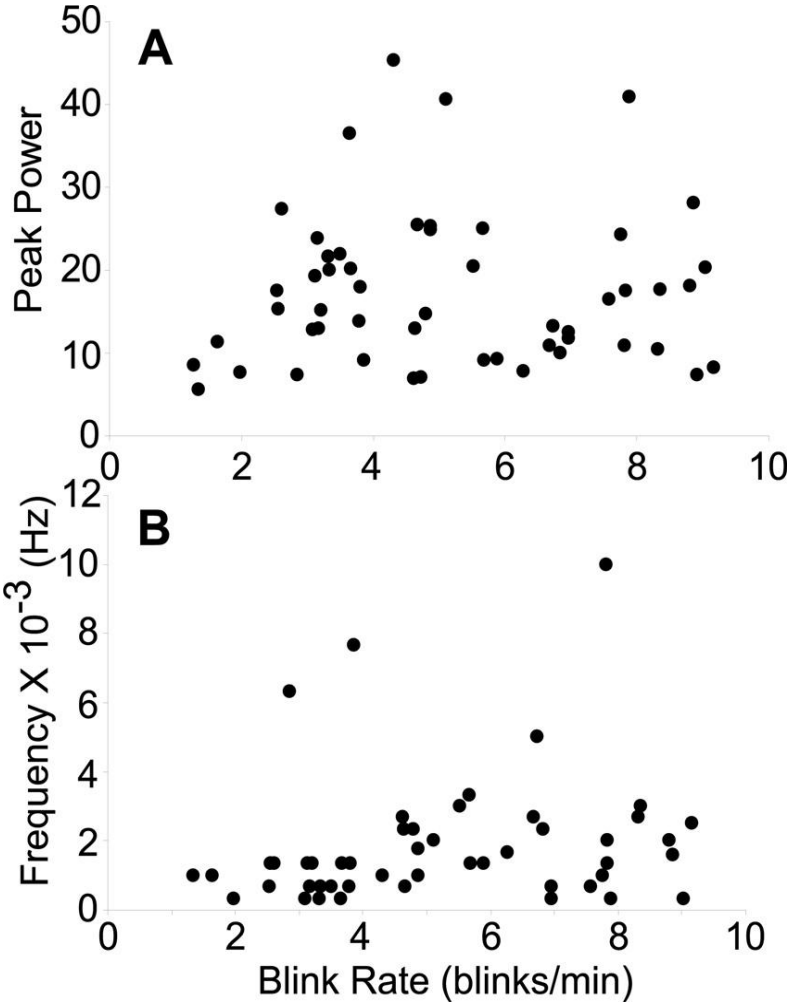


**Figure 12.** (A) Peak power of autocorrelation FFT as a function of blink rate for all rats. Each point is an individual experiment. r2 = 0.001 (B) Frequency at peak power of autocorrelation FFT as a function of blink rate. Each point is an individual experiment. r2 = 0.03.

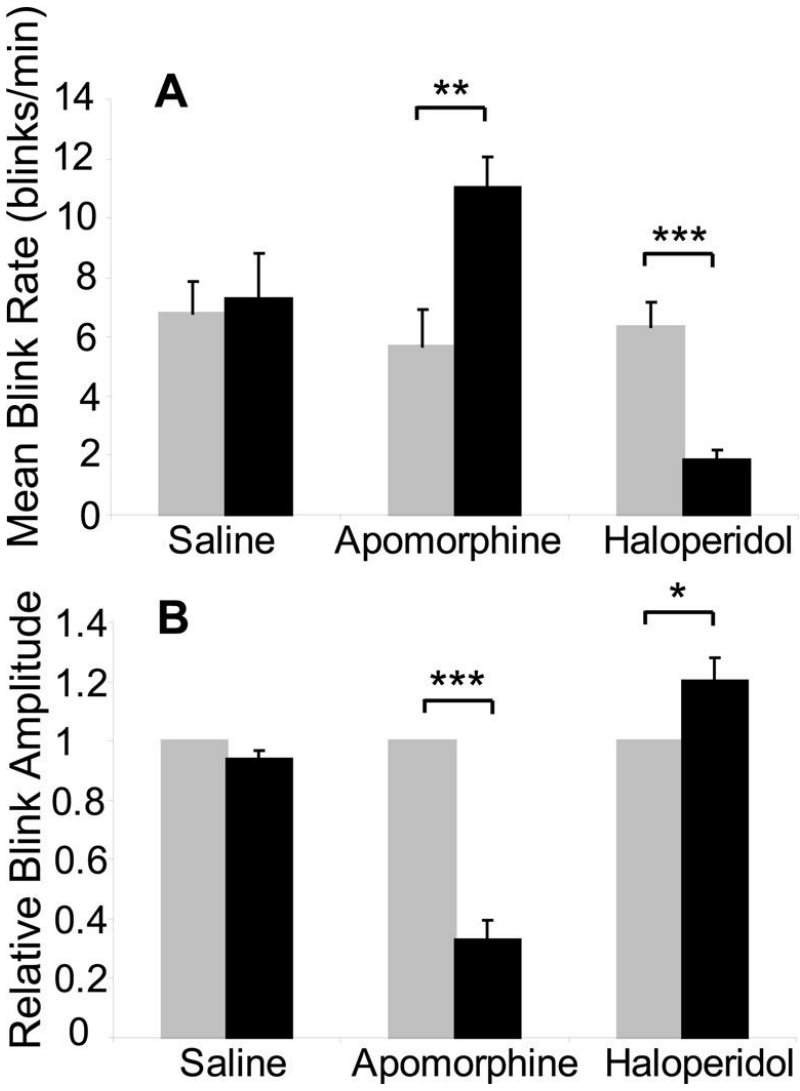


**Figure 13.** Effects of dopaminergic drugs on spontaneous blinking. (A) Mean blink rate before (gray bars) and after (black bars) saline, 1 mg/kg apomorphine, and 0.1 mg/kg haloperidol. (B) Blink amplitude relative to blink amplitude before drug treatment before (gray bars) and after (black bars) saline, 1 mg/kg apomorphine, and 0.1 mg/kg haloperidol. * $p < 0.05$, **$p < 0.01$, *** $p < 0.001$

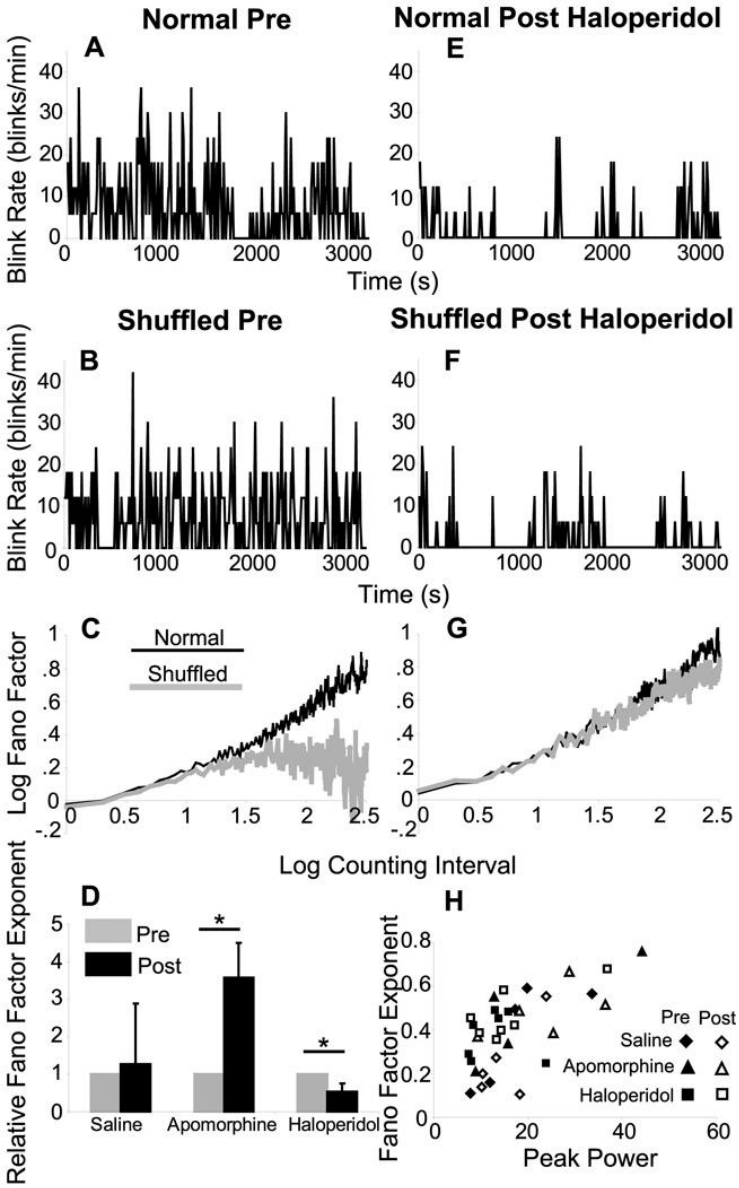


**Figure 14.** Effect of dopaminergic drugs on spontaneous blinking (A) Blink rate in consecutive 10 s bins for 3200 s before treatment. (B) Blink rate in consecutive 10 s bins for 3200 s before treatment after randomly shuffling the IBIs from A. (C) Log Fano factor as a function of log counting interval for the normal data (black line, A) and the shuffled data (gray line, B). (D) Relative Fano factor exponent before (gray bars) and after (black bars) saline, 1 mg/kg apomorphine, and 0.1 mg/kg haloperidol. (E) Blink rate in consecutive 10 s bins for 3200 s after 1 mg/kg haloperidol. (F) Blink rate in consecutive 10 s bins for 3200 s with 1 mg/kg haloperidol treatment after randomly shuffling the IBIs in E. (G) Log Fano factor as a function of log counting interval for the normal data (black line, A) and the shuffled data (gray line, B) after 1 mg/kg haloperidol. (H) Fano factor exponent as a function of peak power of the autocorrelation FFT for all animals and days before (open symbols) and after (filled symbols) treatment with saline(◊), 1 mg/kg apomorphine (∆), or 0.1 mg/kg of haloperidol (□). Records in A, B, C, E, F, and G are data from a single day for one rat. . * $p < 0.05$

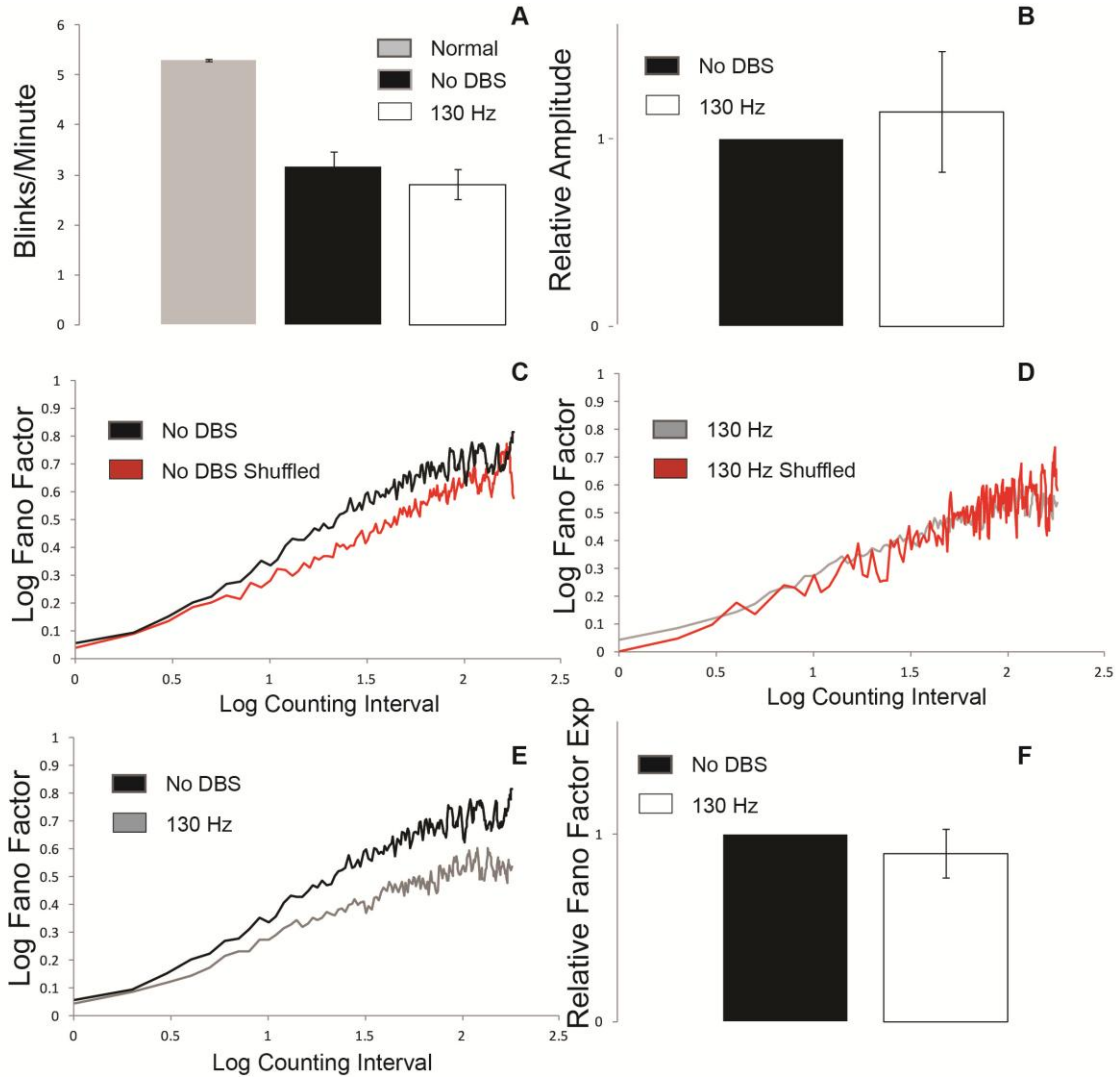


**Figure 15.** Effect of 130 Hz STN DBS on spontaneous blinking in 6-OHDA lesioned rats (A) Average blink rate for 6-OHDA lesioned rats during No DBS and 130 Hz STN DBS conditions compared to the average blink rate for normal rats. (B) Spontaneous blink amplitude for 6-OHDA lesioned rats during 130 Hz STN DBS relative to No DBS. (C) Log Fano factor as a function of log counting interval for the normal No DBS data (black line) and the shuffled No DBS data (red line). (D) Log Fano factor as a function of log counting interval for the normal 130 Hz STN DBS data (grey line) and the shuffled 130 Hz STN DBS data (red line). (E) Log Fano factor as a function of log counting interval for the normal No DBS data (black line) and the normal 130 Hz STN DBS data (gray line). (F) Relative Fano factor exponent with 130 Hz STN DBS relative to the No DBS condition for 6-OHDA lesioned rats. Error bars are SEM.

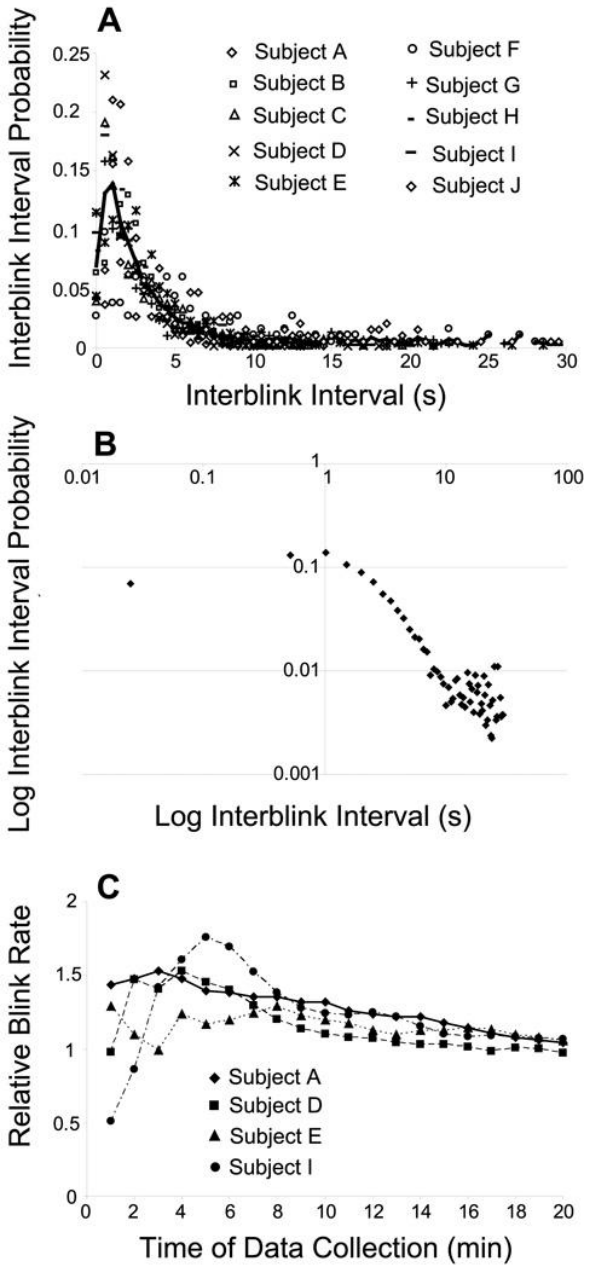


**Figure 16.** (A) Interblink interval probability distribution for 10 human subjects (symbols) and the average probability distribution of all subjects (solid line). (B) Log interblink interval probability of average human data as a function of the log interblink interval. (C) Mean blink rate determined in increments of one minute for four subjects over the first twenty minutes of data collection relative to the mean blink rate in the first twenty-four minutes of data collection.

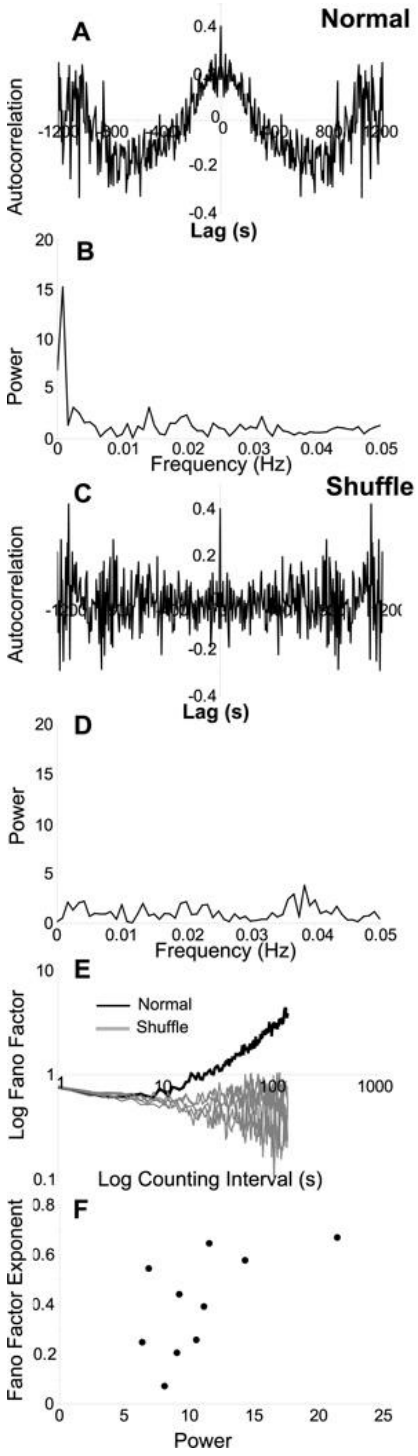


**Figure 17.** (A) Autocorrelation of normal blinking and (B) FFT of the autocorrelation using 6 s bins for subject A. (C) Autocorrelation of shuffled data and (D) FFT of the autocorrelation using 6 s bins. (E) Log Fano factor as a function of the log counting interval for Subject A for normal (black line) and five shuffles (gray lines) of normal data. (F) Fano factor exponent as a function of peak power. Each point is data from a single subject.

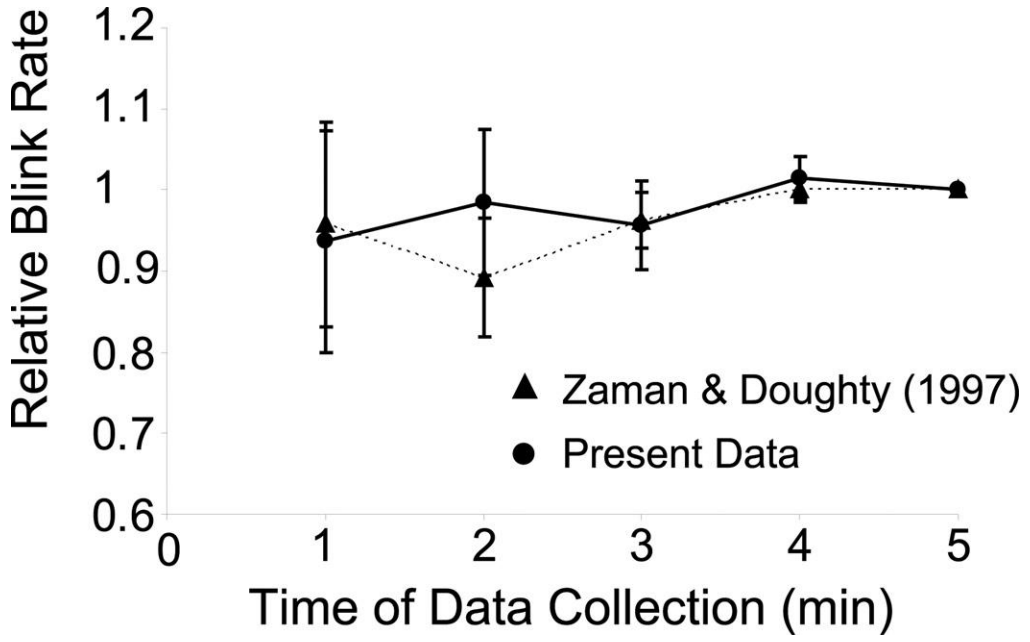


**Figure 18.** Mean blink rate determined in increments of one minute for our ten subjects (●) and fourteen subjects from Zaman & Doughty (1997) (▲) over five minutes of data collection relative to the mean blink rate during the first five minutes of data collection.

# III. DISRUPTING BETA OSCILLATIONS RESTORES NORMAL BLINK REFLEX BEHAVIOR IN 6-OHDA LESIOND RATS

## INTRODUCTION

Parkinson's disease (PD) patients suffer from a wide variety of motor symptoms. Many of these symptoms involve difficulty initiating voluntary movements (*i.e*. bradykinesia and rigidity). Several studies highlight the potential role of exaggerated synchronized beta oscillatory activity present throughout cortical-basal ganglia circuits in PD in creating these symptoms (Brown *et al.*, 2001; Hammond *et al.*, 2007). The strength of this 8 – 35 Hz activity correlates with voluntary movement impairment (Kuhn *et al.*, 2009) and with improvement after dopaminergic treatment and Deep Brain Stimulation (DBS) (Kuhn *et al.*, 2006; Wingeier *et al.*, 2006; Ray *et al.*, 2008). Nevertheless, it has proven difficult to establish a causal role between this activity and the generation of these symptoms because the strength of beta oscillations changes dynamically with stimuli that cue voluntary movements and does not simply reflect motor output (Leventhal *et al.*, 2012). The study of involuntary motor behaviors may clarify the role of the enhanced synchronized beta oscillations present in PD.

In addition to voluntary movement disturbances, PD patients suffer from several reflex abnormalities (Cody *et al.*, 1986; Agostino *et al.*, 1987; Duysens *et al.*, 2010). One of these significantly altered reflexes is the trigeminal blink reflex. This reflex is hyperexcitable in both PD patients (Kimura, 1973; Agostino *et al.*, 1987) and the 6-hydroxydopamine (6-OHDA) rat model of PD (Basso *et al.*, 1993). Additionally, PD patients off L-dopa demonstrate an impaired potentiation of the blink reflex (Battaglia *et al.*, 2006) with a protocol previously developed to examine blink reflex plasticity (Mao & Evinger, 2001). However, considering the enhanced excitability of the blink reflex in PD (Agostino *et al.*, 1987), it is critical to test the ability to

depress the blink reflex in PD in order to conclude that dopamine depletion in PD causes a true impairment in blink reflex plasticity. Thus, I hypothesized that if dopamine depletion causes impairments in blink reflex plasticity, 6-OHDA lesioned rodents will fail to exhibit normal blink depression.

Although we know the circuit through which the basal ganglia modulate the blink reflex (Basso & Evinger, 1996; Basso *et al.*, 1996), it is unknown if beta oscillations are important in these Parkinsonian blink reflex symptoms. Chapter II suggested that beta oscillations are not involved in spontaneous blinking abnormalities in PD, but reflex symptoms likely involve different mechanisms. STN DBS provides a tool to test the role of synchronized beta band activity in these Parkinsonian reflex abnormalities. Therapeutic STN DBS disrupts synchronized beta band activity in PD patients (Wingeier *et al.*, 2006; Ray *et al.*, 2008; Dorval *et al.*, 2010), as well as the 6-OHDA lesioned rat model of PD (McConnell *et al.*, 2012). Importantly, only high frequency STN DBS reduces this activity (Sharott *et al.*, 2005; McConnell *et al.*, 2012). Therefore, I compared the effect of high (130 Hz) and low (16 Hz) frequency STN DBS on Parkinsonian blink reflex symptoms in 6-OHDA lesioned rats to investigate whether reducing beta band activity affects these symptoms.

## MATERIALS AND METHODS

Experiments were performed on ten male Sprague Dawley rats (350-550 g) maintained on a reversed 12 hour light/dark cycle and fed ad libitum. All experiments received approval by the Stony Brook University Institutional Animal Care and Use Committee and complied with all Federal, State, and University regulations and guidelines regarding the use of animals in research.

*Surgery*

Under general anesthesia (ketamine, 90 mg/kg and xylazine, 10 mg/kg), rats were implanted unilaterally with laboratory designed stimulating electrodes in the subthalamic nucleus (STN). Electrodes were implanted into the right STN stereotaxically at AP: -3.8, ML: 2.5 based on the Paxinos and Watson stereotaxic atlas (Paxinos & Watson, 1998). Final STN electrode position was determined by recording through one of the DBS leads to find an increase in neural activity as the electrode moved through the zona incerta into the STN. The stimulation electrodes were 4 twisted stainless steel Teflon coated wires (0.003 inch diameter bare, 0.0055 inch coated; A-M Systems, Everett, WA). After electrode implantation, rats were prepared for chronic recording of the left orbicularis oculi EMG (OOemg) and stimulation of the left supraorbital (SO) branch of the trigeminal nerve (Basso *et al.*, 1993; Evinger *et al.*, 1993). Wires were led subcutaneously to a connector embedded in a dental acrylic platform on the skull. The platform was attached to the skull by four stainless-steel screws. A silver wire connected to one of the stainless-steel screws served as the ground (Evinger & Manning, 1993; Dauvergne & Evinger, 2007).

Immediately before OOemg and SO electrode implantation, rats received unilateral 6-hydroxydopamine (6-OHDA) lesions of the right SNc and medial forebrain bundle (Basso *et al.*,

1993). Rats received analgesic (ketorolac, 7 mg/kg) for at least 24 hrs after the surgery. Rats were alert and eating within 24 hrs of the surgery. The experiments began 10 days after surgery. After completion of experiments all rats were deeply anesthetized and perfused intracardially. The brains were sectioned at 50 µm and every other section was stained with cresyl violet to identify DBS electrode location or immunohistochemically stained for tyrosine hydroxylase to assess lesion magnitude (Basso *et al.*, 1993). Tyrosine hydroxylase staining revealed full unilateral lesions of the SNc in all ten 6-OHDA injected rats. Only rats with correct STN electrode placement were reported in this study.

*Procedures*

In all experiments, the SO stimulus current was relative to the minimum current at which a 100 µs stimulus reliably elicited the R1 component of a reflex blink, threshold (T). This current was determined at the beginning of each day for each rat and was held constant throughout that day's experiment. Threshold varied little across days (Ryan *et al.*, 2014). All data were collected at twice threshold (2T). Typical of all mammals except primates, SO stimulation evoked a strong R1 response and a smaller R2 component that occurred on slightly over half of the trials (Dauvergne & Evinger, 2007; Ryan *et al.*, 2014). STN DBS was a continuous biphasic 100 µA 100µS stimulus delivered at either 130 Hz or 16 Hz. Rats were observed closely for the duration of the day's experiments for signs of abnormal motor behavior. This stimulation intensity did not cause any irregular motor behaviors in the rats or tissue damage in the STN, but affected trigeminal blink behavior.

*Blink Reflex Excitability*

I evaluated the effects of 16 and 130 Hz STN DBS on trigeminal blink excitability using two paradigms, a paired stimulus and a habituation paradigm. For the paired stimulus paradigm,

rats underwent three blocks of twenty trials comprised of pairs of 2T SO with a 100 ms interstimulus interval. During each block, the rat received continuous 16 or 130 Hz STN DBS or No DBS; experiencing all three conditions each day. For the habituation paradigm, rats underwent three blocks of twenty five trials of identical electric pulses to the SO. The interstimulus interval between pulses was 1000 ms. As with the double pulse paradigm, during each block, the rat received continuous 16 or 130 Hz STN DBS or No DBS; experiencing all three conditions each day. Because our previous study (Basso *et al.*, 1993) demonstrated that the side contralateral to the 6-OHDA lesion exhibited the largest changes in blink excitability, I only examined OOemg activity contralateral to the 6-OHDA lesion and STN stimulating electrode.

*Blink Reflex Plasticity*

To investigate the effect of 130 and 16 Hz STN DBS on blink reflex plasticity, I utilized a protocol previously designed to depress blink reflex gain (Mao & Evinger, 2001) that we recently adapted for use in rodents (Ryan *et al.*, 2014) (HFS-B; *Figure 19*). Each day's data collection consisted of five blocks: (1) pre high frequency stimulation; (2) high frequency stimulation (HFS) treatment; (3) immediately; (4) 30 min; and (5) 60 min post HFS. Pre and post HFS blocks were the same for all experiments. In these blocks, rats received 20 trials of a pair of 2T SO stimuli separated by 100 ms with an inter-trial interval of 20 ± 5 s. Each HFS treatment trial consisted of a single SO stimulus at 2T to evoke a reflex blink followed by five, 400Hz 2T SO stimuli delivered before the onset of the R2 component of the OOemg activity (HFS-B). Sixty HFS trials occurred at a 20 ± 5 s interval. In a counterbalanced design, rats received either No STN DBS, 16 Hz, or 130 Hz STN DBS. Rats received were in the same stimulus condition for at least ten days of experiments before switching to a different DBS condition. In these experiments, STN DBS began before testing blink threshold and continued

throughout the entire day's experiment. Three rats also underwent a protocol in which 130 Hz STN DBS was only turned on 5 minutes prior to HFS-B treatment and turned off immediately after HFS-B treatment concluded (130-D). Post HFS data collection began 5 minutes after 130 Hz STN DBS was turned off in this paradigm.

*Data Collection and Analysis*

Reflex blinks were monitored as rats moved freely in their home cage in a darkened room during their subjective night. OOemg signals were amplified, filtered at 0.3–5 kHz, collected at 4 kHz per channel and stored for later offline analysis on laboratory developed software (Dauvergne & Evinger, 2007). Blink amplitude was determined by integrating the rectified OOemg activity of each blink component.

As with clinical analysis of trigeminal blink reflex excitability in PD patients (Agostino *et al.*, 1987), I quantified blink excitability by dividing blink amplitude evoked by the second SO (test response) by the amplitude of the blink elicited by the first SO (condition response). I calculated excitability for the R1 and R2 blink components separately. To quantify habituation, I binned the twenty-five trials into five second bins and averaged the blink amplitude for each bin. Average blink amplitude was compared across bins.

In humans, HFS-B treatment depressed the R2 component of subsequent blinks (Mao & Evinger, 2001). I, however, investigated the effects of HFS-B on the R1 component of the blink reflex as it is the largest component of the blink reflex in non-primate mammals and exhibits the same changes in the HFS-B paradigm as R2 (Ryan *et al.*, 2014). I normalized all within day blink amplitude by dividing OOemg amplitude by the median pre OOemg amplitude. As there was no difference in the post HFS blocks (Ryan *et al.*, 2014), post HFS blink amplitude was averaged over all three post treatment blocks. The normalized mean pre HFS blink amplitude

was subtracted from the normalized mean post blink amplitude. As the blink evoking stimulus remained constant throughout the day, we termed this measure of the change in blink amplitude following HFS *relative gain change*.

I calculated two measures of long-term modifications induced by HFS treatment. For each day of treatment, I determined the mean amplitude of 2T SO evoked blinks pre treatment relative to the median pre treatment amplitude on the first day of testing. If treatment produced a long-term depression in the trigeminal blink amplitude, then pre treatment blink amplitude should decrease across days of HFS treatment. Second, to determine if the HFS treatment created a larger relative change following repeated days of treatment, I plotted the relative change each day as a function of the number of days of treatment. If the treatment became more effective at modifying trigeminal reflex blink gain following repeated treatments, then there should be greater relative change across days of treatment.

Statistical tests of significance ($p < 0.05$) were performed with SPSS software (SPSS, Chicago, IL) using an Analysis of Variance Test (ANOVA) with post hoc Tukey tests, paired or independent t tests. Data are presented as the mean ± SEM. Regressions were performed using linear, power, logarithmic and exponential curves. The function generating the highest correlation was employed to describe the data.

## RESULTS

Parkinsonian patients and 6-OHDA lesion models of PD exhibit increased excitability of the R2, but not the R1 component of the trigeminal reflex blink circuit (Agostino *et al.*, 1987; Basso *et al.*, 1993). If beta band activity plays a role in the expression of this enhanced excitability, then disrupting this activity with 130 Hz STN DBS should reduce the increased R2 excitability exhibited by 6-OHDA lesioned rats. As predicted, 130 Hz STN DBS significantly reduced R2 excitability 50 ± 10 % relative to the No DBS condition ($t_{(6)} = 5.19$, $p < 0.01$; *Figure 20A*) but did not alter R1 excitability ($t_{(6)} = -0.61$, $p > 0.05$; *Figure 20A*). In contrast, low frequency DBS (16 Hz), which does not disrupt beta band activity (McConnell *et al.*, 2012) did not alter the excitability of either the R1 ($t_{(5)} = -0.52$, $p > 0.05$; *Figure 20A*) or R2 ($t_{(5)} = -0.17$, $p > 0.05$; *Figure 20A*) components of the blink reflex relative to No DBS blink excitability. The reduced R2 excitability with 130 Hz STN DBS was not the result of reducing the condition R2 amplitude because 130 Hz STN DBS did not significantly modify the condition R2 blink amplitude ($t_{(7)} = 0.16$, $p > 0.05$). 130 Hz STN DBS exerted its effect on the test R2, reducing it by approximately 35% ($t_{(7)} = 1.87$, $p = 0.052$). This result is also consistent with the finding that 130 Hz STN DBS did not alter the amplitude of reflex blinks in response to a single stimulus in PD patients (Bologna *et al.*, 2012).

In addition to increased excitability of the R2 component of the blink reflex, PD patients and 6-OHDA lesioned rats fail to habituate to repeated presentations of identical blink evoking stimuli (Klawans & Goodwin, 1969; Basso *et al.*, 1993). I replicated this finding as 6-OHDA lesioned rats failed to exhibit a significant decrease in blink amplitude with repeated SO stimulation ($p > 0.05$, *Figure 20B*). If exaggerated beta band activity prevents blink reflex habituation, then 130 Hz STN DBS should restore normal blink reflex habituation in these

dopamine depleted rats, but 16 Hz STN DBS will not have this effect. As predicted, 6-OHDA lesioned rats exhibited significant blink reflex habituation while undergoing 130 Hz STN DBS ($r^2 = .88$, $p < 0.01$). 16 Hz STN DBS, however, failed to reinstate habituation ($p > 0.05$, *Figure 20B*). Thus, disrupting beta band activity with 130 Hz STN DBS in 6-OHDA lesioned rats restored normal blink reflex excitability and habituation.

PD patients off dopamine replacement therapy exhibit impaired potentiation of the blink reflex produced by a pattern of high frequency SO stimulation that increases blink reflex gain (Mao & Evinger, 2001; Battaglia *et al.*, 2006). As hypothesized, 6-OHDA lesioned rats exhibited impaired depression of the blink reflex as HFS-B treatment induced an insignificant 13.6 ± 7.0 % ($t_{(8)} = 2.08$, $p > 0.05$; *Figure 21A*) decrease in blink amplitude. Thus, dopamine depletion impairs the plasticity of the blink reflex.

To investigate the role of beta band activity on blink reflex plasticity, 6-OHDA lesioned rats underwent continuous 130 Hz STN DBS or 16 Hz STN DBS throughout the HFS-B protocol for 10 consecutive days. When rats received 130 Hz STN DBS, HFS-B treatment significantly decreased blink amplitude by 28.4 ± 6.4 % ($t_{(8)} = 4.73$, $p < 0.001$, *Figure 21B*). In contrast, 16 Hz STN DBS reduced the blink depression from HFS-B treatment only 13.7 ± 6.3 % ($t_{(5)} = 2.26$, $p > 0.05$, *Figure 21B*). A repeated measures ANOVA performed on five rats that received all three DBS conditions (No, 130 Hz, and 16 Hz) revealed a significant main effect of treatment condition ($F_{(2, 8)} = 10.60$, $p < 0.01$; *Figure 21B*). Post hoc analyses demonstrated that the No DBS condition differed significantly from the 130 Hz STN DBS condition ($p < 0.05$) as did the 16 Hz STN DBS from the 130 Hz STN DBS condition ($p < 0.05$). There were no significant differences, however, between the No DBS and the 16 Hz STN DBS condition ($p > 0.05$). Therefore, disrupting beta band activity restored blink reflex plasticity in a rat model of PD.

The 130 Hz STN DBS could be critical in restoring acquisition of the HFS induced gain change, maintaining the gain change, or both aspects of this form of motor learning. I investigated this issue by presenting 130 Hz STN DBS only during HFS treatment. If this procedure restores normal motor learning, then the disruption of beta oscillations was only critical during acquisition. Presentation of 130 Hz STN DBS during only the HFS-B (130-D) enable the treatment to produce a significant 29.6 ± 8.0 % ($t_{(2)} = 4.52$, $p < 0.05$, *Figure 21C*) decrease in blink amplitude. A repeated measures ANOVA performed on three rats that received both 130 Hz conditions (130 and 130-D) revealed a significant main effect of treatment condition ($F_{(2, 4)} = 14.68$, $p < 0.01$; *Figure 21B*). Post hoc analyses demonstrated a significant difference between the 130-D and No DBS conditions ($p < 0.01$). As there were no significant differences between 130 and 130-D on relative gain change ($p > 0.05$), the groups were collapsed for subsequent analyses.

Averaging across days obscures long term changes that occur with repeated HFS-B treatment in normal rats. These long-term changes are an increase in the effectiveness of HFS-B treatment in depressing reflex blink gain and a progressive decrease in the amplitude of reflex blinks evoked by the 2T SO stimulus before HFS-B treatment each day (Ryan *et al.*, 2014). Given that 6-OHDA lesioned rats did not show short-term gain changes unless treated with 130 Hz STN DBS (*Figure 21*), I predicted that 6-OHDA lesioned rats would exhibit long-term changes in the effectiveness of HFS-B modification of blink gain only when receiving 130 Hz STN DBS. When rats did not undergo STN DBS, there was no significant difference in the relative gain change between the first three (6.8 ± 4.3 %) and last three (13.3 ± 3.3 %) days of HFS-B treatment ($t_{(2)} = 0.01$, $p > 0.05$) (*Figure 22A*). Likewise, 16 Hz STN DBS had no significant effect on changes in relative gain change across days ($t_{(2)} = 1.40$, $p > 0.05$, *Figure 22*).

With 130 Hz STN DBS, however, relative gain change was significantly larger during the last three days (52.8 ± 10.9 %) than during the first three (22.4 ± 12.6 %) ($t_{(2)} = 4.23$, $p > 0.05$) days of HFS-B treatment (*Figure 22A*). The significant, linear increase of the amount of relative gain decrease with repeated days of HFS-B treatment ($r^2 = .42$, $p < 0.05$, *Figure 22B*) further illustrated the increasing effectiveness of HFS-B treatment across days when 6-OHDA rats received 130 Hz STN DBS. 130 Hz STN DBS also restored the ability of HFS-B treatment to reduce trigeminal drive on blink circuits. Our previous study showed that the threshold for evoking a blink did not change significantly across days (Ryan *et al.*, 2014) and I replicated this result ($r^2 = 0.06$, $p > 0.05$). Thus, the progressive, logarithmic decrease in the Pre R1 amplitude with repeated HFS-B treatment ($r^2 = .56$, $p < 0.01$, *Figure 22C*) demonstrated a long-term reduction in the strength of the trigeminal system drive on blink circuits. This modification in Pre HFS R1 across days did not occur in rats receiving 16 Hz STN DBS or in rats not receiving DBS (*Figure 22C*). These data demonstrated that 130 Hz STN DBS restored the long term changes induced by HFS-B treatment in the rat 6-OHDA model of PD.

## DISCUSSION

These experiments utilized 130 Hz STN DBS to show that disrupting beta oscillations attenuates blink reflex abnormalities in 6-OHDA lesioned rats. PD patients and the 6-OHDA rat model of PD exhibited hyperexcitable trigeminal reflex blinks revealed by elevated Test R2 amplitude in the paired stimulus paradigm and a lack of blink habituation (Klawans & Goodwin, 1969; Kimura, 1973; Agostino *et al.*, 1987; Basso *et al.*, 1993). In addition, dopamine depletion impaired gain modification of trigeminal blinks in the HFS paradigm (Battaglia *et al.*, 2006). I hypothesized that 130 Hz STN DBS, which attenuates beta oscillations in PD (Meissner *et al.*, 2005; Wingeier *et al.*, 2006; Ray *et al.*, 2008) and the rat 6-OHDA model of PD (McConnell *et al.*, 2012; Sutton *et al.*, 2013) would restore normal reflex blink behavior, while low frequency STN DBS that does not diminish beta oscillations (McConnell *et al.*, 2012) would not restore normal behavior. As predicted, 130 Hz STN DBS reduced R2 blink excitability by 50% relative to the No DBS condition in the paired stimulus paradigm (*Figure 20A*). 130 Hz STN DBS also restored normal blink reflex habituation in these 6-OHDA lesioned rats (*Figure 20B*). 16 Hz STN DBS had no significant effect on blink reflex excitability (*Figure 20*). Thus, disrupting beta oscillations with 130 Hz STN DBS alleviated blink excitability displayed by 6-OHDA lesioned rats (Basso *et al.*, 1993).

I also investigated the effect of disrupting beta oscillations on blink reflex plasticity in the 6-OHDA rat model of PD. Healthy people (Mao & Evinger, 2001) and rodents (Ryan *et al.*, 2014) demonstrate significant gain modification of the blink reflex after HFS treatment of the SO. PD patients off their dopamine replacement drugs, however, fail to exhibit gain modification with HFS treatment (Battaglia *et al.*, 2006). 6-OHDA lesioned rats also demonstrate this impairment, as 6-OHDA lesioned rats that did not undergo STN DBS failed to

exhibit significant depression with HFS-B treatment (*Figure 21A*). Treatment with continuous 130 Hz and not 16 Hz STN DBS restored blink reflex plasticity in 6-OHDA lesioned rats (*Figure 21*). 130 Hz STN DBS also restored the long-term changes produced by repeated days of HFS-B treatment, an increased ability of the HFS-B treatment to reduce blink gain and a reduction in the trigeminal drive onto blink circuits (*Figure 22*). 16 Hz STN DBS and No DBS conditions did not enable any long-term modifications with HFS-B treatment. Thus, disrupting beta oscillations during HFS-B treatment is sufficient to restore normal short- and long-term plasticity of the blink reflex in 6-OHDA lesioned rats.

Furthermore, the presentation of 130 Hz STN DBS only during the HFS-B treatment (130-D) restored plasticity to a degree not significantly different than with continuous 130 Hz STN DBS (*Figure 21C*). This result demonstrated that beta oscillations during the acquisition of the gain change were sufficient to block normal plasticity of this reflex in PD. Clearly, normal basal ganglia activity during the HFS treatment is critical for short- and long-term plasticity. This interpretation could explain why other basal ganglia pathologies, like Huntington's disease (Crupi *et al.*, 2008) and Gilles de la Tourette syndrome (Suppa *et al.*, 2011) display a similarly impaired blink reflex plasticity despite having different basal ganglia pathophysiologies. Furthermore, these data indicate that beta oscillations may disrupt normal motor learning and not simply play an antikinetic role in PD.

The circuit through which the basal ganglia modulate the excitability of the blink reflex has been well characterized. Dopamine depletion alters output from the SNr to the spinal trigeminal complex via the SC and the NRM (Basso & Evinger, 1996; Basso *et al.*, 1996). Clearly, the pathological beta activity throughout the basal ganglia in PD disrupts normal nigro-collicular interactions. While these data and several patient studies (Battaglia *et al.*, 2006; Crupi

*et al.*, 2008; Suppa *et al.*, 2011) highlight a critical role of the basal ganglia in normal blink reflex plasticity, it is not well understood how the basal ganglia modulate this behavior. The cerebellum plays an essential role in this type of plasticity (Ryan *et al.*, 2014), and it is possible that pathological beta oscillations in PD disrupt normal basal ganglia-cerebellar interactions (Hoshi *et al.*, 2005; Jinnah & Hess, 2006). Closer examination of the mechanisms of this blink reflex plasticity will provide the opportunity to examine more closely the relationship between basal ganglia-cerebellar interactions under normal and pathological conditions.

Synchronized beta oscillatory activity is prominent throughout the basal ganglia-cortical network in PD patients (Brown *et al.*, 2001; Kuhn *et al.*, 2005) and animal models (Goldberg *et al.*, 2002; Sharott *et al.*, 2005; Mallet *et al.*, 2008; McConnell *et al.*, 2012). A great deal of evidence links increasing beta band activity with the slowing of movement (Chen *et al.*, 2007; Kuhn *et al.*, 2009; Pogosyan *et al.*, 2009). Furthermore, Parkinsonian treatments such as L-dopa and DBS that alleviate voluntary movement abnormalities reduce beta activity (Kuhn *et al.*, 2006; Ray *et al.*, 2008). Although these data are consistent with the hypothesis that beta oscillations suppress voluntary movements in PD, the role of beta activity and voluntary movement appears to be more complicated than beta oscillations performing a simple akinetic role. For example, beta activity changes dynamically with salient cues under normal conditions (Leventhal *et al.*, 2012), and may reflect changes in the likelihood that a new movement will need to be initiated, or "motor readiness." (Jenkinson & Brown, 2011). Furthermore, decreases in beta activity occur with increases in gamma oscillations (> 30 Hz) when initiating a new movement (Lopez-Azcarate *et al.*, 2010; Tan *et al.*, 2013). This complicated relationship has made it difficult to establish unambiguous causal links between synchronized beta oscillatory activity and PD symptoms.

The study of the blink reflex makes this relationship clearer, as we can examine the effect of Parkinson's disease's exaggerated background beta activity independent of specific cues and motor plans. My data demonstrated that disrupting beta oscillations with 130 Hz STN DBS can restore normal behavior to a brainstem reflex. Furthermore, these data showed that beta oscillations can prevent normal plasticity and learning which may extend to circuits and symptoms beyond the brainstem. Overall, these data indicate that beta oscillations play a role in Parkinsonian blink reflex abnormalities.

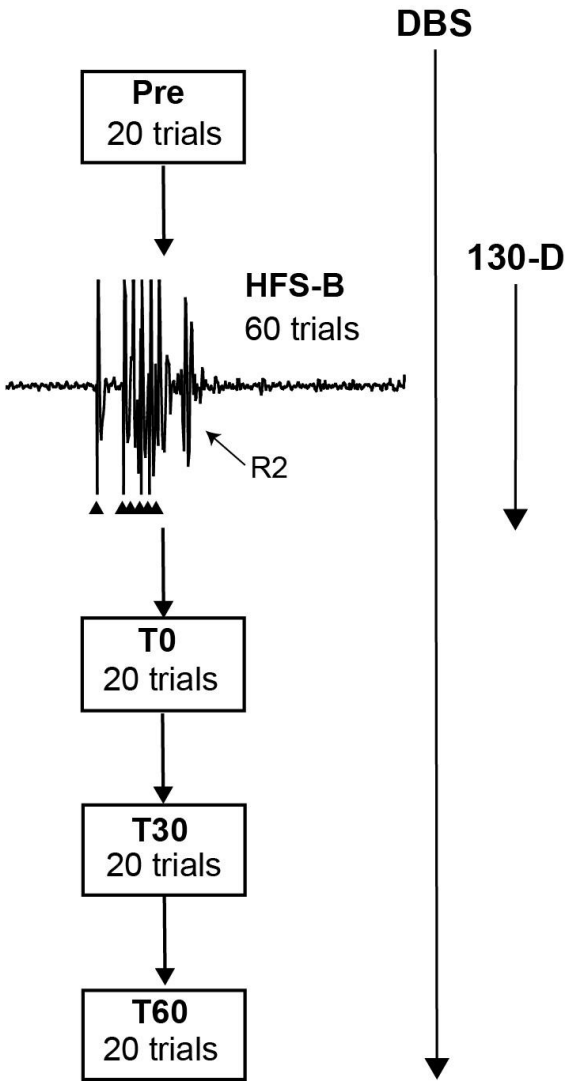


**Figure 19.** HFS-B treatment protocol. Triangles show a twice threshold 100 μs supraorbital nerve stimulus. DBS, deep brain stimulation of the subthalamic nucleus. 130-D, 130 Hz STN DBS presented during HFS-B treatment only.

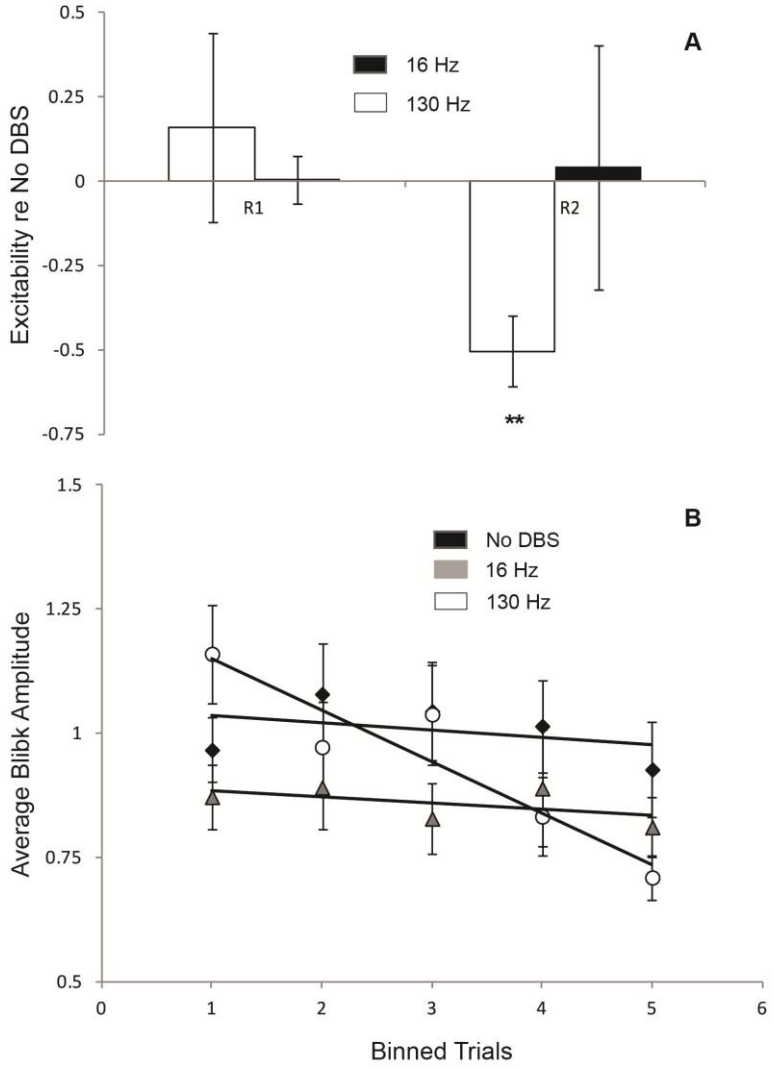


**Figure 20.** (A) Average change in reflex excitability for R1 and R2 components of the blink reflex for 6-OHDA lesioned rats undergoing 16 or 130 Hz STN DBS relative to the No DBS condition. Only 130 Hz STN DBS significantly decreased R2 excitability relative to No DBS. (B) Average change in blink amplitude with a single SO stimulus across 25 trials with a 1 second ISI. Only 130 Hz STN DBS restored normal blink reflex habituation. Lines are linear best fit. Error bars are SEM. ** $p < 0.01$

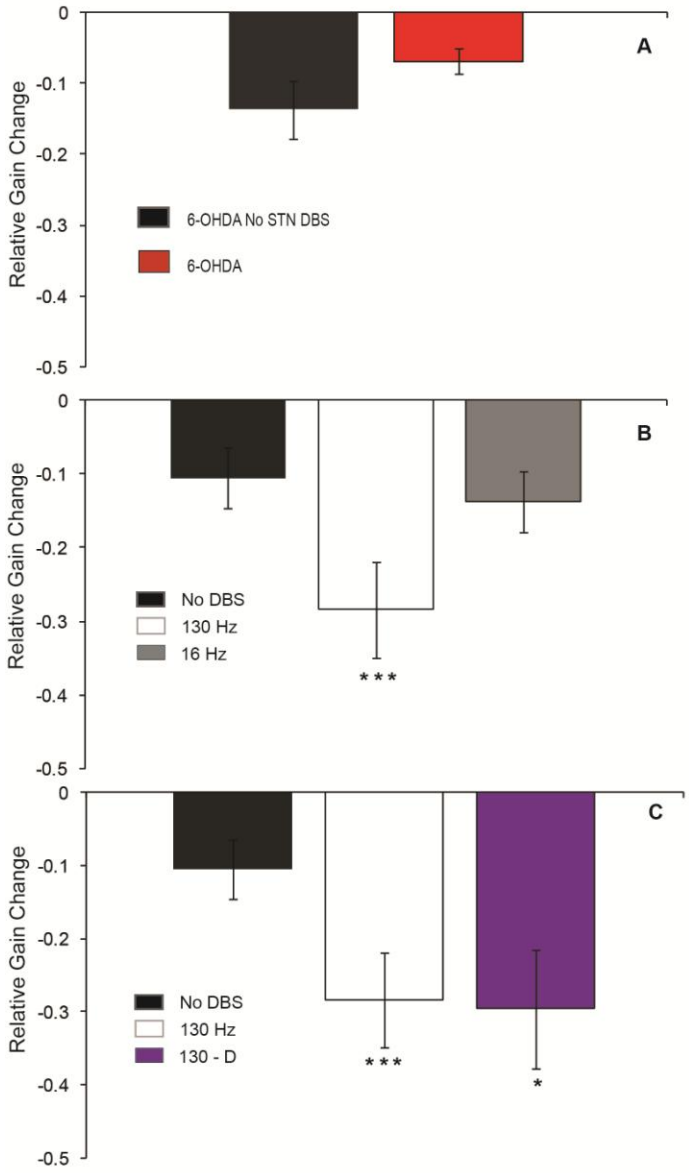


**Figure 21.** (A) Average relative gain change for 6-OHDA lesioned rats without subthalamic stimulating electrodes (red) and 6-OHDA lesioned rats with STN DBS turned off (black). Implantation of STN stimulating electrodes alone did not significantly alter the gain change. (B) Average relative gain change for 6-OHDA lesioned rats receiving No DBS, 130, or 16 Hz STN DBS. Only 130 Hz STN DBS significantly increased the relative gain change. (C) Relative gain change for 6-OHDA lesioned rats receiving No DBS, 130-D, or 130 Hz STN DBS. Both 130 Hz and 130-D treatment significantly increased the relative gain change. Error bars are SEM.
* $p < 0.05$ *** $p < 0.001$

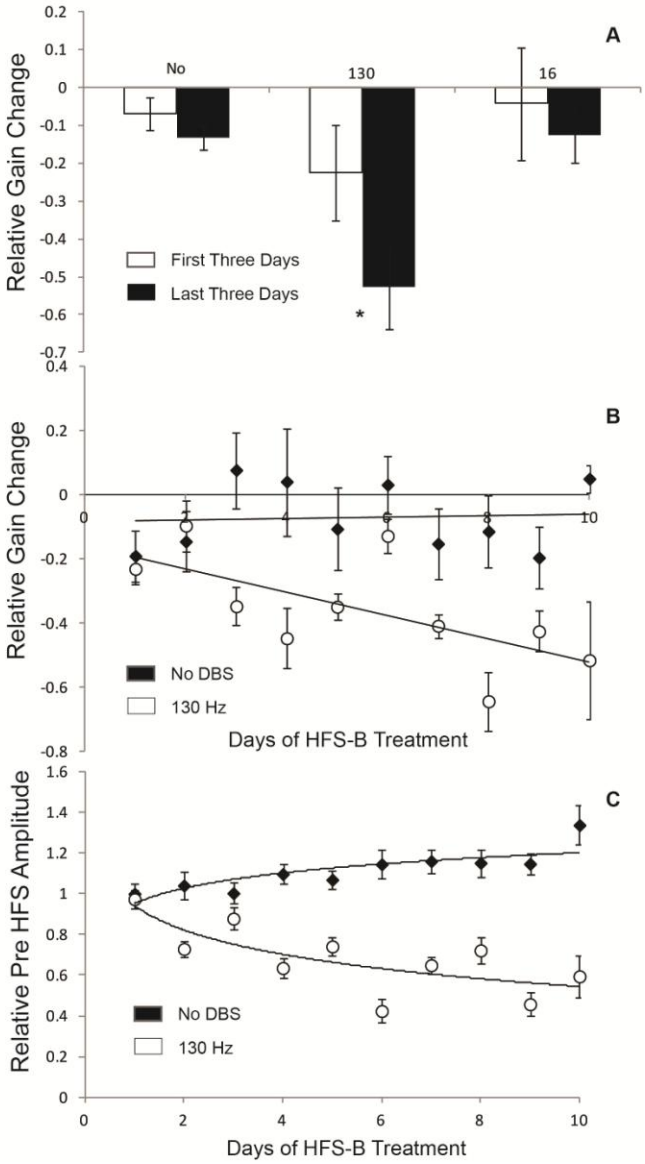


**Figure 22.** (A) Mean relative gain change over the first three and last three days for 6-OHDA lesioned rats receiving consecutive days of No DBS, 130, or 16 Hz STN DBS. When rats received 130 Hz STN DBS they exhibited an increased gain change over the last three days of HFS-B treatment compared to the first three days. (B) Average relative change for 6-OHDA lesioned rats receiving consecutive days of No DBS or 130 Hz STN DBS as a function of days of HFS-B treatment. The average relative gain change increased with days of HFS-B treatment when rats underwent 130 Hz STN DBS. Lines are linear best fits. (C) Average pre-HFS blink amplitude relative to median pre-HFS blink amplitude on *day 1* for 6-OHDA lesioned rats receiving consecutive days of No DBS or 130 Hz STN DBS as a function of days of HFS-B treatment. The average pre HFS blink amplitude decreased with days of HFS-B treatment when rats underwent 130 Hz STN DBS. Rats that Lines are logarithmic best fits. Error bars are SEM. * $p < 0.05$

# IV. BETA SUBTHALAMIC NUCLEUS STIMULATION INDUCES PARKINSONIAN LIKE BLINK REFLEX SYMPTOMS

## INTRODUCTION

Several studies demonstrate the presence of exaggerated, synchronized beta band (10-30 Hz) activity throughout the basal ganglia-thalamo-cortical network in Parkinson's disease (PD) and animal models of PD (Brown *et al.*, 2001; Brown & Williams, 2005; Kuhn *et al.*, 2005; Sharott *et al.*, 2005). Beta band power in PD correlates with voluntary movement impairment (Kuhn *et al.*, 2009) and with improvement after dopaminergic treatment and Deep Brain Stimulation (DBS) (Kuhn *et al.*, 2006; Draganski *et al.*, 2008). The therapeutic effect of DBS appears to result from reducing synchronized beta band activity throughout the basal ganglia-cortical network (Meissner *et al.*, 2005). Only regular, high frequency DBS alleviates motor symptoms in PD (Dorval *et al.*, 2010) as low frequency DBS does not reduce beta band activity or attenuate PD symptoms (McConnell *et al.*, 2012). Indeed, low frequency DBS worsens motor deficits in PD patients (Timmermann *et al.*, 2004; Boeve *et al.*, 2007). Although consistent with synchronized beta band activity creating PD symptoms, these data do not distinguish between exaggerated beta band activity playing a critical role in disrupting movement in PD or being an epiphenomenon of dopamine loss.

PD, however, affects reflex as well as voluntary movements. The strength of beta band oscillation synchrony varies with different components of voluntary movement and with the stimuli cueing the movement (Leventhal *et al.*, 2012). Unlike voluntary movements, however, the timing of reflex stimuli is independent of any variations in beta oscillations. This absence of correlated beta modulation makes it possible to test the effects of beta activity on reflex movements directly in normal animals. This analysis requires an animal model of PD that

accurately mimics abnormalities in basal ganglia activity and reflex motor behavior present in PD. The 6-hydroxydopamine (6-OHDA) lesioned rat model develops the exaggerated beta band oscillations typical of PD patients (Sharott *et al.*, 2005) and identical trigeminal blink abnormalities as PD patients, including hyperexcitable blink reflexes, lack of habituation and impaired prepulse inhibition (Agostino *et al.*, 1987; Basso *et al.*, 1993; Schicatano *et al.*, 2000). Therefore, the trigeminal blink circuit provides an ideal motor system to examine the behavioral effects of inducing beta activity in normal rats. Additionally, PD patients exhibit impaired blink reflex plasticity revealed by a protocol that utilizes high frequency stimulation of the supraorbital branch of the trigeminal nerve (Mao & Evinger, 2001; Battaglia *et al.*, 2006; Ryan *et al.*, 2014). Chapter III demonstrated that 6-OHDA lesioned rats exhibit these same blink reflex disturbances that can be normalized by disrupting beta oscillations with 130 Hz STN DBS. Clearly, the blink reflex in 6-OHDA lesioned rats provides a great system to examine a causal role of beta activity in the generation of Parkinsonian motor symptoms.

These experiments test the effects of STN DBS at beta (16 Hz), theta (7 Hz), and 130 Hz frequencies on blink reflex behavior and plasticity of normal rats. I compared these data with those from the 6-OHDA lesion model of PD. I posit that if exaggerated beta band activity is critical in the generation of Parkinsonian reflex and plasticity abnormalities, then only 16 Hz stimulation will induce a PD-like impairment in blink reflex behavior in normal rats.

## MATERIALS AND METHODS

Experiments were performed on fifteen male Sprague Dawley rats (350-550 g) maintained on a reversed 12 hour light/dark cycle and fed ad libitum. All experiments received approval by the Stony Brook University Institutional Animal Care and Use Committee and complied with all Federal, State, and University regulations and guidelines regarding the use of animals in research.

*Surgery*

Under general anesthesia (ketamine, 90 mg/kg and xylazine, 10 mg/kg), eight of the fifteen rats were implanted unilaterally with laboratory designed stimulating electrodes in the subthalamic nucleus (STN). Electrodes were implanted into the right STN stereotaxically at AP: -3.8, ML: 2.5 based on the Paxinos and Watson stereotaxic atlas (Paxinos & Watson, 1998). Final STN electrode position was determined by recording through one of the DBS leads to find an increase in background activity as the electrode moved through the zona incerta into the STN. The stimulation electrodes were 4 twisted stainless steel Teflon coated wires (0.003 inch diameter bare, 0.0055 inch coated; A-M Systems, Everett, WA). After electrode implantation, rats were prepared for chronic recording of the left orbicularis oculi EMG (OOemg) and stimulation of the left supraorbital (SO) branch of the trigeminal nerve (Basso *et al.*, 1993; Evinger *et al.*, 1993). Wires were led subcutaneously to a connector embedded in a dental acrylic platform on the skull. The platform was attached to the skull by four stainless-steel screws. A silver wire connected to one of the stainless-steel screws served as the ground (Evinger & Manning, 1993; Dauvergne & Evinger, 2007).

Immediately before OOemg and SO electrode implantation, seven of the 15 rats received unilateral 6-hydroxydopamine (6-OHDA) lesions of the right SNc and medial forebrain bundle

(Basso *et al.*, 1993). Rats received analgesic (ketorolac, 7 mg/kg) for at least 24 hrs after the surgery. Rats were alert and eating within 24 hrs of the surgery. The experiments began 10 days after surgery for the 6-OHDA injected rats and at least 1 week after the surgery for all other rats. After completion of experiments all rats were deeply anesthetized and perfused intracardially. The brains of rats with DBS electrodes were sectioned at 100 µm and stained with cresyl violet to identify electrode location. The brains of rats with 6-OHDA lesions were sectioned at 40 µm and immunohistochemically stained for tyrosine hydroxylase to assess lesion magnitude (Basso *et al.*, 1993). Tryosine hydroxylase staining revealed full unilateral lesions of the SNc in all seven 6-OHDA injected rats. Only rats with correct STN electrode placement were reported in this study.

*Procedures*

In all experiments, the SO stimulus current was relative to the minimum current at which a 100 µs stimulus reliably elicited the R1 component of a reflex blink, threshold (T). This current was determined at the beginning of each day for each rat and was held constant throughout that day's experiment. Threshold varied little across days (Ryan *et al.*, 2014). All data were collected at twice threshold (2T). Typical of all mammals except primates, this stimulus intensity evoked a strong R1 response and a smaller R2 component that occurred on slightly over half of the trials (Dauvergne & Evinger, 2007; Ryan *et al.*, 2014).

Rats underwent continuous biphasic 100 µA 100µS STN DBS that began five minutes prior to threshold testing each day. Rats were observed closely for the duration of the day's experiments for signs of abnormal motor behavior. This stimulation intensity did not cause any irregular motor behaviors in the rats or tissue damage in the STN, but affected trigeminal blink behavior. STN DBS was continued until the completion of that day's experiment.

I evaluated the effects of 16 and 130 Hz STN DBS on trigeminal blink excitability and amplitude in eight normal rats. Each day, rats underwent three blocks of twenty trials comprised of pairs of 2T SO with a 100 ms interstimulus interval (See *Figure 5*). During each block, the rat received continuous 16 or 130 Hz STN DBS or No DBS; experiencing all three conditions each day. As with clinical analysis of trigeminal blink reflex excitability in PD patients (Agostino *et al.*, 1987), I quantified blink excitability by dividing blink amplitude evoked by the second SO by the amplitude of the blink elicited by the first SO. Because our previous study (Basso *et al.*, 1993) demonstrated that the side contralateral to the 6-OHDA lesion exhibited the largest changes in blink excitability, I only examined OOemg activity contralateral to the STN stimulating electrode.

To examine blink reflex plasticity I utilized a protocol previously designed to modify blink reflex gain (Mao & Evinger, 2001) that we recently adapted for use in rodents (Ryan *et al.*, 2014) and delivered only a single frequency of STN DBS each day (*Figure 19*). Each day's data collection consisted of five blocks: (1) pre high frequency stimulation; (2) high frequency stimulation (HFS) treatment; (3) immediately; (4) 30 min; and (5) 60 min post HFS. Pre and post HFS blocks were the same for all experiments. In these blocks, rats received 20 trials of a pair of 2T SO stimuli separated by 100 ms with an inter-trial interval of 20 ± 5 s. Each HFS treatment trial consisted of a single SO stimulus at 2T to evoke a reflex blink followed by five, 400Hz 2T SO stimuli delivered <u>before</u> the onset of the R2 component of the OOemg activity (HFS-B). HFS trials also occurred at a 20 ± 5 s interval. 6-OHDA lesioned rats underwent this learning protocol without DBS for at least eight consecutive days. Non-lesioned rats received either No STN DBS, 7 Hz, 16 Hz, or 130 Hz STN DBS in a counterbalanced design for at least eight days per condition.

*Data Collection and Analysis*

Reflex blinks were monitored as rats moved freely in their home cage in a darkened room during their subjective night. OOemg signals were amplified, filtered at 0.3–5 kHz, collected at 4 kHz per channel and stored for later offline analysis on laboratory developed software (Dauvergne & Evinger, 2007). Blink amplitude was determined by integrating the rectified OOemg activity of each blink component.

In humans, HFS-B treatment depressed the R2 component of subsequent blinks (Mao & Evinger, 2001). I investigated the effects of HFS-B on the R1 component of the blink reflex, however, as it is the largest component of the blink reflex in non-primate mammals and exhibits the same changes in the HFS-B paradigm as R2 (Ryan *et al.*, 2014). I normalized all within day blink amplitude by dividing EMG amplitude by the median pre EMG amplitude. As there was no difference in the post HFS blocks (Ryan *et al.*, 2014), post HFS blink amplitude was averaged over all three post treatment blocks. The normalized mean pre HFS blink amplitude was subtracted from the normalized mean post blink amplitude. As the blink evoking stimulus remained constant throughout the day, we termed this measure of the change in blink amplitude following HFS *relative gain change*.

Statistical tests of significance ($p < 0.05$) were performed with SPSS software (SPSS, Chicago, IL) using an Analysis of Variance Test (ANOVA) with post hoc Tukey tests, paired or independent t tests. Data are presented as the mean ± SEM.

## RESULTS

Patients with PD and 6-OHDA lesion models of PD exhibit increased excitability of the R2, but not the R1 component of trigeminal reflex blink (Agostino *et al.*, 1987; Basso *et al.*, 1993). If 16 Hz STN DBS creates a PD-like condition in reflex blinking in normal rats, then R2, but not R1 excitability should increase with 16 Hz STN DBS compared with the No DBS condition. As predicted, 16 Hz STN DBS significantly enhanced R2 excitability 153 ± 42 % relative to the No DBS condition ($t_{(6)} = -4.0$, $p < 0.01$) but did not alter R1 excitability ($t_{(6)} = -0.81$, $p > 0.05$; *Figure 23A*). This increase in excitability is similar to that observed in the 6-OHDA lesioned rats when compared to normal rats (Powers *et al.*, 1997). In contrast, R1 and R2 excitability during 130 Hz STN DBS were not significantly different than during the No DBS condition (R1, $t_{(6)} = 1.35$ $p > 0.05$; R2, $t_{(6)} = -1.5$ $p > 0.05$, *Figure 23A*). Likewise, 7 Hz STN DBS significantly increased R2 excitability ($t_{(2)} = -3.0$, $p < 0.05$), but not R1 excitability ($t_{(2)} = 1.0$, $p > 0.05$; *Figure 23A*). Thus, 7 Hz STN DBS increased R2 excitability as occurs in the focal dystonia benign essential blepharospasm (Berardelli *et al.*, 1985) and 16 Hz STN DBS increased the R2 excitability of trigeminal blink reflexes of normal rats as occurs in PD patients (Kimura, 1973; Agostino *et al.*, 1987) and dopamine depleted rodents (Basso *et al.*, 1993). Similar to the reduction in blink amplitude created by PD (Korosec *et al.*, 2006), 16 Hz STN DBS significantly reduced the amplitude of reflex blinks in normal rats (*Figure 23B*). 16 Hz STN DBS reduced R2 amplitude by 16.1 ± 6.9 % ($t_{(9)} = 2.4$, $p < 0.05$) and R1 amplitude by 25.6 ± 11.7% ($t_{(7)} = 2.3$, $p < 0.05$) relative to the No DBS condition. As with excitability, 130 Hz STN DBS did not significantly affect trigeminal reflex blink amplitude (R1, $t_{(11)} = 1$, $p > 0.05$; R2, $t_{(10)} = 0.09$, $p > 0.05$). 7 Hz STN DBS, a theta oscillation associated with dystonia (Silberstein *et al.*, 2003; Chen *et al.*, 2006), served as a test of whether the 16 Hz induced changes in reflex blink amplitude

were frequency specific or a generalized effect of low frequency STN DBS. 7 Hz STN DBS caused an insignificant elevation of 10.8 ± 9% in R1 blink amplitude ($t_{(5)} = -1.3$, $p > 0.05$), but a significant increase of 77.2 ± 39% in R2 blink amplitude ($t_{(4)} = 2.2$, $p < 0.05$). The increased R2, but not R1, matches the change in blink amplitude caused by benign essential blepharospasm (Berardelli *et al.*, 1985).

High frequency SO stimulation modifies blink reflex gain in both humans (Mao & Evinger, 2001) and rodents (Ryan *et al.*, 2014). PD, however, impairs this reflex plasticity in patients off dopaminergic replacement therapy (Battaglia *et al.*, 2006). If 16 Hz STN DBS creates PD-like abnormalities in blink plasticity, then normal rats undergoing this stimulation frequency should fail to exhibit blink plasticity in this paradigm. In contrast, as the focal dystonia benign essential blepharospasm enhances gain adaptation in this paradigm (Quartarone *et al.*, 2006a), STN DBS at a theta frequency typical of dystonia (Silberstein *et al.*, 2003; Tsang *et al.*, 2012) should exaggerate blink plasticity.

A normal rat that received eight days of all four treatment conditions illustrated the effects of different frequencies of STN DBS on blink reflex plasticity (*Figure 24A*). Similar to Ryan *et al*, 2014, HFS-B treatment in the No DBS condition produced a 24.9 ± 6.1 % relative gain change. HFS-B treatment during 130 Hz STN DBS caused a similar 30.1 ± 5.9 % depression. In contrast, 16 Hz STN DBS reduced the blink depression from HFS-B treatment to a mere 4.2 ± 10.7 % and 7 Hz STN DBS theta band stimulation enabled HFS-B treatment to exaggerate the relative gain change to 63.4 ± 5.8%.

Averaging across all normal rats and days produced the same results in reflex plasticity as shown by the exemplar rat ($F_{(3, 258)} = 13.52$, $p < .001$; *Figure 24B*). In the No DBS condition, HFS-B treatment significantly depressed subsequent R1 responses of normal rats by 23.3 ± 3.0

% ($t_{(80)}$ = 7.02, $p < 0.001$). During 130 Hz STN DBS, HFS-B treatment also significantly decreased blink amplitude by 19.7 ± 3.0 % ($t_{(59)}$ = 6.92, $p < 0.001$), which was not significantly different from normal rats that did not receive any STN DBS ($p > 0.05$). In normal rats, 16 Hz STN DBS reduced blink depression from HFS-B treatment to an insignificant 2.0 ± 3.5 % ($p > 0.05$). This depression was not significantly different than the 6.6 ± 4.4% depression in blink gain observed in the separate group of rats with a 6-OHDA lesion ($p > 0.05$). Thus, HFS-B treatment created significantly more blink depression in the No DBS and 130 Hz STN DBS conditions than with 16 Hz STN DBS ($p < 0.001$ and $p < 0.05$, respectively). Rats that received 7 Hz STN DBS during HFS-B treatment exhibited an enhanced relative gain change of 36.7 ± 3.9 % ($t_{(56)}$ = 9.40, $p < 0.001$) that was significantly more than exhibited during No DBS ($p < 0.001$), 130 Hz STN DBS ($p < 0.001$) or 16 Hz STN DBS ($p < 0.001$). Thus, 16 Hz STN DBS in normal rats impaired blink reflex plasticity as occurs in PD patients (Battaglia *et al.*, 2006), whereas 7 Hz STN DBS exaggerated blink plasticity as occurs in the focal dystonia, blepharospasm (Quartarone *et al.*, 2006a).

## DISCUSSION

These experiments employed continuous STN DBS to demonstrate that specific frequencies of basal ganglia stimulation significantly altered reflex behavior in normal rats. I hypothesized that stimulation of the STN in normal rats at a beta band frequency typical of PD (Brown *et al.*, 2001; Brown & Williams, 2005; Kuhn *et al.*, 2005; Sharott *et al.*, 2005) would create the same blink reflex abnormalities present in PD patients and a rat model of PD, including, blink hyperexcitability, reduced blink amplitude, and impaired blink reflex plasticity. As predicted, 16 Hz STN DBS increased R2 blink excitability 153% relative to the No DBS condition (*Figure 23A*). 16 Hz STN DBS also reduced trigeminal reflex blink amplitude by 25.6 and 16.1% for the R1 and R2 respectively (*Figure 23B*). Thus, 16 Hz STN DBS created the same trigeminal blink reflex abnormalities as occur in the 6-OHDA lesion model of PD and PD patients.

I investigated blink reflex plasticity with a paradigm previously used to evaluate gain modification in normal subjects (Mao & Evinger, 2001) and patients with basal ganglia related disorders. In PD (Battaglia *et al.*, 2006), Huntington's disease (Crupi *et al.*, 2008), and Gilles de la Torrette syndrome (Suppa *et al.*, 2011), this paradigm revealed impaired gain modification, but in patients with the focal dystonia, benign essential blepharospasm, HFS treatment enhanced this plasticity (Quartarone *et al.*, 2006a). I hypothesized that activating the basal ganglia at a beta band frequency characteristic of PD (Brown & Williams, 2005; Sharott *et al.*, 2005) would impair blink plasticity in a normal rat, whereas theta band activation typical of dystonia (Chen *et al.*, 2006; Tsang *et al.*, 2012) would enhance gain modification. I further predicted that stimulation at 130 Hz would not affect blink reflex plasticity in normal rats, as this frequency of STN DBS alleviates motor symptoms in PD and dystonic patients by reducing exaggerated low

frequency basal ganglia oscillations (Dorval *et al.*, 2010; McConnell *et al.*, 2012). Activating the STN with a beta band frequency impaired blink plasticity in normal rats as occurred in PD patients (Battaglia *et al.*, 2006) and a rat model of PD (*Figure 24B*). In contrast, driving the STN at a theta band frequency exaggerated gain adaptation in normal rats as occurred with the focal dystonia, benign essential blepharospasm (Quartarone *et al.*, 2006a).

The changes in blinking were not a generalized effect of STN DBS because they were frequency specific. 130 Hz STN DBS, a commonly used frequency to treat movement disorders (Meissner *et al.*, 2005; Dorval *et al.*, 2010), did not affect blink excitability, amplitude, or plasticity (*Figure 23*, *24*). Stimulation at a theta band frequency typical of dystonia (Silberstein *et al.*, 2003; Chen *et al.*, 2006), however, increased R2 blink amplitude (*Figure 23B*) and exaggerated blink plasticity (*Figure 24B*) as occurs in the focal dystonia, benign essential blepharospasm (Quartarone *et al.*, 2006a). R2 blink excitability increased with both 7 Hz and 16 Hz STN DBS as occurred with blepharospasm (Berardelli *et al.*, 1985), PD (Agostino *et al.*, 1987), and animal models of PD (Basso *et al.*, 1993). Thus, the effects of STN DBS were frequency specific. Depending upon the frequency of STN DBS, the same rat could exhibit normal behavior, PD blink abnormalities, or blepharospasm-like blink characteristics.

Although the circuit through which the basal ganglia modulate the excitability of the blink reflex is known (Basso & Evinger, 1996; Basso *et al.*, 1996), how the basal ganglia affects blink reflex plasticity is less understood. The cerebellum plays a significant role in gain changes for many brainstem reflexes, including the blink reflex (Pellegrini & Evinger, 1997; Blazquez *et al.*, 2004; Ryan *et al.*, 2014). It may be that synchronized beta and theta band oscillations in the basal ganglia disrupt normal cerebellar-basal ganglia interactions (Hoshi *et al.*, 2005; Jinnah & Hess, 2006). The stark difference in plasticity we observed between 7 Hz and 16 Hz STN DBS

treatment support evidence that oscillatory activity at beta and theta band frequencies present in the basal ganglia with PD (Brown & Williams, 2005; Kuhn *et al.*, 2005) and dystonic patients (Silberstein *et al.*, 2003; Chen *et al.*, 2006; Tsang *et al.*, 2012), respectively, play a critical function in modulating this form of gain adaptation.

Synchronized beta band oscillatory activity has been postulated to play a role in PD motor symptoms, as this activity is present throughout the basal ganglia-cortical network in PD patients (Brown & Williams, 2005; Kuhn *et al.*, 2005) and animal models (Goldberg *et al.*, 2002; Sharott *et al.*, 2005). This aberrant activity correlates with symptom severity (Kuhn *et al.*, 2009; Pogosyan *et al.*, 2009) and movement effective treatments reduce the strength of beta band oscillations (Kuhn *et al.*, 2006; Ray *et al.*, 2008). The efficacy of DBS depends on the reduction of beta band activity as DBS frequencies that do not attenuate beta band activity also fail to ease Parkinsonian motor symptoms (McConnell *et al.*, 2012). Indeed, low frequency STN DBS worsens PD symptoms (Timmermann *et al.*, 2004; Chen *et al.*, 2007; Eusebio *et al.*, 2008). All of this evidence, however, is correlational.

Data from healthy humans support a causal relationship between beta band activity and voluntary motor performance. Using transcranial alternating current stimulation (TACS) in normal humans, investigators report that 20 Hz beta band stimulation significantly slowed the initial and peak velocity of voluntary movements, although there were no significant effects on reaction time or mean velocity (Pogosyan *et al.*, 2009). A later study reports that 20 Hz TACS reduced force development and peak force in a Go/No-Go task, while gamma stimulation (70 Hz) had the opposite effect (Joundi *et al.*, 2012). Although the effects were subtle, these experiments provided causal evidence for an antikinetic role of beta band activity in movement. Nevertheless, not all data support this antikinetic role for beta oscillations in PD. The report that

1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine injected monkeys become severely bradykinetic prior to the development of synchronized beta activity in the basal ganglia (Leblois *et al.,* 2007) is inconsistent with beta oscillations causing the bradykinesia of PD. Furthermore, delivering a 23 Hz oscillating current to the STN of normal rats and non human primates failed to create Parkinsonian-like motor abnormalities (Syed *et al.,* 2012). The focus of these studies, however, was on locomotion and arm movements, voluntary actions in which beta oscillations appear to play multiple roles (Leventhal *et al.,* 2012). Reflexes with PD, however, occur in an environment of exaggerated beta oscillations whose modulation is unrelated to the occurrence of reflex evoking stimuli. Driving the STN at a beta frequency in normal rats mimics the background upon which reflexes occur in PD or animal models of PD. Consistent with this situation, trigeminal reflex blink abnormalities with 16 Hz STN DBS are the same as with PD or rodent models of PD. This similar pattern of abnormalities in rodent models and patients probably results from the highly conserved organization of trigeminal blink circuits across mammals.

The current study utilized STN DBS to induce different frequency activity in the basal ganglia of normal rodents. Using this approach, I found that different frequencies created PD and dystonic blink plasticity abnormalities in the same rat. Thus, this approach offers the ability to reproduce Parkinsonian- and dystonic-like patterns of behavior without destroying dopaminergic neurons or blocking dopamine receptors. This technique may offer a novel opportunity to investigate the neural bases of basal ganglia disorders.

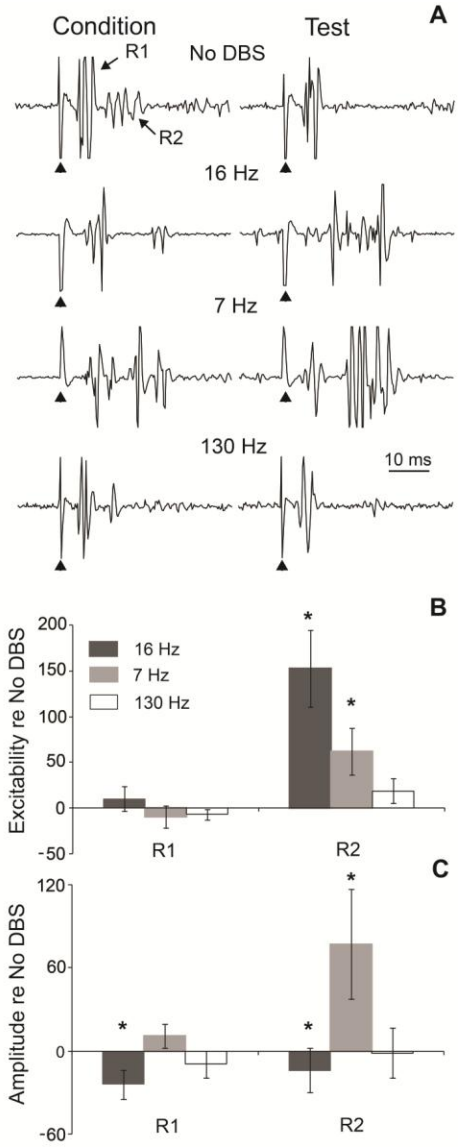


**Figure 23.** (A) Individual trials of the paired stimulus paradigm for a single rat without DBS (No DBS), 16 Hz STN DBS (16 Hz), 7 Hz STN DBS (7 Hz), and 130 Hz STN DBS (130 Hz). 7 and 16 Hz STN DBS increased the R2 test response. 130 Hz STN DBS had no effect. Triangles show SO stimulus artifact and arrows show R1 and R2 components. (B) Average percent change in reflex excitability for R1 and R2 components of the blink reflex for normal rats undergoing 16, 7 or 130 Hz STN DBS relative to the No DBS condition. 7 and 16 Hz STN DBS significantly increased the R2 excitability. 130 Hz STN DBS did not alter R2 excitability. None of the stimulation frequencies affected R1 excitability. (C) Average percent R1 and R2 Condition reflex blink amplitude relative to the No DBS condition with 16, 7, and 130 Hz STN DBS. 16 Hz STN DBS significantly reduced the R1 and R2 components of the blink reflex while 7 Hz STN DBS significantly increased the R2 response of the blink reflex. Error bars are SEM. * $p < 0.05$

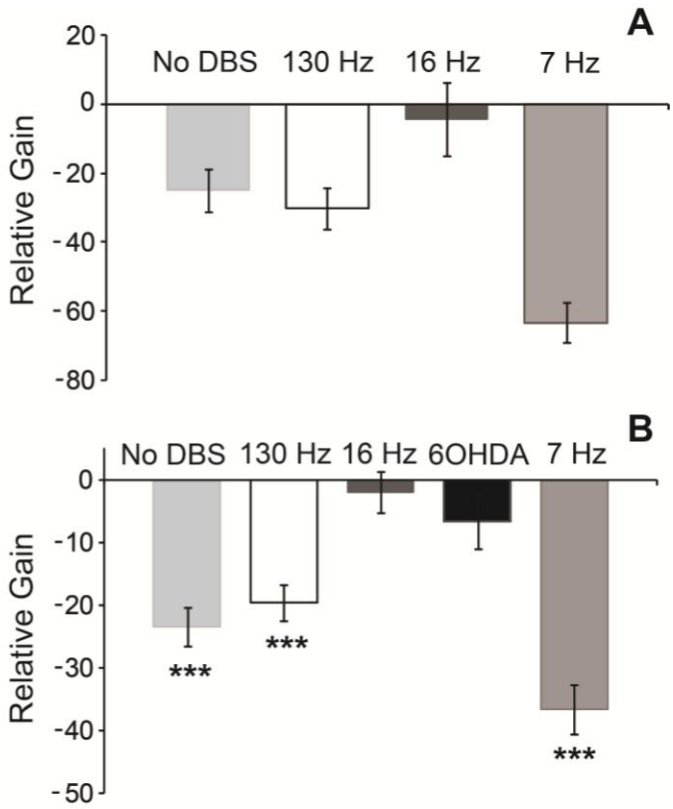


**Figure 24.** (A) Average percent relative gain change for a single rat with No DBS, 130, 16 and 7 Hz STN DBS. 16 Hz STN DBS reduced the relative gain change while 7 Hz STN DBS increased the relative gain change in response to HFS-B treatment. 130 Hz STN DBS had no effect on the relative gain change. (B) Average percent relative gain change for all normal rats receiving No DBS or 130, 16 or 7 Hz STN DBS. Rats that received 16 Hz STN DBS exhibited a significantly impaired relative gain similar to a group of 6-OHDA lesioned rats. Rats that received 7 Hz STN DBS exhibited an exaggerated gain change with HFS-B treatment. Error bars are SEM. *** $p < 0.001$

# V. GENERAL DISCUSSION

My experiments utilized the blink system to investigate the role of beta oscillations in Parkinsonian motor symptoms. Overall, these data demonstrate that basal ganglia beta oscillations play a critical role in the modification of blink reflex behavior and plasticity in PD. My data also reveal that the dopamine depletion, but not the exaggerated beta oscillations of PD, are responsible for the reduced spontaneous blink rate of PD patients. These data support the proposal that the blink system provides an extremely valuable tool to study the mechanisms of movement disorders and basal ganglia pathologies.

*General Summary*

Chapter II characterized the spontaneous blink generator as well as the dopaminergic influence on spontaneous blinking. My experiments demonstrated that although the spontaneous blink rates are different, both rats and humans display a similar temporal organization to their spontaneous blinking. For both species, the pattern of spontaneous blinking revealed long-term periodicity composed of episodes of frequent blinking interspersed with interludes of few blinks, independent of the spontaneous blink rate. The similarities of the temporal organization of blinking between species indicated that the rat provides an apropriate model to examine the neural basis of spontaneous blinking and also points to the presense of an endogenous spontaneous blink generator in mammals.

Although, I did not aim to determine the location of the spontaneous blink generator in these studies, it is clearly modulated by central dopamine levels. My data are consistent with other primate data that demonstrate treatment with dopaminergic agonists increases the spontaneous blink rate, while dopamine depletion or receptor blockade reduces the spontaneous blink rate (Karson *et al.*, 1982b; Lawrence & Redmond, 1991; Taylor *et al.*, 1999; Bologna *et al.*, 2012). Haloperidol injections (*Figure 13A*) and 6-OHDA lesions (*Figure 15A*) reduced the

spontaneous blink rate while apomorphine treatment increased the spontaneous blink rate (*Figure 13A*). These drugs also affected the pattern of spontaneous blinking in rats. Haloperidol and 6-OHDA lesions increased the temporal variability of spontaneous blinking (*Figure 14D*), while apomorphine reduced the variability of this behavior (*Figure 14D*). This reduced periodicity was seen in other motor behaviors with dopamine depletion (Leblois *et al.*, 2010).

My last experiments in Chapter II examined the role of beta oscillations in spontaneous blink abnormalities in PD. I utilized 130 Hz STN DBS as a tool to disrupt beta oscillations present in 6-OHDA lesioned rats (Meissner *et al.*, 2005; Sharott *et al.*, 2005; Kuhn *et al.*, 2008; Dorval *et al.*, 2010; McConnell *et al.*, 2012). 130 Hz STN DBS did not affect spontaneous blinking in 6-OHDA lesioned rats, as it neither increased the spontaneous blink rate nor reduced the variability of spontaneous blinking (*Figure 15*). Although dopamine clearly modulates the spontaneous blink generator, my data suggested that synchronized beta oscillations are not the mechanisms through which dopamine depletion alters spontaneous blinking.

In Chapters III and IV, I investigated the role of beta oscillations in blink reflex excitability. In both PD patients (Kimura, 1973; Agostino *et al.*, 1987) and the 6-OHDA lesioned rat model of PD (Basso *et al.*, 1993) the blink reflex is hyperexcitable. This hyperexcitability was due to altered nigro-collicular interactions that modified the excitability of the spinal trigeminal complex (Basso & Evinger, 1996; Basso *et al.*, 1996). In Chapter III, I determined whether 130 Hz STN DBS that reduces beta oscillations in the 6-OHDA rat model of PD blocked trigeminal blink hyperexcitability. In Chapter IV, I determined whether inducing beta oscillations in a **normal** rat created reflex blink hyperexcitability. My data demonstrated that disrupting beta oscillations in 6-OHDA lesioned rats with 130 Hz STN DBS restored habituation (*Figure 20B*) and normal excitability in the paired stimulus paradigm (*Figure 20A*).

16 Hz STN DBS that does not disrupt beta oscillations did not affect blink reflex excitability in 6-OHDA lesioned rats (*Figure 20*). Furthermore, my data demonstrated that beta stimulation of the STN with 16 Hz STN DBS is sufficient to induce hyperexcitability in normal rats. These data demonstrate the criticial role of beta oscillations in the generation of Parkinsonian blink reflex hyperexcitability.

Additionally, Chapters III and IVinvestigated the role of beta oscillations in blink reflex plasticity. Battaglia et al (2006) established that PD patients off their L-dopa medication did not exhibit trigeminal reflex blink gain modifcations (Battaglia *et al.*, 2006). I demonstrated that 6-OHDA lesioned rats also failed to show eithr short- and long term plasticity of the blink reflex (*Figures 21, 22*). 130 Hz STN DBS restored normal blink reflex plasticity in these Parkinsonian-like rats suggesting a role for synchronized beta oscillations in the impairment of blink plasticity (*Figures 21, 22*). My demonstration that presentation of 130 Hz STN DBS only during the HFS-B treatment (130-D) was sufficient to improve blink reflex plasticity (*Figure 21C*) revealed that the exaggerated beta oscillations of PD blocked acquisition of the gain modification. Again, 16 Hz STN DBS did not significantly alter blink reflex plasticty in 6-OHDA lesioned rats (*Figures 21, 22*).

While my data from Chapter III was consistent with beta oscillations being responsible for impaired blink reflex plasticity in PD, Chapter IV provided causal evidence. My data showed that stimulation of the STN at a beta frequency impaired blink reflex plasticity in normal rats as occurs in 6-OHDA lesioned rats (*Figure 24*) and PD patients (Battaglia *et al.*, 2006). This effect was specific to beta stimulation, as 130 Hz STN DBS did not affect blink reflex plasticity in normal rats. Moreover, theta stimulation (7 Hz) actually enhanced blink reflex plasticity as is observed with dystonic patients (Quartarone *et al.*, 2006a) (*Figure 24*). Together my data from

Chapters III and IV indicate that basal ganglia beta oscillations play a critical role in the development of blink reflex abormalities in PD.

*Implications*

The major findings of my experiments are that beta oscillations play an important role in Parkinsonian blink reflex abnormalities but not spontaneous blink symptoms. Although one group found a very miminal effect of STN DBS on the spontaneous blink rate in PD patients (Bologna *et al.*, 2012), my data clearly demonstrate that STN DBS has no effect on spontaneous blink rate or variability in Parkinsonian-like rats (Chapter II). Furthermore, my data demonstrate the importance of beta oscillations in Parkinsonian blink reflex abnormalities (Chapters III and IV). This strongly suggests that the basal ganglia modulate spontaneous blinking and reflex blinking via different mechansims.

Significant evidence points to the existence of an endogenous spontaneous blink generator (Stern *et al.*, 1984; Karson, 1988; Naase *et al.*, 2005; Kaminer *et al.*, 2011), although we still do not know its location. My data confirm evidence from MPTP monkeys (Taylor *et al.*, 1999) and PD patients (Karson *et al.*, 1982b; Bologna *et al.*, 2012) that demonstrate basal ganglia dopamine depletion significantly alters the spontaneous blink generator (*Figures 13, 14, 15*). Clearly, the basal ganglia modulate the spontaneous blink generator, although we have yet to elicidate the neural mechanism. It might be expected that STN DBS does not affect spontaneous blinking in PD, as STN DBS does not consistently restore other central pattern generator behaviors, like locomotion in PD (Moreau *et al.*, 2008; Hausdorff *et al.*, 2009; St George *et al.*, 2010). In fact peduculopontine (PPT) DBS has been shown to be more effective at treating gait disturbances in PD than STN DBS (Stefani *et al.*, 2007; Lozano & Snyder, 2008). The basal ganglia has strong connections to the PPT (Redgrave & Coizet, 2007), but it is possible that patholol gical interactions between the basal ganglia and PPT in PD are not

characterized by synchronized beta oscillations. If the spontaneous blink generator is altered by a similar mechansim to the locomotion in PD, PPT DBS may better for restoring spontaneous blink function in PD.

On the other hand, my data demonstrated that beta activity plays a causal role in the development of a cardial Parkinsonian motor symptom shared across all mammals – the hyperexcitable blink reflex. Previous work in our lab established that aberrant nigro-collicular interactions are responsible for blink reflex hyperexcitability in PD (Basso & Evinger, 1996; Basso *et al.*, 1996). While the rate model would predict that increased GABABergic activity from the SNr with dopamine depletion would cause this symptom, my data demonstrate that basal ganglia beta oscillations are critical in the development of this symptom. Significant correlational evidence has implicated beta oscillations in the generation of PD symptoms (Kuhn *et al.*, 2006; Kuhn *et al.*, 2009), but the few studies that have sought to establish a causal relationship with beta activity and voluntary movement disturbances have found subtle effects at best (Chen *et al.*, 2007; Pogosyan *et al.*, 2009; Syed *et al.*, 2012). This is likely due to the complicated relationship between beta activity and voluntary movement under normal conditions (Lopez-Azcarate *et al.*, 2010; Leventhal *et al.*, 2012). The study of this simple Parkinsonian reflex symptom in my experiments allowed me to establish a clear causal relationship. Furthermore, my data suggest that synchronized beta oscillations in the basal ganglia network not only affect basal ganglia-cortico behaviors like voluntary movements, but also impact involuntary behaviors. Synchronized beta oscillations could therefore be responsible for many other involuntary symptoms that occur in PD patients.

My data not only highlight the important role of beta activity in the expression of simple reflex abnormalities, but also suggest that beta oscillations disrupt normal learning and plasticity.

My study replicated a finding that dopamine depletion impaired plasticity of the blink reflex (Battaglia *et al.*, 2006), and further demonstrated that beta oscillations during the presentation of the HFS stimuli were responsible for this impairment (*Figure 21C*). I also observed long term deficits of this plasticity in 6-OHDA lesioned rats (*Figure 22*), which shows that beta oscillations provide much more than an "antikinetic" neural signal and are involved with complex tasks like learning.

Additionally, my data highlight the importance of theta oscillations (4-10 Hz) in dystonia. As with beta oscillations in PD, theta oscillations have been observed in dytonic patients (Silberstein *et al.*, 2003; Chen *et al.*, 2006), although a causal relationship between these oscillations and dystonic symptoms has not yet been estabished. My studies demonstrated that theta stimulation of the STN with 7 Hz STN DBS exaggerated blink reflex plasticity in normal rats (*Figure 24*) to a degree previously displayed by patients with the focal dystonia, blepharospasm (Quartarone *et al.*, 2006a). While the neural mechanisms underlying focal dystonias are less well understood, my data support the theory that it involves maladaptive plasticity (Quartarone *et al.*, 2006b; Quartarone *et al.*, 2008; Quartarone & Pisani, 2011).

While we do not know the exact neural mechanism for normal plasticity of the blink reflex, previously work in our lab has demonstrated that it is cerebellar dependent (Ryan *et al.*, 2014). Exaggerated synchronized basal ganglia oscillations likely disrupt normal basal ganglia-cerebellar interactions (Bostan & Strick, 2010). The exact neural circuits for these abormalities in PD and dystonia are not yet known although several possibilities exist. For example, there is a disynaptic projection from the STN to the cerebellar cortex via the pontine nucleus, as well as interactions between the striatum and cerebellum via the thalamus (Bostan & Strick, 2010). Interestingly, STN DBS restores a normal activity pattern in the cerebellum of PD patients

(Grafton *et al.*, 2006). Future studies could utilize beta or theta STN DBS to increase the strength of this oscillatatory activity in normal rodents to assess the effects on cerebellar activity and behavior.

Overall, these experiments have significant implications for our understanding of brain oscillations in Parkinson's disease and dystonia. It is clear that exaggerated basal ganglia beta oscillations are not merely an epiphenomon of dopamine loss in PD, but are actually pathological and responsible for at least some Parkinsonian symptoms (*i.e*. blink reflex). However, my data also show beta oscillations are not responsible for all PD symptoms (*i.e*. spontaneous blinking). This highlights the complexity of Parkinson's disease, as dopamine depletion clearly affects separate circuits via different neural mechanisms. This is especially important when we consider the wide range of Parkinsonian motor and nonmotor symtpoms and how antiparkinsonian treatments work on these symptoms with a varying degree of efficacy. Furthermore, these experiments present a simple technique that can be used to further test the importance of oscillations in other prominent Parkinsonian symptoms, for example bradykinesia and cognitive deficits.

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