NEUROPHYSIOLOGICAL MECHANISMS
UNDERLYING DEEP BRAIN STIMULATION FOR
THE TREATMENT OF MOVEMENT DISORDERS

by

Luka Milosevic

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Institute of Biomaterials & Biomedical Engineering

University of Toronto

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University of Toronto

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Abstract

Deep brain stimulation (DBS) is an invasive neuromodulation therapy for management of the motor symptoms of several movement disorders; however, its mechanisms are complex, varied, and remain unclear. We employed a dual-microelectrode methodology for intraoperative measurement of neuronal activity and synaptic events, in order to assess the physiological response properties of different subcortical DBS target structures to electrical stimulation at different settings. A total of 50 patients with Parkinson’s disease undergoing DBS surgery of the subthalamic nucleus (STN; n=40), globus pallidus internus (GPi; n=8), or ventral intermediate nucleus (Vim; n=2); and nine patients with essential tremor undergoing DBS surgery of the Vim (n=9) participated in the studies. We found that the STN required higher frequencies for inhibition during stimulation compared to the substantia nigra par reticulata (SNr), likely due to a higher prevalence of GABAergic terminals on SNr somas; although both structures receive
predominantly GABAergic inputs. In the Vim, a structure with predominantly glutamatergic synapses, higher (200Hz) frequencies were required for neuronal inhibition compared to the STN (100Hz) and SNr (50Hz). Additionally, unlike the STN and SNr, a transient period of neuronal excitation occurred prior to inhibition, reflective of the glutamatergic predominance. Thus, the effects of electrical stimulation are site specific and dependent on the weighted composition of inhibitory and excitatory inputs. We furthermore determined that an enhancement of inhibitory synaptic plasticity occurred in the SNr and GPi after continuous high frequency stimulation (HFS) at 100Hz; demonstrated by increased amplitudes of extracellular inhibitory field evoked potentials (fEPs). We found greater plasticity in the GPi compared to SNr, and that intraoperative administration of levodopa had a potent effect on SNr plasticity. Further, we determined that during stimulation at ≥30Hz, synaptic depression occurred in the SNr; demonstrated by rapid attenuation of successive fEPs. Clinically, we determined that lower levels of SNr plasticity were related to higher severity axial and global motor symptoms, and that neuronal inhibition in the Vim was necessary for tremor suppression. These findings expand upon the understanding of the physiological stimulation response properties of different brain structures in movement disorders patients, and may have implications for advancing DBS technologies.
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**Abbreviations**

6-OHDA: 6-hydroxydopamine;  
AC: anterior commissure  
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid  
ANOVA: analysis of variance  
ATP: adenosine-triphosphate  
CaMKII: Ca2+ calmodulin dependent kinase II  
cAMP: cyclic adenosine monophosphate  
DBS: deep brain stimulation  
ECAPs: evoked compound action potentials  
EPSP: excitatory postsynaptic potential  
fEP: focal evoked field potential  
GABA: gamma-Aminobutyric acid  
GABAARs: GABA\textsubscript{A} receptors  
GABARAP: GABA\textsubscript{A} receptor associated protein  
GPe: globus pallidus external segment  
GPi: globus pallidus internal segment  
GRIP: glutamate receptor interacting protein  
HFS: high frequency stimulation  
IF50: 50% inhibitory frequency  
IPSP: inhibitory postsynaptic potential  
LFP: local field potential  
MCP: mid-commissural point
MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MRI: magnetic resonance imaging

MSE: mean squared error

mUPDRS: motor subscore from Part III of the Unified Parkinson’s Disease Rating Scale

NMDA: N-methyl-d-aspartate

PC: posterior commissure

PKA: protein kinase A

PKC: protein kinase C

SNr: substantia nigra pars reticulata

STN: subthalamic nucleus

TMS: transcranial magnetic stimulation

UPDRS: Unified Parkinson’s Disease Rating Scale

Thalamic nuclei:

VA: ventral anterior nucleus

Vc: ventral caudal nucleus

Vim: ventral intermediate nucleus

VL: ventral lateral nucleus

VLa: anterior ventral lateral nucleus

VLp: posterior ventral lateral nucleus

Voa: ventral oral anterior nucleus

Vop: ventral oral posterior nucleus
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CHAPTER 1

1 – Introduction

1.1 – Thesis framework

1.1.1 – Problem statement and motivation

Deep brain stimulation (DBS) has emerged as a widely accepted invasive neuromodulation therapy for the management of the symptoms of various movement disorders including Parkinson’s disease (DBS of the subthalamic nucleus; STN; or globus pallidus internus; GPi), essential tremor or tremor-dominant Parkinson’s disease (DBS of the thalamic ventral intermediate nucleus; Vim), and cervical dystonia (GPi-DBS). Despite its clinical efficacy for the motor symptoms of such disorders, the underlying mechanisms of action are yet to be fully elucidated. The goal of DBS is to maximize stimulation delivery of the target neuron types with minimal activation of the non-target neuron types, using the least amount of energy possible (McIntyre & Hahn, 2010). In order to do this, a thorough understanding of exactly how electrical stimulation delivery interacts with the stimulated brain tissue is necessary. Converging evidence from neuroanatomical, electrophysiological, neurochemical, and neuroimaging studies
has revealed that the mechanisms of DBS are far more complex than originally believed. A detailed understanding of the underlying mechanisms of DBS could not only improve outcomes and reduce adverse effects, but also lead to further understanding of the disorders to which it is applied, optimize current implementations, and expand/ensure successful implementation for other pathologies. In the work presented in this thesis, we employed a unique methodology (Levy et al., 2007) for measuring neuronal activity and synaptic events in different subcortical target structures during electrical stimulation at different settings. We also investigated the relevance of patient pathology and clinical symptomology, with respect to these physiological responses.

1.1.2 – Thesis objectives and hypotheses

The main objectives of the works in this thesis were to:

**Objective 1**: Investigate the frequency-dependent responses of electrical stimulation on neuronal activity and synaptic events in two different basal ganglia surgical target structures, the STN and substantia nigra pars reticulata (SNr), in patients with Parkinson’s disease (Chapter 3).

**Background**: Previous work investigated the stimulus frequency response properties of GPi neurons in patients with various dystonic phenotypes (Liu et al., 2012). These neurons were found to be partially inhibited during stimulation at low frequencies (≤30Hz), and completely silenced during a short train of 100Hz stimulation.
Furthermore, the amplitudes extracellularly recorded inhibitory focal evoked field potentials (fEPs; analogous to inhibitory postsynaptic synaptic potentials; IPSPs) were found to initially increase and then decrease with increases in stimulation frequency. An objective of this study was to extend these findings to neurons of the STN in patients with Parkinson’s disease, the most common indication for DBS; as well as neurons of the SNr in patients with Parkinson’s disease, a recent promising DBS indication for treatment of resistant axial motor symptoms of Parkinson’s disease (Weiss et al., 2013). STN neurons have previously been shown to have an excitatory rebound burst response following a short inhibitory period after the cessation of high frequency stimulation (HFS; Filali et al, 2004). This acute response is different from the pure inhibitory response of neurons of the SNr and Gpi. Thus, we sought to measure and compare the frequency-dependent responsiveness of STN and SNr neurons during stimulation trains at various frequencies.

**Hypotheses**: (i) Since GPi and SNr share anatomical, predominantly inhibitory, afferent innervations, rapid successive activation of inhibitory inputs will produce neuronal silencing in the SNr. (ii) Since STN neurons have shown mixed inhibitory and excitatory responses following short trains of HFS, the stimulus frequency response function will be less sensitive to inhibition than that of the SNr.

**Objective 2**: Investigate the clinical relevance of the frequency-dependence of electrical stimulation by measuring neuronal activity in Vim, while concurrently measuring a robust, real-time symptomatic correlate (postural tremor), during trains of stimulation at
different frequencies in patients with essential tremor and patients with tremor-dominant Parkinson’s disease (Chapter 4).

**Background:** Benabid’s (1987) initial observations that HFS through a thalamotomy lesioning electrode (1-2mm tip) could arrest tremor without making a permanent thermo-lesion ushered in the lesion-free era of neuromodulation. Various studies have described the tremor-related (3-6Hz) behaviour of Vim neurons (ex. Lenz et al., 1988), as well as described the clinical benefits of Vim-DBS for tremor reduction (ex. Benabid et al. 1991). However, the stimulus response properties of Vim neurons during HFS have never been explored, nor any associations made about the physiological mechanistic action of HFS and how it may lead to tremor reduction. Thus, we were interested in investigating the effects on neuronal activity and on tremor, measured concurrently. We tested 100Hz and 200Hz stimulation because clinical studies have suggested that Vim-DBS produces better tremor benefit with higher programmed stimulation frequencies than typically used for STN (≥185 Hz vs ~130 Hz; Earhart et al., 2007). This project was additionally inspired by an intraoperative observation that a long train of HFS in the Vim resulted in an initial short burst of neuronal excitation followed by inhibition.

**Hypotheses:** (i) The frequency-dependent responses to electrical stimulation of the Vim, a structure with predominantly glutamatergic afferent innervation, will differ (excitatory followed by inhibitory) from those of the predominantly GABAergic structures of the basal ganglia (purely inhibitory) that have been previously studied. (ii) Higher stimulation frequencies (200Hz compared to 100Hz) will be more effective at suppressing tremor.
**Objective 3:** Investigate the pulse width-dependent effects of electrical stimulation on the two basal ganglia output structures (SNr and GPi), classify differences in synaptic plasticity between the two structures, and investigate the effects of levodopa administration on changes to synaptic plasticity in patients with Parkinson’s disease (Chapter 5).

**Background:** Prescott et al. (2009) reported a dopamine-dependent increase in inhibitory synaptic plasticity in the SNr using a standard tetanizing protocol of intermittent trains of HFS, commonly used in hippocampal slice experiments. This intermittent stimulation protocol failed to elicit changes to plasticity in the absence of exogenous dopamine. Conventional DBS therapy is not intermittent, but rather HFS is delivered continuously. Thus, the objective of this study was to investigate the effects of a continuous train of HFS; more comparable to the therapeutic stimulation delivery of DBS; on synaptic plasticity within the SNr and GPi. The findings described in Chapter 3 (Objective 1) indicate that a continuous train of HFS does indeed lead to a significant increase in inhibitory synaptic plasticity in the absence of exogenous dopamine. Therefore, in this study we sought to expand upon the findings in Chapter 3, and characterize this phenomenon by systematically studying a range of pulse widths before and after a train of continuous HFS in the presence and absence of exogenous dopamine. Additionally, we wanted to investigate similarities or differences of this response in a basal ganglia structure with similar anatomical inputs, the GPi. Lastly, due to recent promising reports of the efficacy of SNr-DBS for treatment of resistant axial motor impairment of
Parkinson’s disease (Weiss et al., 2013), an additional objective of this study was to
determine if any correlations existed between plasticity measurements in the SNr and
patients’ axial motor symptoms.

**Hypotheses:** (i) Due to a larger volume of tissue activation, longer stimulation pulse
widths will activate more presynaptic terminals. (ii) Enhancements of inhibitory synaptic
plasticity after a train of continuous HFS will be similarly modulated (similar increases
in fEP amplitudes) between the SNr and GPi, due to their similar composition of
inhibitory and excitatory afferent inputs. (iii) Exogenous levodopa will enhance the
efficacy of GABAergic transmission.

### 1.2 – Parkinson’s disease

Parkinson’s disease was first described by James Parkinson in 1817. Parkinson’s disease
is a progressive hypokinetic movement disorder which is characterized by a loss of
dopaminergic projections from the substantia nigra pars compacta (SNc) to the striatum
(more on basal ganglia anatomy and physiology in Section 1.4 – Basal ganglia). By the
time symptoms being to arise, it is estimated that 60-70% of dopaminergic neurons are
already lost (Kish et al., 1988; Lang and Lozano, 1998a). The reduced dopaminergic
input to the striatum is believed to give rise to increased neuronal firing of the inhibitory
basal ganglia output structures (Albin et al., 1989; DeLong, 1990), and to disturbed firing
patterns with increased synchronization (Levy et al., 2002; Brown, 2003).
1.2.1 – Prevalence

In North America alone, more than one million people have been diagnosed with Parkinson’s disease (Lang & Lozano, 1998a). The prevalence of Parkinson’s disease increases with age. The mean age of onset is approximately 60 years old, but 5-10% of patients are diagnosed before the age of 40 (Lang & Lozano, 1998a). Parkinson’s disease is highly prevalent, but the global prevalence varies widely and there are evident difficulties to provide exact estimates of the incidence and prevalence at the global scale. Reliable estimates are difficult, due to different methodology and the development of healthcare systems in different countries (Muangpaisan, et al, 2009). Despite variations, a recent rigorous meta-analysis of 47 international studies that include Parkinson’s disease patients of different ages and sex in different geographic locations, found that the overall prevalence is 315 per 100,000 (Pringsheim, et al, 2014). The same study found an increasing prevalence of Parkinson’s disease with age (41 in ages 40-49; 107 in ages 50-59; 173 in ages 55-64; 428 in ages 60-69; 425 in ages 65-74; 1087 in ages ≥80, per 100,000). Studies have further determined a high (~0.2%) prevalence rate of Parkinson’s disease in Canada (Pohar & Jones, 2009). This prevalence rate is similar to a previous Canadian study which found an overall crude prevalence rate of 248.9 per 100,000 for males and 239.8 per 100,000 for females (Svenson, et al, 1993).

1.2.2 – Clinical features

Parkinson’s disease is clinically diagnosed based on the presence of several cardinal motor symptoms, as there is no biological marker that can otherwise confirm its
presentation (Lang and Lozano, 1998b). These cardinal motor features include tremor (typically at rest), muscular rigidity, bradykinesia (slowness of movement), akinesia (paucity of movement), and/or postural instability (Lang and Lozano, 1998a,b; Jankovic, 2008). In addition to these cardinal symptoms, Parkinson’s disease can also present with other gait and postural symptoms including freezing of gait and festination of gait (rapid shortening of steps and quickening of gait; Jankovic, 2008), as well as certain non-motor features including dementia, executive dysfunction, hyposmia, sleep disturbances, autonomic failure, amongst others (Dirnberger et al., 2005; Hely et al., 2005; Gallagher et al., 2010). Motor symptoms typically begin unilaterally, and progress bilaterally, and both motor and non-motor symptoms worsen as the disease progresses (Foltynie et al., 2004).

Typically regarded as the most noticeable sign of Parkinson’s disease – although not always the most debilitating – is the parkinsonian rest tremor. It manifests as 4-6Hz rhythmic muscle contractions which occur when a limb is resting in a relaxed state and ceases during voluntary movements (Deuschl et al., 1998; Jankovic, 2008). Tremor prevails in the distal parts of the upper limbs, but can also involve the legs, lips, chin, and jaw. Less commonly, patients can present with a postural, and/or kinetic tremor as well. Tremor is often regarded as the symptom which is most difficult to treat in Parkinson’s disease as it may not respond well to dopamine replacement therapy (Ondo et al., 1998; Fishman, 2008). The central origin of parkinsonian tremor remains unknown, however, there is notable evidence which suggests that the pathophysiology of parkinsonian tremor
transcends the basal ganglia and additionally involves the cerebellum and thalamus (more on this in Section 1.5 – Tremor circuits: Cerebellum, basal ganglia and the motor thalamus).

Muscular rigidity in Parkinson’s disease occurs as stiffness due to an increased muscular tone at rest, as well as a resistance to passive movements of various body parts (Jankovic, 2008). It can occur proximally, involving the neck, shoulders, and hips; distally, involving the wrists and ankles; and as the disease progresses it has been shown to occur axially, causing postural deformity (Jankovic, 2008). Voluntary movement of the contralateral limb can exacerbate, or unmask latent rigidity, implying that the pathophysiology may involve a distributed brain network (Broussolle et al., 2007), and much like parkinsonian tremor, is difficult to attribute to a single locus (Baradaran et al., 2013).

Bradykinesia and akinesia in Parkinson’s disease together result in a reduction in the velocity and amplitude of the initiation and execution of voluntary movements. More specifically, bradykinesia is classified as a slowness of a performed (voluntary) movement, and in particular difficulties with execution of sequential and/or simultaneous movements, whereas in akinesia the execution of spontaneous (facial expression) or associated (arm swing during gait) movements is hindered (Berardelli et al., 2001; Mazzoni et al., 2007; Jankovic, 2008). An additional non-cardinal symptom of Parkinson’s disease that is often associated with bradykinesia and akinesia is
hypokinesia; which refers to the clinical presentation of movements being smaller than desired (as in micrographia; Evarts et al., 1981). Bradykinesia is believed to arise from a disruption of motor cortical activity, attributed to reduced dopaminergic function within the basal ganglia (Jankovic, 2008). As such, it appears to correlate with striatal dopamine deficiency (Vingerhoets et al., 1997), as well as with pathological synchronization within the basal ganglia (Kühn, 2009).

Postural instability in Parkinson’s disease is associated with a flexed posture and leads to impaired balance. This symptom represents one of the most debilitating and detrimental symptoms of Parkinson’s disease as it is associated with increased falls and loss of independence (Jankovic, 2008; Kim et al., 2013). In addition to this, it is one of the more pharmacologically difficult to treat symptoms, as levodopa replacement therapy has proven to be only marginally effective (Marsden, 1994), and in a subset of patients has even been shown to accentuate this symptom (Bronte-Stewart, 2002). Likewise, stimulation of the STN (more on this in Section 1.6 – Deep brain stimulation) also only has marginal therapeutic effect on axial stiffness and on the velocity and frequency of postural swaying, while worsening sway amplitude (Maurer et al., 2003). As such, recent studies have placed new focus on a potential complementary stimulation target within the basal ganglia – the substantia nigra pars reticulata (SNr; Chastan et al., 2009; Weiss et al., 2011a,b, 2013) – in an attempt to address the difficulties in treating axial motor symptoms, as the SNr has been implicated in the (patho-)physiology of both postural muscle tone and locomotion (Takakusaki et al., 2003).
1.2.3 – Dopamine replacement therapy: Levodopa

When enough dopaminergic SNC neurons degenerate, Parkinson’s disease symptoms begin to appear (Bezard & Gross, 1998). Levodopa is an oral dopamine precursor and is currently the most effective drug therapy in the management of Parkinson’s disease symptoms (Lang & Lozano, 1998a). However, patients who undergo long-term levodopa treatment often develop motor fluctuations, where the effects of levodopa switch rapidly and unpredictably from no effect, to positive effect, to uncontrollable involuntary movements. These involuntary movements are known as levodopa-induced dyskinesias, which manifest as hyperkinetic movements resembling chorea and dystonia. Medication-induced motor fluctuations are difficult to treat and become a large contributor to disability in patients (Schrag & Quinn, 2000). Thus, patients who develop levodopa-induced dyskinesias become candidates for DBS therapy.

Studies of rat corticostriatal slices have suggested that levodopa-induced dyskinesias may be the result of alterations to synaptic plasticity at the dopamine-receiving basal ganglia input (Picconi et al., 2003; Picconi et al., 2008). These studies demonstrated that long-term potentiation, induced by HFS, is absent in dopamine lesioned (6-hydroxydopamine; 6-OHDA) rats, but can be restored with chronic levodopa treatment. Furthermore, Morgante et al. (2006) demonstrated that paired-associative stimulation (a single pulse of transcranial magnetic stimulation (TMS) of the motor cortical hand representation followed by a single pulse of median nerve stimulation 21.5ms later; capable of
producing long-term potentiation (LTP) -like changes in the sensorimotor system in humans; Ziemann et al., 2004) increased motor evoked potential amplitudes in healthy controls, but not in patients with Parkinson’s disease off medication. However, levodopa administration restored the potentiation of motor evoked potential amplitudes in non-dyskinetic patients, but not dyskinetic patients. Taken together, these studies suggest that impaired synaptic plasticity may play a role in the pathophysiology of Parkinson’s disease, and levodopa therapy may act to restore these impairments.

1.3 – Essential tremor

1.3.1 – Prevalence

There is a general agreement among scholars that essential tremor is the most prevalent neurological movement disorder (Findley, 2000; Sullivan, et al., 2004; Elble & Deuschl, 2011; Clark & Louis, 2018). Early studies of the prevalence ranged widely (Louis et al., 1998), due to inconsistent methodologies and standardized classifications of the disorder (Findley, 2000). More recent meta-analytical studies of the prevalence of essential tremor based on data from 28 population-based studies in 19 countries found that the prevalence of essential tremor in the general population (all ages) was 0.9%, while among persons aged 65 years and older was 4.6% (Louis and Ferreira, 2010). Most research would indeed agree that the prevalence increases substantially with age (Rajput et al., 1984; Benito-León et al., 2005; Deuschl et al., 2011). Approximately 50% of all patients have a family history of essential tremor (Whaley et al., 2007), often inherited in a mendelian-
dominant fashion (Lorenz et al., 2004). However, to date, no specific gene has been found to be associated with the disorder (Deng et al., 2007).

1.3.2 – Clinical features and treatment

Tremor is defined as an involuntary, rhythmic, oscillatory movement of a body part produced by alternating or simultaneous contraction of agonist and antagonist muscles (Bhatia et al., 2018). In at least 95% of essential tremor patients, tremor affects the upper limbs (Whaley et al., 2007; Elble and Deuschl, 2009). It is also common for essential tremor to affect the head (≥34%), lower limbs (~30%), tongue (~30%), voice (≥12%), face/jaw (~7%), and trunk (~5%). An important indication of essential tremor, is that it occurs during posture and movement in any/all affected body parts. While rest tremor may also develop in advanced cases, this may be an indication of Parkinson’s disease comorbidity (Djaldetti et al., 2008), or it is often mistaken for a postural tremor that occurs due to incomplete muscle relaxation (Elble and Deuschl, 2009; Rajput et al., 2004). Patients with advanced essential tremor also often present with intention tremor (a crescendo hand tremor upon approach of the intended target), which is indistinguishable from the intention tremor caused by lesions of deep cerebellar nuclei. An indication that essential tremor may be associated with cerebellar pathology (more on this in Section 1.5 – Tremor circuits: Cerebellum, basal ganglia and the motor thalamus), is that some patients present with impaired tandem walking (Stolze et al., 2001). Although essential tremor is classically believed not to be associated with any other abnormal nonmotor
signs, recent studies have indicated potential associations with deficits in frontal
cognitive function (Higginson et al., 2008).

While many patients experience substantial improvements to their tremor in response to
ethanol/alcohol (Growdon et al., 1975; Koller et al., 1984), propranolol and primidone
are the two most effective medications for managing essential tremor, however, only
about 50% of patients experience benefit from one or both drugs (Zesiewicz et al., 2005).
Moreover, treatment with these medications often only suppresses tremor by 50% and is
largely limited to hand tremor. Surgical treatment (lesioning or DBS of the ventral
intermediate nucleus of the thalamus) is the current most effective treatment but is often
only reserved for severe drug-resistant tremor (Elble and Deuschl, 2009).

1.4 – Basal ganglia

1.4.1 – Overview

The basal ganglia are a group of subcortical nuclei which play a major role in the control
of voluntary movements as well as other functions such as procedural learning, routine
behaviors, eye movements, cognition, and emotion (Alexander et al., 1991). The
principle nuclei of the basal ganglia include the striatum (consisting of the caudate and
putamen), globus pallidus (internus; GPi, and externus; GPe), subthalamic nucleus
(STN), and substantia nigra (pars compacta; SNc, and pars reticulata; SNr). With respect
to voluntary movements, unlike most other structures of the motor system (ex. motor
cortical areas, cerebellum, red nucleus), the basal nuclei do not have direct input or output connections with the spinal cord. In the basal ganglia motor circuit, the striatum (input tier of the basal ganglia), receives excitatory cortical afferents from the primary motor cortex, somatosensory cortex, premotor cortex, and supplementary motor cortex (Yeterian and Van Hoesen, 1979). The striatum also receives inputs from dopaminergic neurons of the SNC, and ventral tegmental area. These projections contain two types of dopamine receptors; D1 receptors, which cause depolarization of striatal medium spiny neurons, and D2 receptors, which hyperpolarize them (Sealfon and Olanow, 2000). The primary target of the inhibitory basal ganglia output nuclei (the SNr and GPi), via ventral lateral and ventral anterior nuclei (VA/VL complex) of the thalamus (Schell and Strick, 1984), is the supplementary motor cortex (Utter and Basso, 2008). Other projections of the output structures include the pedunculopontine nucleus, superior colliculus, reticular formation, and lateral habenula.

1.4.2 – Rate model

The rate model (Fig. 1.1) was introduced by Albin et al. (1989) and Delong (1990) after key advances in the knowledge of the functional organization of the basal ganglia nuclei, in both healthy and diseased animal models. The rate model implies that cortical information received by the striatum is transmitted to the output nuclei (SNr and GPi) either via a “direct” or “indirect” pathway (Alexander and Crutcher, 1990).

The striatum projects directly to the output nuclei in the direct pathway of the rate model. The striatal projection is inhibitory (GABAergic) and reduces the activity of the SNr and
Figure 1.1 – Functional connectivity of basal ganglia nuclei. The direct pathway: inhibitory projections from the striatum disinhibit tonically active inhibitory neurons of the SNr and GPi, which project in turn to the motor thalamus (VA/VL complex; or Voa/Vop). The indirect pathway: transiently active inhibitory projections from the striatum project to the tonically active inhibitory neurons of the GPe, consequently disinhibiting the STN. The STN in turn projects to the SNr and GPi, transiently opposing the disinhibition of the direct pathway. The SNc upregulates the direct pathway via excitatory D1 receptor mediated projections, and downregulates the indirect pathway via D2 receptor mediated projections. Figure adapted from Smith et al. (1998). (GPe = globus pallidus externus; GPi = globus pallidus internus; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus). Excitatory cortico-subthalamic hyperdirect pathway (Nambu et al., 2000) omitted from figure.
GPI. By nature, these two structures provide tonic inhibition of premotor centers, thus the inhibitory striatal projection leads to disinhibition of premotor centers, allowing for volitional drive of movements. In the indirect pathway, the striatum sends inhibitory projections to the GPe, which disinhibits the STN and in turn, the STN sends excitatory (glutamatergic) projections to the SNr and GPI. These excitatory projections allow the SNr and GPI to transiently inhibit projections to premotor centers. Thus, the indirect pathway antagonizes the activity of the direct pathway, suppressing the volitional drive of movements. It should be noted that the STN also sends excitatory projections back to the GPe. Activation of the direct and indirect pathways are counteractive; in both their projections to the target regions (inhibitory vs excitatory), as well as function (facilitation vs suppression of movements; Smith et al., 1998).

The two striatal output pathways are modulated by dopaminergic projections from the SNc. Striatal projections of the direct pathway have D1 dopamine receptors which facilitate transmission, while projections of the indirect pathway have D2 receptors which reduce transmission. Therefore, the dopaminergic regulation of basal ganglia function favours the facilitation of movements initiated in the cortex via disinhibition. In the pathological case of Parkinson’s disease, due to the depletion of dopaminergic neurons in the SNc, there is a lack of up-regulation of the direct pathway and down-regulation of the indirect pathway. Consequently, the over-activated indirect pathway (and under-activated direct pathway) gives reason as to why Parkinson’s disease is
classified as a hypokinetic disorder which suppresses motor activity. The rate model provided the justification of therapies and surgical approaches for treatments of Parkinson’s disease, including dopamine replacement therapy, surgical lesioning, and deep brain stimulation (Smith et al, 1998).

1.4.3 – Oscillation model

Neural oscillation (i.e. neuronal or subthreshold membrane oscillation) is a fundamental feature of the brain, which allows populations of neurons from different brain regions to become linked despite the limited interconnections between regions (Buzsáki & Draguhn, 2004). The neural binding hypothesis states that diverse neural information can be integrated, or paired, through synchronized neuronal oscillations to allow for flexibility and nuanced response to a variety of context-dependent stimuli (Senkowski et al., 2008). The basal ganglia oscillation model assumes that the pathophysiology of Parkinson’s disease consists of both an increase in firing rate of neurons as proposed in the rate model, as well as a change in the pattern of synchronization of discharges between neurons (Brown, 2003). This synchronized activity produces oscillations in local field potentials (LFPs). LFPs are generated by action potentials, as well as in large part by the cumulative pre- and postsynaptic potentials related to the synaptic activity of neurons (Buzsáki et al., 2012). Notable frequency bands of oscillations include theta (4-7Hz), alpha (8-12Hz), beta (13-30Hz), and gamma (30-100Hz) (Brown, 2006). While oscillation can be physiologically beneficial for integration of information (as in the binding hypothesis), pathologically synchronized activity may result in the loss of
neuronal selectivity, or less efficient rate coding of information (Bevan, et al, 2002), which is otherwise transmitted/processed in the absence of synchronized oscillation.

In particular, the parkinsonian oscillation model assumes that oscillations in the beta frequency band are considered antikinetic, whereas gamma oscillations are prokinetic. These two rhythms are antagonistic of each other and consequently, an increased tendency of synchronized oscillation in the beta frequency range within the basal ganglia has been found to be associated with Parkinson’s disease (Brown, 2003; Kühn et al., 2006). LFP recording studies have demonstrated that beta synchrony in the STN is associated with an akinetic-rigid state in Parkinson’s disease patients (Hammond et al., 2007; Kühn, et al, 2009) and parkinsonian animal models (Costa et al., 2006; Mallet et al., 2008a, 2008b). Furthermore, desynchronization of the beta frequency is observed following levodopa administration (Brown et al., 2001; Levy et al., 2002), during motor preparation and execution (Kühn et al., 2009; Yugeta, et al., 2013), and during high frequency stimulation with DBS (Kühn et al., 2008); supporting its antikinetic nature.

Additionally, studies have found low-frequency (4-10Hz) LFP activity to be associated with dystonic symptoms (in the GPi; Tang et al., 2007), and levodopa-induced dyskinesia (in the STN; Alonso-Frech et al., 2006).

1.5 – Tremor circuits: Cerebellum, basal ganglia, and motor thalamus
1.5.1 – Functional anatomy

The pathophysiological generation of tremor in both Parkinson’s disease and essential tremor remains in question. Research suggests that tremor is associated with dysfunction of the motor circuits of the basal ganglia, the cerebellum, as well as the neurotransmitter systems within these networks (Helmich et al., 2013). The basal ganglia and cerebellum each have projections to the motor thalamus, but to separate thalamic subnuclei. The GPi sends inhibitory (GABAergic) projections to the anterior ventral lateral thalamus (VLa), while the cerebellar output nuclei send excitatory (glutamatergic) projections to the posterior ventral lateral nucleus (VLp); by the nomenclature of Hirai and Jones (1989). However, the Schaltenbrand and Wahren (1977) human stereotactic atlas utilizes Hassler’s (1959) nomenclature, and due to its widespread use in DBS literature (Krack et al., 2002) the pallidal receiving nucleus (VLa) will henceforth be referred to as the ventral oral anterior and posterior nuclei (Voa and Vop), and the cerebellar receiving nucleus will be referred to as the ventral intermediate nucleus (Vim). Due to their functionally opposite projections (excitatory cerebellar projections to the Vim vs inhibitory pallidal projections to the Voa/Vop), the cerebellar output nuclei facilitate motor cortical activity (Horne & Butler, 1995), while the GPi inhibits it (Mink, 1996). The cerebellar output nuclei also send a second, inhibitory projection to the inferior olive, which sends excitatory projections back to the cerebellar climbing fibers and deep cerebellar nuclei. Finally, Bostan et al. (2010) have shown that the STN has anatomical projections to the cerebellar cortex, via the pons, which link the circuits of the basal ganglia and cerebellum.
1.5.2 – Parkinsonian tremor models

In Parkinson’s disease, the dopaminergic denervation of the striatum and subsequent disrupted processing of basal ganglia pathways explains hypokinetic symptoms, but not tremor. Rather, rest tremor has been attributed to interactions of the basal ganglia with the cerebello-thalamo-cortical circuit. As such, studies have reported the presence of a pathological tremor-related oscillation frequency between 4 and 6Hz, which has been observed in the pallidum (Hutchison et al., 1997; Raz et al., 2000), STN (Hutchison et al., 1998) and Vim (Lenz et al., 1994).

Early work in parkinsonian 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; a neurotoxin which destroys dopaminergic neurons of the substantia nigra, causing parkinsonian symptoms) monkeys by Wichmann et al. (1994) gave rise to a thalamo-centric model of tremor genesis. The work suggested that abnormal hyperpolarizing 12-15Hz oscillations of pallidal output were converted into 4-6Hz tremor oscillations within the thalamus by intrinsic membrane hysteresis, which gave rise to tremor. However, a pallido-centric theory was later developed, termed the dimmer-switch hypothesis (Helmich et al., 2011), which was rationalized by the subsequently discovered existence of tremor-related activity within the human basal ganglia of in patients with Parkinson’s disease. This theory suggests rather that Parkinson’s disease tremor is initiated in the basal ganglia (GPi; switch), and its amplitude is modulated by the cerebello–thalamo–cortical network (the dimmer). However, this theory fails to explain why the Vim (rather
than the Voa/Vop) is the optimal target for surgical interventions, or why it is found to be more synchronously coherent with tremor (Magnin, et al., 2000; Atkinson, et al., 2002; Timmermann et al, 2002). Thus, it was postulated that the pathological tremor-related signals from the GPi might interact with the cerebello-thalamo-cortical network via the motor cortex (Hoover et al., 1999), through the pallido-thalamo-cortical network projections.

1.5.3 – Essential tremor models

Essential tremor is regarded as a disorder of the cerebellum, most commonly attributed to a GABAergic channelopathy (Kralic et al., 2005; Boecker et al., 2010; Paris-Robidas et al., 2012). Reduced inhibitory tone of Purkinje cells causes increased disinhibition of the deep cerebellar neurons, which subsequently facilitate the activity of the cerebello-thalamo-cortical network. Like the early thalamo-centric theories of parkinsonian tremor genesis, it was hypothesized that the generation of tremor-related synchronization within the thalamus was a result of unique ion channel dynamics (Jahnsen and Llinás 1984a; Jahnsen and Llinás 1984b; Llinás, 1988). It was postulated that movement-related activation of nucleo-olivary cells caused Purkinje cells to synchronously inhibit deep cerebellar nuclei which generated oscillatory rebound potentials (via T-type Ca$^{2+}$ currents) that made their way through the cerebello-thalamo-cortical network. Indeed, the pathophysiology of essential tremor has been assumed to be attributed to disrupted transmission through the cerebello-thalamo-cortical pathway due to abnormal cerebellar afferent input from areas such as the inferior olive (Pinto et al., 2002). Furthermore,
studies have revealed distinct tremor-related LFP clusters (Pedrosa et al., 2012) and single-unit tremor-related activity (Lenz et al. 1988) within the in Vim.

1.6 – Deep brain stimulation

1.6.1 – Overview

DBS was introduced as a reversible alternative to surgical ablation/lesioning of brain tissue. DBS uses battery-operated, surgically-implanted electrodes to deliver electrical stimulation (typically continuous stimulation at high frequencies) to target structures within the specific area of the brain implicated in the relevant pathology. The electrode lead is connected with an extension wire to a programmable pulse generator that is implanted below the clavicle. The stimulation parameters are programmed using non-invasive radio-telemetry (Lemaire, et al, 2007). In the early era of DBS, systematic exploration of stimulation parameters and their various clinical effects was carried out, and included examining the effects of stimulation intensity, frequency, and pulse-width (Rizzone et al., 2001; Moro et al., 2002). However, the direct physiological effects of varying stimulation parameters have not been elucidated.

An implementation of DBS was first adapted therapeutically as a means to treat intractable tremor in Parkinson’s disease (Benabid et al., 1987) by delivering continuous high-frequency stimulation (HFS) to the Vim; as an alternative to surgical lesioning. Since then, it has rapidly surpassed lesioning, and become a widely adapted surgical
option for treating the motor symptoms of various movement disorders (Lozano et al., 2017); typically, these would include STN- or GPi-DBS for Parkinson’s disease (Benabid et al., 1994; Limousin et al., 1995; Kumar et al., 1998, 2000), Vim-DBS for parkinsonian tremor or essential tremor (Benabid et al., 1991, 1993, 1996; Nguyen and Degos, 1993; Deiber et al., 1993), and GPi-DBS for dystonia (Kumar et al., 1999; Coubes et al., 2004; Hung et al., 2007). Its application is rapidly expending beyond these and is currently under consideration for various off-label uses including treatment of Tourette’s syndrome (Maciunas et al., 2007), epilepsy (Hodaie et al., 2002), chronic pain (Bittar et al., 2005; Rasche et al., 2006), obsessive-compulsive disorder (Lipsman et al., 2007), major depressive disorder (Mayberg et al., 2005; Hardesty and Sackeim, 2007; Schlaepfer et al., 2008), Alzheimer’s disease (Lozano, 2016), amongst others.

Early hypotheses suggested that the mechanism of action of DBS was shared with that of lesioning (i.e. mimicking ablation), as it was found to mimic the effects of beneficial lesions (Bergman et al., 1990; Aziz et al., 1991; Heywood and Gill, 1997) as well physiological inactivation by injections of muscimol (GABA agonist) and lidocaine (Wichmann et al., 1994; Levy et al., 2001). Despite – and perhaps because of – its history of success for the treatment of Parkinson’s and essential tremor, its clinical application has preceded a sound understanding of its fundamental mechanisms of action which underly its therapeutic benefit. In addition, it is not precisely known how varying stimulation parameters of DBS impacts physiologically upon the target structures being stimulated.
1.6.2 – Proposed mechanisms of action

One hypothesis which is in line with the early hypotheses of neuronal inactivation is depolarization blockade. Beurrier et al. (2001) demonstrated a six-minute long blockade of action potential generation in the STN of rat brain slice preparations, which outlasted a one-minute train of HFS. They hypothesized that HFS in fact led a to blockage of voltage-sensitive Na\(^+\) channels, and that the effect was non-synaptic. Their conclusions came from the observations that the HFS-induced inhibition was persistent in the presence of blockers of ionotopic GABA and glutamate receptors. However, their study was limited by a lack of assessment of synaptic function during HFS. Subsequently, Magarinos-Ascone et al. (2002) demonstrated that sustained HFS of the STN in rat brain slices led to an initial depolarization, followed by bursting, followed by complete inhibition. Although they did not investigate the postsynaptic mechanisms underlying the silencing, they hypothesized based on the findings of Beurrier et al. (2001) that the mechanism “probably” involved gradual inactivation of Na\(^+\)- and Ca\(^{2+}\)-mediated voltage-gated conductances, which underlie intrinsic rhythmic discharge of STN neurons. Shin et al. (2007) demonstrated that in the entopeduncular nucleus (homolog of the human GPi) of rat brain slices, elevated K\(^+\) concentrations depressed neuronal activity, which is in line with the findings in hippocampal rat brain slices by Bikson et al., (2001) that HFS results in increased extracellular K\(^+\) concentrations (perhaps partially mediated by Glial cells), which depolarize the membrane sufficiently enough to inactivate voltage-gated Na\(^+\) channels.
Indeed, DBS-glial interaction has also been considered as a mechanism. Tawfik et al. (2010) demonstrated that HFS leads to release of both glutamate and adenosine within thalamic ferret brain slices; thus, they postulate that astrocytic neurotransmitter release may be an important mechanism of DBS, which works by moderating thalamic glutamate release (Cunha, 2005). Accordingly, Bekar et al. (2008) found that in thalamic rat brain slices, DBS led to increased concentrations of extracellular ATP and adenosine, as well as release of glutamate. They postulated that the effect of astrocytic activation was a suppression of glutamatergic excitatory drive in the thalamus during HFS, which they hypothesize is necessary for suppression of tremor.

Another leading hypothesis for the mechanism of DBS is that HFS-induced neurotransmitter release may cause “jamming” of pathophysiological activity, either by way of normalization of otherwise pathological firing rate/pattern, or by desynchronization of pathological oscillations (Montgomery and Baker, 2000; Kang and Lowery, 2013). As such, it has been demonstrated that STN-DBS suppresses beta activity during (Eusebio et al., 2011) and immediately after cessation of HFS (Wingeier et al., 2006), and that this suppression/desynchronization is correlated with improvements in rigidity and bradykinesia, but not tremor (Kühn et al., 2008). Furthermore, it has been demonstrated that enhanced coherence in the beta rhythm between the subthalamic nucleus and cortex correlates with parkinsonian symptoms and is reduced by HFS (Devos et al., 2004); and also, that HFS leads to a disruption of the
enhanced phase-amplitude coupling interaction between the STN and primary motor cortex (de Hemptinne et al., 2015).

The theory of synaptic modulation suggests that DBS activates (as opposed to early hypotheses of ablation) stimulated synapses as well as fibers of passage within the vicinity of the DBS electrode (Chiken and Nambu, 2014). Synaptically, the theory depends on the composition of inhibitory and excitatory inputs of stimulated structures. In DBS surgical patients, Dostrovsky et al. (2000) have demonstrated that brief microstimulation of a GPi neuron causes a poststimulation period of inhibition that corresponds to that of a typical GABAergic IPSP. In the STN of rat slice preparations, Lee et al. (2004) demonstrated that HFS elicited either IPSPs or EPSPs, and corresponding inhibition or excitation or neuronal firing, and that both types of postsynaptic potentials could be blocked by bath application of glutamate and GABA antagonists. This bimodal behaviour is reflective of the mixed GABAergic and glutamatergic afferent innervation of the STN (Kita et al., 1983; Fujimoto et al., 1993).

The synaptic modulation theory however also suggests that DBS may activate efferent projections of stimulated target structures. As such, Maurice et al. (2003) have demonstrated excitation of SNr neurons in response to STN stimulation, likely due to the activation of glutamatergic subthalamonigral projections. Indeed, computational modelling studies suggest that that DBS may cause inhibition of somatic activity and a concurrent increase in axonal firing (McIntyre et al., 2004a, b).
Lastly, a hypothesis of particular relevance to this thesis is synaptic depression. While the synaptic modulation hypothesis suggests that electrical stimulation causes neurotransmitter release, synaptic depression implies that presynaptic terminals are unable to sustain the high rate of neurotransmitter release provoked by HFS (Zucker and Regehr, 2002). The reason for this is that presynaptic terminals contain a readily releasable pool of neurotransmitter vesicles. When consecutive stimuli are delivered at a rate faster than the rate of replenishment of this pool, fewer vesicles are released by each successive stimulus, eventually depleting the pool (Rosenmund and Stevens 1996).

Iremonger et al. (2006) found a lack of sustained depolarization/neuronal excitation in response to HFS of glutamatergic thalamocortical axons projecting to the primary motor cortex in rat brain slices. They postulated that HFS caused a “functional deafferentation” by way of neurotransmitter depletion. Furthermore, Cabin et al. (2002) found a deficiency of docked vesicles and impaired synaptic glutamate release in response to HFS in hippocampal slices of α-synuclein knockout mice. α-Synuclein enables neurotransmitter release at presynaptic terminals, and also happens to be implicated in the etiology and pathogenesis of Parkinson’s disease (Stefanis, 2012).

1.6.3 – Future directions

Despite clinical benefits, current DBS systems still possess inefficiencies. DBS settings are manually adjusted and the programming is time consuming for clinicians and patients. Furthermore, open-loop strategies utilize continuous, fixed stimulation settings and as a result, do not control parkinsonian motor fluctuations or rapid symptom
variations (Priori, et al, 2013). A study by Graupe et al. (2010) has demonstrated that often more than 50% of continuously delivered stimulation is unnecessary in essential tremor (due to an absence of symptoms), and that the stimulation could have been switched off. Hence, recent research has been focused on the development of closed-loop DBS systems, which would stimulate only when necessary. The major hinderance to the development of such systems is selection of a robust feedback parameter which can reliably characterize symptoms, which are known to fluctuate on a moment by moment basis based on cognitive and motor loads, as well as concurrent drug therapy (Little and Brown, 2013). If a suitable feedback parameter can be selected, it might be possible to improve therapeutic efficacy while preserving battery life and limiting side effects (Modolo et al., 2012; Priori, et al, 2013). An implementation of a brain computer interface-controlled closed-loop system has been experimentally tested, using local filed potentials in the beta frequency to turn stimulation on or off, with promising results (Little et al., 2013). However, the reliability of beta frequency oscillations as a robust control parameter is still in question (Arlotti et al., 2016). Beyond the development of novel stimulation paradigms, technological advancements in lead design (Deeb et al., 2016) are increasing the spatial resolution of stimulation delivery and allowing for current steering (Pollo et al., 2014) and dual-target stimulation (Weiss et al., 2011).

1.7 – Synaptic plasticity

1.7.1 – Glutamatergic plasticity
Long-term potentiation (LTP) is described as a sustained increase in synaptic strength following high-frequency activation; causing a long-lasting increase in synaptic transmission between two neurons (Cooke and Bliss, 2006). LTP was first observed in the hippocampus of anesthetized rabbits by Terje Lømo in 1966, and later characterized by Bliss and Lømo (1973). The original characterization, termed long-lasting potentiation, was that excitatory postsynaptic synaptic potentials (EPSPs) in the dentate gyrus of the hippocampus elicited by single-pulse stimulation could be enhanced for a long period of time after delivery of HFS to the presynaptic perforant pathway fibers. Subsequent single-pulse stimuli produced enhanced and prolonged EPSPs in the postsynaptic cell population. LTP at glutamatergic synapses implies activation of Ca\(^{2+}\) (and Na\(^{+}\)) -permissive NMDA receptors (NMDARs), which are innately inactive due to a Mg\(^{2+}\) block (Nicoll and Malenka, 1999). With enough glutamate-mediated depolarization of the postsynaptic membrane via AMPA receptors, the Mg\(^{2+}\) block is expelled, allowing entry of Ca\(^{2+}\) and Na\(^{+}\) ions, which depolarize the postsynaptic membrane further. In addition, Ca\(^{2+}\) ions act as second messengers to activate protein kinases (such as Ca\(^{2+}\) calmodulin dependent protein kinase II; CaMKII) which trigger a signalling cascade to cause insertion of new AMPA receptors into the postsynaptic membrane. These changes to the postsynaptic membrane occur rapidly and can last for hours to days. The result of these changes is an increased postsynaptic sensitivity to glutamate (Malenka and Bear, 2004); i.e. a stimulus of equal strength (prior to these changes) results in a larger/enhanced response. In addition to these postsynaptic changes to receptor function and expression, postsynaptic Ca\(^{2+}\) can facilitate the release of neurotransmitter from the presynaptic terminal via retrograde signals, such as nitric oxide (O’dell et al., 1991).
Much like LTP, long-term depression (LTD) research has primarily been done in the CA1 region of the hippocampal dentate gyrus; in addition to cerebellar Purkinje neurons. While LTP serves to enhance transmission between two neurons, LTD is a mechanism by which the signal transmission is reduced; or, the synaptic strength between two neurons is weakened. While LTP requires brief HFS, NMDA receptor dependent LTD is induced by longer trains of low frequency stimulation (0.5-5Hz, ≥900 stimulation pulses; Malenka and Bear, 2004). Both LTP and LTD are dependent on NMDA receptor activation (Cummings et al., 1996), however their actions are opposite. It is purported that in LTD, distinct NMDA receptor subpopulations contribute to LTP or LTD (Hrabetova et al., 2000). In LTD, Ca\(^{2+}\) activates protein phosphatases, which dephosphorylate certain protein kinases (such as protein kinase A; PKA; and protein kinase C; PKC), resulting either in reduction of AMPA receptor channel opening (Banke et al., 2000), or rapid internalization of AMPA receptors (Malinow and Malenka, 2002); thereby reducing postsynaptic sensitivity to glutamate.

**1.7.2 – GABAergic plasticity**

While much less is known about the mechanisms of GABAergic plasticity compared to glutamatergic, there has been substantial progress in this field of research. GABAergic plasticity has been attributed to such things as changes to presynaptic GABA release, or changes to postsynaptic GABA\(_A\) receptor expression and/or sensitivity (Castillo et al., 2011). One mechanism of presynaptic GABAergic modification is associated with
changes in the number of functionally active release sites (Caillard et al., 1999). This study performed in CA3 hippocampal rat slices demonstrated that GABAergic LTD was expressed as a decrease in the probability of GABA release, which was dependent on synergistic activation of both postsynaptic GABA<sub>A</sub> and NMDA receptors. Gaiarsa et al. (2002) postulate that a presynaptic mechanism of GABAergic plasticity may involve switching between an on or off state of functionally active neurotransmitter release, since GABA<sub>A</sub> receptor clusters are often found in the presence of presynaptically silent GABAergic synapses (Kannenberg et al., 1999). Postsynaptically, changes in GABAergic signal transmission (like glutamatergic plasticity) have been shown to be largely dependent on changes in postsynaptic intracellular Ca<sup>2+</sup>; and subsequent signalling cascades (Gaiarsa et al., 2002). Once such study has demonstrated that in CA1 hippocampal adult guinea pig slices, HFS induces an NMDA-dependent LTP of glutamatergic signal transmission, and a concurrent decrease in GABAergic signal transduction; demonstrating shared Ca<sup>2+</sup> signalling pathways between these forms of plasticity (Wang and Stelzer, 1996). Another postsynaptic mechanism of GABAergic plasticity involves changes to the number of available GABA receptor on the postsynaptic membrane. A study by Nusser et al. (1998) demonstrated that the functional strength of GABAergic synapses is proportional to the number of GABA<sub>A</sub> receptors on the postsynaptic membrane. Accordingly, Marsden et al. (2007) demonstrated in rat slices that activation of NMDA receptors (and subsequent Ca<sup>2+</sup> signalling cascades), that induces excitatory LTD through AMPA endocytosis, simultaneously increases the expression of GABA<sub>A</sub> receptors at the dendritic surface of hippocampal neurons. Indeed, Kittler et al. (2000) have suggested that GABA<sub>A</sub> receptors cycle between the synaptic
membrane and intracellular sites. In Chapters 3 (SNr) and 5 (SNr and GPi) of this thesis, we have demonstrated an enhancement of GABAergic signal transmission following a train of continuous HFS. This enhancement is demonstrated by larger amplitude extracellular fEPs and longer durations of neuronal inhibition (occurring concurrently; elicited by single pulses of stimulation) post-HFS compared to pre-HFS. Mechanisms for the enhancement of GABAergic plasticity are discussed in greater detail in Chapter 5.

1.7.3 – Short-term plasticity

Unlike long-term plasticity, short-term acts on a much shorter time scale, typically on the order of hundreds to thousands of milliseconds; but at most up to several minutes (Zucker and Regehr, 2000). However, short-term plasticity can also act to either strengthen or weaken synaptic signal transmission. Short-term plasticity can work fundamentally in two ways; through synaptic depression, or synaptic enhancement (which can be in the form of facilitation, augmentation, or post-tetanic potentiation). Synaptic depression (also termed synaptic fatigue) is most often attributed to a decrease in neurotransmitter release (Fioravante and Regehr, 2011). This reduced release can be a result of decreased presynaptic Ca\(^{2+}\) (which is a requirement for vesicle fusion; Forsythe et al., 1998; Xu and Wu, 2005; Neher and Sakaba, 2008), neurotransmitter vesicle depletion, and/or inactivation of release sites. When repeated stimuli are delivered at short time intervals, subsequent stimuli delivered before vesicle replenishment will release fewer vesicles, eventually depleting the “readily releasable” pool (Zucker, 1989; Rosenmund and Stevens, 1996). Indeed, rat brain slice studies have shown that synaptic
depression increases with the frequency of activation in cerebellar synapses (Dittman and Regehr, 1998) and cortico-thalamic synapses (Ran et al., 2009). Inactivation of release sites has been attributed to inhibition of subsequent vesicle fusion as a result of the time it takes to clear vesicular membrane proteins from the release site, which get incorporated into the plasma membrane upon vesicle fusion (Neher and Sakaba, 2008). The various forms of synaptic enhancement are most often attributed to the effects of a residual elevation in presynaptic Ca$^{2+}$, which triggers the release of transmitters. Synaptic facilitation (alternatively known as paired pulse facilitation) occurs at the onset of repeated stimulation and is expressed as a short-lived enhancement of synaptic function, believed to occur by increased presynaptic Ca$^{2+}$ (Katz and Miledi, 1968). The mechanism for facilitation is believed to involve residual Ca$^{2+}$ that persists in the presynaptic terminal following the initial synaptic activation (Zucker and Regehr, 2000). Post-tetanic potentiation is described as a presynaptic form of synaptic enhancement that occurs after sustained high-frequency synaptic activation (Schlapfer et al. 1976; Baxter et al. 1985), which becomes longer lasting when the stimulus frequency and duration are increased (Fioravante and Regehr, 2011). Post-tetanic potentiation is believed to work by increasing the probability of neurotransmitter release either as a result of an increase in Ca$^{2+}$ influx (Habets and Borst, 2006), and/or by a PKC-dependent increase in the Ca$^{2+}$ sensitivity of vesicle fusion (Korogod et al., 2007). Augmentation is often difficult to differentiate from post-tetanic potentiation (including its dependence on Ca$^{2+}$), and in fact, can occur simultaneously. However, augmentation occurs and decays with a time constant of ~5–10 s, whereas post-tetanic potentiation lasts for ~30s to several minutes (Thomson, 2000; Zucker and Regehr, 2000).
CHAPTER 2

2 – General methods

Outlined in this Chapter are the general methods used throughout this thesis. Detailed methods pertaining to the specific experiments are described in each of the subsequent Chapters (3-5).

2.1 – Patients and consent

A total of 50 patients with Parkinson’s disease undergoing DBS surgery of the STN (n=40), GPi (n=8), or Vim (n=2); and nine patients with essential tremor undergoing DBS surgery of the Vim (n=9) participated in the studies (described in detail in Chapters 3-5). Electrophysiological data were obtained from DBS target locations (and surrounding structures; such as SNr) during the intraoperative neurophysiological mapping/targeting procedure, which is used to localize the target location prior to the final (unilateral or bilateral) implantation of the DBS macroelectrode, or thermo-lesion being made. Clinical assessments of patients were performed pre- and post-operatively by a movement disorder specialist at the Toronto Western Hospital; specifically, the
Unified Parkinson’s disease rating scale (UPDRS) was used to assess patients with Parkinson’s disease (Chapters 3 and 5). Assessments of tremor were done offline in Spike 2 (Cambridge Electronic Design, Cambridge, UK), by the author, using tremor amplitude and frequency of accelerometry data obtained intraoperatively from patients undergoing Vim-DBS surgery for the treatment of essential tremor, or the tremor component of Parkinson’s disease (Chapter 4). All of the conducted experiments conformed to the guidelines set by the Tri-Council Policy on Ethical Conduct for Research Involving Humans and were approved by the University Health Network Research Ethics Board. Furthermore, all of the patients in this study provided written, informed consent prior to taking part in the study.

2.2 – DBS surgical procedure and microelectrode recording setup

2.2.1 – Surgical procedure

Stereotactic coordinates of the anterior commissure (AC) and posterior commissure (PC) were determined using a T1-T2 fusion MRI (Signa, 1.5T or 3T, General Electric, Milwaukee, USA) on a surgical neuronavigation workstation (StealthStation, Medtronic, Minneapolis, USA), in reference to the coordinates of the stereotactic frame (Leksell G, Elekta Inc., Atlanta, USA) affixed to the patient’s head. The tentative target position of the DBS macroelectrode is determined by direct visualization of the target structure, or indirect targeting by estimation of the location of the STN (x=12mm lateral, y=3mm posterior to the midcommissural point, z=3mm inferior to AC-PC), GPi (x=20mm lateral, y= midcommissural point, z=5mm inferior to AC-PC), or Vim (x=14.5mm
lateral, y=6mm anterior to PC, z=midcommissural point). Under local anesthetic, an incision is made to the scalp and Burr holes are drilled into the patient’s skull. The microelectrode headstage assembly (which holds the microelectrodes used for recording) is placed on the stereotactic frame arch and the microelectrodes (via a guidetube) are guided through a DBS cannula to 10mm above the planned target location. Two independently-driven microelectrodes are then advanced from 10mm above to 5mm below the planned target, and the target nuclei are delineated by their characteristic electrophysiological firing rates, patterns, and responses to microstimulation (described in Section 2.3 – Neurophysiological targeting). Once the target location is confirmed, the microelectrode recording assembly is removed, and the DBS macroelectrode is implanted.

2.2.2 – Microelectrode recording and stimulation setup

Two microelectrodes are each backloaded into a dual-barrel stainless steel intracranial guidetube (Levy et al., 2007); allowing for a ~600μm medial-lateral separation (Fig 2.1A). The intracranial guidetube is affixed to the microelectrode headstage assembly that gets placed on the stereotactic frame arch (with the guidetube being inserted into the standard DBS cannula) when it is time to perform the electrophysiological targeting procedure. The microelectrodes (FHC Inc., Bowdoine, USA) are made of tungsten and insulated with Parylene-C, with an uninsulated tip of ~15-25μm and an uninsulated back end onto which recording wires are attached. The microelectrodes share a common earth ground, and are referenced with respect to the intracranial guidetube (each having a
Figure 2.1 – Dual-microelectrode recording setup and headstage assembly. (A) The two independently driven microelectrodes (~600µm medial-lateral separation) are advanced through a dual-barrel stainless steel intracranial guidetube. (B) The intracranial guidetube and micro-drives (to which the microelectrodes are attached) are affixed to the headstage apparatus, which gets placed on the stereotactic frame arch when it is time to perform the electrophysiological mapping procedure. The microelectrode recording wires (red) and reference wires (black) are connected to the recording amplifier (monopolar recording configuration) and share a common (green).
assembly). The microelectrode tips are electro-plated inhouse with platinum (YSI 3140 Platinizing solution, YSI Inc., Yellow Springs, USA) and gold (Gold electroplating solution 24- karat yellow, Krohn Technical Products, Carlstadt, USA) in order to achieve an optimal impedance of ~0.2-0.5MΩ.

Open filter recordings (5-3,000Hz) were amplified 5,000 times and sampled at 12.5kHz using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, USA). The recordings were digitized using a CED 1401 (Cambridge Electronic Design, Cambridge, UK) data acquisition system, and monitored online using Spike2 (Cambridge Electronic Design, Cambridge, UK) software. Microstimulation for electrophysiological targeting as well as for experimental protocols is delivered using an isolated constant-current Neuro-Amp1A (Axon Instruments, Union City, USA) stimulator with biphasic pulses (cathodal followed by anodal). Detailed descriptions of microstimulation protocols and parameters pertaining to each study are described in each of the subsequent Chapters (3-5), in their respective Methods subsections.

2.3 – Neurophysiological targeting

Techniques for electrophysiological identification of STN and SNr (Hutchison et al., 1997), GPi (Hutchison et al., 1994), and thalamic sub-nuclei (Lenz et al., 1988) have been previously described; however, targeting techniques are summarized below.
2.3.1 – STN (and SNr) trajectories

A sagittal section (12mm lateral to the midline) of the STN and SNr can be seen in Fig. 3.1A. Trajectories targeting STN typically start with units of the thalamic reticular nucleus (slow, burst firing). After a quiescent period, entry into the STN is confirmed based on an increase in background noise, and recording of units with firing rates of approximately 20-40Hz, irregular firing patterns, periods of beta activity, and/or periods of tremor-related activity, as well as movement-related responses. Microstimulation-induced motor contractions can be observed if the trajectory is too far anterior, by stimulation of the posterior region of the anterior limb of the internal capsule; whereas microstimulation-induced paresthesia indicates that the trajectory is too far posterior, through stimulation of fibers of the medial lemniscus. After 4-6mm advancement though the STN, decreases in spike incidence signify exit from the ventral border of the STN. Entry into the SNr is characterized by lower amplitude units with fast rates (80-100Hz) and regular firing patterns, as well as brief periods of inhibition following a short-train of low-amplitude HFS (5µA, 200Hz, 0.5s).

2.3.2 – GPi trajectories

A sagittal section (20mm lateral to the midline) of the GPi can be seen in Fig. 5.1C. GPi trajectories typically start within the GPi, and are confirmed based on presence of irregular, high frequency discharge (HFD; 50-90Hz) neurons with responsiveness to movements, as well as border cells with highly regular (20-60Hz) firing rates. GPi HFD units (but not border cells), like SNr units, are also briefly inhibited following a short-
train of low-amplitude HFS (5µA, 200Hz, 0.5s). After 10-12mm advancement through the GPi, decreases in spike incidence signify exit from the ventral border, and the optic tract is confirmed based on visually evoked potentials elicited by brief flashes of light in the visual field, as well as patient reports of microstimulation-induced phosphenes in the contralateral visual hemifield.

2.3.3 – Vim trajectories

A sagittal section (14.5mm lateral to the midline) of the thalamic sub-nuclei with a typical microelectrode recording trajectory can be seen in Fig. 4.1B. Single units are tested for responses to passive and active movements of the wrist, elbow, and shoulder (Molnar et al., 2005). Units with movement related responsiveness are considered movement-related cells of the motor thalamus (Vop/Vim). Units of the Vop (basal-ganglia-receiving neurons) respond to internally-generated movements, whereas units of the Vim (cerebellar-receiving neurons) respond to passive, externally-generated movements but not voluntary movements (Ohye et al., 1989; Lenz et al., 1990). Units with tremor-related activity (synchronous bursting at 4-6Hz) are present in the Vop, but more commonly in the Vim. Vim recording sites are also confirmed by the presence of beta oscillatory activity in the absence of tremor, which is not otherwise found in surrounding structures (Basha et al., 2014). Microstimulation (100µA, 200Hz, 1-5s) is performed every 1 mm along the trajectory to coarsely delineate Vim from Vop based on stimulation-induced tremor reduction or tremor arrest. Low-threshold microstimulation-induced (<50µA, 200Hz, 0.5s) paraesthesia is considered to be in the vicinity of the
anterior border of the ventral caudal nucleus (Vc), as well as neuronal responsiveness to light touch; whereas microstimulation resulting in high-threshold (>50µA, 200Hz, 0.5s) hemi-body paresthesia is indicative of pre-lemniscal radiations.
CHAPTER 3

3 – Neuronal inhibition and synaptic plasticity of basal ganglia neurons in Parkinson's disease

The material presented in this chapter has been published in the article:


NOTE: The content of this chapter is identical to the material presented in the publication except for the text formatting which was done according to University of Toronto requirements.
Deep brain stimulation of the subthalamic nucleus is an effective treatment for Parkinson’s disease symptoms. The therapeutic benefits of deep brain stimulation are frequency-dependent, but the underlying physiological mechanisms remain unclear. In order to advance deep brain stimulation therapy an understanding of fundamental mechanisms is critical. The objectives of this study were to (i) compare the frequency-dependent effects on cell firing in subthalamic nucleus and substantia nigra pars reticulata, (ii) quantify frequency-dependent effects on short-term plasticity in substantia nigra pars reticulata, and (iii) investigate effects of continuous long-train high frequency stimulation (comparable to conventional deep brain stimulation) on synaptic plasticity.

Two closely spaced (600um) microelectrodes were advanced into the subthalamic nucleus (n=27) and substantia nigra pars reticulata (n=14) of 22 patients undergoing deep brain stimulation surgery for Parkinson’s disease. Cell firing and evoked field potentials were recorded with one microelectrode during stimulation trains from the adjacent microelectrode across a range of frequencies (1-100Hz, 100uA, 0.3ms, 50-60 pulses).

Subthalamic firing attenuated with ≥20Hz (P<0.01) stimulation (silenced at 100Hz), while substantia nigra pars reticulata decreased with ≥3Hz (P<0.05) (silenced at 50Hz). Substantia nigra pars reticulata also exhibited a more prominent increase in transient silent period following stimulation. Patients with longer silent periods after 100Hz stimulation in the subthalamic nucleus tended to have better clinical outcome after DBS.

At ≥30Hz the first evoked field potential of the stimulation train in substantia nigra pars reticulata was potentiated (P<0.05), however, the average amplitude of the subsequent potentials was rapidly attenuated (P<0.01). This is suggestive of synaptic facilitation.
followed by rapid depression. Paired pulse ratios calculated at the beginning of the train revealed that 20Hz (P<0.05) was the minimum frequency required to induce synaptic depression. Lastly, the average amplitude of evoked field potentials during 1Hz pulses showed significant inhibitory synaptic potentiation after long-train high frequency stimulation (P<0.001) and these increases were coupled with increased durations of neuronal inhibition (P<0.01). The subthalamic nucleus exhibited a higher frequency threshold for stimulation-induced inhibition than the substantia nigra pars reticulata likely due to differing ratios of GABA:glutamate terminals on the soma and/or the nature of their GABAergic inputs (pallidal vs striatal). We suggest that enhancement of inhibitory synaptic plasticity, and frequency-dependent potentiation and depression are putative mechanisms of deep brain stimulation. Furthermore, we foresee that future closed-loop deep brain stimulation systems (with more frequent OFF stimulation periods) may benefit from inhibitory synaptic potentiation that occurs after high frequency stimulation.

**Keywords:** clinical neurophysiology; deep brain stimulation; neurosurgery; Parkinson’s disease; subthalamic nucleus
3.2 – Introduction

Parkinson’s disease is a hypokinetic movement disorder characterized by a loss of dopaminergic projections from the substantia nigra pars compacta (SNc) to the input of the basal ganglia: striatum. The prevailing model of Parkinson’s disease suggests that the downstream effect of this dopaminergic denervation is a loss of inhibitory tone on the subthalamic nucleus (STN), due to excessive inhibition of neurons in the globus pallidus externus (GPe) by the overactive striatal indirect pathway neurons. The loss of inhibition subsequently leads to hyperactivity of the STN (Albin et al. 1989; DeLong 1990) which, in turn, contributes to increased neuronal firing of inhibitory basal ganglia outputs: the globus pallidus internus (GPi) and substantia nigra pars reticulata (SNr). This is accompanied by disrupted firing patterns and increased synchronization, and these changes are ultimately believed to be the cause of Parkinson’s disease symptoms (Albin et al., 1989; DeLong, 1990; Levy et al., 2002; Brown, 2003). It is believed that decreasing the hyperactivity of the STN should alleviate Parkinson’s disease motor symptoms. However, recent optogenetic findings in mice have challenged the canonical model (that the direct and indirect pathways are selectively active), suggesting that these pathways may be concurrently active (during movements) or inactive (at rest) (Cui et al., 2013). The lack of consensus (Kravitz et al., 2010) suggests that further research is required to better understand the neurocircuitry and treatment of Parkinson’s disease.

Nevertheless, deep brain stimulation (DBS) has been widely adapted as a conventional treatment method of Parkinson’s disease. DBS delivers continuous high frequency
stimulation (HFS; ~130Hz) to target structures through chronic indwelling electrodes (Benabid et al. 1987; Starr et al. 1998; Perlmutter and Mink 2006; Wichmann and DeLong 2006). DBS of the STN mimics the effect of beneficial lesions (Bergman et al. 1990; Aziz et al. 1991; Heywood and Gill 1997) or inactivation by injections of muscimol (GABA agonist) and lidocaine, suggesting that DBS may inhibit STN activity (Wichmann et al. 1994; Levy et al. 2001). Thus, STN-DBS has proven to be remarkably efficacious as a symptomatic treatment modality for Parkinson’s disease (Benabid et al. 1994; Limousin et al. 1995; Kumar et al. 1998; Kleiner-Fisman et al., 2006; Perlmutter and Mink, 2006). Also, there have been recent promising reports on the efficacy of SNr-DBS for gait (Chastan et al., 2009; Weiss et al., 2011b; Weiss et al., 2013), and combined STN-/SNr-DBS for improvements in freezing of gait (Weiss et al., 2011a), although little is known about the effects of electrical stimulation on responses of neurons and synaptic events in the SNr in human. Furthermore, despite the known frequency-dependence of STN DBS on therapeutic outcome (Moro et al., 2002; Timmermann et al., 2004), the effects of different stimulation frequencies on neurophysiology within STN and SNr are yet to be elucidated (di Biase et al., 2016). The objectives of this study were to (i) compare the effects of stimulation over a range of frequencies on cell firing in STN and SNr, (ii) quantify the frequency-dependent effects on short-term plasticity (facilitation and depression) in SNr, and (iii) investigate the effects of continuous, long-train high-frequency stimulation (HFS; 100Hz, 0.3ms, 10s) on short-term plasticity.
Studies from our group have shown that focal microstimulation leads to inhibition of neuronal firing and it has been suggested that GABA release at afferent terminals may be involved in the mechanism of action (Dostrovsky et al. 2000; Wu et al. 2001; Filali et al. 2004; Lafreniere-Roula et al. 2010; Liu et al. 2012). Other studies have suggested that DBS may work by exciting efferent fiber outputs, as well as fibers of passage (Hashimoto et al., 2003; Bar-Gad et al., 2004; Johnson and McIntyre 2008; Xu et al. 2008), and/or by activating the cortex through antidromic firing of the cortico-STN hyperdirect pathway (Gradinaru et al., 2009; Kuriakose et al., 2010; Walker et al., 2012). The downstream excitatory effects may lead to a more regular pattern of firing from STN efferent fibers, which would reduce and/or replace the irregular, pathological neuronal activity (Benazzouz and Hallett, 1999; Garcia et al., 2005; Kringelbach et al., 2007).

In addition to the disturbed firing patterns within the circuitry, corticostriatal slice work has suggested that abnormal involuntary movements, such as dyskinesias, are the result of alterations in synaptic plasticity (Picconi et al., 2003; Picconi et al., 2008; Calabresi et al., 2016). These studies have shown that long term potentiation in the corticostriatal synapse can be induced with HFS, and reversed with low frequency stimulation in healthy adult Wistar rats. A study from our group (Prescott et al., 2009) demonstrated that synaptic plasticity in the SNr is enhanced with L-dopa treatment after four short-trains of HFS, implicating a lack of plasticity in the pathophysiology of Parkinson’s disease. In the current study we investigated the effects of continuous, long-train HFS
(comparable to conventional DBS) on short- and long-term synaptic plasticity in the SNr in the absence of exogenous dopamine in Parkinson’s disease patients.

3.3 – Methods and Materials

3.3.1 – Patients

STN and SNr recording sites were investigated during microelectrode-guided placement of DBS electrodes in a total of 22 patients with Parkinson’s disease, after overnight withdrawal of medication. For all patients we also determined the motor subscore from Part III of the Unified Parkinson’s Disease Rating Scale (mUPDRS) in 12hr OFF drug state with DBS ON and OFF to assess degree of symptomatic improvement with DBS. All of the conducted experiments conformed to the guidelines set by the Tri-Council Policy on Ethical Conduct for Research Involving Humans and were approved by the University Health Network Research Ethics Board. Furthermore, all of the patients in this study provided written, informed consent prior to taking part in the study.

3.3.2 – Microelectrode recording procedure

Techniques used for electrophysiological identification of the STN during stereotactic and functional neurosurgery have been previously published (Hutchison et al., 1998). Briefly, stereotactic coordinates of the anterior comissure and posterior comissure were determined using MRI (Signa, 3T, General Electric, Milwaukee, WI) and used to estimate the location of the STN based on the 12.0mm sagittal section of the
Schaltenbrand and Wahren’s standard atlas (Schaltenbrand and Wahren, 1977) (Fig. 3.1A). Direct visualization of the target and the trajectory of approach was carried out with commercial planning software on T1-T2 fused images (Stealth Workstation, Medtronic). Two microelectrodes were advanced in the dorsoventral direction beginning 10mm above the planned target. Recordings from both electrodes typically began with activity from reticular thalamic cells (slow bursting firing pattern). After a section of no activity, entry into the STN was confirmed based on cell firing rates of approximately 20-40Hz, irregular firing patterns with periods of beta activity, and responsiveness to movements (Hutchison et al., 1998). After 4-6mm advancement, decreases in spike incidence signified exit from the ventral border of the STN and entry into the SNr was characterized by fast (80-100Hz), regular firing patterns. The tentative target for the lowest contact of the DBS macroelectrode was at the ventral border of the STN.

### 3.3.3 – Data acquisition

Two independently driven microelectrodes (25μm tip lengths, 600μm apart, 0.2-0.4MΩ impedances, at 12.5kHz), which share a common ground on a stainless-steel intracranial guidetube were used for recordings and microstimulation (Fig. 3.1B). The open filter recordings (5-3,000Hz) were amplified 5,000 times using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, CA), digitized using a CED 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK), and monitored using Spike2 software (Cambridge Electronic Design). Microstimulation was done using an
Figure 3.1 – Experimental recording location, stimulation protocol, and an example of a neuronal recording in human STN. A: A representative microelectrode track of the STN and SNr, in which recordings and experimental protocols were executed. B: Our custom dual-microelectrode recording assembly with ~600µm mediolateral spacing. Upon locating a well isolated spike on one electrode, the adjacent microelectrode was used to deliver stimulation. C: Timeline of the stimulation protocol for frequency-dependent studies of neuronal firing, silent period, and short-term plasticity. All stimulation protocols used 100uA and 0.3ms biphasic pulse widths. Note that only one block of 10s is shown for 1, 2, 3, and 5Hz stimulation trains. In fact, each of these stimulation trains was delivered for 10s. Intervals between stimulation trains were ~5-10s. D: A template matched spike (top trace) from a raw recording (bottom trace) obtained in the STN of showing inhibition of neuronal firing during 20, 30, 50, and 100Hz stimulation trains, and a prolonged silent period after 100Hz.
isolated constant-current stimulator (Neuro-Amp1A, Axon Instruments) with symmetric, 0.3ms biphasic pulses (cathodal followed by anodal).

### 3.3.4 – Stimulation Protocols

Upon locating a well-isolated single unit (spike) within the STN or SNr, data were collected for a 20-30s OFF-stimulation baseline firing rate measurement with one microelectrode. The adjacent microelectrode (600μm away in the mediolateral direction) was used to deliver stimulation trains. For investigating frequency-dependent responses of neuronal firing (inhibition during the stimulation train) and silent periods (the time between the last stimulus pulse in the stimulation train to the time of occurrence of the first spike) in both STN (recording sites, n=27; number of patients, nₚ=16) and SNr (n=14; nₚ=9) neurons, we delivered stimulation trains separated by 5-10s at increasing frequencies (1, 2, 3, 5, 10, 20, 30, 50, and 100Hz, 100µA, 0.3ms biphasic pulse width, for a total of 10, 20, and 30 pulses per train for the first three stimulation frequencies respectively, and 50–60 pulses per train for the remainder; see Fig. 3.1C). All stimulation was done at 100µA and 0.3ms biphasic pulse width. The same SNr recording sites (n=14; nₚ=9) were used for investigating the frequency-dependent effects on short-term plasticity in SNr. We chose the ascending series for the stimulation protocol to avoid hysteresis of the evoked field potential (fEP) potentiation induced by higher frequency stimulation trains (Liu et al., 2012). We have shown that 20, 30, 50, or 100Hz stimulation are capable of potentiating the fEP, thus, if the stimulation frequencies were randomized and the series began with any of those frequencies, plasticity would be
induced from the beginning, and would obscure our objective of determining the frequency threshold. To further justify privileging the ascending series, we performed a descending series (i.e. starting at 100Hz) in the SNr with a separate patient (Supplementary Fig. 3.1), and as predicted, it considerably obscured the incremental, frequency-dependent increases in the first-fEP amplitude (hysteresis).

In order to further investigate the effect of fEP potentiation on neuronal inhibition at SNr recording sites, a separate protocol was used in a subset of patients (n=12, n_p=8): HFS-induced synaptic plasticity. Upon locating a well-isolated spike, 1Hz stimulation (100uA, 10s) “test pulses” were delivered to measure the baseline amplitude of the fEP, followed by “long-train” HFS (100Hz, 100uA, 0.3ms, 10s), followed by another set of post-HFS test pulses. Inhibitory synaptic plasticity was quantified using fEP peak amplitudes, and the duration of neuronal inhibition after each stimulation pulse.

3.3.5 – Offline analysis of neuronal activity

Data were not included for analyses if the monitored unit was lost or dropped below a 2:1 signal to noise ratio before completion of the stimulation protocol (two units were lost during the HFS-induced synaptic plasticity protocol). For measurement of firing rates, stimulus artifacts were removed from the signal starting at the onset of the stimulation pulse, to its end. The removed area was substituted with an equivalent period of neural data immediately prior to the stimulus artifact. Single-units were discriminated using the wavemark template matching tool in Spike2. Since each STN and SNr cell had a unique
baseline firing rate, the firing rates throughout the stimulation trains were normalized and represented as a percentage value of a randomly selected 10s pre-stimulation baseline. The silent period was measured offline in Spike2 as the duration in time from the last stimulation pulse in the train, to the return of the first spike. Amplitudes of the fEP were measured as peak voltage deflections from the pre-stimulus baseline. For the frequency-dependent fEP amplitudes during the stimulation train (see Fig. 3.5A), the average-, first-, and last-fEP amplitude were normalized with respect to the “baseline” fEP amplitude measured during the 1Hz stimulation train. Furthermore, paired pulse ratios were measured using the first two fEPs in each stimulus train, where the interstimulus interval was taken as the inverse of the stimulation frequency. Lastly, for the HFS-induced inhibitory synaptic plasticity protocol, the post-HFS potentiation was quantified as the average fEP peak amplitude percentage increase, as well as the associated percentage increase in silent period following each stimulation test-pulse.

3.3.6 – Statistical Analysis

Stimulation frequency-dependent effects on neuronal firing rate and silent period at increasing frequencies were analyzed using split-plot ANOVA (repeated measures ANOVA with between subject factor) to compare STN (n=27) and SNr (n=14) recording sites. Additionally, one-way repeated measures ANOVA were used to analyze the overall stimulation frequency-dependent effects on each nucleus individually with respect to “baseline”. For silent periods, the baseline was taken as the silent period measurement following the 1Hz stimulation train. To analyze the stimulation frequency-dependent
effects on the average fEP during stimulation trains at SNr recording sites (n=14), one-way ANOVA was used. Two-way repeated measures ANOVA with within subject factor was used to compare the amplitude of the first- and last-fEP during stimulation trains. One-way ANOVA was used to analyze each of those measurements individually. One-way ANOVA was also additionally used to analyze the paired pulse ratios. Significant results on the ANOVA tests were followed up with post-hoc multiple comparisons with a Bonferroni correction. Finally, for the HFS-induced synaptic potentiation protocol at SNr recordings sites (n=12), t-tests were used to analyze the post-HFS fEP amplitude and average silent period increases during 1Hz test-pulses.

3.4 – Results

3.4.1 – Distinct frequency-dependent effects on firing rate of STN and SNr neurons

Fig. 3.2A demonstrates that in both STN (n=27) and SNr (n=14) neurons, the firing rate decreased as the stimulation frequency was increased. Repeated measures ANOVA post hoc pairwise comparison t-tests for STN [F(9,234)=51.866, MSE=2.805, P<0.001] revealed that the firing rate significantly decreased from baseline at a stimulation frequency of 20Hz (P<0.01), and even further at frequencies above 20Hz (P<0.001). For SNr [F(9,117)=145.428, MSE=2.302, P<0.001], the firing rates decreased from baseline already at 3Hz (P<0.05), 5Hz (P<0.01), and at all frequencies above 5Hz (P<0.001). In STN, neuronal firing rates were silenced at 100Hz, while in SNr they were silenced at 50Hz. Analyses revealed a significant main effect of the nucleus [F(1,39)=603.092, MSE=139.874, P<0.001] and frequency [F(9,351)=110.386, MSE=4.562, P<0.001].
Moreover, the interaction of frequency and nucleus was significant [F(9,351)=9.317, MSE=0.385, P<0.001], suggesting that stimulation frequency induced dissimilar responses in the firing rate between the two anatomical structures, with SNr exhibiting a more sensitive response. The STN log stimulus response function was a negative hyperbolic curve, whereas the SNR was sigmoid.

3.4.2 – Frequency-dependent effects on post-stimulation silent period of STN and SNr neurons

Fig. 3.2B shows that the average time of the transient silent period following stimulation trains at different frequencies in STN (n=27) and SNr (n=14) neurons was only prolonged after high frequencies. After the 100Hz stimulation train, the silent period (mean ± standard error) in STN was 211.6 ± 28.23ms (baseline: 54.85 ± 9.910ms), and 483.8 ± 138.8ms (baseline: 48.75 ± 4.450ms) in SNr. Repeated measures ANOVA post-hoc pairwise comparison t-tests for STN [F(8,200)=7.346, MSE=0.074, P<0.001] indicated that the silent period only prolonged significantly from baseline after 100Hz (P<0.001) stimulation. However, for SNr, despite a significant main effect of frequency [F(8,200)=7.346, MSE=0.074, P<0.001] and a greater increase in the mean value, the post-hoc t-test results showed a non-significant difference from baseline at all frequencies, presumably due to higher variability in values at higher frequencies (as suggested by larger standard errors for frequencies above 10Hz). Regardless, our analyses revealed a significant main effect of nucleus [F(1,38)=4.449, MSE=0.426,
Figure 3.2 – Frequency-dependent responses of average firing rates and silent periods of STN and SNr neurons. A: The firing rates decreased as the stimulation frequency was increased in both STN and SNr neurons. SNr neurons appeared to have stronger inhibitory response to stimulation frequency and were silenced at 50Hz in most cases, while STN neurons were only silenced with 100Hz stimulation. STN neuronal firing rates differed significantly from pre-stimulation baseline (dashed line) at 20Hz or greater, while SNr neurons began to differ from baseline at 3Hz. B: The silent period is the time between the last pulse of a stimulation train and the return of the first spike after cessation of stimulation. In both STN and SNr neurons, the silent period was not modulated with lower frequencies of stimulation. At frequencies of 20Hz and greater, the silent period began to increase, having a seemingly larger response in SNr neurons, however with a higher variability. With 100Hz stimulation, the silent period increased to 211.6 ± 28.23ms in STN (baseline: 54.85 ± 9.910ms), and 483.8 ± 138.8ms (baseline: 48.75 ± 4.450ms) in SNr. *(P<0.05), **(P<0.01), †(P<0.001).
P<0.05], frequency [F(8,304)=19.537, MSE=.356, P<0.001], and the interaction of these two factors [F(9,22)=5.303, MSE=0.361, P<0.01].

3.4.3 – HFS-induced synaptic plasticity in the SNr: Effects on single neurons

In the SNr, single pulse stimulation (and higher frequency stimulation trains) produced robust, positive-going, short latency “evoked field potentials” (fEPs) immediately after each stimulation pulse (STN recording sites very rarely exhibited this response and therefore the analysis of STN was limited to firing rates). Fig. 3.3A shows a sample raw microelectrode recording of the firing of an individual SNr neuron and the fEP immediately following a single 1Hz test pulse, before and after potentiation with HFS (100Hz, 100uA, 0.3ms, 10s). At all (n=12) recording sites, HFS produced a very robust response. The average amplitude of the fEP, and the average time delay in return of firing of the spike during 1Hz test pulses (100uA, 0.3ms, 10s) increased after HFS. The post-HFS fEP amplitude increased by a factor of 1.72 (P<0.001), while the time delay between the stimulation pulse and first spike increased by 1.88 (P<0.01) (Fig. 3.3B). The silent period increased from 46.45 ± 4.650ms to 89.79 ± 21.11ms (mean ± standard error; P<0.05; Fig. 3.4). All 12 recording sites in SNr showed the same, highly reproducible effect on fEP amplitude and inhibition of firing.
**Figure 3.3 – HFS-induced inhibitory synaptic plasticity at SNr recording sites.** Stimulation with 1Hz (100μA, 100μA, 0.3ms, 10s) test-pulses revealed an inhibitory evoked field potential (fEP). 

- **A:** A raw trace (5000x gain) of the fEP peak amplitude and transient inhibition of neuronal firing during a single pre-, and a single post-HFS test pulse.
- **B:** After HFS (100Hz, 100μA, 0.3ms, 10s), both the fEP and the transient silent period were increased significantly. The fEP increased by an average factor of 1.72 (P<0.001), while the silent period increased by factor of 1.88 (P<0.01) from baseline. ***(P<0.01), †(P<0.001).**

**Figure 3.4 – HFS-induced increase in SNr silent period during 1Hz test pulses.** The figure shows the 10th and 90th percentiles, first and third quartiles, and median of the silent period during 1Hz test pulses pre- and post-HFS. The mean silent period increased from 46.45 ± 4.65ms (mean ± standard error) to 89.79 ± 21.11ms after HFS. **(P<0.05).
3.4.4 – Frequency-dependent effects on fEP amplitude in SNr neurons: Short term plasticity

When stimulating at ≥30Hz, the fEP demonstrated an initial increase in amplitude (a potentiation caused by the previous stimulation train), followed by a rapid attenuation (depression) throughout the rest of the train (see Fig. 3.5A). Fig. 3.5B shows that the average-fEP closely followed the curve of the amplitude of the last-fEP. Post-hoc pairwise t-tests showed that the average amplitude of the fEP throughout the stimulation train was significantly attenuated at 30Hz (P<0.05) and frequencies above 30Hz (P<0.001) (Fig. 3.5B), and while the amplitude of the last-fEP also showed significant attenuation at ≥30Hz (P<0.001), the amplitude of the first-fEP was potentiated at 30Hz (P<0.01), 50Hz (P<0.05), and 100Hz (P<0.01). Furthermore, the potentiation and depression of the first- and last-fEPs respectively appeared to have ceiling/floor effects at 50Hz. There were significant main effects of frequency on the average-fEP [F(8,96)=48.989, MSE=1.360, P<0.001], and on both the first-fEP [F(8,96)=14.314, MSE=0.730, P<0.001] and last-fEP [F(8,96)=40.973, MSE=1.796, P<0.001] within stimulation trains, as well as a significant main effect of fEP timing (first-/last-) [F(1,96)=131.761, MSE=13.786, P<0.001], and a significant interaction of the fEP timing and stimulation frequency [F(8,96)=64.112, MSE=2.369, P<0.001].

Furthermore, the paired pulse ratio curve (Fig. 3.6), shows that there was significant attenuation of the synaptic response at interstimulus intervals of 50 (P<0.05), 33 (P<0.001), 20 (P<0.001), and 10ms (P<0.001), indicating that 20Hz was the minimum
Figure 3.5 – Frequency-dependent responses (short-term plasticity) of the average, first-, and last-fEP during stimulation trains at SNr recording sites. A: Sample raw traces from the same patient showing the first three and last three post-stimulus fEPs induced during a 30Hz stimulation train and the first and last five fEPs induced during a 50Hz stimulation train. The dashed line represents the “baseline” fEP amplitude during the 1Hz stimulation train, which remained constant. B: Frequency-dependent responses in first-, last-, and average fEP amplitudes during stimulation trains. As the frequency of stimulation was increased, there was a rapid attenuation of the amplitude of the average-fEP. However, the first-fEP within each train became potentiated as the stimulation frequency increased. The inhibitory potentiation (facilitation) effect was induced by the stimulus delivered by the previous stimulation train. As the facilitation increased, the synaptic depression effect correspondingly increased as well, demonstrated conjointly by the attenuation of average and last-fEP amplitudes. These phenomena are believed to be, in part, modulated by depletion of releasable stores of GABA within presynaptic terminals. *(P<0.05), **(P<0.01), †(P<0.001).
stimulation frequency required to induce paired pulse depression. There was a significant main effect of interstimulus interval [F(9, 108) = 108.51, MSE=1.781, P<0.001].

**Figure 3.6 – Paired pulse depression of the fEP in SNr.** The data was obtained by measuring the paired pulse ratio between the first two pulses in each of the stimulation trains delivered. The interstimulus interval is the inverse of the stimulation frequency. The paired pulse ratio significantly differed from baseline at interstimulus intervals of 50, 33, 20, and 10ms. This tells us that 20Hz stimulation (50ms interstimulus interval) was the minimum frequency required to induce a depression of the synaptic response. Data for intervals greater than 200ms was not significant and excluded from the figure. *P<0.05, †P<0.001.


3.4.5 – Clinical correlations

In order to determine the clinical significance of the findings we compared the frequency at which the firing rate in STN was reduced by 50%, (“50% inhibitory frequency”, IF50) for each patient, to the degree of improvement in patient’s mUPDRS scores (Fig. 3.7A). Although our sample size was small, we found a trend for patients that had lower IF50 values in STN (i.e. more sensitive to stimulation frequency) to be those that obtained greater clinical improvement from DBS ($R^2=0.15$, $P=0.13$). Furthermore, we correlated the silent period of STN neurons after 100Hz to the degree of improvement in mUPDRS after DBS (Fig. 3.7B) and similarly found a trend for longer inhibition to be associated with better improvement with DBS ($R^2=0.20$, $P<0.1$). However, we failed to find any correlation between the baseline firing of STN neurons and the clinical improvement after DBS ($R^2=0.02$, $P=0.61$), or pre-DBS mUPDRS scores ($R^2=0.01$, $P=0.71$).

3.5 – Discussion

One of the most controversial aspects of DBS that is yet to be elucidated is the mechanism of action of electrical stimulation and its effects on the physiology/pathophysiology of the target structures (Kringelbach et al., 2010). It is unlikely that DBS works by one single mechanism, and our study suggests that at least three mechanisms are involved:
Figure 3.7 – Clinical correlations with inhibition of neuronal activity in STN. The difference in pre-post OFF drug mUPDRS subscores (depicting clinical improvement) was correlated with the silent period after the 100Hz stimulation train (A), and the frequency at which the firing rate in STN was reduced by 50% (IF50) (B) for each patient. A: we found a trend for patients with longer STN silent period values after 100Hz stimulation to be those that obtained greater clinical improvement from DBS ($R^2=0.20$, $P<0.1$). B: Furthermore, those with lower IF50 values (i.e. more sensitive to stimulation frequency) appeared to be associated with better clinical improvement with DBS ($R^2=0.15$, $P=0.13$). The easier it was to inhibit STN neuronal firing rates, and the longer they were inhibited for after HFS, the better the clinical improvement was for patients.
(1) *Inhibition of activity of target neurons:* electrical stimulation led to release of neurotransmitters from the pre-synaptic terminals of afferent projections, which cause hyperpolarization of neuronal cell bodies due to the high prevalence of GABAergic terminals in STN and SNr.

(2) *Inhibitory synaptic potentiation occurs after HFS:* we found that the amplitude of the inhibitory fEP and the associated duration of neuronal inhibition were increased after continuous HFS (during which synaptic depletion occurs).

(3) *Continuous HFS causes synaptic depletion:* this phenomenon can occur because HFS does not allow the synapse enough time to recover after release of its “releasable stores” of neurotransmitter, or HFS could be decreasing presynaptic Ca\(^{2+}\) conductance and inhibiting further release of neurotransmitters (one such cause of that could be activation of presynaptic GABA\(_B\) receptors).

Although we do not discount the possibility of other important mechanisms, such as effects on voltage-gated channels (Wilson and Bevan, 2011), activation of efferent projections, or other (see Lozano and Lipsman, 2013), this study was able to provide evidence supporting the aforementioned mechanisms. The justifications for these mechanisms and their implications are discussed below.
Inhibition of activity of DBS target neurons

The STN and SNr receive projections from a number of sources, both inhibitory (GABAergic) and excitatory (glutamatergic). The STN receives excitatory input from the cortex, and inhibitory input from the GPe. In turn, the STN sends excitatory projections to the SNr (and GPi). While the SNr receives some inhibitory afferents from the GPe, the primary contributor of inhibitory GABAergic projections are the medium spiny neurons of the striatum (Smith and Bolam, 1989; Parent and Hazrati, 1995a, b; Bolam et al., 2000). Although both the STN and SNr have a mix of inhibitory and excitatory synaptic terminals, in both nuclei GABAergic terminals represent the majority of boutons on the somata, however, the relative distributions differ. GABAergic terminals on the somata account for 60% of the total number of boutons in the STN, while in the SNr they account for nearly 90% (Rinvik and Otterson, 1993; Parent and Hazrati, 1995b). Under the assumption that electrical stimulation leads to excitation of pre-synaptic terminals on the somata (Ribak et al. 1979, 1981; Dostrovsky et al. 2000; Wu et al. 2001; Filali et al. 2004; Galvan et al. 2006; Lafreniere-Roula et al. 2010), and since GABAergic afferents comprise the majority of these terminals, this could explain why STN and SNr neuronal firing rates were both attenuated as the stimulation frequency was increased (Fig. 3.2A), since GABA release leads to hyperpolarization of the postsynaptic membrane. Furthermore, the higher prevalence of GABA synapses in SNr likely explains why SNr neurons exhibited a greater inhibitory response to electrical stimulation than STN neurons.
Baufreton et al. (2005) have shown that one function of the inhibitory input to STN from GPe is to enable increased excitatory post-synaptic potential (EPSP) –spike coupling so that during stimulation-induced inhibition, STN firing becomes phase locked and therefore more regular and actually enhances Na\(^+\) channel deinactivation. This may allow the STN to follow beta activity coming from the motor cortex along the hyperdirect pathway. Indeed, we found STN silent periods (after 1-50Hz stimulations) to be in the order of 50-60ms, which corresponds to a spike return frequency of 15-20Hz, falling in the beta band range. However, at 100Hz stimulation (which we know with conventional DBS to be therapeutically beneficial), the silent period becomes significantly prolonged and is no longer permissive of transmission in the beta range.

Our analyses showed that at higher stimulation frequencies, the post-stimulation silent periods increased substantially (Fig. 3.2B). This effect may be a putative mechanism for therapeutic benefit of HFS. While temporal summation of GABA\(_A\)-mediated (~50ms) inhibitory post-synaptic potentials (IPSPs) may account for the inhibitory effect observed during stimulation trains at lower frequencies, it is less likely to explain the prolonged (>100ms) inhibition observed after the stimulation train at higher frequencies (Dostrovsky et al. 2000; Lafreniere-Roula et al. 2010). The longer duration inhibition following stimulation at higher frequencies (≥50Hz) could be due to activation of metabotropic GABA\(_B\) receptors which produce longer lasting inhibition. In animal studies, GABA\(_B\) receptors have been implicated in GABAergic striatonigral, pallidonigral, and pallidosubthalamic transmission (Chan et al., 1998; Charara et al.,
2000; Smith et al., 2001; Boyes and Bolam, 2003; Galvan et al., 2005; Hallworth and Bevan, 2005; Kaneda and Kita 2005). Furthermore, rat brain slice preparations have shown that HFS was necessary to elicit GABA_B-mediated responses in STN (slow IPSPs), while single pulses elicited GABA_A-mediated responses only (Hallworth and Bevan, 2005; Kaneda and Kita 2005; Kita et al., 2006).

Our findings suggest that the frequency-dependent inhibitory effects of electrical stimulation are more complex than simple temporal summation of inhibitory responses, and that HFS may be eliciting the action of presynaptic GABA_B receptors leading to prolonged inhibition. Presynaptic GABA_B receptors decrease Ca^{2+} conductance on autoreceptors of GABA releasing terminals, and on heteroreceptors in neighbouring terminals, including glutamatergic ones (Dutar and Nicoll, 1988; Mintz and Bean, 1993; Dolphin, 2003; Bettler et al., 2004). Synaptic transmission is highly dependent on presynaptic Ca^{2+} conductance, so this action would cause inhibition of further release of neurotransmitters. In support of this claim, physiological, electrophysiological and neurochemical studies have suggested that GABA_B receptors mainly participate presynaptically while the role of postsynaptic GABA_B receptors in SNr cells is minimal (Floran et al., 1988; Rick and Lacey, 1994; Chan and Yung, 1999).

3.5.2 – Inhibitory synaptic potentiation occurs after HFS

Despite the presence of GABAergic afferents in both STN and SNr, we only observed stimulation-induced fEPs in the SNr (eg. Fig. 3.3A). This is also possibly attributable to
the more balanced representation of glutamatergic (40%) to GABAergic (60%) synapses present on the somata of STN neurons. Studies have suggested that the glutamatergic response overlaps with the GABAergic effects, which could explain how in the STN, the glutamatergic response from cortical afferents may be shunting the GABAergic response and thus, not inducing an fEP (Rodriguez-Moreno et al., 1997; Kaneda and Kita 2005).

Since it is not possible to block GABAergic responses in humans, for obvious reasons, our discussion of the fEP and its implications will be henceforth limited to SNr neurons.

Based on aforementioned observations, we believe our stimulation protocol is primarily activating inhibitory GABAergic projections in SNr, either from the striatum or the GPe. This is consistent with the observation that all of our extracellular fEP measurements are positive (eg. Fig. 3.3A), indicating an intracellular hyperpolarization (such as a GABA$_A$-mediated Cl$^-$ influx). Previous animal studies have demonstrated the inhibitory nature of a caudate-evoked GABAergic positive field in the SNr (Precht and Yoshida, 1971; Yoshida and Precht, 1971). The studies demonstrated that the time course of an intracellular IPSP was equivalent to the extracellular positive field, and that the field could be blocked in its entirety by picrotoxin (GABA antagonist). In our study, complete inhibition of SNr firing was observed during the time course of the fEP, suggesting that the stimulation-evoked positive field is associated with a hyperpolarizing event, likely local GABA release. Furthermore, we have shown that the amplitude of the stimulation-evoked fEP is directly associated with prolonged inhibition of neuronal firing and that both responses are conjointly enhanced after HFS (Fig. 3.3). We have demonstrated
enhancement of inhibitory synaptic plasticity by observing that both the peak amplitude 
of the fEP, and the associated inhibition of neuronal firing increased by an average factor 
of 1.72 (P<0.001), and 1.88 (P<0.01) respectively following HFS (100Hz, 100uA, 0.3ms, 
10s). The proportionality between the responses further supports the hypothesis that 
stimulation is evoking an inhibitory, GABA-related phenomenon.

Although potentiation after HFS may not be a current putative mechanism of DBS, since 
continuous DBS is always ON, this knowledge can be used to justify the purported 
increased efficacy of the novel applications of DBS (adaptive or closed-loop systems; 
Little et al., 2013) which have frequent OFF stimulation periods, and thus frequent 
enhancement of the efficacy of inhibitory synapses.

3.5.3 – Continuous HFS causes synaptic depletion

We also investigated the dynamics of the fEP by measuring the amplitude of the fEP 
after each pulse during the stimulation train at different frequencies (Fig. 3.5). By 
observing the behaviour of the average-fEP amplitude, it would appear that the fEP was 
simply being attenuated with increasing frequency, indicating a depletion of the 
GABAergic response. However, looking at the amplitude of the first- and last-fEP within 
each train uncovered a more complex behaviour. While the last-fEP within each train 
was being attenuated much like the average-fEP, the first-fEP was contrarily being 
potentiated (synaptic facilitation). This implies that there was a rapid attenuation of 
successive fEPs (refractory depression) throughout the stimulation train after the initial
potentiated fEP (see Fig. 3.5A). This initial potentiation was the consequence of the electrical stimulation from the previous stimulation train (e.g., prior effect of 20Hz stimulation train produced a potentiated first-fEP during the 30Hz train). Thus, the gradual increase in first-fEP amplitude demonstrates a frequency-dependent potentiation which began after the 20Hz train, with an apparent ceiling effect occurring at 50Hz stimulation. The rapid attenuation of the successive fEPs after the first was a result of synaptic depression (fatigue) presumably due to vesicle depletion from presynaptic terminals induced by HFS (≥30Hz). Furthermore, the paired pulse ratio curve (Fig. 3.6) demonstrates that 20Hz (50ms interstimulus interval) is the minimum stimulation frequency required to induce depression of the synaptic response (significant attenuation of consecutive fEPs). Hippocampal slice preparations have shown that GABAergic IPSPs are “labile” and attenuate after repetitive stimulation due to a reduction of driving force (McCarren and Alger 1985; Huguenard and Alger 1986), and a decrease in synaptic release (Thompson and Gahwiler 1989). It is believed that synaptic depression is caused by depletion of a limited store of releasable transmitter by repetitive stimulation, which is not instantaneously replenished (Zucker, 1989). Thus, our results are able to discern the time course of GABA vesicle recovery. Synaptic depression begins at approximately 20-30Hz stimulation, this implies that the minimum time required for transmitter reuptake is between 100-200ms, since stimulation frequencies between 5-10Hz failed to induce synaptic fatigue. Furthermore, the gradual decrease in last-fEP amplitude demonstrates a frequency-dependent synaptic depression, with an apparent floor effect occurring at 50Hz stimulation.
Synaptic depression can be classified as a transient decrease in synaptic strength; this is hypothesized to occur by way of vesicle depletion and/or decreased presynaptic Ca\textsuperscript{2+} influx (Zucker and Regher, 2002; Fioravante and Regehr, 2011). When a stimulus releases a large fraction of the readily releasable pool of neurotransmitter vesicles, subsequent stimuli delivered before replenishment will release fewer vesicles. Modelling studies have predicted that depression will increase when the initial release probability and the frequency of activation are increased (Dittman and Regehr, 2000; Zucker and Regher, 2002; Rizzoli and Betz, 2005; Fioravante and Regehr, 2011). Even small decreases in Ca\textsuperscript{2+} influx can lead to significant presynaptic plasticity due to neurotransmitter release having a strong dependence on Ca\textsuperscript{2+} (Neher and Sakaba, 2008).

One possible mechanism of altering Ca\textsuperscript{2+} entry into the presynaptic terminal, as discussed above, is activation of presynaptic GABA\textsubscript{B} receptors which would prevent further transmitter release. Several studies have implicated that high-frequency presynaptic activity leads to an accumulation of residual Ca\textsuperscript{2+}, and that this phenomenon is involved in various forms of short-term plasticity including facilitation (Katz and Miledi, 1968; Zucker and Stockbridge, 1983; Kamiya and Zucker, 1994; Atluri and Regehr, 1996), post-tetanic potentiation (Delaney et al., 1989), and recovery from presynaptic depression (Dittman and Regehr, 1998; Stevens and Wesseling, 1998; Wang and Kaczmarek, 1998). These studies are consistent with our findings; facilitation of the fEP (increase in the amplitude of the first-fEP) occurred at the stimulation frequency which also induced synaptic depression (where the successive fEP amplitudes were attenuated). From Fig. 3.5B, we can clearly see that the first- and last-fEP have a close inverse proportionality. The synapses increasingly enhanced their initial response.
(facilitation), coupled with increasing refractory depression. Furthermore, when increasing the stimulation frequency failed to induce further facilitation of the first-fEP (ceiling effect), synaptic depression appeared to level off accordingly (floor effect). Higher frequencies of stimulation would be required to test this hypothesis further.

These results provide further evidence that inhibitory synaptic plasticity is enhanced after HFS, and that the effect appears to be frequency-dependent. These findings support the hypothesis that HFS induces non-specific synaptic depletion, and this is likely a putative therapeutic mechanism of action of DBS (Rosenbaum et al., 2014). Although we are unable to provide explicit evidence of glutamatergic synaptic depletion, we hypothesize that HFS also depresses these synapses. This hypothesis may be evidenced by the fact that neuronal firing continued to be increasingly inhibited (Figs. 1&2) despite an increasing depletion of GABAergic synapses (Fig. 3.5).

3.5.4 – Functional Implications

According the canonical rate model, higher firing rates in STN are thought to give rise to worse motor symptoms in Parkinson’s disease (Delong, 1990). But, both in our study and as reported by Chesselet et al., (1996), we did not find any relationship between the baseline firing rate of STN neurons and symptom severity or clinical improvement with DBS. However, our findings did suggest that the easier it was to suppress the firing of STN neurons, and the longer they were inhibited for after clinically relevant HFS (100Hz), the better the clinical improvement was for patients after STN DBS therapy.
Presently, the ideal stimulation parameters for SNr DBS are unknown, however, our study suggests that the SNr can be inhibited using lower frequencies than the STN. This supports the notion that the optimal contacts for 60Hz stimulation for axial symptoms (including balance and gait disturbances) in Parkinson’s disease are situated more ventrally than those for 130Hz (Khoo et al., 2014). Furthermore, our SNr findings with respect to inhibitory plasticity (and this finding’s implications for novel DBS technologies) may be applicable to GPi (Liu et al., 2012), a conventional DBS target for Parkinson’s disease and cervical dystonia.

3.5.5 – Limitations

A limitation of the current study, compared to animal studies, was the inability to use pharmacological interventions to help elucidate the specific molecular mechanisms. Additionally, we were limited to applying only short duration stimulation trains compared to that of days (or longer) in clinical applications. It is also important to consider the fact that a DBS macroelectrode would stimulate a much larger population of neurons than a microelectrode, with a current density that is capable of spreading up to 2mm from the center of a contact (Wu et al., 2001; Erez et al. 2009), although previous studies have shown that STN neuronal firing is inhibited with a DBS macroelectrode as well (ex. Filali et al., 2004; Toleikis et al., 2012). Considering this and the fact that DBS also excites efferent axons, it is highly likely that STN-DBS produces a high-frequency activation of subthalamic nigral axons as well. However, in the case of clinically beneficially stimulation frequencies (~130Hz), if the axon is following the frequency, the
downstream synapses may also be subject to neurotransmitter depletion, although this was not directly investigated here. With respect to the glutamatergic subthalamonomigral projections, these only account for ~10% of the terminals located on SNr neurons. Thus, a potential therapeutic mechanism of STN DBS could be a “selective” downregulation of downstream structures. Indeed, studies in non-human primates have shown that GP neuronal firing is not entrained to high frequency STN DBS (Agnesi et al., 2015; Zimnik et al., 2015), and may act as an “information filter”, downstream, by blocking the resting pathologic firing characteristics in Parkinson’s disease, but allowing transmission of task-related information (Zimnik et al., 2015), which presumably involves other pathways (i.e. the direct pathway). It would be valuable to further study these downstream effects of electrical stimulation in humans (ex. subthalamanigral projections).

3.6 – Conclusions

The findings of this study indicate that stimulation likely induces pre-synaptic neurotransmitter release which regulates the level of signal transmission, as well as various forms of synaptic plasticity. These phenomena were differentially modulated in STN and SNr, likely due to the higher prevalence of GABAergic terminals on SNr somata, although both structures contain primarily GABAergic synapses. A direct result of presynaptic transmitter release was frequency-dependent inhibition of activity of the target neurons. However, by looking at the silent period after the cessation of stimulation, it is clear that the mechanism of DBS is not a simple temporal summation of inhibitory
responses, implying that HFS modulates the behaviour of synaptic transmission. We further showed that stimulation induces synaptic potentiation after HFS, but during HFS, there is a rapid synaptic depletion, which is likely a putative therapeutic mechanism of DBS. The purported increased efficacy of novel applications of DBS technology (adaptive or closed loop systems; Little et al., 2013) may be benefiting from potentiation of inhibitory responses that occurs after HFS because of more frequent OFF stimulation periods.

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3.9 – Disclosures

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3.10 – Supplementary material

Supplementary Figure 3.1 – Frequency-dependent responses of the average, first-, and last-fEP during a descending series of stimulation trains in an SNr recording site. Since 20, 30, 50, or 100Hz stimulation are capable of enhancing the fEP and producing plasticity, a randomized stimulation series beginning with any of those frequencies would induce hysteresis of the initial potentiation response. In this example, we delivered a descending series (beginning at 100Hz) in a separate patient. The baseline measurement of the fEP using 1Hz “test pulses” is denoted by the horizontal dashed line. All of the first-fEPs at lower frequencies are already potentiated, i.e., the effect of potentiation was saturated.
CHAPTER 4

4 – Physiological mechanisms of thalamic ventral intermediate nucleus stimulation for tremor suppression

The material presented in this chapter has been published in the article:


NOTE: The content of this chapter is identical to the material presented in the publication except for the text formatting which was done according to University of Toronto requirements.
4.1 - Abstract

Ventral intermediate thalamic deep brain stimulation is a standard therapy for the treatment of medically refractory essential tremor and tremor-dominant Parkinson’s disease. Despite the therapeutic benefits, the mechanisms of action are varied and complex, and the pathophysiology and genesis of tremor remain unsubstantiated. This intraoperative study investigated the effects of high frequency microstimulation on both neuronal firing and tremor suppression simultaneously. In each of nine essential tremor and two Parkinson’s disease patients who underwent stereotactic neurosurgery, two closely spaced (600µm) microelectrodes were advanced into the ventral intermediate nucleus. One microelectrode recorded action potential firing while the adjacent electrode delivered stimulation trains at 100Hz and 200Hz (2-5s, 100µA, 150µs). A tri-axial accelerometer was used to measure postural tremor of the contralateral hand. At 200Hz, stimulation led to 68±8% (P<0.001) inhibition of neuronal firing and a 53±5% (P<0.001) reduction in tremor, while 100Hz reduced firing by 26±12% (not significant) with a 17±6% (P<0.05) tremor reduction. The degree of cell inhibition and tremor suppression were significantly correlated (P<0.001). We also found that the most ventroposterior stimulation sites, closest to the border of the ventral caudal nucleus, had the best effect on tremor. Finally, prior to the inhibition of neuronal firing, microstimulation caused a transient driving of neuronal activity at stimulus onset (61% of sites), which gave rise to a tremor phase reset (73% of these sites). This was likely due to activation of the excitatory glutamatergic cortical and cerebellar afferents to the ventral intermediate nucleus. Temporal characteristics of the driving responses (duration, number of spikes, and onset latency) significantly differed between 100Hz and 200Hz.
stimulation trains. The subsequent inhibition of neuronal activity was likely due to synaptic fatigue. Thalamic neuronal inhibition seems necessary for tremor reduction and may function in effect as a thalamic filter to uncouple thalamo-cortical from cortico-spinal reflex loops. Additionally, our findings shed light on the gating properties of the ventral intermediate nucleus within the cerebello-thalamo-cortical tremor network, provide insight for the optimization of deep brain stimulation technologies, and may inform controlled clinical studies for assessing optimal target locations for the treatment of tremor.

*Keywords*: clinical neurophysiology; deep brain stimulation; neurosurgery; tremor; Parkinson’s disease
4.2 – Introduction

Tremor is characterized by involuntary rhythmic muscle contractions that can occur in one or more body parts. It can occur alone as in essential tremor, or with other motor symptoms as in Parkinson’s disease and occasionally dystonia. Essential tremor is currently the most prevalent movement disorder in man (Louis et al., 1998), and three out of four Parkinson’s disease patients develop tremor at some point during the disease process (Hughes et al., 1993). In Parkinson’s disease, tremor is typically present at rest, while essential tremor patients possess postural or kinetic tremor (Deuschl et al., 1998; Elble & Deuschl, 2009). Tremor is regarded as the most difficult to treat symptom of Parkinson’s disease as it may not respond well to dopamine replacement therapy, and essential tremor has also proven quite intractable to treat pharmaceutically in a subset of patients (Goldman et al., 1992; Koller et al., 1994; Ondo et al., 1998; Fishman, 2008).

Deep brain stimulation (DBS) of the thalamic ventral intermediate nucleus (Vim) is an efficacious and reversible standard of care that has largely replaced Vim thalamotomy for the amelioration of tremor (Benabid et al., 1991, 1993, 1996; Nguyen and Degos, 1993; Deiber et al., 1993). Numerous studies have supported the central origin of tremor by hypothesizing the presence of a single pathological oscillation frequency between 4 and 6 Hz (Rajput et al., 1991; Deuschl et al., 1998; Llinas et al., 2005).

In Parkinson’s disease, an early thalamo-centric theory of tremor genesis stated that 12-15Hz oscillations in pallidal output found in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys were converted into 4-6Hz tremor oscillations by intrinsic
thalamic membrane hysteresis (Llinas, 1995). A more recent pallido-centric theory (Helmich et al., 2011), termed the dimmer-switch hypothesis, suggests that Parkinson’s disease tremor is initiated by the basal ganglia (the switch) and its amplitude is modulated by the cerebello–thalamo–cortical network (the dimmer). Indeed, single neurons with 4-6Hz tremor oscillations are present in the human globus pallidus internus (GPi; Hutchison et al., 1997). This theory suggests that the GPi sends tremorganic output to the thalamus which then ascends through the thalamo-cortical network. However, that would suggest a predominant role for the pallidal thalamic input nuclei, ventral oral anterior and posterior (Voa, Vop), in tremor-genesis, but this does not fit with DBS intraoperative findings which show that intervention of the cerebellar thalamus (Vim) is superior for treating tremor (Atkinson, et al., 2002), or that there are more “tremor cells” in the Vim than in Vop/Voa (Magnin, et al., 2000). However, studies (reviewed in Duval, et al., 2016) suggest that bursting activity can propagate to different nuclei within the thalamus by way of relay nuclei that can either induce bursting activity in neighboring neurons, or simply relay bursting activity that is already present. Furthermore, burst firing of thalamic neurons has been demonstrated to provide a nonlinear amplification of sensory signals (Guido and Weyand, 1995). Thus, periodic oscillations at tremor frequency could be amplified in cortical regions. The same cortical regions which receive this thalamic input exhibit oscillatory tremor-related activity, and send projections to the striatum (Volkmann et al., 1996), as well as direct projections to the subthalamic nucleus (STN; Monakow et al., 1978; Nambu et al., 1996; Mathai and Smith, 2011), which could explain the presence of tremor-related oscillations within the basal ganglia.
Essential tremor is regarded as a disorder of the cerebellum. Postmortem studies have described various levels of neurodegeneration in essential tremor patients including Purkinje cell loss and Purkinje cell axonal swelling in the neocerebellum and vermis (Louis et al., 2007; Axelrad et al., 2008; Yu et al., 2011; Louis et al., 2011; Shill et al., 2008). However, other studies have not found neurodegenerative changes, rather that there is neurophysiological evidence of a reduction in GABAergic tone. In the dentate nucleus of essential tremor patients, postmortem studies have revealed lower levels of GABA-A and GABA-B receptors compared to controls (Paris-Robidas et al., 2012).

Thus, the restricted inhibitory influence of Purkinje cells may result in increased disinhibition of deep cerebellar neurons, and the subsequent over-activity may spread through the cerebello-thalamo-cortical network. Indeed, the Vim has a distinct role within essential tremor pathophysiology. DBS studies have demonstrated tremor-related local field potential clusters (Pedrosa et al., 2012) and intraoperative studies have shown single-unit tremor-related discharges (tremor cells; Lenz et al. 1988; Takahashi et al., 1998) in Vim that are coherent with tremor. What drives these oscillatory networks is still unsubstantiated. Early theories hypothesize that unique ion channel dynamics in the thalamus, inferior olive, and cerebellum can generate oscillations (Jahnsen and Llinas 1984a; Jahnsen and Llinas 1984b; Llinas, 1988). Movement-related activation of nucleo-olivary cells may cause Purkinje cells to synchronously inhibit deep cerebellar nuclei which generate oscillatory rebound potentials (inhibition-induced excitation) that make their way through the cerebello-thalamo-cortical network. However, studies (reviewed in Helmich et al., 2013) have moved away from single oscillator hypotheses, and suggest
that there may be shifting modes of cooperation in all nodes of the tremor network, and that all components are capable of acting as resonators and entraining each other.

In this study, we set out to elucidate how electrical stimulation interacts with the brain on a physiological level during therapeutic high-frequency stimulation (HFS) and how it leads to clinical benefit. While modelling studies (Meijer et al., 2011; Kuncel et al., 2012; Birdno et al., 2014) have been used to predict the effects of thalamic DBS on neuronal firing, our unique intraoperative dual-microelectrode assembly allows us to record the activity of single neurons during stimulation from a nearby electrode while simultaneously quantifying effects on tremor. Our findings suggest that tremor reduction was associated with inhibition of neuronal firing, which occurred after a transient driving of neuronal activity. Additionally, our findings shed light on the complex pathophysiology of tremor-genesis, and could also provide insight for the optimization of DBS technology for the treatment of tremor.

4.3 – Methods and materials

4.3.1 – Patients

A total of 21 Vim sites were investigated during microelectrode-guided placement of DBS electrodes in 11 patients; nine with essential tremor and two with Parkinson’s disease (who had an additional postural tremor component). The experiment conformed to the guidelines set by the Tri-Council Policy on Ethical Conduct for Research
Involving Humans and were approved by the University Health Network Research Ethics Board. Furthermore, all of the patients in this study provided written, informed consent prior to taking part in the study.

4.3.2 – Data acquisition

Two independently driven microelectrodes (25μm tip lengths, 600μm apart, 0.2-0.4MΩ impedances, sampled at 12.5kHz), which share a common ground on a stainless-steel intracranial guidetube were used for recordings and microstimulation (Fig. 4.1A). Open filter recordings (5-3,000Hz) were amplified 5,000 times using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, USA), digitized using a CED 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK), and monitored using Spike2 software (Cambridge Electronic Design). Microstimulation was done using one of the two isolated constant-current stimulators (Neuro-Amp1A, Axon Instruments, USA) with square wave, 0.3ms biphasic pulses (cathodal followed by anodal).

4.3.3 – Microelectrode recording procedure

Techniques used for intraoperative electrophysiological identification of Vim have been previously published (Lenz et al., 1988; Ohye et al., 1989; Tasker et al., 1998). Briefly, stereotactic coordinates of the anterior commissure and posterior commissure were determined using a T1-T2 fusion MRI (Signa, 1.5T or 3T, General Electric, Milwaukee, USA) on a surgical neuronavigation workstation (Mach 4.1, StealthStation, Medtronic,
Minneapolis, USA), in addition to an estimation of the location of Vim based on the 14.5mm sagittal section of the Schaltenbrand and Wahren (1977) standard atlas. The two microelectrodes were advanced through a tentative trajectory through the thalamus in an anterodorsal to ventroposterior direction towards coordinates of x=14.5mm (or 11 mm lateral to the 3rd ventricle), y=6mm anterior to the posterior commissure and z=0mm from the mid-commissural point (Fig. 4.1B). Several techniques were used for the delineation of thalamic sub-nuclei. Single units were tested for responses to passive and active movements of the wrist, elbow, and shoulder. Units with movement-related responses were considered cells of the motor thalamus: Vop/Vim (Molnar et al., 2005). Microstimulation (100-200Hz, 100µA, 2-5s, 0.3ms pulse width) was delivered every 1mm along the trajectory to coarsely delineate Vim from Vop based on tremor reduction or tremor arrest. The first site along the trajectory with stimulation-induced paresthesia was considered to be in the vicinity of the anterior border of the ventral caudal nucleus (Vc). We also confirm Vim recording sites by the presence of beta oscillatory activity in the absence of tremor, which is not otherwise found in surrounding structures (Basha et al., 2014).

4.3.4 – Experimental protocol

Based on the above criteria, the protocol was undertaken in recording sites that were determined to be in the Vim (maximum 5mm away from Vc). Upon locating a well isolated single unit (cell), patients were asked to maintain a tremorgenic posture by holding up a bottle of iso-propyl alcohol (filled to ~150mL), while a tri-axial
Figure 4.1 – Experimental setup. A: Our custom dual-microelectrode recording assembly with ~600um mediolateral spacing between adjacent microelectrodes. Upon locating a well isolated spike on one microelectrode, the adjacent microelectrode was used to deliver stimulation trains at the same depth. B: Representative microelectrode track of the Vim and surrounding structures, thalamic sub-nuclei, and fibers (Voa = ventral oral anterior; Vop = ventral oral posterior; Vim = ventral intermediate; Vc = ventral caudal; Vcpc = ventral caudal parvocellular; RaPrl = prelemniscal radiations; STN = subthalamic nucleus; SNr = substantia nigra pars reticulata).

Accelerometer (Crossbow Technology Inc., San Jose, USA) was used to measure the scalar sum of accelerations on the wrist of the contralateral hand. In two patients we also obtained electromyography (Intronix Technologies, Bolton, Canada) from the wrist extensor muscle. When stable tremor was present, stimulation trains at 100Hz and 200Hz were delivered (2-5s, 100μA, 150μs) from the adjacent microelectrode (600μm away in the mediolateral direction). A total of 88 stimulation trains were delivered (40 at 100Hz and 48 at 200Hz, at least one of each per stimulation site). At three recording sites only tremor reduction was measured as the units were lost (excluded from correlations).
4.3.5 – Offline analyses and statistics

To measure firing rates during stimulation trains, stimulus artifacts (0.3ms pulse duration) were removed offline from the signal starting at the onset of the stimulation pulse to its end. Single-units were discriminated using the waveform template matching tool in Spike2. Cell inhibition was measured as the ratio of the firing rate during the stimulation train to a 10s pre-stimulation baseline firing rate of the cell. This value was subtracted from 1 and multiplied by 100 to get “% cell inhibition” (i.e. a value of 100 represents complete inhibition). In recordings sites that had an initial transient driving of neuronal activity at stimulation onset (ex. Fig. 4.5), the cell inhibition was measured after the initial burst. In these recording sites, we measured the burst duration (ms), firing rate (Hz), number of spikes, and onset latency (ms; from the first pulse of the stimulation train). For tremor reduction, the root mean square amplitude (0.2s time constant) of the accelerometer signal was measured. A ratio was taken between the waveform averages during the tremor reduction period compared to a pre-stimulation baseline period immediately before the stimulation train. This value was subtracted from 1 and multiplied by 100 to get “% tremor reduction” (i.e. a value of 100 represents complete tremor arrest). The duration of both the tremor reduction period and pre-stimulation baseline were equivalent to the duration of the stimulation train. However, we measured the maximal tremor reduction period, which always had a delay with respect to the stimulation train onset, as seen in Fig. 4.2. The average delay between stimulation onset and maximal tremor reduction period was 466±24ms (average±standard error). Tremor phase resets were determined by comparing the instantaneous frequency of each phase of the tremor cycle before stimulation, to the instantaneous frequency immediately after
Collectively, the figures show that 200Hz stimulation led to near complete cell inhibition and tremor reduction, while 100Hz was insufficient for achieving these phenomena. The bottom-most trace in each figure is a raw microelectrode recording during stimulation from the adjacent microelectrode. Above that is the artifact-removed, template-matched spike, which shows the neuronal activity during the stimulation train. The spectrogram demonstrates the frequency of the spike bursting (depicting a 5Hz synchronous discharge of the neuronal firing; tremor cell), and shows that at 200Hz (when spike firing is mostly inhibited) the 5Hz tremor-related activity is desynchronized, but at 100Hz (when spike firing is persistent) the 5Hz activity is still present. The top-most trace in each figure is the accelerometer signal during postural tremor of the contralateral hand.

Figure 4.2 – Sample data during 100Hz (A) and 200Hz (B) stimulations from a single patient.
onset of the stimulation (see Fig. 4.6). Paired sample t-tests (2-tailed) were used to
determine whether stimulation trains had a significant effect on tremor reduction and
neuronal inhibition compared to baseline for each of the frequencies. To compare the
effect of stimulation frequency on cell inhibition, tremor reduction, and the transient
driving response variables (listed above), paired sample t-tests (1-tailed) were used,
under the hypothesis that 200Hz had a greater effect on each of the parameters than
100Hz. A second-order polynomial regression line was fit to the correlation between cell
inhibition and tremor reduction, and a Pearson’s coefficient of correlation was calculated.
To determine the effect of tremor reduction as a function of depth though the trajectory at
100Hz and 200Hz, linear regression lines were fit and Pearson’s coefficients of
correlation were calculated.

4.4 – Results

4.4.1 – Vim recording sites

The average pre-stimulation baseline firing rate of all recorded neurons was 48±8Hz
(average ± standard error). Of the recorded neurons, 56% (10/18) were tremor cells that
exhibited 4-6Hz tremor-related burst firing (with an average intra-burst firing rate of
88±12Hz) and movement-related responses (ex. Fig. 4.7). In 61% (11/18) of the neurons,
we recorded transient stimulation-induced driving of neuronal activity that was limited to
the start of the stimulation trains (ex. Fig. 4.5A). In 57% (12/21) of all recordings sites, a
tremor phase reset occurred at the start of the stimulation trains (ex. Fig. 4.6). Eight of
the 11 (73%) neurons with transient driving responses had phase resets. Our
electromyography recordings from the wrist extensor muscles showed an average fast latency muscle activation of 62±4ms from the start of the stimulation train during phase resets.

4.4.2 – Tremor reduction and cell inhibition during stimulation

Higher neuronal inhibition was associated with improved tremor reduction, which was more prominent at 200Hz. Fig. 4.3A shows that 200Hz stimulation led to 68±8% (P<0.001) inhibition of neuronal firing compared to baseline, and a 53±5% (P<0.001) reduction in tremor, while 100Hz only reduced firing by 26±12% (not significant) with a 17±6% (P<0.05) tremor reduction. At 200Hz, both the cell inhibition (P<0.001) and tremor reduction (P<0.001) were significantly higher than at 100Hz. Fig. 4.3B shows that the degree of neuronal inhibition and tremor reduction were significantly correlated with a second-order polynomial fit (R²=0.28, P<0.001), most representative of the relationship. There was also a significant linear correlation (R²=0.28, P<0.001; not shown in the figure).
**Figure 4.3 – Neuronal inhibition and tremor reduction.** A: Shows the degree of cell inhibition and tremor reduction during stimulation trains at 100Hz and 200Hz compared to baseline for stimulations across all recording sites. At 200Hz, there was significantly more cell inhibition and tremor reduction compared to 100Hz. B: Shows the correlation between cell inhibition and tremor reduction across all recording sites, fitted with a second order polynomial. *P<0.05, †P<0.001

### 4.4.3 – Spatial distribution of tremor reduction

The most ventroposterior stimulation sites, closest to the Vim-Vc border, had the best effect on tremor. Fig. 4.4 shows that tremor reduction and proximity to the Vim-Vc border were significantly correlated at both 100Hz (R²=0.17, P<0.05) and 200Hz (R²=0.33, P<0.001). At 200Hz, stimulation sites within 1mm of the Vim-Vc border led to a tremor reduction of 70±4%.
Figure 4.4 – Tremor reduction with respect to distance from Vc. The correlation suggests that clinical benefit was maximal at recording sites closest to the Vim-Vc border. The 0mm mark is the first location with patient-reported paresthesia. This does not imply that the recorded neuron at that site was in Vc, but rather that the stimulation has begun to spread into Vc.

4.4.4 – Transient stimulation-induced driving of neuronal activity

In all recording sites with transient driving responses, the bursts were present during both 100Hz and 200Hz stimulations. Fig. 4.5B shows that the duration of the bursts at 100Hz (421±24ms) was significantly longer ($P<0.001$) than at 200Hz (194±21ms), there were significantly more ($P<0.01$) spikes per burst at 100Hz (71±11) compared to 200Hz (30±4), the latency from stimulation onset to burst onset was significantly longer ($P<0.05$) at 100Hz (36±4ms) compared to 200Hz (24±3ms), but there was no significant difference between the burst firing rates between 100Hz (166±21Hz) and 200Hz (154±16Hz), likely due to the refractory period of spike firing.
Figure 4.5 – Transient stimulation-induced driving of neuronal activity. A: Representative example of the transient driving of neuronal activity at the start of a 100Hz and 200Hz stimulation train at a recording site in a single patient (with stimulus artifacts removed and represented with shaded box). B: Box-and-whisker plots describing the transient driving responses. The figures show the 10\(^{th}\) and 90\(^{th}\) percentiles, first and third quartiles, and median of the firing rate, duration, number of spikes, and onset latency of the driving responses. There was a significant difference in all values except firing rate. *\(P<0.05\), **\(P<0.05\), †\(P<0.001\).

4.5 – Discussion

A major finding of the present study is that – following an initial transient driving response – both the firing of Vim neurons and contralateral hand tremor were strongly suppressed during 200Hz microstimulation, and not affected or only partially reduced during 100Hz. Therefore, thalamic neuronal inhibition seems necessary for tremor
reduction and may function as a thalamic filter to uncouple thalamo-cortical from cortico-spinal reflex loops.

The likely reason for this pattern of brief excitation followed by inhibition is the activation of afferent inputs to the neurons. The Vim is primarily innervated by excitatory glutamatergic projections from both the dentate nucleus of the cerebellum (Asanuma et al., 1983; Anderson and Turner, 1991; Kultas-Illinsky and Illinsky, 1991; Kuramoto et al., 2011) and the cerebral cortex (Bromberg et al. 1981; Sherman and Guillery, 1996). The less prominent afferent inputs are the inhibitory GABAergic thalamic reticular projections (Ambardekar et al. 1999, Ilinsky et al. 1999; Kuramoto et al., 2011). The activation of glutamatergic presynaptic terminals by electrical stimulation would explain why the somadendritic part of the neurons produced the initial burst of action potentials. It may also explain why Vim neurons were not as prone to inhibition compared to neurons in the STN, substantia nigra pars reticulata (SNr), and GPi that we have previously studied (Liu et al., 2012; Milosevic et al., 2017). The predominant afferent inputs of these basal ganglia structures are GABAergic (Rinvik and Ottersen, 1993; Parent & Hazrati, 1995a. 1995b), and we found that 100Hz stimulation was effective at completely silencing neuronal firing in the STN, while SNr and GPi could be silenced with an even lower frequency of 50Hz. Furthermore, neither transient nor tonic excitatory responses occurred in those structures, unlike in Vim. This suggests that the mechanism of action of electrical stimulation is dependent on the underlying microcircuit anatomy of the target structure.
4.5.1 – Initial burst and subsequent inhibition during HFS

A modelling study by Kuncel et al. (2012) predicted that with 125Hz Vim-DBS, neuronal firing is either entirely inhibited, or exhibits a sustained entrainment. However, our findings showed that there is a bi-modal response, and appear to support the theory by Dittman et al. (2000) that there may be interplay between facilitation and depression. In many synapses (especially glutamatergic, due to their lower probabilities of neurotransmitter release) there is a “short-lived” synaptic facilitation that occurs at the onset of repeated stimulation, believed to occur by increased pre-synaptic calcium (Katz and Miledi, 1968). The facilitation is followed in short order by synaptic depression (Katz, 1966; Malenka and Sieglbaum, 2001; Fioravante and Regehr, 2011), believed to occur by vesicle depletion and/or decreased presynaptic calcium (Zucker and Regher, 2002; Fioravante and Regehr, 2011). When a rapid stimulus results in release of a readily releasable pool of neurotransmitter vesicles, subsequent stimuli delivered before replenishment will release fewer vesicles, eventually depleting the pool (Zucker, 1989; Rosenmund and Stevens, 1996). Modelling studies have shown that synaptic depression increases when the initial release probability and/or frequency of activation are increased (Dittman and Regehr, 1998; Zucker and Regher, 2002; Rizzoli and Betz, 2005; Fioravante and Regher, 2011). Indeed, these findings have been found to hold true in glutamatergic cortico-thalamic synapses in a rat brain slices (Ran et al., 2009).
With lower stimulation frequencies, which would allow sufficient time for vesicle replenishment, the driving response should be sustained (ex. Supplementary Fig. 4.1). Although we were not able to measure synaptic field potentials, previous studies from our group (Liu et al., 2012; Milosevic et al., 2017) have shown that the rate of attenuation of extracellular IPSPs in SNr and GPi increases as stimulation frequency is increased, indicative of frequency-dependent neurotransmitter depletion/synaptic depression as a mechanism of HFS.

An intracellular sensorimotor thalamic rat brain slice study by Anderson et al., (2004) has indeed shown that HFS leads to an initial transient depolarization, characterized by a burst of action potentials. Following the initial burst, the neurons were either quickly repolarized and returned to a quiescent baseline, or maintained some level of membrane depolarization, with or without spike firing. Reduction in the initial depolarization was achieved with application of kynurenate, a non-specific antagonist of ionotropic glutamate receptors, as well as with application of NMDA receptor blocker, and sodium channel blocker. This suggests that the HFS-induced depolarization was primarily mediated by glutamate. Furthermore, blockade of voltage-dependent calcium channels, which reversibly inhibited the depolarization, suggested that the depolarization was mediated primarily through pre-synaptic calcium channels (Anderson et al., 2004), which are known to facilitate transmitter release (Zucker and Regher, 2002). Thus, Anderson et al. (2004) hypothesize that HFS in the ventral thalamus disrupts local synaptic function and neuronal firing thereby leading to a “functional deafferentation.”
Alternatively, other post-synaptic mechanisms may underlie the stimulation induced burst at the onset of HFS. When thalamic neurons are hyperpolarized for 50-100ms, incoming excitatory synaptic potentials trigger activation of T-type Ca\(^{2+}\) currents (Jahnsen and Llinas, 1984a), which causes the cell to fire a burst of action potentials. This leads to further calcium channel openings, which eventually trigger calcium-activated potassium currents, which quickly hyperpolarize the cell and reset it for another cycle of bursting. While these mechanisms may explain the generation of rhythmic bursts (i.e. tremor cells), they are less likely to explain the lack of continued bursting (/sustained inhibition) that we have shown here occurs during HFS. The more likely involvement of the T-current is that the initial excitatory response (via glutamate release) leads to inactivation of T-type Ca\(^{2+}\) channels, thereby preventing bursting activity. Beurrier et al. (2001) have shown that in the STN of rat brain slices, there is an inhibition of neuronal activity that outlasts a one minute train of HFS. They found that (L- and) T-type Ca\(^{2+}\) currents were indeed transiently depressed during the HFS-induced silence. Additionally, they found that the HFS-induced inhibition was persistent in the presence of blockers of ionotropic GABA and glutamate receptors, and suggest that the inhibition was non-synaptic. However, they did not study the synaptic function during HFS. Thus, neurotransmitter blockers would not affect the persistent inhibition if synaptic function was already depressed due to the HFS.
Furthermore, thalamic inhibition has been linked to the activity of neuromodulators. Bekar et al. (2008) found that in rodent thalamic slices, DBS caused increased levels of adenosine, which they hypothesized led to neuronal inhibition that was necessary for suppression of tremor. Additionally, Dirkx et al. (2014) showed that the treatment of Parkinson’s tremor with levopoda was associated with increased thalamic self-inhibition, which may be a physiological mechanism that protects the thalamus from a permanent oscillatory state.

4.5.2 – Thalamic gating

This study offers mechanistic insight on the gating properties of the Vim and its thalamo-cortical projection. The Vim sends excitatory glutamatergic projections to cortical motor regions in order to modulate movements (Rouiller et al., 1994). In this study, we have identified five different types of Vim firing patterns that corresponded to different motor states. Firstly, there were three previously described in the literature that occurred in the absence of electrical stimulation, exemplified in Fig. 4.7. When the patient was at rest with no tremor, the neurons exhibited (i) tonic irregular firing. Both passive and voluntary manipulations of the limb led to (ii) kinesthetic movement-related responses (Ohye and Narabayashi, 1979; Lenz et al., 1990). When the patient had tremor, the neuron exhibited (iii) tremor-related (4-6Hz) bursting (Albe-Fessard et al., 1963). The significance of these classifications is the potential to use this real-time information in an application of closed-loop DBS (Priori et al., 2013; Arlotti et al., 2016) for the control of tremor.
A novel finding of this study was the stimulation induced (iv) transient driving of Vim neurons that reset the regular periodic rhythmicity of the tremor (Fig. 4.6). The most likely explanation of this is that the transient neuronal driving response leads to an activation of thalamo-cortical motor neurons either in the primary or supplementary motor cortical areas (Rouiller et al., 1994) via collaterals that give rise to the transcortical reflex that then quickly activate the forearm muscles. Our electromyography results showed a fast latency muscle activation that is consistent with thalamo-cortical activation of the transcortical reflex. In many simple laboratory models of central pattern generators, such as the locust thoracic ganglion motor neuron recordings, a very similar phenomenon of rhythmic reset is observed with short train out-of-phase stimulation of the isolated proprio-sensory input from the wing to the central pattern generator (Pearson, 1991; Marder and Bucher, 2001). In fictive locomotion induced by mesencephalic locomotor region stimulation in the decerebrate paralyzed cat, a prominent reset of the step cycle is produced by brief out-of-phase 100Hz stimulation of the Group I muscle spindle afferents (Guertin, et al. 1995; Hiebert et al., 1996). This would suggest that tremor reset and tremor reduction is due to interruption of the pacing of proprioceptive input in human thalamus, which is found near the Vim-Vc border that receives input from deep muscles (Tasker et al., 1987; Vitek et al., 1994). Indeed, our results show that more efficacious tremor reduction was at stimulation sites closest to the Vim-Vc border.
Figure 4.6 – Representative example of tremor phase resets at the start of a 100Hz (A) and 200Hz (B) stimulation train. A tremor phase reset is present at the start of the stimulation train, which closely follows the initial stimulation-induced neuronal driving response of the cell. This is likely due to a thalamo-cortical activation of motor cortical areas during the driving response, before the subsequent neuronal inhibition (and tremor suppression) occurs.

While the phase reset demonstrates that a transient excitatory neuronal response in Vim would facilitate a brief movement, the subsequent (v) inhibition of neuronal activity was associated with a reduction of tremor. This finding supports the hypothesis (Anderson et al., 2004) that DBS at a high frequency may in effect function as a reversible lesion,
which disrupts the pathological tremor-genic rhythmicity of Vim (see Fig. 4.2B). Indeed, we have found that at a lower stimulation frequency (100Hz) that is less effective at inhibiting the firing of Vim neurons, the tremor and tremor-related bursting persist (see Fig. 4.2A). These findings support recent functional magnetic resonance imaging findings by Dirkx et al. (2017), which suggest that efficacious treatment of tremor with levodopa may act by increasing thalamic self-inhibition. However, it is unlikely that the stimulation-induced inhibition of Vim only effects tremor, but may also be associated with a more widespread inhibition of movements. The continuous inhibition of neuronal activity in this area may explain the commonly reported adverse effects on other motor functions such as gait disturbances and ataxia (Cury et al., 2007), or less commonly weakness/uncertainty of the treated limbs (Takahashi et al., 1998). With respect to the gating function of Vim, it supports the notion that inhibition of neuronal activity has a role in down-regulation of movements, including perhaps non-pathological (Strafella et al., 1997). This would further justify the need for a closed-loop system to selectively control tremor, in order to offset the chronic adverse effects of unnecessary continuous stimulation.

Taken together, these observations support the theory that the Vim acts as a gate for incoming information required to trigger movements. Depending on the input it receives (inhibitory, excitatory, rhythmic, etc.), its thalamo-cortical projection gives rise to an appropriate motor action. It also shows that the Vim can be selectively modulated by external stimuli. This likely explains why HFS relieves tremor, low frequency
stimulation has been shown to induce or worsen tremor (Hassler et al., 1960; Barnikol et al., 2008; Pedrosa et al., 2013) likely due to persistent driving/entrainment of neuronal activity (ex. Supplementary Fig. 4.1), and also why additional incoming proprioceptive information may de-synchronize tremor-related activity (Naros et al., 2018). It may also explain why anti-phasic rhythmic stimulation has been reported to be efficacious for suppressing tremor (Cagnan et al., 2013), which likely works by regularizing the overall neuronal firing in Vim by producing short excitations between tremor bursts, rather than by overall inhibition which we have shown here appears to be the mechanism of continuous HFS.

4.5.3 – Clinical utility

We found that the degree of cell inhibition was correlated to the degree of tremor reduction, suggesting that suppression of neuronal firing in the Vim is likely an important mechanism of DBS for the control of tremor. Our finding of better tremor suppression with 200Hz supports clinical studies (Blomstedt et al., 2007; Earhart et al., 2007; Kuncel et al., 2012) which suggest that Vim-DBS produces better tremor benefit with higher programmed stimulation frequencies than typically used for STN (~185Hz vs. ~130Hz). Single and multicenter studies have reported an average tremor reduction of ~80% with Vim-DBS in essential tremor patients (Koller et al. 1999; Ondo et al.1998; Rehncrona et al. 2003). We found a reduction of 53±5% with microstimulation at 200Hz, which is likely due to stimulating a much smaller population of neurons as well as testing less effective sites dorsoanterior to the tentative target site. The most effective sites for tremor
reduction were in close proximity to the Vim-Vc border (Fig. 4.4). At stimulation sites within 1mm of the Vim-Vc border, 200Hz microstimulation led to a tremor reduction of 70±4%, comparable to that of the reported benefit of DBS macro-stimulation. This finding is important in informing surgical electrode placement, which can be accounted for intraoperatively with micro-recording and stimulation. It also supports neurosurgical observations that the ideal location for a Vim thalamotomy is the small section of Vim near Vc that receives proprioceptive input (Tasker et al., 1987). A recent study identified that more posterior DBS electrode placements were associated with failure of benefit, and more anterior placements were optimal (Sandoe et al., 2018). Our study shows that microstimulation of the ventroposterior region of Vim (i.e. as close to Vc as possible, without inducing paresthesia) yielded the best tremor reduction, within the standard Vim-DBS trajectory. This is likely due to the larger size of DBS electrodes and the contacts being too close to Vc, producing paresthesias that limit the current density required for tremor reduction. In the advent of novel “current-steering” electrodes, this finding may be able to inform stimulation delivery, i.e. placement of the DBS electrode near the Vim/Vc border, but directing the current away from Vc.

4.5.4 – Functional implications

Additionally, sub-optimal electrode placement can be clinically compensated for by increasing the volume of tissue activation. However, this increases the risk of stimulating different neuro-circuits that lack relevance to the patient pathology, which likely gives rise to side effects such as paresthesia and dysarthria (Cury et al., 2007). Our study
confirms the existence of an optimal site within the standard Vim trajectory, just anterior to the Vim-Vc border. At sites within 1mm of the Vim-Vc border, 200Hz microstimulation led to comparable long-term benefit of previously reported DBS macrostimulation, despite stimulating a smaller population of neurons. This demonstrates the potential for improving therapeutic window by (i) minimizing the volume of tissue activation (reduces risk of side-effects), and (ii) minimizing the size of the stimulating electrode to have a more focal target (reduces risk of edema, hemorrhage, micro-lesion, etc.).

Additionally, having an embedded electrode with a significantly smaller effective contact size can allow for the possibility to chronically record single neurons (DBS macroelectrodes are limited to local field potentials). Although ambitious, DBS technologies are evolving more rapidly than ever (Arlotti et al., 2016). This would allow for measurement of tremor-related neuronal activity to be used as a control parameter for adaptive DBS systems. Since tremor amplitude and prevalence can fluctuate over time, within seconds or minutes (Beuter and Vasilakos, 1995a; Beuter and Vasilakos, 1995b), continuous open-loop strategies present an inefficient solution. Closed-loop DBS has been explored in Parkinson’s disease using beta (12-35Hz) oscillations (ex. Little et al., 2013; Little and Brown, 2013), but tremor-related activity in Vim may be a more robust and promising symptomatic correlate (see Fig. 4.7).
Figure 4.7 – Tremor- and movement-related spiking in Vim in a single neuron. A: 5Hz spiking activity is present during a slight rest tremor. When the patient was asked to raise their arm (to begin our experimental protocol), there is a voluntary movement-related kinesthetic response and an interruption in the 5Hz tremor-related activity. This is followed by a re-emergence of tremor bursting with the maintenance of a tremorgenic posture (postural tremor). B: At rest with no tremor, the neuron had irregular tonic firing, and the emergence of 5Hz bursting was robustly measurable even with the slightest tremor onset.

Finally, we have shown that HFS can downregulate activity, which is important in essential tremor (where Vim receives pathophysiological input from cerebellum) and Parkinson’s disease (where the STN is believed to be overactive; Delong et al., 1990).
However, we propose that stimulation at lower frequencies (conducive to excitation, but insufficient for neuronal inhibition) may be able to persistently drive/entrain neuronal firing in a target structure with predominantly glutamatergic inputs (ex. Supplementary Fig. 4.1). This could have implications for upregulating activity in pathologies where structures may be underactive.

4.5.5 – Limitations

One limitation of human intraoperative studies is the inability to use pharmacological agents to elucidate specific synaptic mechanisms. In contrast, these studies have the advantage over animal studies in that it is not known how well animal models correspond to human conditions, or anatomy. Furthermore, DBS is delivered chronically over a long period of time, while the time course of our intraoperative stimulation is limited. DBS macroelectrodes also stimulate a much larger population of neurons, with a current density that is capable of spreading up to 2mm from the center of a contact (Wu et al., 2001; Erez et al. 2009). Despite the short durations of stimulation and smaller volume of tissue activation with a microelectrode, we were still able to produce marked therapeutic symptomatic benefit, especially when delivered to the optimal location. Thus, our findings should be applicable to understanding the mechanisms that might be involved in Vim-DBS. A future study to validate our findings within the Vim, would be the demonstration of tremor reduction in response to direct activation of the afferent dentatothalamic tracts (Coenen et al., 2014). While our results suggest that HFS of the Vim, and in particular ventroposterior Vim/Vc border region, does lead to marked tremor
reduction, it would also be of interest to compare our results to other targets implicated in
tremor suppression, such as caudal zona incerta, prelemniscal radiations, or subthalamic
nucleus, as outlined in Elble & Deuschl (2011), which may have stronger effects.
Another interesting prospective study would be the investigation of the effect of low
frequency stimulation on Vim neuronal activity, and the potential relationship with the
purported worsening of tremor.

4.6 – Conclusions

Our study shows that the degree of neuronal inhibition in the Vim is associated with the
degree of tremor suppression. The predominance of glutamatergic boutons located on
somas of Vim neurons may explain why Vim was more resistant to neuronal inhibition
than structures such as STN, SNr and GPi, which have predominantly GABAergic
inputs. Hence, the mechanism of action of electrical stimulation is dependent on the
underlying anatomical and physiological properties of the stimulated target structures.
The transient excitatory responses at the onset of stimulation likely reflect those
 glutamatergic inputs, whereas the subsequent inhibition may be due to synaptic fatigue.
Furthermore, we have shown that the location for maximal tremor suppression within the
Vim is the ventroposterior region proximal to the Vim-Vc border. Finally, some of the
response properties described in this study can help guide advancement of DBS therapy.
Firstly, the potential for using Vim tremor-related spike bursting as a robust, real time
predictor of tremor onset and occurrence, and secondly, the potential for using electrical
stimulation to upregulate neuronal activity.
4.7 – Acknowledgments

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4.8 – Funding

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4.9 – Conflict of Interest

S.K.K., M.H., A.M.L., and W.D.H. have received honoraria, travel funds, and/or grant support from Medtronic. M.R.P. is a shareholder in MyndTec Inc. and an advisor to Myant Inc. A.M.L. is a co-founder of Functional Neuromodulation Ltd.
4.10 – Supplementary material

For illustrative purposes, we present one additional Parkinson’s disease patient who received 5Hz and 100Hz (10s, 100µA, 150µs) microstimulation while recording from a Voa neuron during an STN-DBS implantation procedure. Like Vim, Voa and Vop receive considerable glutamatergic input from the cerebral cortex (Bromberg et al. 1981; Sherman and Guillery, 1996). During the 5Hz stimulation train, each stimulation pulse produced firing of several (4.9±0.04) action potentials. Throughout the entirety of the 5Hz train, there was a robust and sustained entrainment of neuronal activity (Supplementary Fig. 4.1A), which happened to mimic the rhythmic tremor-related activity found in Vim (ex. Fig. 4.2&4.8). A 100Hz stimulation train yielded a response similar to HFS in Vim; a transient driving of neuronal activity limited to the start of the stimulation train (184Hz firing rate, 461ms duration, 84 spikes, 24ms latency), succeed by complete inhibition (Supplementary Fig. 4.1B). This may further support our hypothesis of HFS-induced synaptic fatigue. Additionally, these findings demonstrate that low frequency stimulation in the thalamus (with predominantly glutamatergic inputs) can persistently drive/entrain neuronal activity, unlike in STN, SNr, or GPi (with predominantly GABAergic input), indicating that different subcortical structures have dissimilar responses to electrical stimulation that depend on their underlying microcircuit anatomy. In effect, this also likely explains why low stimulation frequencies in the Vim have been shown to induce or worsen tremor. Finally, it demonstrates that a synchronized excitatory input (ex. cerebellar) to the thalamus can give rise to rhythmic spike bursting (ex. tremor cells).
Supplementary Figure 4.1 – Low-frequency entrainment and high-frequency inhibition of a Voa neuron in a Parkinson’s disease patient. A: Each stimulation pulse produced firing of several action potentials. The filtered, artifact-removed spike trace shows robust, sustained entrainment to the 5Hz stimulation, which looks similar to that of tremor-related activity present in Vim. B: During a 100Hz stimulation train, there was a transient driving of neuronal activity at stimulus onset followed by inhibition of neuronal firing, as occurs in Vim.
CHAPTER 5

Inhibitory plasticity of basal ganglia output nuclei in Parkinson’s disease: stimulation and levodopa

The material in this chapter will be submitted for publishing under the title:


NOTE: The content of this chapter is identical to the material which will be submitted for publication except for the text formatting which was done according to University of Toronto requirements.
5.1 – Abstract

Objectives: While deep brain stimulation of certain target structures within the basal ganglia is an effective therapy for the management of the motor symptoms of Parkinson’s disease, its mechanisms are varied and complex. We sought to investigate the stimulation and levodopa induced effects on inhibitory synaptic plasticity in the basal ganglia output nuclei, and to determine the clinical relevance of altered plasticity with respect to patients’ symptoms.

Methods: Two closely spaced microelectrodes were advanced into the substantia nigra pars reticulata and/or globus pallidus internus in each of 28 Parkinson’s disease patients undergoing subthalamic and/or pallidal deep brain stimulation surgery. Sets of 1Hz test-pulses were delivered at different cathodal pulse widths in randomized order, before and after a train of continuous high frequency stimulation at 100Hz.

Results: Increasing the pulse width led to progressive increases in both the amplitudes of focally evoked inhibitory field potentials and durations of neuronal silent periods. Both of these effects were augmented after a train of continuous high frequency stimulation. We found greater enhancements in the globus pallidus internus compared to the substantia nigra pars reticulata, and that levodopa administration had a potent effect on the enhancement of nigral plasticity. We also found that lower levels of nigral plasticity were associated with higher severity motor symptoms.

Interpretation: The findings of this study demonstrate that the efficacy of inhibitory synaptic transmission may be involved in the pathophysiology of Parkinson’s disease,
and furthermore may have implications for the development of novel stimulation protocols, and advancement of DBS technologies.

**Keywords:** basal ganglia; deep brain stimulation; GABA; globus pallidus; levodopa; Parkinson’s disease; substantia nigra; synaptic plasticity
5.2 – Introduction

Parkinson’s disease is a hypokinetic movement disorder characterized by a loss of dopaminergic projections from the substantia nigra pars compacta (SNc) to the striatum; the input of the basal ganglia. The canonical parkinsonian model of basal ganglia neurocircuitry (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990) suggests that the downstream effects of this dopaminergic denervation lead to increased neuronal activity of the inhibitory basal ganglia output nuclei; the substantia nigra pars reticulata (SNr) and globus pallidus internus (GPi). This occurs as a result of a loss of inhibitory tone on the subthalamic nucleus (STN) due to excessive striatal inhibition of the inhibitory globus pallidus externus (GPe) via the D2-receptor-mediated indirect pathway, and a concurrent reduced striatal inhibitory tone on the SNr and GPi via the D1-receptor-mediated direct pathway (Surmeier et al., 2007). These changes to the neuronal firing rates, as well as changes in firing patterns (Levy et al., 2002; Brown, 2003), are believed to give rise to the symptoms of Parkinson’s disease.

Currently, dopamine replacement by levodopa administration remains the most effective therapy for the management of Parkinson’s disease symptoms. However, long-term therapy is complicated by motor fluctuations and levodopa-induced dyskinesias, which represent a substantial source of disability in some patients (Nutt, 1990; Obeso et al., 2000). Subsequently, deep brain stimulation (DBS) of the STN and/or GPi has been widely adapted as a conventional alternative management option (Benabid et al., 1994; Limousin et al., 1995; Kumar et al., 1998, 2000; Kleiner-Fisman et al., 2006; Perlmutter...
and Mink, 2006; Lozano et al., 2017). DBS works by delivering continuous high frequency stimulation (HFS) to the target structures through chronic indwelling electrodes (Starr et al., 1998; Perlmutter and Mink, 2006; Wichmann and DeLong, 2006), which mimics the effect of beneficial lesions (Bergman et al., 1990; Aziz et al., 1991; Heywood and Gill, 1997) or inactivation by injections of muscimol and lidocaine (Wichmann et al., 1994; Levy et al., 2001), suggesting that DBS may work by inhibition of neuronal activity. There have also been recent promising studies demonstrating the efficacy of SNr-DBS, or combined STN/SNr-DBS for the treatment of axial motor symptoms in Parkinson’s disease (Chastan et al., 2009; Weiss et al., 2011a, b, 2013); however, little is known about the effects of electrical stimulation of SNr neurons.

The precise physiological mechanisms that give rise to Parkinson’s disease symptoms, as well as the therapeutic mechanisms of action of DBS are complex and varied, and the effects of pulse width on neuronal activity have not been systematically studied. Although stimulation amplitude and frequency are well known to impact on therapeutic results, several clinical studies have suggested that varying the pulse width can also impact outcome (Rizzone et al., 2001; Moro et al., 2002; Reich et al., 2015). In this intraoperative study, we investigated the effects of single pulses of electrical stimulation at different pulse widths on the neuronal activity and synaptic events at recording sites of the basal ganglia output nuclei (SNr and GPi), before and after a train of continuous HFS. This study employed a unique methodology for eliciting and measuring focal evoked potentials (fEPs) in SNr and GPi using two closely-spaced microelectrodes.
The objectives of this study were to (i) investigate the pulse-width dependent effects of electrical stimulation on fEP amplitudes and neuronal firing at SNr and GPi recording sites, (ii) compare the changes in synaptic plasticity after a train of continuous HFS between the two output structures, (iii) investigate the effects of exogenous dopamine on synaptic plasticity in the SNr, and (iv) determine if a relationship exists between synaptic plasticity in the SNr and patients’ axial motor symptoms. It has been hypothesized that persistent changes to synaptic plasticity plays a role in both adaptive and maladaptive responses to different forms of pathology and injury of the central nervous system (Kullmann et al., 2012). However, little is known about the mechanisms of inhibitory (GABAergic) synaptic plasticity, compared to that evoked at excitatory synapses. The findings of this study demonstrate and implicate the involvement of inhibitory synaptic plasticity in Parkinson’s disease, and furthermore may have implications for DBS programming, and advancement of DBS technologies.

5.3 – Methods and materials

5.3.1 – Patients

A total of 28 patients with Parkinson’s disease undergoing microelectrode-guided placement of DBS electrodes into either the STN or GPi participated in this study, after overnight withdrawal from medication (“OFF”). In these patients, a total of 23 SNr ($n_{\text{patients}}=20$; in patients undergoing STN surgery) and 11 GPi ($n_{\text{patients}}=8$) recording sites were investigated. A subset of (STN/) SNr patients ($n_{\text{patients}}=7$) were each given oral
administration of 100mg of levodopa (Sinemet 100/25) after completion of the recordings on the first side, thus, the second side was considered “ON” (i.e. an additional 7 recording sites ON). There was an average of 39.5±9.4min between levodopa administration and recording from the SNr on the second side. For each patient we determined the Unified Parkinson’s Disease Rating Scale (UPDRS) total motor subscore and axial subscore (sum of speech, facial expression, arising from chair, posture, gait, postural stability, body bradykinesia, and neck rigidity) in OFF and ON conditions. All of the conducted experiments conformed to the guidelines set by the Tri-Council Policy on Ethical Conduct for Research Involving Humans and were approved by the University Health Network Research Ethics Board. Furthermore, all of the patients in this study provided written, informed consent prior to taking part in the study.

5.3.2 – Data acquisition

Two independently driven microelectrodes (25μm tip lengths, 600μm apart, 0.2-0.4MΩ impedances, at 12.5kHz), which share a common ground on a stainless-steel intracranial guidetube were used for recordings and microstimulation (Fig. 4.1A). Open filter recordings (5-3,000Hz) were amplified 5,000 times using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, USA), digitized using a CED 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK), and monitored using Spike2 software (Cambridge Electronic Design). Microstimulation was delivered using an isolated constant-current stimulator (Neuro-Amp1A, Axon Instruments) with biphasic pulses (cathodal followed by anodal).
5.3.3 – Microelectrode recording procedures

Techniques for electrophysiological identification of STN/SNr (Hutchison et al., 1998) and GPi (Hutchison et al., 1994) have been previously published. Briefly, stereotactic coordinates of the anterior commissure and posterior commissures were determined using a T1-T2 fusion MRI (Signa, 1.5T or 3T, General Electric, Milwaukee, USA) on a surgical neuronavigation workstation (StealthStation, Medtronic, Minneapolis, USA). Additionally, estimation of the location of the STN (x=12mm lateral, y=3mm posterior to MCP, z=3mm inferior to AC-PC) or GPi (x=20mm lateral, y=MCP, z=5mm inferior to AC-PC) was done based on the Schaltenbrand and Wahren (1977) standard atlas (Fig. 5.1B/C). Two microelectrodes were advanced in the dorsoventral direction beginning 10mm above the planned target. For STN/SNr trajectories, entry into the STN was confirmed based on an increase in background noise, and recording of single units with firing rates of approximately 20-40Hz, irregular firing patterns with periods of beta activity, and kinesthetic single units. After 4-6mm advancement, decreases in spike incidence signified exit from the ventral border of the STN and entry into the SNr was characterized by lower amplitude units with fast rates (80-100Hz) and regular firing patterns. For GPi trajectories, recording sites were confirmed based on presence of irregular, high frequency discharge (50-90Hz) neurons with responsiveness to movements, as well as border cells with highly regular 20-60Hz firing rates. After 10-12mm advancement, decreases in spike incidence signified exit from the ventral border, and the optic tract was confirmed based on visually evoked potentials elicited by brief
flashes of light in the visual field as well as patients’ reports of stimulation-induced phosphenes in the contralateral visual hemifield.

5.3.4 – Stimulation protocol

At SNr and/or GPi recordings sites, stimulation trains were delivered from a single microelectrode to elicit fEPs, which were recorded by the adjacent microelectrode (600µm apart) at the same depth. Five sets of 1Hz “test pulses” (100µA for 10s; 10 pulses per set) were delivered at different cathodal pulse widths in randomized order (25, 50, 100, 150, 250µs), separated by 5-10s. This was followed by a “long-train” of HFS (100Hz, 100µA, 150µs pulse width, 10s), followed by another five sets of post-HFS “test pulses” (see Fig. 5.1C). In the SNr OFF/ON levodopa patient subset, the first side was considered OFF, and the second side was considered ON.

5.3.5 – Offline analyses and statistics

Amplitudes of the fEP were measured from the pre-stimulus baseline to the peak voltage deflections after each stimulation pulse (Prescott et al., 2009; Liu et al., 2012; Milosevic et al., 2017). Pre- and post-HFS fEP amplitudes were normalized with respect to the average fEP amplitude of the pre-HFS 150µs pulse width (i.e. all fEP measurements at a single recording site were divided by the average fEP amplitude of the pre-HFS 150µs pulse width train at the same recording site). The “silent period” was measured as the duration of neuronal inhibition after each stimulation pulse (i.e. between the stimulation
Figure 5.1 – Microelectrode configuration, experimental recording locations, stimulation protocol, and sample data. (A) Custom dual-microelectrode assembly with ~600µm mediolateral spacing. Representative (B) STN/SNr and (C) Gpi microelectrode recording trajectories for electrophysiological mapping of the DBS target location. (D) Schematic of the stimulation protocol performed at SNr and Gpi recording sites; five sets of 1Hz (100µA, 10s) “test-pulses” at different pulse widths (25, 50, 100, 150, 250µs in randomized order) were delivered before and after HFS (100Hz, 100µA, 10s, 150µs pulse width). (E) Sample data of a single pre- and post-HFS fEP and associated durations of neuronal inhibition (silent periods) elicited by a 50µs pulse (left) and a 150µs pulse (right). fEP amplitudes and silent periods were greater with larger pulse widths, and both variables were further enhanced after HFS.
pulse and the return of the first spike). To measure the effects of pulse width and HFS on fEP amplitudes (Fig. 5.2A) as well as on silent periods (Fig. 5.2B) in the SNr, two-way repeated measures ANOVA (within subject factors) was used. A post-stimulus plot of the average firing rate of all SNr neurons was constructed (10ms bins) to demonstrate the slow return to baseline firing with increasing pulse widths (only 50, 150, and 250µs depicted), before and after HFS (Fig. 5.2C). Additionally, Pearson coefficients of correlation and p-values were obtained between normalized fEP amplitudes and silent periods pre- and post-HFS (Fig. 5.3). To determine whether there was a difference in the amount of plasticity after HFS between SNr OFF and SNr ON (Fig. 5.4A), two-way split-plot ANOVA was used (within subject factor: pulse width; between subject factor: medication) to compare the post-HFS amplitudes across pulse widths. The same (within subject factor: pulse width; between subject factor: location) was done to compare post-HFS fEP amplitudes between SNr OFF and GPI (Fig. 5.4B). All ANOVAs were followed up with posthoc pairwise comparison t-tests with Bonferonni correction. The data were not normally distributed and were log transformed prior to ANOVAs. The 25µs pulse width often failed to elicit an fEP, thus was not included in ANOVAs (a value of zero cannot be log transformed). Additionally, we obtained Pearson coefficients of correlation between SNr plasticity (i.e. increase in normalized fEP amplitude, calculated as the average of the pre-HFS normalized fEP amplitude subtracted from the average post-HFS normalized fEP amplitude at the 150µs pulse width), and both the UPDRSIII total motor subscore (Fig. 5.5A), and the axial symptom subscore (Fig. 5.5B).
5.4 – Results

5.4.1 – SNr plasticity OFF medication: fEP amplitudes and silent periods

We found that at SNr recording sites, both fEP amplitudes (Fig. 5.2A) and durations of neuronal inhibition (silent periods; Fig. 5.2B) progressively increased as the pulse width was increased, and that measurements of both variables were greater after HFS (HFS-induced synaptic plasticity). For fEP amplitudes ($n_{\text{recording-sites}}=23$) the main effects of pulse width [$F(3,66)=155.442$, $P<0.001$] and HFS [$F(1,22)=61.825$, $P<0.001$] were significant, with a significant interaction between the factors [$F(3,66)=4.954$, $P<0.01$]. Posthoc pairwise comparisons indicated significant ($P<0.001$) differences across all pulse widths and pre-/post HFS. For silent periods ($n_{\text{recording-sites}}=13$), the main effects of pulse width [$F(3,36)=27.291$, $P<0.001$] and HFS [$F(1,12)=28.647$, $P<0.001$] were also significant, with no interaction between factors. Posthoc pairwise comparisons indicated significant differences pre-/post-HFS ($P<0.001$), and across all pulse widths ($P<0.01$); except between 100 and 150µs, and 150 and 250µs.

We further demonstrated that the fEP amplitudes and silent periods were positively correlated (i.e. a larger fEP led to a longer silent period) both pre-HFS ($R^2=0.1275$, $P<0.001$; Fig. 5.3A) and post-HFS ($R^2=0.2810$, $P<0.001$; Fig. 5.3B), and in both cases the effects scaled linearly with the pulse width.
HFS-induced inhibitory plasticity in SNr (OFF)

A: fEP

B: silent period

C: slow return of firing rate

Figure 5.2 – HFS-induced inhibitory synaptic plasticity in SNr (OFF), and pulse-width dependent effects. Larger stimulation pulse widths elicited larger (A) fEP amplitudes (n recording sites=23), and longer (B) silent periods (durations of neuronal inhibition; n recording sites=13). Furthermore, after a 10s train of HFS, both fEP amplitudes and durations of neuronal inhibition were greater. Taken together, these findings are indicative of HFS-induced enhancement of inhibitory synaptic plasticity, which scaled with pulse width. For fEP amplitudes, we found significant main effects of both pulse width [F(3,66)=155.442, P<0.001] and HFS [F(1,22)=61.825, P<0.001]. Likewise for silent periods, we found significant main effects of pulse width [F(3,36)=27.291, P<0.001] and HFS [F(1,12)=28.647, P<0.001]. Detailed statistics can be found in Results section. Additionally, (C) the stimulation pulse width augmented the average latency of the return to baseline firing rate of neurons, before and after HFS.
Figure 5.3 – fEP and silent period correlations. Larger fEP amplitudes were associated with longer durations of the neuronal inhibition (silent periods). The silent period was linearly correlated with fEP amplitude both (A) pre-HFS (P<0.001) and (B) post-HFS (P<0.001). Furthermore, fEP amplitudes and silent period durations scaled with the stimulation pulse width.

5.4.2 – Plasticity and levodopa (SNr OFF/ON): fEP amplitudes

Like SNr OFF medication, we demonstrated that at SNr recording sites ON medication (n_recording-sites=7) fEP amplitudes progressively increased as the pulse width was increased, and were larger after HFS. The main effects of pulse width [F(3,18)=43.512, P<0.001] and HFS [F(1,6)=14.224, P<0.01] were significant, with a significant interaction between factors [F(3,18)=5.439, P<0.01]. Posthoc pairwise comparisons indicated significant
differences pre-/post-HFS (P<0.001), and across all pulse widths (P<0.05); except between 150µs and 250µs.

Our results further imply that there was a greater synaptic potentiation at SNr recordings sites ON medication, compared to OFF medication. When comparing only post-HFS fEP amplitudes between SNr ON and SNr OFF, the main effect of medication [F(1,28)=5.354, P<0.05] was significant. The main effect of pulse width [F(3,84)=108.538, P<0.001] was also significant, with no interaction between factors. Posthoc pairwise comparisons indicated significant (P<0.05) differences across all pulse widths and ON/OFF medication.

5.4.3 – Plasticity in different basal ganglia output nuclei (GPi/SNr OFF): fEP amplitudes

At GPi recording sites, fEP amplitudes also progressively increased as the pulse width was increased, and were larger after HFS (nrecording-sites=11; Fig. 5.4B). The main effects of pulse width [F(3,30)=129.737, P<0.001] and HFS [F(1,10)=24.006, P=0.001] were significant, with no interaction between factors. Posthoc pairwise comparisons indicated significant (P<0.001) differences across all pulse widths and pre-/post HFS.

Additionally, our results imply that there was a greater synaptic potentiation at GPi recordings sites, compared to SNr OFF medication. When comparing only post-HFS fEP
amplitudes between GPi and SNr OFF, the main effect of location \([F(1,32)=5.677, P<0.05]\) was significant, as was the main effect of pulse width \([F(3,96)=193.059, P<0.001]\), with no interaction between factors. Posthoc pairwise comparisons indicated significant \((P<0.05)\) differences across all pulse widths and between locations.

Figure 5.4 – (A) Effects of levodopa on SNr plasticity, and (B) differential modulation of plasticity between SNr and GPi OFF medication. Like SNr OFF medication, the fEP amplitudes in (A) SNr ON medication \((n_{\text{recording-sites}}=7)\), and in (B) GPi OFF \((n_{\text{recording-sites}}=11)\) increased as the pulse width was increased, and were further enhanced after HFS. Our results also indicated that levodopa further enhanced synaptic plasticity in the SNr, and that synaptic plasticity in the GPi was greater than in SNr. 2-way split-plot ANOVAs comparing post-HFS fEP amplitudes between SNr OFF and (A) SNr ON, as well as comparing SNr OFF and (B) GPi OFF, revealed significant main effects of (A) medication \([F(1,28)=5.354, P<0.05]\), and (B) location \([F(1,32)=5.677, P<0.05]\). Detailed statistics can be found in Results section.
5.4.4 – Clinical correlations

In Fig. 5.5A/B, we demonstrated that patients with higher severity UPDRSIII OFF medication scores had less plasticity (smaller increases in fEP amplitudes following HFS). Accordingly, we found significant inverse linear correlations between plasticity after HFS and both the UPDRSIII total motor subscore ($R^2=0.4766$, $P=0.0015$; Fig. 5.5A) and axial subscore ($R^2=0.4303$, $P=0.0031$; Fig. 5.5B).

In Fig. 5.5C/D, we demonstrated the concurrent improvement of patients’ clinical scores from OFF to ON medication with the increases in plasticity (in all but one patient) after oral administration of one tablet of Sinemet (100/25) in the ON/OFF patient subset. The average values of plasticity and symptom severity in OFF and ON conditions were calculated and displayed in each figure to demonstrate the mean improvement of both variables. The patient that did not have an increase in plasticity had the shortest time between levodopa administration and performing of the stimulation protocol on the second side (26min), therefore there may not have been sufficient time to produce the ON condition (Lewitt et al., 2012; Hauser et al., 2018).

5.5 – Discussion

One main finding of this study is that increasing the stimulation pulse width progressively increased both the amplitude of extracellular inhibitory fEPs, and the durations of neuronal inhibition in the SNr and GPi. The concurrent increases of these
**Figure 5.5 – SNr synaptic plasticity and clinical symptom correlations.** The top figures demonstrate that patients with higher severity UPDRSIII OFF medication scores had less plasticity. There were significant inverse linear correlations between the amount of synaptic plasticity (the difference between pre- and post-HFS fEP amplitudes) and UPDRSIII (A) total motor subscore, and (B) axial symptoms subscore. The bottom figures demonstrate concurrent improvements in clinical symptom severity when the patients were ON, with increases in synaptic plasticity after administration of one tablet of Sinemet (100/25). The bold arrows represent the average changes (from OFF to ON) in both the amount of synaptic plasticity, and UPDRSIII (C) total motor subscore, and (D) axial symptoms subscore.
responses are indicative of a pulse-width-dependent membrane hyperpolarization, likely due to activation of presynaptic terminals and a consequent inhibitory response via GABA\textsubscript{A}-mediated Cl\textsuperscript{-} influx (Dostrovsky et al., 2000; Wu et al., 2001; Filali et al., 2004; Lafreniere-Roula et al., 2010; Milosevic et al., 2017). Since increases in pulse width lead to a larger volume of tissue activation (Butson and McIntyre, 2008), the amount of the hyperpolarization likely increased due to activation of more inhibitory presynaptic terminals. While the fEP amplitudes continued to rise with the pulse width, the silent period appeared to plateau at 150\(\mu\)s. The fEP is a continually increasing population response, while the silent period reflects the focal effects on a single neuron, and synaptically released neurotransmitters are known to saturate their receptors (Clements et al., 1996).

Our findings are consistent with the canonical findings of concurrent suppression of spontaneous neuronal activity with extracellularly recorded positive field potentials elicited by stimulation of caudato-nigral fibers in the substantia nigra of anesthetized cats (Yoshida and Precht, 1971). Precht and Yoshida (1971) further demonstrated the blockage of these responses by picrotoxin, implicating the involvement of GABA. Accordingly, histological findings have confirmed that the predominant afferent innervations of the SNr and GPi are the direct pathway GABAergic projections from the medium spiny neurons of the striatum (Ribak et al., 1979, 1981; Smith and Bolam, 1989; Rinvik and Otterson, 1993; Shink and Smith 1995; Parent and Hazrati, 1995a, b; Bolam et al., 2000; Smith et al. 2001). To a lesser extent, the afferents also include GABAergic
projections from the GPe, and direct pathway glutamatergic projections from the STN. Only ~10% of the total synapses on SNr somata are glutamatergic, while GABAergic projections account for the remaining ~90% (Rinvik and Otterson, 1993; Parent and Hazrati, 1995b). While the stimulation pulses delivered in this study likely led to a nonspecific activation of presynaptic terminals, the results appear to reflect the predominant GABAergic afferent innervation.

The other major novel findings of this study are the elucidations of HFS- and medication-induced enhancements of inhibitory synaptic plasticity in the SNr; which scaled with stimulation pulse width. Our results further indicated that plasticity was differentially modulated in the SNr and GPi. The canonical Parkinsonian “rate model” states that the inhibitory indirect (striatio-nigral/-pallidal) pathway is underactive due to a degenerated dopaminergic system. Here, we demonstrate that after HFS the efficacy of this pathway is enhanced, which may have implications as a compensatory mechanism in the absence of dopamine. We also demonstrated that the efficacy of the pathway is further enhanced in the presence of dopamine after administration of one tablet of Sinemet (100/25). While little is known about the mechanisms of inhibitory (GABAergic) synaptic plasticity, compared to excitatory (involving NMDA and AMPA receptors), there has been substantial progress in this field of research in recent years (see reviews Castillo et al., 2011; Luscher et al., 2011; Kullmann et al., 2012). Potential mechanisms, the involvement of dopamine in plasticity, and the clinical/functional relevance of our findings are discussed below.
5.5.1 – Mechanisms of inhibitory synaptic plasticity

Experimental studies (Clements, 1996) have demonstrated that synaptically released neurotransmitters saturate their receptors; subsequently, the functional strength of GABAergic synapses is proportional to the number of GABA\(_A\) receptors (GABA\(_A\)Rs) on the postsynaptic membrane (Otis et al., 1994; Nusser et al., 1997). Recent research has demonstrated mechanisms of adjustment of GABAergic transmission, with a focus on GABA\(_A\)R-trafficking-mediated plasticity of inhibitory synapses (see Luscher et al., 2011). Studies have revealed that overexpression of the GABA\(_A\)R associated protein (GABARAP; Wang et al., 1999; Chen and Olsen, 2007) facilitates the translocation of GABA\(_A\)Rs to the cell surface of hippocampal neurons (Leil et al., 2004). A study by Marsden et al. (2007) demonstrated in rat slices that activation of NMDA receptors, that induces excitatory long-term depression (LTD) through AMPA endocytosis, simultaneously increases the expression of GABA\(_A\)Rs at the dendritic surface of hippocampal neurons; subsequently increasing amplitudes of miniature IPSPs. The study demonstrated that regulated trafficking of GABA\(_A\)Rs was dependent on GABARAP, as well as on the glutamate receptor interacting protein (GRIP), and Ca\(^{2+}\) calmodulin dependent kinase II (CaMKII). This data reveals that bidirectional trafficking of AMPA receptors and GABA\(_A\)Rs was driven by a single glutamatergic stimulation, which provided a potent postsynaptic mechanism for modulation of synaptic transmission (enhancement of inhibitory transmission).
It is possible then, that the stimulation-induced enhancements of inhibitory plasticity demonstrated in our study were also dependent on the presence and activation of glutamatergic subthalamic afferents of the indirect pathway. Although these glutamatergic afferents only account for ~10%, Marsden et al. (2010) demonstrated that only “moderate” chemical stimulation of NMDA receptors causes exocytosis of GABA\(_{\AA}\)Rs and potentiation of miniature IPSCs. Under such conditions, CaMKII translocates to inhibitory (but not excitatory) synapses that trigger GABA\(_{\AA}\)R insertion and enhance inhibitory transmission. Following “strong” glutamatergic stimuli, CaMKII translocates to excitatory synapses inducing NMDA-receptor-dependent long-term potentiation (LTP). The selective targeting of CaMKII to glutamatergic or GABAergic synapses provides neurons with a mechanism whereby synaptic activity can selectively potentiate excitation or inhibition. In the context of our findings, this may explain why patients with less plasticity had more severe symptoms. The canonical parkinsonian basal ganglia model suggests that symptoms arise from more degenerated GABAergic (direct pathway), and more pronounced glutamatergic (indirect pathway) innervation of the basal ganglia output nuclei (SNr and GPi; Albin et al., 1989; DeLong, 1990). Thus, less plasticity may be observed due to a “stronger” glutamatergic activation which may limit inhibitory potentiation, and would furthermore be associated with more severe symptoms.

Another form of inhibitory LTP has been described in the rat cerebellum at synapses between inhibitory stellate cells and Purkinje cells, termed rebound potentiation, which
leads to a long-lasting (up to 75min) potentiation of GABA_{A}R-mediated IPSCs (Kano et al., 1992; Hirano 2016). Rebound potentiation is a heterosynaptic form of plasticity that is induced by depolarization of excitatory (glutamatergic) climbing fiber synapses. This activation causes an increase in extracellular Ca^{2+} concentration, and a subsequent activation of CaMKII, leading to an upregulation of postsynaptic GABA_{A}R function (Kawaguchi and Hirano, 2000, 2002, 2007; Kitagawa et al., 2009). While rebound potentiation has been found to be critically dependent on CaMKII-dependent alteration of GABARAP, and on GABARAP binding to GABA_{A}Rs, it occurs without measurable changes in cellular distribution or surface expression of GABA_{A}Rs (Kawaguchi and Hirano, 2007).

Finally, one of the first forms of short-term synaptic plasticity to be described may also be relevant; post-tetanic potentiation. However, studies in this domain of research are limited to non-GABAergic synapses (ex. motor end-plates and neuromuscular junctions, calyx of Held, pyramidal neurons, etc.). Nevertheless, post-tetanic potentiation is described as a presynaptic form of synaptic enhancement that occurs after sustained high-frequency synaptic activation (Schlapfer et al 1976; Baxter et al 1985, Wojtowicz & Atwood 1986; Zucker 1989; Zucker and Regehr, 2002; Balakrishnan et al., 2010). It can last several minutes, and becomes longer lasting when the stimulus frequency and duration are increased (Fioravante and Regehr, 2011). Post-tetanic potentiation is believed to work by increasing the probability of neurotransmitter release either as a result of an increase in Ca^{2+} influx (Habets and Borst, 2006), and/or by a protein kinase C
(PKC) -dependent increase in the Ca$^{2+}$ sensitivity of vesicle fusion (Korogod et al., 2007). Of particular relevance to our findings is that post-tetanic potentiation is often only observed following recovery from prolonged stimulation that is accompanied by synaptic depression (Borst et al., 1995; Zucker and Regher, 2002). We have previously demonstrated (Milosevic, et al., 2017) that indeed there is a rapid attenuation of fEPs that occurs during HFS at 100Hz in the SNr (also robustly observed in this study), after which synaptic potentiation occurs, while lower stimulation frequencies that failed to induce depression also failed to induce post-HFS potentiation.

5.5.2 – Dopamine and plasticity

It has been well established that dopaminergic regulation (or lack thereof) of neuronal activity of the basal ganglia direct and indirect pathways plays an important role in Parkinson’s disease. As such, Barone et al. (1987) have provided direct evidence of the presence of D1 dopamine receptors in the terminals of striatal projections to both the SNr and entopeduncular nucleus (homologous to the primate GPi), with GABA being the main neurotransmitter of these projections (Scheel-Krüger, 1986). Rat brain slice studies (6-hydroxy-dopamine; 6-OHDA lesion) have indeed demonstrated that D1 receptor activation by endogenous dopamine (Aceves et al., 1995), exogenous dopamine (Floran et al, 1990), and dopamine synthesized from levodopa (Aceves et al., 1991) enhances GABA release from both striatal projections to the SNr and entopeduncular nucleus. Furthermore, it has been demonstrated that D1 activation and the subsequent facilitated GABA release leads to enhancement of GABAergic IPSCs (Radnikow and Misgeld,
1998), as well as reductions in neuronal firing rates (Timmerman and Abercrombie, 1996) in SNr neurons. D1 receptor activity has been shown to be coupled with the formation of the secondary messenger cyclic adenosine monophosphate (cAMP) in the presynaptic terminals of SNr neurons (Jaber et al., 1996). Subsequently, Arias-Montano et al. (2007) have demonstrated that cAMP formation leads to activation of protein kinase A (PKA), which enhances exocytosis of GABA vesicles by increased presynaptic Ca\(^{2+}\) influx through the involvement of P/Q-type voltage-activated Ca\(^{2+}\) channels.

Immunohistochemical studies have also provided evidence that the primate GPi receives “massive” dopaminergic innervation (Parent and Smith, 1987; Lavoie et al., 1989). Our finding of greater enhancement of fEP amplitudes in GPi compared to SNr may reflect higher levels of endogenous dopamine at GPi terminals. To that point, in the absence of exogenous levodopa, it remains unresolved exactly how HFS elicits changes to synaptic efficacy in the SNr and GPi; whether by inducing postsynaptic changes to the efficacy or surface expression of GABA\(_A\)Rs, or by activation of D1 receptors on presynaptic terminals by endogenous dopamine.

5.5.3 – Clinical and functional implications

Although the SNr is not currently a conventional DBS target, studies (Chastan et al., 2009; Weiss et al., 2011a, 2013) have implicated it as an emerging complementary therapeutic target in Parkinson’s disease patients for the treatment of axial motor symptoms including gait and balance disorders. Our clinical findings suggest that the
SNr may indeed have a pathophysiological role in mediating axial motor symptoms. To that effect, Takakusaki et al. (2003) have demonstrated modulations of both postural muscle tone and locomotion induced by HFS (100Hz) of the SNr in unanesthetized acutely decerebrate cats. Additionally, Burbaud et al. (1998) have demonstrated severe axial postural anomalies induced by microinjections of GABAergic blockers into the SNr of normal primates, whereas Henderson et al. (2005) demonstrated that SNr lesions reversed postural abnormalities in hemiparkinsonian (6-OHDA lesioned) primates, without improvements in bradykinesia. Indeed, GABAergic SNr efferents descend to the brainstem ponto-mesencephalic area, including the pedunculopontine nucleus and mesencephalic locomotor region which are known to be involved in locomotion and postural control (Aziz et al., 1998; Pahapill and Lozano, 2000; Takakusaki et al., 2003, 2005).

In this study, we also demonstrated a direct physiological phase-amplitude relationship between the durations of neuronal inhibition and the amplitudes of fEPs elicited by single pulses. Specifically, the amplitude of the fEP modulates the duration of the hyperpolarization (Fig. 5.2B), and rate of return to baseline firing of the neurons (Fig. 5.2C); which were augmented by pulse width. A study by de Hemptinne et al. (2015) has suggests that one potential therapeutic mechanism of STN-DBS is the disruption of phase-amplitude coupling within the primary motor cortex. We have previously shown (also robustly observed in this study) that delivery of consecutive stimulation pulses (i.e. HFS; ≥30Hz) results in a rapid attenuation of fEP amplitudes, demonstrative of synaptic
fatigue (Milosevic et al., 2017). However, despite a reduction in fEP amplitudes, the neuronal inhibition persists, thus demonstrating a disruption of the local phase-amplitude relationship during HFS, and a subsequent enhancement after cessation.

The results of this study furthermore have implications for a novel control parameter for closed-loop DBS; fEP amplitudes. Since the fEP amplitude scales with medication state, it would be able to inform the DBS system about ON/OFF fluctuations. The system would thereby be able to reduce stimulation intensity when the patient is ON, and increase it when they are OFF or experiencing peak-dosage levodopa induced-dyskinesias. Furthermore, fEP amplitudes are able to inform the system about the efficacy of the inhibitory synapses. As such, if the efficacy drops below a pre-determined threshold, the system could selectively re-potentiate synapses by delivering a train of HFS. Although this would require a permanent embedded system to measure fEP amplitudes, DBS technologies are evolving more rapidly than ever (Arlotti et al., 2016). Russo et al. (2018) have demonstrated an efficacious implementation of closed-loop spinal cord stimulation which controls stimulation dose by measuring the recruitment of fibers in the dorsal column and by using the amplitude of evoked compound action potentials (ECAPs) to treat individuals with chronic pain.

Finally, in Fig. 5.6 we propose a relationship between stimulation pulse width and stimulation frequency for achieving neuronal inhibition by temporal summation of the silent period. With a shorter pulse width, higher stimulation frequencies would be
Figure 5.6 – Proposed relationship between stimulation pulse width and frequency required for neuronal silencing. (A) The figure demonstrates that after HFS, the duration of neuronal inhibition is prolonged, thus, the frequency required for complete neuronal inhibition (by temporal summation) can be reduced. (B) The table displays the frequency required for complete inhibition of neuronal firing across all investigated pulse widths, before and after HFS. The values displayed are the inverses of the average silent period at each pulse width. A hypothesis that stems from this study is that when a patient is ON medication the frequency required for neuronal silencing after HFS would be lower than when they are OFF. This is assumed since we have demonstrated that levodopa enhances fEP amplitudes and that fEP amplitudes were correlated with silent periods.

<table>
<thead>
<tr>
<th>pulse (µs)</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-HFS</td>
<td>43.36</td>
<td>26.44</td>
<td>22.48</td>
<td>20.89</td>
<td>18.20</td>
</tr>
<tr>
<td>post-HFS</td>
<td>54.35</td>
<td>19.67</td>
<td>17.00</td>
<td>14.69</td>
<td>14.06</td>
</tr>
<tr>
<td>ON post-HFS?</td>
<td>&lt;50</td>
<td>&lt;19</td>
<td>&lt;17</td>
<td>&lt;14</td>
<td>&lt;14</td>
</tr>
</tbody>
</table>
how the enhancement of inhibitory synaptic efficacy can be utilized to reduce electrical energy; after HFS-induced potentiation a lower stimulation frequency can be used. Or, if it is preferable to maintain the stimulation frequency, then the pulse width could be reduced instead (from Fig. 5.6; the same frequency can be used with a pre-HFS pulse width of 150µs, as can be used with a post-HFS pulse width of 50µs). This may have implications for moderating DBS side effects, for example, to compensate for sub-optimal electrode placements in order to reduce stimulation of surrounding pathways (i.e. internal capsule or medial lemniscus fibers). Intermittently enhancing the efficacy of inhibitory synapses can allow for a reduction in the volume of tissue activation while maintaining neuronal inhibition, thereby widening the therapeutic window (Reich et al., 2015). With GPi already being a conventional DBS target and recent interest in SNr; we foresee that the enhancement of synaptic efficacy that occurs after HFS will become especially relevant in future applications of DBS with frequent OFF stimulation periods (i.e. closed-loop, intermittent, or cycling-DBS).

5.5.4 – Limitations

A limitation of the present intraoperative study was the inability to use pharmacological interventions to help elucidate specific molecular mechanisms, or the exact pre- or postsynaptic nature of the enhancement of synaptic plasticity. On the contrary, human studies have advantages over animal studies in that it is not known how well animal models correspond to human pathology, or anatomy. In lieu of a control population, our study compared different anatomical locations (SNr/GPi), as well as different medication
states (ON/OFF) within the same disease population. An additional pragmatic limitation was the inability to obtain measurements of synaptic plasticity at the same recording location under both medication states. This would excessively prolong intraoperative time; however, obtaining such measurements would be an interesting prospective study in an appropriate animal model. An additional prospective study would be to investigate the effects of exogenous levodopa on plasticity in the Gpi, as well as to preform clinical correlations in a larger Gpi patient cohort.

5.6 – Acknowledgments

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5.8 – Conflict of Interest

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CHAPTER 6

6 – General discussion and conclusions

In the late 1980’s Benabid et al. (1987) made the observation that Parkinson’s tremor was arrested with high frequency electrical stimulation performed as a test prior to placing a permanent thermo-lesion in the motor thalamus. Shortly thereafter, this observation evolved into today’s well-established DBS therapy. While DBS is now a widely accepted therapy for the management of the motor symptoms of various movement disorders, its early success preceded a detailed scientific understanding of its underlying mechanisms of action. In the work presented in this thesis, we employed a unique dual-microelectrode methodology for measuring neuronal activity (firing rates and silent periods) and synaptic events (extracellularly recorded inhibitory evoked field potentials) in different subcortical target structures during electrical stimulation at different settings with the objective to elucidate the underlying mechanisms of DBS action. Specifically, we sought to elucidate the clinical relevance of these physiological responses by connecting them to patient-specific symptomology and clinical outcomes. A detailed understanding of the underlying mechanisms of DBS could not only improve outcomes and reduce adverse effects, but also lead to further understanding of the
disorders to which it is applied, optimize current implementations, and expand/ensure successful implementation for other pathologies.

6.1 – Scientific contributions

From Project 1 (Chapter 3) we conclude that stimulation induces presynaptic neurotransmitter release, which regulates the level of signal transmission, as well as various forms of synaptic plasticity. We demonstrated that the "inhibitability" of STN and SNr neurons is differentially modulated, likely due to the higher prevalence of GABAergic terminals on SNr somata, although both structures contain primarily GABAergic synapses. Furthermore, the frequency-dependent increases in silent periods indicate that the mechanism of DBS is not a simple temporal summation of inhibitory responses; implying that HFS modulates the behaviour of synaptic transmission. As such, we demonstrated that a robust synaptic potentiation occurred after a 10sec train of continuous stimulation at 100Hz; however, during stimulation ≥30Hz synaptic depression occurred. Although we were unable to record evoked potentials at recording sites within the STN (likely shunted due to the more balanced ratio of glutamatergic to GABAergic synapses present on the somata), a substantial increase in the post-stimulus silent period of STN neurons indicates that HFS modulates the levels of inhibitory signal transmission in the STN as well. Finally, we determined that the "inhibitability" of STN neurons was associated with better clinical outcomes after DBS surgery.
In Project 2 (Chapter 4), we sought to add direct clinical significance to the physiological frequency-dependence of electrical stimulation. We measured neuronal activity in the Vim, while concurrently measuring a robust, real-time symptomatic correlate (tremor) during trains of stimulation at 100Hz and 200Hz. We found that 100Hz was only partially effective at ameliorating tremor and suppressing neuronal firing, while 200Hz had a marked effect on both measurements, and that the amount of neuronal inhibition was correlated with the degree of tremor suppression; concluding that neuronal inhibition was necessary for therapeutic benefit. This idea was further supported by the finding that a brief period of neuronal excitation occurred at the start of stimulation trains, which gave rise to a tremor phase reset / transient exacerbation of tremor. This transient excitation is reflective of the predominance of glutamatergic boutons located on somata of Vim neurons, while the subsequent inhibition of neuronal activity was likely attributable to synaptic depression. As such, the predominance of glutamatergic boutons, and the differences in their transmitter release and reuptake dynamics compared to GABAergic synapses, is the likely explanation for the higher stimulation frequency selectivity of Vim-DBS compared to that of STN- or GPi-DBS.

In Project 3 (Chapter 5), we were interested in expanding upon the findings related to HFS-induced enhancement of synaptic plasticity from Project 2, as well as investigating the stimulation pulse width-dependent effects of electrical stimulation. Indeed, we found that stimulation pulse width augmented the strength of the hyperpolarization of neurons in both the SNr and GPi, and that HFS-induced potentiation occurred in both of these
structures. We further demonstrated that administration of one tablet of levodopa had a potent effect on the enhancement of inhibitory synaptic plasticity at SNr recording sites, and that in the absence of exogenous dopamine, the plasticity was greater at GPi recording sites compared to SNr. This was perhaps due to higher levels of endogenous dopamine at GPi terminals. Finally, we demonstrated that a lack of plasticity in the SNr was associated with higher severity motor symptoms; likely due to more degenerated GABAergic striatino-nigral (indirect pathway) projections.

Taken together, the findings of these studies suggest that several proposed mechanisms of action of DBS co-exist, depending on how stimulation is applied. We’ve demonstrated that singe pulses of electrical stimulation do indeed fit the synaptic modulation hypothesis; activation of inhibitory synapses results in local synaptic inhibition through GABA release (Johnson et al., 2008), whereas activation of excitatory synapses results in local synaptic excitation through glutamate release (Agnesi et al., 2009). However, based on our findings from stimulation of several target structures, it appears that the effects of stimulation are dependent on the weighted composition of inhibitory and excitatory inputs. Thus, the effects of electrical stimulation are site specific, and dependent upon the underlying microcircuit anatomy of the stimulated structure. However, a mechanism which is perhaps more relevant for explaining the therapeutic efficacy of existing DBS implementations is synaptic depression via HFS. It appears as though the selective modulation of inhibitory and/or excitatory afferent inputs becomes less relevant as the stimulation frequency is increased. The overall non-specific effect of increasing the
stimulation frequency is a depletion of synaptic vesicles that exceeds the rate of replenishment, thus inhibiting local neuronal activity. As such, the studies presented in this thesis have not only provided a physiological justification for the DBS frequency selectivity (Earhart et al., 2007) in different applications of the therapy (ex. Vim- vs. STN-DBS), but can also contribute to future implementations in other target structures for other pathologies.

In addition, we have provided insights into a novel DBS mechanism; enhancement of inhibitory synaptic plasticity which occurs after HFS. It has been suggested that persistent changes to synaptic plasticity may play a role in both adaptive and maladaptive forms of pathology of the central nervous system (Kullmann et al., 2012). However, a suitable methodology for direct measures of synaptic plasticity in the human central nervous system has been lacking (Cooke and Bliss, 2006). The studies presented in the works of this thesis represent the very few marquee studies related to direct measurements and descriptions of alterations of synaptic plasticity in Parkinson’s disease, as well as its involvement in the mechanisms of DBS, in an area of vast therapeutic interest; the basal ganglia. Several studies have used paired associative stimulation to investigate plasticity within the primary motor cortex of Parkinson’s disease patients; a paradigm which consists of repetitive low-frequency median nerve stimulation combined with TMS of the contralateral motor cortex. Ueki et al. (2006) demonstrated that dopaminergic medications may restore altered plasticity in Parkinson’s disease; however, in the later stages of the disease, deficient plasticity in patients with
levodopa-induced dyskinesias was not restored by dopaminergic medications (Morgante et al., 2006). The general observation of such studies is that impairment of motor cortical plasticity may play a role in the pathophysiology of Parkinson’s disease. Indeed, we found (see Chapter 4) that lower levels of synaptic plasticity within the SNr were associated with higher severity axial, and global motor symptoms, and in Chapter 5 we demonstrated that administration of low doses of levodopa indeed has a potent effect on the enhancement of plasticity in the SNr. Furthermore, it has been demonstrated that pairing STN-DBS and motor cortical TMS at specific latencies could increase motor cortical excitability (Kuriakose et al., 2009). Similarly, repetitive pairs of GPi-DBS pulse with motor cortical TMS at two specific time intervals produced long term potentiation-like effects in the motor cortex (Ni et al., 2018). As such, these studies postulate that modulation of motor cortical plasticity could be one of the mechanisms of action of DBS. In Chapters 3 and 5 of this thesis, we have provided direct physiological evidence that a train of continuous HFS stimulation in the SNr and/or GPi indeed enhances inhibitory synaptic plasticity within the respective target neurons.

As such, this modulation of synaptic plasticity can have implications for advancing the application of DBS. Enhancement of inhibitory synaptic plasticity can be used to the clinician’s advantage. Periodic enhancement of the efficacy of inhibitory synapses can allow for a reduction in the volume of tissue activation by a reduction in electrical energy delivery (proposed in Chapter 5; Fig. 5.6). This can be used to widen the therapeutic window, for example, by compensating for suboptimal DBS electrode placements in
order to avoid stimulation of non-target neurons, as well contribute to energy savings. We foresee that whether by intention or not, novel applications of DBS technologies (adaptive and closed loop systems) with more frequent OFF stimulation periods will benefit from the synaptic potentiation of inhibitory responses that occurs after HFS. To that point, in the work of this thesis we have proposed two novel control parameters for the control of such closed loop systems; one being Vim tremor cells to selectively suppress tremor (Chapter 4; Section 4.5.3 – Clinical Utility; Fig. 4.7), and the other being fEP amplitudes, which scale with medication and can inform the system about ON/OFF fluctuations (Chapter 5; Section 5.5.3 – Clinical and functional implications). The ability to measure these variables in real-time, however, is hindered by current DBS technology. Successful implementation of these variables as control parameters would require chronic embedded micro-recording capability in future evolutions of DBS technologies. Nevertheless, both of these proposed control parameters have robust and specific clinical correlates. Implementations of adaptive and closed-loop DBS systems have been a topic of interest for over a decade, chiefly focused on the measurement of beta frequency LFP activity. For example, Little et al. (2013) have demonstrated a proof-of-principle implementation of adaptive DBS in a small patient cohort, which measures beta activity in real-time and stimulates only when the amplitude of the beta-power surpasses a pre-determined threshold. The study further found that this beta-dependent adaptive stimulation delivery led to better clinical outcomes than both continuous, and random-intermittent deliveries. Ultimately though, the reliability of beta activity as a robust predictor of clinical symptomology remains in question (Arlotti et al., 2016) and has hindered its widespread implementation. Thus, the control parameters proposed in this
thesis, namely fEP amplitudes, can be of great interest in this field of research; which has largely lacked novel direction.

Furthermore, some of the findings within this thesis may have important clinical implications. Patient-specific stimulation parameters of implanted DBS systems are programmed by neurologists in rather empirical fashion. While stimulation frequency is well known to impact on therapeutic results (Moro et al., 2002) and clinical studies have recently suggested that varying the pulse width can also have an impact on outcome (Reich et al., 2015), the physiology underlying these effects is not well known. The physiological understanding of the frequency-dependent (Chapters 3 and 4) and pulse width-dependent (Chapter 5) effects on neuronal activity within different target structures may be able to help guide programming, as well as provide justification for parameter selections. Furthermore, while SNr-DBS is not a current conventional therapy, recent studies have explored its potential use for the treatment of resistant axial motor symptoms in Parkinson’s disease (Weiss et al., 2013); however, the physiological mechanisms underlying stimulation of SNr neurons are not well studied. We have provided descriptions of the frequency- and pulse width-dependent modulations of neuronal activity and various forms of synaptic plasticity within the SNr. Additionally, we have demonstrated that altered synaptic plasticity within the SNr was significantly correlated with both global, and axial motor symptoms (Chapter 5; Fig. 5.5). Thus, these studies shed light on the underlying mechanisms of action involved in stimulation of the SNr and represent a possible (patho-) physiological justification for its potential more
widespread future implementation. Lastly, we have shown that the location for maximal
tremor suppression within the Vim is the ventroposterior region proximal to the Vim-Vc
border. This finding can help guide neurosurgical planning for DBS electrode placement,
as well as inform the spatial navigation of stimulation delivery in the advent of novel
current steering technologies.

6.2 – Future directions

While the works of this thesis have shed light on several novel physiological mechanisms
of action, there is certainly work to be done. One concept that must be considered, in
addition to the local synaptic activation, is that stimulation of axons can orthodromically
activate downstream synapses (Anderson et al., 2003; McIntyre et al., 2004b).
Simplistically, this is evidenced by the fact that stimulation of medial lemniscus
(afferent) or internal capsule (efferent) fibers has a "downstream" effect; which are
activation of sensory thalamus to produce paresthesia, or activation of lower motor
neurons to produce motor contractions, respectively; two common side-effects of DBS
during the screening process. Thus, in the case of STN-DBS, the stimulation would drive
a high-frequency activation of downstream subthalamo-nigral/pallidal axons. As such, in
using clinically beneficial stimulation frequencies (~130Hz), the downstream neurons
may also be subject to synaptic depression; a novel hypothesis that arises from the work
of this thesis. In that case, STN-DBS would act as a means of “selectively” modulating
the activity of the basal ganglia output structures (Fig. 6.1A and B portray the canonical
basal ganglia rate model in healthy and parkinsonian brains, respectively) by suppressing
the glutamatergic indirect pathway projections only, which are hypothesized to be overactive, and leaving the inhibitory prokinetic direct pathway striato-nigral/pallidal projections (albeit downregulated) intact; acting to “filter” out the pathological overactive STN activity. This might imply then that DBS is most effectively applied to a “gateway” structure within a pathological neurocircuit, in the same way that the Vim is not the origin of pathological tremor-related activity, nor the endpoint, but rather a means to an end; a gate (refer to Chapter 4; Section 4.5.2 – Thalamic gating). Thus, it would be valuable to study these downstream effects of electrical stimulation further. For example, a microelectrode could be used to record activity within the SNr, while stimulating with a macroelectrode in the STN, as these structures are functionally connected, and can easily be simultaneously accessed for stimulation/recording during a typical STN-DBS implantation surgery (see Fig. 3.1). If indeed continuous HFS depresses the downstream glutamatergic projections from the STN to the SNr (/GPi), as suggested above, then one would expect that the output of the basal ganglia would be reduced (i.e. returned closer to normal; as proposed in Fig. 6.1C) from its pathologically overactive state (seen in Fig. 6.2B) since only the inhibitory direct pathway projections would be left intact. Other specific future “translational” research studies can also be pursued based on the findings of the studies in this thesis. For example, a study could be done to clinically determine if the inhibitory potentiation of SNr or GPi neurons may have an effect on motor performance. This could be done by measuring patient performance on certain mUPDRS tasks before and after potentiation. One might expect that enhancing the efficacy of the pathologically downregulated striato-nigral-/pallidal inhibitory projections may also work to reduce the output of the basal ganglia (i.e. return the output closer to normal;
Figure 6.1 – Modulation of the basal ganglia neurocircuit. (A) Neurocircuity of the basal ganglia with a “healthy” output, mediated by dopaminergic projections from the SNc. (B) In the absence of dopamine, the output of the basal ganglia is increased due to an underactive D1-mediated direct (inhibitory) pathway and an overactive D2-mediated indirect (excitatory) pathway. (C) STN-DBS may work by depressing both STN afferents and efferents; thus, leaving only inhibitory direct pathway projections (albeit downregulated) intact. (D) Enhancement of inhibitory synaptic plasticity of the basal ganglia output structures (SNr or GPi), which happens after a train of continuous HFS, may also work to reduce the pathologically overactive basal ganglia output. Excitatory cortico-subthalamic hyperdirect pathway (Nambu et al., 2000) omitted from figures.
as proposed in Fig. 6.1D). An additional prospective study that arises from the work in this thesis is the development of online intraoperative proof-of-principle closed-loop stimulation paradigms which can measure and use neuronal activity as a feedback parameter; such as tremor-related spike bursting in the Vim, to selectively abolish tremor. Finally, the techniques used in this thesis to measure neuronal activity and synaptic events in subcortical structures during stimulation at different parameters can certainly be applied to various other structures in the brain that may be involved in pathology of other disorders. The ability to discern the physiological response properties of various neurons in the brain may be of great importance for developing novel indications for DBS and/or other forms of neuromodulation.

6.3 – Limitations

Electrophysiological studies are traditionally explored with the use of appropriate animal models due to the relative experimental difficulties in obtaining such measurements in humans, the inability to use pharmacological agents to elucidate specific molecular mechanisms, and a lack of healthy controls. Nonetheless, human studies have advantages of their own, most notably due to the lack of association between animal models and human pathology (Wichmann, 2008). For example, neurotoxin animal models such as 6-OHDA and MPTP produce acute degeneration over days or weeks whereas idiopathic Parkinson’s disease develops over years and decades. As such, experiments such as the ones presented in this thesis provide invaluable insights into precise (patho-) physiological properties which can translate directly into the advancement of DBS
technology and patient care. An additional limitation of intraoperative human studies is the relatively short periods of time that experiments can be feasibly carried out. As such, we were unable to determine the time course of the potentiation that occurred after HFS in the SNr and GPi to its full extent; thus, it could not be classified with certainty as LTP. Additionally, microstimulation studies such as these are performed in the range of several seconds to minutes, whereas chronic DBS is continuous. This limitation will remain until conventional DBS technologies evolve enough to contain embedded systems which allow for the ability to record data reliably over long periods of time. Finally, human intraoperative studies are limited by the few recording sites per patient, relative to the number of recording sites that can be obtained in animal studies, over a much larger period of time.

6.4 – Closing remarks

Knowledge of physiological stimulation response properties and astute selection of stimulation target structures can allow for the expansion of DBS applications and the development of novel stimulation paradigms to selectively suppress (by synaptic depression; refer to Chapter 3), facilitate (by synaptic modulation; refer to Chapter 4, Section 4.10 – Supplementary Material), and/or enhance (by augmenting synaptic plasticity; refer to Chapters 3 and 5) transmission of various neurocircuits.
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