SYNAPTIC PLASTICITY IN BASAL GANGLIA OUTPUT NEURONS IN PARKINSON’S DISEASE PATIENTS

by

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ABSTRACT

Parkinson’s disease (PD) is characterized by the loss of dopamine in the basal ganglia and leads to paucity of movements, rigidity of the limbs, and rest tremor. Synaptic plasticity was characterized in the substantia nigra pars reticulata (SNr), a basal ganglia output structure, in 18 PD patients undergoing implantation of deep brain stimulating electrodes. Field evoked potentials (fEPs) in SNr were measured with one microelectrode using single pulses from a second microelectrode ~ 1 mm away. High frequency stimulation (HFS – 4 trains of 2s at 100Hz) in the SNr failed to induce a lasting change in test fEPs amplitudes in patients OFF medication. Following L-Dopa, HFS induced a potentiation of the fEPs that lasted more than 150s. Our findings suggest that extrastriatal dopamine modulates activity dependent synaptic plasticity at basal ganglia output neurons. Dopamine medication state clearly impacts fEP amplitude, and the lasting nature of the increase is reminiscent of LTP-like changes, indicating that aberrant synaptic plasticity may play a role in the pathophysiology of PD.
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LIST OF FIGURES

Fig. 1 Direct and Indirect Pathways of the Basal Ganglia ......................................................5
Fig. 2 Centre-Surround Model of Basal Ganglia Function ..........................................................11
Fig. 3 Basal Ganglia Dysfunction in PD as Predicted by the Rate Model .................................18
Fig. 4 Mechanisms for Changes in Synaptic Transmission during LTP .....................................28
Fig. 5 Microelectrode Apparatus ..............................................................................................47
Fig. 6 Example Neuronal Traces .............................................................................................48
Fig. 7 HFS Stimulation Protocol ............................................................................................50
Fig. 8 Field Amplitude Test Locations ....................................................................................54
Fig. 9 Depth Profile of SNr in PD Patient while ON Medication .............................................55
Fig. 10 Post Stimulus Time Histograms of SNr Neuronal Firing in PD .................................57
Fig. 11 Firing Rate of a SNr Cell during fEP Amplitude Measures .......................................58
Fig. 12 Paired Pulse Measures ...............................................................................................60
Fig. 13 L-DOPA treatment of a Parkinsonian Patient Restores Plasticity ..............................62
Fig. 14 L-DOPA treatment of a Parkinsonian Patient Enhances Plasticity ............................63
Fig. 15 Dopamine Enhances Synaptic Plasticity, Population Data .........................................65
Fig. 16 Plasticity at Basal Ganglia Output ..............................................................................70
Fig. 17 Aberrant Plasticity in L-Dopa-Induced dyskinesia ......................................................78

LIST OF TABLES

Tbl. 1 Patient Characteristics .................................................................................................43
Abbreviations

cAMP – Cyclic Adenosine Monophosphate
CRE – Ca2+/cAMP responsive element
CREB - Ca2+/cAMP responsive element binding protein
DBS – Deep Brain Stimulation
EPSC – Excitatory Post Synaptic Current
fEP – Field Evoked Potential
GABA – Gamma-Aminobutyric Acid
GPe – External Segment of the Globus Pallidus
GPi – Internal Segment of the Globus Pallidus
HFS – High Frequency Stimulation
L-Dopa - Levodopa
LFS – Low Frequency Stimulation
LIDs – Levodopa-Induced Dyskinesia
LTD – Long Term Depression
LTP – Long Term Potentiation
MEP – Motor Evoked Potential
MSN – Medium Spiny Neurons
PAS – Paired Associative Stimulation
PD – Parkinson’s Disease
PPN – Pedunculopontine Nucleus
SC – Superior Colliculus
STN – Subthalamic Nucleus
SM - Sensorimotor
SNARE – soluble N-ethylmaleimide attachment protein receptor
SNC – Substantia Nigra Pars Compacta
SNr – Substantia Nigra Pars Reticulata
UPDRS – Unified Parkinson’s Disease Rating Scale
## 1 INTRODUCTION

### 1.1 General Introduction

### 1.2 Basal Ganglia

#### 1.2.1 Direct Pathway

#### 1.2.2 Indirect Pathway

##### 1.2.2.1 Substantia nigra pars reticulata

#### 1.2.3 Additional Models of Basal Ganglia Function

##### 1.2.3.1 Centre-Surround

##### 1.2.3.2 Connectivity Model

#### 1.2.4 Movement Disorders

### 1.3 Parkinson’s Disease

#### 1.3.1 Etiology

#### 1.3.2 Models of Parkinson’s Disease

##### 1.3.2.1 Rate Model

##### 1.3.2.2 Oscillatory Model

#### 1.3.3 Current Treatments

##### 1.3.3.1 Dopamine Therapy

##### 1.3.3.2 Deep Brain Stimulation

### 1.4 Synaptic Plasticity

#### 1.4.1 Long-term potentiation

##### 1.4.1.1 LTP Mechanisms

#### 1.4.2 Long-term Depression

##### 1.4.2.1 LTD Mechanisms

#### 1.4.3 GABAergic Plasticity

#### 1.4.4 Synaptic Plasticity in the Basal Ganglia

#### 1.4.5 Measuring Plasticity in Human Subjects

#### 1.4.6 LTP and LTD as Models for Behaviour

### 2 OBJECTIVE & HYPOTHESIS

### 3 METHODS

#### 3.1 Patients

#### 3.2 Surgery

#### 3.3 Intraoperative Microelectrode Field Evoked Potentials & Neuronal Recordings

#### 3.4 Stimulation

#### 3.5 Analysis of Neuronal Activity

#### 3.6 Statistics

### 4 RESULTS

#### 4.1 fEP Test Sites

#### 4.2 Field Potential Characteristics

#### 4.3 Paired Pulse Response

#### 4.4 DA Modulation of Synaptic Plasticity in the SNr in PD Patients

### 5 DISCUSSION

#### 5.1 Inhibitory Nature of the Field
5.2 Dopamine and GABA Release ................................................................. 67
5.3 Dopamine and Plasticity at the Basal Ganglia Output .................... 68
5.4 Plasticity and Motor Behaviour ............................................................ 71
5.5 Possible Mechanism for Dopaminergic Modulation of Plasticity in SNr . 72
5.6 Applicability of Findings to the GPi ...................................................... 74

6 CONCLUSION .......................................................................................... 76

7 FUTURE STUDIES .................................................................................. 76
1 INTRODUCTION

1.1 General Introduction

Parkinson’s disease (PD) is a hypokinetic movement disorder characterized by the loss of dopaminergic projections from the substantia nigra pars compacta (SNC) to various targets, including the striatum, the input of the basal ganglia. Reduced dopaminergic input to the striatum is thought to ultimately result in increased neuronal firing of the inhibitory basal ganglia output and disturbed firing patterns with increased synchronization (Albin et al., 1989; Brown, 2003; DeLong, 1990; Levy et al., 2002). Such changes bring about bradykinesia, rigidity, tremor, and postural instability, although the underlying mechanisms leading to these symptoms are still poorly understood. Currently, levodopa (L-Dopa) administration is the most common and effective therapeutic treatment. However, long-term L-Dopa treatment is not without its own serious side effects. Abnormal involuntary movements (dyskinesias) are motor complications that develop following prolonged treatment in the majority of PD patients (Obeso et al., 2000a; Obeso et al., 2000b).

In addition to its dopaminergic nigrostriatal projections, the SNC also sends ventrally projecting dendrites to the SNr (Cheramy et al., 1981; Geffen et al., 1976; Korf et al., 1976; Robertson et al., 1991). However, little is known of the effects of dopamine released from these ventral SNC projections, either in animal models or humans, despite the fact that basal ganglia output structures seem intimately tied to dyskinesia. Deep brain stimulation (DBS) in the subthalamic nucleus (STN), a basal ganglia structure that sends glutamatergic projections to the SNr and GPi, has proven remarkably efficacious as a
treatment of PD and L-Dopa induced dyskinesia (Kleiner-Fisman et al., 2006; Perlmutter and Mink, 2006). While STN DBS does not provide a greater degree of benefit for PD symptoms than optimal therapy with L-Dopa (Krack et al., 2003; Pahwa et al., 2005), it does lessen the time a patient spends in the “OFF” state when the benefit from an individual dose of medication has diminished, and permits the reduction of dopaminergic medications and their adverse side effects including dyskinesia (Jaggi et al., 2004; Kleiner-Fisman et al., 2006; Moro et al., 1999). DBS appears to mimic the effect of beneficial lesions instead of exacerbating the hyperactivity in the basal ganglia output neurons; however, the mechanism of action remains unclear.

Neurophysiological studies in corticostriatal slice suggest that abnormal involuntary movements such as dyskinesia are the result of alterations to synaptic plasticity at the basal ganglia input. Long term potentiation (LTP) at the corticostriatal synapse can be induced with high frequency stimulation (HFS) and reversed with low frequency stimulation (LFS) in healthy adult Wister rats (Picconi et al., 2003; Picconi et al., 2008). LTP is absent in dopamine lesioned (6-OHDA) rats, but can be restored with chronic L-Dopa treatment. Additionally, several paired associative stimulation (PAS) studies have shown that motor evoked potential (MEP) amplitudes in the motor cortex of PD patients are modulated by dopaminergic medication state and that these changes are LTP-like in nature (Morgante et al., 2006; Ueki et al., 2006). PAS increased MEP amplitude in controls but not in patients OFF medication irrespective of their dyskinesia state. L-Dopa administration restored the potentiation of MEP amplitudes by PAS in non-dyskinetic but not dyskinetic patients (Morgante et al., 2006). These findings indicate that LTP-like plasticity is absent from the motor cortex in a dopamine deprived state and,
taken together, these studies in cortex and striatum suggest that a lack of LTP-like plasticity caused by the absence of dopamine may play an important role in mediating the disabling motor symptoms of PD. However, to this point, a suitable methodology for direct measurement of synaptic plasticity in the human central nervous system has been lacking (Cooke and Bliss, 2006).

The aim of this study was to characterize synaptic plasticity at the basal ganglia output during in-vivo recordings in PD patients undergoing implantation of DBS electrodes in the STN. Employing a novel methodology for evoking and measuring field evoked potentials (fEPs) in SNr using a pair of microelectrodes, we found that the amplitude of these positive fEPs was modulated both by tetanizing trains and L-dopa, implicating extrastriatal dopamine actions in the pathophysiology of PD.

1.2 Basal Ganglia

The basal ganglia consist of a group of interconnected subcortical nuclei that function in critical motivation, motor planning, and procedural learning functions (Graybiel et al., 1994; Hikosaka et al., 2000; Yin et al., 2006). Neural circuits within these nuclei form an integral part of the extrapyramidal motor system, and dysfunction of these circuits is associated with many prominent neurological disorders including Parkinson’s disease and Huntington’s disease (Albin et al., 1989; DeLong, 1990), as well as psychiatric disorders such as obsessive-compulsive disorder (Aouizerate et al., 2004).

These subcortical nuclei, organized in a network fashion of stimulatory and inhibitory connections and mediated by a range of neurotransmitters, are responsible for
the processing of cortical input. The primary structures are the substantia nigra pars compacta (SNc) and pars reticulata (SNr), the striatum, the internal and external segments of the globus pallidus (GPi and GPe), and the subthalamic nucleus (STN).

The most prominent model of basal ganglia motor circuit function was originally proposed by (Albin et al., 1989) and (DeLong, 1990) and termed the rate model. Derived from studying human movement disorders, this model is based on the segregation of information processing into direct and indirect pathways, which act in opposing ways to control movement (Figure 1). It describes two parallel cortico-basal ganglia-thalamo-cortical loops that diverge within the striatum and are differentially modulated by dopamine.
Figure 1 Direct and Indirect Pathways of the Basal Ganglia. In the direct pathway, transiently inhibitory projections from the striatum project to tonically active inhibitory neurons of the SNr and GPi, which project in turn to the VA/VL complex of the thalamus. In this pathway the striatum receives transiently excitatory projections from the cortex and substantia nigra. In the indirect pathway, transiently active inhibitory projections from the striatum project to the tonically active inhibitory neurons of the GPe. The influence of the nigral input the striatum is inhibitory in this pathway. The GPe projects to the STN which also receives an excitatory input from the cortex. The STN in turn projects to the GPi, where it transiently acts to oppose the disinhibition of the direct pathway,
1.2.1 Direct Pathway

Sensorimotor cortex (SM) activation results in excitation of the input structure of the basal ganglia, the striatum, via glutamatergic corticostriatal projections. In the direct pathway, the striatum, in turn, sends inhibitory gamma-aminobutyric acid (GABA) projections to the output nuclei of the basal ganglia, the internal segment of the globus pallidus and the substantia nigra pars reticulata (Figure 1). The direct pathway of the basal ganglia is so termed because it directly links the input and output of the basal ganglia with a single GABAergic projection. These output nuclei then send GABAergic efferents to the ventrolateral thalamus, a structure responsible for motor control (Dostrovsky et al., 2002; Parent and Hazrati, 1995a). Thus, SM cortical activity results in excitation of striatal neurons, inhibition of the GPi and SNr, and disinhibition of the motor thalamus since diminished output nuclei activity results in less inhibitory drive to the thalamus.

In addition to glutamatergic afferents from the cortex, the striatum also receives dopaminergic projections from the SNc. Dopamine released in this region binds to the dopamine D1 and D2 receptors, which are anatomically and functionally segregated (Wooten, 2001) and involved in the direct and indirect pathways respectively. In the direct pathway, binding of dopamine to D1 receptors has an excitatory effect on striatal medium spiny neurons projecting to the output nuclei of the basal ganglia (Figure 1).

The D1 receptor subtype is a G-protein coupled receptor, and its activation stimulates adenylate cyclase which in turn activates cyclic adenosine monophosphate (cAMP) and associated cAMP-dependent protein kinases (Missale et al., 1998).
The GPi and SNr also send efferent projections to targets such as the superior
colliculus (SC), involved in oculomotor control (Sparks and Mays, 1990a) (Hikosaka et
al., 2000) and the pedunculopontine nucleus (PPN), a structure increasingly thought to be
involved in movement control (Pahapill and Lozano, 2000; Weinberger et al., 2008a).

1.2.2 Indirect Pathway

As its name implies, the indirect pathway connects the input of the basal ganglia
to the output via two secondary structures: the GPe and STN. In addition to the
striatonigral projections of the direct pathway, the striatum sends GABAergic efferents to
the GPe, which has inhibitory GABA projections to the STN (Figure 1). STN excitation
results in activation of glutamatergic efferents to the GPe and GPi (Kita et al.,
2004; Nambu et al., 2000). Thus, in this pathway, SM activation excites inhibitory striatal
projections to the GPe, which results in less GPe imposed inhibition on the STN,
allowing the STN to excite the output nuclei of the basal ganglia, thereby inhibiting the
premotor centres. Therefore the indirect pathway acts to inhibit movements and is in
opposition to the direct pathway.

As in the direct pathway, dopamine plays an important role in regulating activity
of the indirect pathway. However, unlike the direct pathway, dopamine has an inhibitory
effect on striatal medium spiny neurons projecting to the GPe. Binding of dopamine to
D2 receptors on striatal neurons that project to GPe results in cessation of GABA release.
Like its D1 counterpart, the D2 receptor subunit is a metabotropic G-protein coupled
receptor. In contrast with D1, activation of D2 receptors inhibits the formation of cAMP by inhibiting adenylate cyclase (Missale et al., 1998)

One can thereby envisage that under normal conditions, the direct pathway serves to inhibit the GPi/SNr and facilitate movement, while the indirect pathway tends to prevent or slow movement. Cooperatively the two pathways are thought to regulate thalamocortical neurons and allow movement to be controlled (Parent and Hazrati, 1995a).

### 1.2.2.1 Substantia nigra pars reticulata

The substantia nigra is a brain structure located in the mesencephalon that plays an important role in reward, addiction, and movement. *Substantia nigra* is Latin for "black substance", as parts of the substantia nigra appear darker than neighbouring areas due to high levels of melanin in the dopaminergic pars compacta neurons (Francois et al., 1984).

The pars reticulata of the substantia nigra (SNr), along with the internal segment of the globus pallidus (GPi), are the major output nuclei of the basal ganglia. These cell groups are primarily composed of GABAergic neurons and they integrate inputs from all upstream basal ganglia structures (striatum, GPe, and STN). From rodents to primates (including humans), the SNr and GPi innervate thalamic and brain stem nuclei connected to motor, prefrontal, parietal and temporal associative cortical areas (see review by Deniau et al., 2007) thereby allowing the basal ganglia access to control of motor, cognitive, and emotional/motivational processes (Bar-Gad et al., 2003; Francois et al.,
1984; Francois et al., 2002; Takakusaki et al., 2003). As mentioned above, the SNr also projects to the superior colliculus, which is implicated in orienting behaviour and oculomotor functions (Hikosaka et al., 2000; Nakamura and Hikosaka, 2006a; Nakamura and Hikosaka, 2006b; Sparks and Mays, 1990b), and to regions of the pontine tegmentum, controlling postural tone and locomotion (Grofova and Zhou, 1998; Pahapill and Lozano, 2000; Takakusaki et al., 2004; Weinberger et al., 2008b). Although the precise input–output relationships of SNr neurons remain to be clarified, the spatial distribution of neurons within the SNr and the topographic organization of cortico-striato-nigral projections suggest that the neuronal architecture of SNr provides a mechanism allowing defined corticostriatal inputs to be directed to specific and functionally associated sites in the thalamus, superior colliculus and tegmentum (Deniau et al., 2007).

Inactivation studies have tested how altered SNr activity contributes to parkinsonian motor signs. Interestingly, intra-SNr injections of muscimol, a GABA_A receptor agonist, in the centrolateral region of the SNr improved limb akinesia and bradykinesia in MPTP monkeys whereas injections in the medial region induced saccadic eye movements (Wichmann et al., 2001). Additional results from this group have demonstrated that, while neuronal responses in this region do not always respond directly to passive or active movements, 21% of neurons show movement-related responses. Further, a large proportion of neurons show responses that may be related to memory, attention, and movement preparation (Wichmann and Kliem, 2004). Finally, additional confirmation of the role of the SNr in movement comes from recent clinical DBS studies. SNr stimulation in PD patients has been shown to significantly improve gait and balance (Chastan et al., 2009).
1.2.3 Additional Models of Basal Ganglia Function

1.2.3.1 Centre-Surround

Mink has proposed a model in which the basal ganglia functions through a centre-surround mechanism (Figure 2). This hypothesis is similar to the rate model in that it supports the view that the basal ganglia can be separated into segregated direct and indirect circuits (Mink, 2003). This model posits that the role of the indirect pathway is to broadly inhibit basal ganglia output, while direct pathway activation is more specific and leads to a focused facilitation and surround inhibition of motor programs in thalamus, brainstem and cortex. The anatomical basis for this model is based on the observance of a broad divergence of a single STN neuron onto many GPi/SNr neurons. When voluntary movement is generated, cortical motor areas send a corollary signal to the STN which causes widespread excitation of the GPi and SNr and subsequent inhibition of motor pattern generators for competing postures and movements. Simultaneously, the motor cortex sends signals to the striatum which filters and transforms those signals in a context-dependent manner and then focally inhibits GPi and SNr to remove tonic inhibition from motor pattern generators involved in the desired movement. The output of the basal ganglia acts to focally select desired motor mechanisms and broadly inhibit competing motor mechanisms to allow movement to proceed without interference (Mink, 1996).
Figure 2 Centre-Surround Model of Basal Ganglia Function. Excitatory (green) and inhibitory (red) projections are shown. Relative neuronal efferent activity is shown by thick (high) and thin (low) lines. Input to the striatum or the globus pallidus internal segment (GPi) or the substantia nigra pars reticulata (SNr) can either inhibit (grey) or excite (white) efferent inhibitory neurons. The action of subthalamic nucleus (STN) is also shown. Adapted from (Mink, 2003).
1.2.3.2 Connectivity Model

Using computational network models of the STN and GPe in the indirect pathway, Terman et al. (2002) highlighted the role of the coupling architecture in the network, and associated synaptic conductances, in modulating activity patterns displayed within the network (Terman et al., 2002). In this connectivity model, depending on the arrangements and strengths of synaptic connections within and between cellular populations, different cell firing patterns emerge. These patterns can include clustering, propagating waves, and repetitive spiking. The network can be switched from irregular uncorrelated spiking to correlated rhythmic patterns by increasing striatal input while at the same time weakening intrapallidal inhibition (Bevan et al., 2002; Terman et al., 2002). Therefore, altering the dopamine level could have profound effects on network activity since it could directly alter striatal activity. A shortcoming of this model is that it is limited to a small sub-circuit of the basal ganglia, and as such, fails to predict how changes in synaptic weights (e.g. caused by changes in DA levels) would affect the output of the basal ganglia.

1.2.4 Movement Disorders

Much of the insight into basal ganglia function has been provided from studying human neurological diseases that involve the basal ganglia. Movement disorders that result from basal ganglia damage and/or dysfunction are often dramatic and, depending on the site of the lesion, can cause extreme slowness of movement and rigidity, or
uncontrollable involuntary postures and movements (Mink, 1996). These diseases include degenerative diseases like Parkinson’s or Huntington’s disease, destructive lesions resulting from vascular accidents like hemiballismus, and a variety of conditions of unknown cause that may arise through, for example, changes in synaptic communication.

1.3 Parkinson’s Disease

First described by James Parkinson in 1817, Parkinson’s disease is a progressive neurological disease that affects multiple brain systems that regulate motor function, mood, perception and cognition (Cummings, 1999; Parkinson, 1817). Affecting in excess of 3% of people over the age of 65 worldwide (Zhang and Roman, 1993), PD has a mean age of onset of approximately 60 years (Hughes et al., 1993) with 90-95% of PD cases first manifesting symptoms after age 40 (Lang and Lozano, 1998a).

PD is characterized by the loss of dopaminergic projections from the SNc to various targets, including the striatum, the input of the basal ganglia. Reduced dopaminergic input to the striatum is thought to result in increased neuronal firing of the inhibitory basal ganglia output and disturbed firing patterns with increased synchronization (Albin et al., 1989; Brown, 2003; DeLong, 1990; Levy et al., 2002). It is estimated that 60-70% of dopaminergic neurons are lost by the onset of symptoms (Kish et al., 1988; Lang and Lozano, 1998a).

The main symptoms of PD include: i) tremor at rest; ii) slowness of movement (bradykinesia); iii) paucity of movement (akinesia); iv) muscular rigidity; and, v) abnormally flexed posture with postural instability (Lang and Lozano, 1998a; Lang and
Lozano, 1998b). The manifestation of PD symptoms can be highly variable from one individual to the next. Some may have severe bradykinesia with minimal rigidity, others may have the opposite and still others may have both equally. The diagnosis of PD is made on the basis of clinical criteria that evaluate the presence and asymmetry of these symptoms and a good response to levodopa. However, definitive diagnosis of PD can only be made through a post-mortem neuropathological examination, as there is no biological marker to date that unequivocally confirms the presence of the disease (Lang and Lozano, 1998b).

1.3.1 Etiology

The pathogenesis of PD remains unknown, but pathological, genetic and epidemiologic evidence suggests that several etiologies may result in the PD phenotype.

Lewy bodies are eosinophilic hyaline inclusions that have been suggested to be pathogenic because they are consistently observed post-mortem in selectively vulnerable neuronal populations (Lang and Lozano, 1998a). However, some have argued that Lewy body formation is not specific to PD and the pervasiveness of Lewy bodies has been found to increase in non-PD brains with age (Gibb and Lees, 1988). This would seem to argue against a causal relationship to PD, but it’s possible that Lewy bodies in non-PD brains are a precursor to disease.

Excitotoxic mechanisms have also been implicated in SNc degeneration. Excessive N-methyl-d-aspartate (NMDA) receptor activation can trigger, through a cascade of events, augmented intracellular calcium concentrations, mitochondrial DNA
damage, and eventually cell death (Dawson and Dawson, 2004). These models are consistent with findings of selective preservations of SNC dopamine neurons that are capable of buffering changes in intracellular calcium levels (Damier et al., 1999; Lang and Lozano, 1998a).

Other PD-pathogenic models propose that dysfunction in the electron transport chain in mitochondria leads to reduced energy production and eventually to cell death. This predisposes dopamine neurons to toxic insults or genetic deficiency and increases the vulnerability of these neurons to apoptosis (Lang and Lozano, 1998a). These models are supported by evidence that 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin known to selectively kill dopamine neurons, inhibits complex 1 of the electron transport chain (Beal, 2003). Furthermore, PD subjects show a 30-40 % decrease in complex 1 activity in SNC (Mann et al., 1992).

Evidence supporting a genetic etiology for PD is derived from genotyping kindreds with rare inherited forms of the disease. Certain gene mutations are associated with PD, i.e. α-synuclein, LRRK2, parkin, DJ-1 and PINK. Autosomal dominant mutations in α-synuclein lead to aggregation of α-synuclein proteins and is thought to be the precursor for Lewy body formation (Cookson, 2005). Also inherited in an autosomal dominant manner, LRRK2 is a protein kinase and overactivity of protein kinases can mediate neurotoxicity (Klein and Lohmann-Hedrich, 2007). Parkin is an E3 ubiquitin ligase and a parkin mutation is thought to impair the ubiquitin-proteosome-mediated hydrolysis of damaged or misfolded proteins (Cookson, 2005; Eriksen et al., 2005). Therefore mutations in the parkin gene might cause accumulation of proteins, some of which may be neurotoxic, and may also be causal for Lewy body formation. The gene
product functions of DJ-1 and PINK remain unclear but both appear to protect against mitochondrial damage (Martella et al., 2009). Mutations in DJ-1 or PINK would render dopamine neurons susceptible to mitochondrial damage, leading to cell death.

Finally, epidemiological studies have reported that living in a rural area, drinking well water, farming, and exposure to pesticides may be risk factors for developing PD (Priyadarshi et al., 2001), suggesting there are environmental factors found in rural environments that may cause PD.

Despite a large body of work, PD remains an incurable, progressive, idiopathic movement disorder with several suspected or implicated etiologies, the hallmark of which is loss of dopamine neurons from the basal ganglia.

1.3.2 Models of Parkinson’s Disease

1.3.2.1 Rate Model

As mentioned above, the rate model of basal ganglia activity is based on the segregation of information processing into direct and indirect pathways, which act in opposing ways to control movement (Figure 1). Originally proposed by (Albin et al., 1989) and (DeLong, 1990), the rate model was derived from studying animal models of movement disorders and describes two parallel cortico-basal ganglia-thalamo-cortical loops that diverge within the striatum and are differentially modulated by dopamine. In PD, the rate model postulates that with the loss of dopaminergic input to the striatum, there is a reduced drive in the direct pathway from the striatum to the output nuclei of the basal ganglia, and an increased drive in the indirect pathway through GPe and STN.
These alterations in activity result in the increased activity of basal ganglia output nuclei, ultimately resulting in the excess inhibition of thalamic and cortical activity which impairs voluntary movements (Figure 2). This model predicts that parkinsonian symptoms should be improved by the ablation or inactivation of the STN and GPi. When this was proven to be the case in MPTP-treated monkeys (Aziz et al., 1992; Bergman et al., 1990), these structure became key targets for DBS therapy (discussed in more detail below).

However, the rate model appears incomplete as it fails to explain why a pallidotomy, which reduces the inhibitory basal ganglia output to the thalamus, improves symptoms for both hypokinetic and hyperkinetic movement disorders (Marsden and Obeso, 1994). Additionally, anatomical studies in the basal ganglia have revealed i) a subpopulation of MSNs that co-express D1 and D2 receptors (Surmeier et al., 1996), and ii) striatal neurons projecting to the GPi and SNr can also send axon collaterals to the GPe (Kawaguchi et al., 1990), suggesting that the direct and indirect pathways are not completely segregated.

Furthermore, dopamine can have dramatic effects in regions of the basal ganglia other than the striatum. Indeed, nigral dopamine depletion has been shown to impair motor performance independent of striatal dopamine neurotransmission, while increased nigral dopamine release can counteract striatal dopamine impairments (Andersson et al., 2006).
Figure 3 Basal Ganglia dysfunction in Parkinson’s disease as predicted by the rate model. In PD, the dopaminergic inputs provided by the SNC are diminished (thinner arrows), making it difficult to generate the transient inhibition from the striatum. This ultimately results in an increased activity in the output nuclei leading to increased inhibition on the glutamatergic excitation of the motor cortex and a subsequent reduction in movement, observed in patients as bradykinesia.
1.3.2.2 Oscillatory Model

Popularized by Brown’s group in London, the oscillatory model of PD postulates that excessive and oscillatory synchronization of neuronal activity occurs in the basal ganglia in PD and that this activity has a predilection for the beta frequency band centred around 20 Hz (Brown, 2003). This hypothesis has gained strength on the support of intra-operative recordings in PD patients that demonstrate locking of neuronal discharges in the STN to beta oscillatory local field potentials (Kuhn et al., 2005; Weinberger et al., 2006; Weinberger et al., 2009). How this synchronization ultimately impairs motor function is unclear but one idea is the “noisy signal hypothesis”. In the parkinsonian state, only partial processing is possible in the basal ganglia as the synchronous activity effectively acts as a disruptive ‘noisy signal’ and is worse than a fixed and unfamiliar patterning of activity when passed on to other processing units like the cortex (Brown and Eusebio, 2008).

Since this model’s inception, it has become clear that beta synchrony may relate to some but not all elements of motor impairment in Parkinson's disease, and the jury is still out on its quantitative importance and the means by which it might disturb motor processing (Eusebio and Brown, 2009). One thing that seems reasonably clear, however, is that beta synchrony is a good biomarker of the conventional akinetic-rigid state in both patients (Hammond et al., 2007) and many animal models of parkinsonism (Costa et al., 2006; Mallet et al., 2008b; Mallet et al., 2008a; Sharott et al., 2005).

An unresolved question is whether there is something particularly important about certain frequencies of pathologically synchronized oscillation or whether it is the
oscillatory synchronization per se, rather than the precise frequency that is more relevant. Most patients show evidence of synchronization in the beta frequency band, but this tells us more about the resonance frequencies of circuits in the absence of dopaminergic input than whether synchronization with a lower or higher frequency might be just as pathogenic if it were to occur. Indeed, one report suggests that within certain limits (8–35 Hz), changes in synchronization rather than frequency correlate better with levodopa-induced improvement in bradykinesia and rigidity (Kühn et al., 2009). At even higher frequencies, however, there seems to be no antikinetic effect, but rather a possible favouring of movement (Brown, 2003).

1.3.3 Current Treatments
1.3.3.1 Dopamine Therapy

The dopamine precursor levodopa (L-Dopa) was discovered in the 1960’s (Cotzias et al., 1967) and remains the most effective drug for controlling PD symptomatology. L-Dopa is typically administered orally in combination with a dopa-decarboxylase inhibitor (such as benserazide or carbidopa) to prevent its metabolism prior to crossing the blood brain barrier (Boshes, 1981). Once across this barrier, L-Dopa is internalized by residual nigral dopaminergic neurons and converted to dopamine by aromatic L-amino-acid dopa-decarboxylase. Once converted and packaged in vesicles, dopamine can be released to stimulate dopamine receptors on post-synaptic striatal cells (Thanvi and Lo, 2004) and restore some semblance of its original function. L-Dopa significantly improves bradykinesia and akinesia (Vingerhoets et al., 1997) and also
improves, to varying degrees, rigidity and tremor, hypometria, the performance of complex tasks, and the generation of internally cued movements (Beckley et al., 1995; Benecke et al., 1987; Burleigh-Jacobs et al., 1997; Yuill, 1976).

An unfortunate side effect of L-Dopa usage is that patients typically develop severe and uncontrollable motor fluctuations, called dyskinesias, after prolonged exposure (Obeso et al., 2000a; Obeso et al., 2000b). L-Dopa-induced dyskinesias are observed in the majority of patients who have been treated for 5–10 years with L-Dopa (Schrag and Quinn, 2000). These motor complications are difficult to treat and become a major contributor to disability in some patients. Why long-term use of L-Dopa results in dyskinesias remains unclear (Dunnett, 2003). Dyskinesias could be the result of strong compensatory processes by which the striatum adapts to disruption of the nigral dopaminergic projections to the striatum (Zigmond et al., 1990), such as increasing presynaptic dopamine release and the sensitivity of postsynaptic dopamine receptors on striatal neurons (Ungerstedt, 1971; Zigmond et al., 1990). In this scenario, dyskinesias would arise when agonist drugs or L-Dopa interact with sensitized receptors. However, dyskinesias can develop independent of the level of denervation and receptor sensitivity (Dunnett, 2003). Alternatively, dyskinesia may be the result of an aberrant form of synaptic plasticity in striatal neurons, related to L-Dopa acting on the dopaminergic regulation of plasticity at corticostriatal synapses (Calabresi et al., 2007; Centonze et al., 1999; Picconi et al., 2003; Picconi et al., 2005), a hypothesis that will be expanded upon in later sections.

One strategy to reduce the risk for motor complications is the use of dopamine receptor agonists which have been proven safe and effective as initial therapy in early
stages of Parkinson's disease. However, it is still controversial whether the use of these agonists must be started early, as opposed to initiation only after the L-Dopa complications develop (Ahlskog, 2003). Dopamine agonists such as bromocriptine, pergolide and apomorphine have a longer half life than levodopa and bind at post-synaptic receptor sites independently of the dopamine terminal (Junghanns et al., 2004), thereby reducing receptor sensitivity and the development of motor complications. However, dopamine agonists are not without their own adverse effects, including nausea, hypotension, hallucinations and edema.

1.3.3.2 Deep Brain Stimulation

Surgical treatment of PD using deep brain stimulation (DBS) can provide additional help for selected patients whose symptoms are not controlled sufficiently by medication. DBS has progressively replaced brain lesioning, such as thalamotomies and pallidotomies, over the last 20 years (Limousin and Martinez-Torres, 2008). DBS in the ventro-intermediate nucleus of the thalamus was the target for these early procedures, and was performed contralateral to thalamotomies to reduce morbidity of bilateral procedures, primarily on speech and balance (Benabid et al., 1987).

The procedure involves implantation of an electrode into the target region using stereotactic neurosurgical techniques (detailed in (Lemaire et al., 2007). The electrode lead is then connected with an extension wire to a programmable pulse generator that is implanted below the clavicle. The stimulation parameters are programmed to achieve maximal clinical benefit using non-invasive radio-telemetry. The clear advantage of DBS
over lesioning is that there is minimal destruction of brain tissue and the electrode can be potentially removed or repositioned without creating permanent damage (Lozano and Mahant, 2004).

While thalamic DBS provided a positive effect on tremor, it provided a limited effect on other cardinal PD symptoms (Limousin and Martinez-Torres, 2008). This limited effectiveness led to the application of the DBS procedure on new targets, the STN and G Pi. The G Pi was chosen based on the noted similarities of the effect of a lesion and HFS to the thalamus and the familiarity on the effect of pallidotomies (Laitinen et al., 1992). The STN was chosen based on research on MPTP-treated monkeys; these animals exhibited excessive STN activity (Bergman et al., 1994) and improvement of parkinsonian symptoms with lesions or STN HFS (Aziz et al., 1992; Bergman et al., 1990).

STN has increasingly become the preferred target for DBS for PD as it has been found to have a positive effect on a wide range of symptoms. OFF motor symptoms can show a dramatic improvement of 40%-60% (Limousin and Martinez-Torres, 2008) while bradykinesia, rigidity, tremor, and axial symptoms also improve (Kleiner-Fisman et al., 2003; Krack et al., 2003; Schupbach et al., 2005). L-Dopa-induced-dyskinesias also improve over time, mostly due to a reduction in medication dosage in the range of 30% to 50% (Limousin and Martinez-Torres, 2008).

While the therapeutic benefits of DBS are clear, its mechanism of action remains debatable. It is unclear whether the therapeutic effects are local or system-wide, or even whether the effects are related to inhibition or excitation. Several studies have reported inhibition of neuronal activity locally during HFS in the G Pi (Dostrovsky et al., 2000)
and STN (Filali et al., 2004) of humans, and Gpi in primates (Boraud et al., 1996). Other studies have examined the effect of HFS on downstream targets, and the findings suggest an activation of efferent axons either directly or through activation of local cell bodies to axon initial segments (Anderson et al., 2003; Hashimoto et al., 2003). Recent work has shown that HFS affects other properties of firing patterns such as oscillatory and burst activity (Brown et al., 2004; Dorval et al., 2008; Kuhn et al., 2006; Xu et al., 2008) suggesting that DBS may suppress the proposed pathological patterns of activity in the basal ganglia.

Presently, there is no surgical alternative to DBS. Dopaminergic cell transplant and intraputaminal delivery of glial cell line-derived neurotrophic factor have shown some improvement in a limited patient set, but these procedures have been stopped because of side effects, such as worsening dyskinesia, and inconsistencies of the treatment effect (Gill et al., 2003). Other forms of restorative treatment, in particular gene therapy, are under investigation (Kaplitt et al., 2007). These approaches might someday replace DBS for many patients.

1.4 Synaptic Plasticity

Neurons connect to one another via synapses, which are the primary site of information transmission in the nervous system. Information storage, including memory and behavioural adaptation, is believed to emerge from changes in neuronal transmission, both over short- and long-term time frames, a property known as synaptic plasticity. The earliest hypotheses on mechanisms of information storage date back to the late 19th century, where theorists proposed that increased utilization of circuits either strengthened
existing circuits or promoted the formation of new circuits (Tanzi, 1893). Hebb famously put forward the modern formulation of this theory stating that “when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased” (Hebb, 1949).

We now know that synaptic plasticity takes many forms and occurs in many regions of the central nervous system. Plastic change can occur and decay rapidly and involves post-translational modifications of synaptic proteins while being protein-synthesis independent (Raymond, 2007). Additionally, some forms of plasticity can be dependent on protein synthesis but independent of gene transcription, while other forms can be more persistent and depend on both gene transcription and protein synthesis (Raymond, 2007). It is a highly regulated process emerging from complex interactions occurring not just at the synapse, but also on a molecular, cellular, and system level.

1.4.1 Long-term potentiation

The central nervous system uses short- and long-term changes in synaptic strength in neuronal circuits to process large amounts of information. One such change, or activity-dependent modification, is long term potentiation (LTP). Studied extensively in the hippocampus, LTP is a sustained increase in synaptic strength that is elicited following short trains of high frequency or patterned stimulation. LTP has long been thought to play a crucial role in memory formation and learning as a result of its properties (rapid generation, input specificity, associativity, and long lasting nature)
(Bliss and Lomo, 1973; Nicoll et al., 1988) and the observation that LTP-like activity occurs in brains of animals learning a behavioural task (Berger, 1984; Moser et al., 1994). Further evidence for a link between LTP and learning and memory is provided by studies where pharmacological blockade of LTP disrupts behavioural learning (Davis et al., 1992).

1.4.1.1 LTP Mechanisms

Classically, activity-dependent LTP occurs at glutamatergic synapses containing the NMDA receptor. This receptor is permeable to $\text{Ca}^{2+}$, but is blocked by physiological concentrations of $\text{Mg}^{2+}$. Depolarization expels $\text{Mg}^{2+}$ from the NMDA receptor channel which in turn allows $\text{Na}^{+}$ and $\text{Ca}^{2+}$ ions to pass into the postsynaptic cell (Nicoll, 1998). The $\text{Ca}^{2+}$ ions that enter the cell serve as a second messenger and activate postsynaptic protein kinases (Nicoll and Malenka, 1999). Through a cascade of events, these kinases can act postsynaptically to cause insertion of new AMPA receptors into the postsynaptic spine, thereby increasing the postsynaptic cell’s sensitivity to glutamate (Malenka and Bear, 2004). These changes occur rapidly, are post-translational in nature, and can last for hours to days (Malenka and Bear, 2004) (Figure 4).

Ultimately, the changes resulting from the interactions of various signal transduction pathways can be translated into even longer-term effects by downstream changes in gene expression. This late phase of LTP is initiated by transcription factors such as CREB, which mediates gene expression through a $\text{Ca}^{2+}$/cAMP responsive element (CRE) on target genes (Squire and Kandel, 1999). CREB is continuously
expressed but can only bind CRE and influence transcription upon its phosphorylation (Gonzalez and Montminy, 1989; Montminy and Bilezikjian, 1987; Yamamoto et al., 1988). Phosphorylation of CREB occurs via multiple pathways; activated PKA can rapidly phosphorylate CREB (Gonzalez and Montminy, 1989), as can multiple Ca\(^{2+}\)/calmodulin-dependent kinases (CaMKs) (Bito et al., 1996; Dash et al., 1991; Kasahara et al., 2001). Functionally, by altering gene expression, this late phase LTP can produce additional transcriptional regulators and even aid new synapse construction (Engert and Bonhoeffer, 1999) (Figure 4).
Figure 4 Mechanisms responsible for long-lasting changes in synaptic transmission during LTP. A) Depolarization removes the Mg$^{2+}$ block from the NMDA receptor and allows Ca$^{2+}$ entry. Ca$^{2+}$ activates postsynaptic protein kinases which, through a cascade of events, can act to insert new AMPA receptor into the postsynaptic terminal. B) The late component of LTP alters gene expression. PKA or CaMKs phosphorylate and activate CREB, a transcriptional regulator, which can turn on specific genes that can alter synapse structure and affect the activity of various transcriptional regulators. (Adapted from Squire and Kandel, 1999)

In addition to postsynaptic changes, presynaptic changes can cause increased or decreased synaptic activity. By altering the release properties of vesicles carrying neurotransmitter, the synapse has an additional site and mechanism for regulation. Best understood in the gill withdrawal response of Aplysia, presynaptic facilitation can produce enhanced synaptic activity independent of the postsynaptic LTP response discussed above. A tail shock activates sensory neurons which in turn excite serotonergic
interneurons (Abrams, 1985). Serotonin release activates adenyl cyclase and produces cAMP (Abrams, 1985). cAMP then activates PKA which phosphorylates key targets involved with exocytosis, such as P/Q-type voltage-activated Ca\(^{2+}\) channels (to enhance Ca\(^{2+}\) influx), synapsins (to enhance vesicle trafficking), and soluble N-ethylmaleimide attachment protein receptor (SNARE) (to enhance vesicle docking, priming, and fusion) (Arias-Montano et al., 2007). As in the general LTP mechanism discussed above, prolonged serotonergic stimulation and subsequent PKA activation can result in changes in gene expression during presynaptic facilitation (Pittenger and Kandel, 2003). PKA activates CREB, and it can also activate another kinase, p42 MAPK (Martin et al., 1997). Both CREB and p42 MAPK can move to the nucleus of the presynaptic cell and bind / phosphorylate key targets which have various downstream effects, including alterations of PKA activity and synapse growth.

Another cAMP-dependent presynaptic form of LTP has been described in the hippocampus at mossy fiber synapses, the junction between the axons of dentate gyrus granule cells and the proximal apical dendrites of CA3 pyramidal cells. Similar to the gill withdrawal response in Aplysia, mossy fiber LTP involves a PKA-dependent, long-lasting modification of the presynaptic release machinery, ultimately causing an increased probability of transmitter release as well as the recruitment of new or previously silent release sites (Reid et al., 2004; Tong et al., 1996). This form of LTP does not require the activation of NMDARs. Rather, it relies on an activity dependent rise in intracellular calcium concentration in presynaptic terminals and it appears that activation of presynaptic kainate receptors by endogenous glutamate plays a role in facilitating mossy fiber LTP (Contractor et al., 2001; Lauri et al., 2003; Schmitz et al., 2003).
1.4.2 Long-term Depression

For changes in synaptic plasticity to be useful, processes other than LTP must exist that serve to selectively weaken synapses. Long-term depression (LTD) has been described as one such way to decrease synaptic strength. Like LTP, LTD has been studied extensively in the CA1 region of the hippocampus. Much study has also focused on LTD at Purkinje cells of the cerebellum. While the LTD mechanisms are not simply the mechanistic reversal of LTP, the net effect at excitatory synapses is the internalization of AMPA receptors on the postsynaptic membrane, thereby decreasing the postsynaptic cell’s sensitivity to glutamate (Malinow and Malenka, 2002). Although some collective processes exist, molecular, biochemical, electrophysiological and pharmacological studies all point to several distinct induction and maintenance mechanisms for this form of synaptic plasticity (Braunewell and Manahan-Vaughan, 2001).

1.4.2.1 LTD Mechanisms

LTD is best understood in terms of two mechanisms; LTD triggered by NMDAR activation, and LTD triggered by mGluR activation. Whereas NMDAR dependent LTP in the CA1 region requires brief high frequency stimulation, NMDAR dependent LTD in this region is induced by much longer trains (>900 stimuli) of low frequency stimulation (0.5-5 Hz) (Malenka and Bear, 2004). Inhibition of NMDARs blocks LTD (Dudek and Bear, 1992), and activation of NMDARs induces it (Cummings et al., 1996; Kandler et al., 1998; Li et al., 2004). As mentioned above in the LTP discussion, NMDARs allow
Ca2+ entry into the cell. Furthermore, intracellular uncaging of Ca2+ via photolysis is sufficient to induce LTD (Yang et al., 1999). Therefore, Ca2+ entering the postsynaptic cell through the NMDAR as a trigger for LTD emerged as a model. Like LTP, however, the quantitative characteristics of the postsynaptic Ca2+ signal required to trigger LTD remain to be determined (Malenka and Bear, 2004). Once inside the postsynaptic cell, Ca2+ activates protein phosphatases. These phosphatases act to dephosphorylate key targets, including PKC and PKA substrates (Hrabetova and Sacktor, 2001; Kameyama et al., 1998; van Dam et al., 2002) and AMPARs (Lee et al., 1998; Lee et al., 2000). This dephosphorylation ultimately leads to either lower AMPAR channel opening probability (Banke et al., 2000) or rapid internalization of AMPARs (Banke et al., 2000; Beattie et al., 2000; Carroll et al., 1999; Lee et al., 2002).

Mechanistically distinct forms of mGluR-dependent LTD are also found in the CA1 region of the hippocampus (Oliet et al., 1997), as well as in Purkinje cells of the cerebellum (Ito et al., 1982). In these cases, glutamate binds and activates mGluRs on the postsynaptic membrane, a G-protein coupled receptor capable of inducing signal transduction cascades. mGluR activation produces several second messengers which in turn activate PKC. PKC then phosphorylates key substrates on certain AMPA receptors leading to endocytosis of AMPA receptors comprised of the subunits GluR2 and GluR3 (Chung et al., 2003; Wang and Linden, 2000).

A presynaptically expressed form of mGluR-dependent LTP has also been described in the hippocampus. Treatment with the mGluR agonist DHPG [(RS)-3,5-dihydroxyphenylglycine] causes a long-lasting increase in paired-pulse ratios (paired pulse facilitation) and decrease in the success rate of dendritically recorded EPSCs.
without affecting their potency (Fitzjohn et al., 2001). The phenomena of paired-pulse facilitation and depression are well known forms of synaptic plasticity. They are expressed in electrophysiological experiments as changes in the amplitude of a test EPSC evoked by a second presynaptic spike that follows the first (conditioning) one in the paired-pulse paradigm (Zucker and Regehr, 2002).

Further experiments found that presynaptic vesicle release was reduced under these conditions (Zakharenko et al., 2002), while the postsynaptic sensitivity to AMPA and glutamate remained unaltered (Rammes et al., 2003; Tan et al., 2003).

1.4.3 GABAergic Plasticity

Like excitatory connections, many recent studies have indicated that activity-dependent forms of synaptic plasticity, such as LTP and LTD, can play a role in the establishment and regulation of functional inhibitory synaptic connections (Gaiarsa, 2004). As described above, long term changes in the strength of synaptic efficacy at excitatory synapses can be accounted for by at least three non exclusive mechanisms: i) modifications in the probability of transmitter release, ii) modifications in the number or properties of receptors at functional synapses, and iii) modifications in the number of functional synapses through either pre- or post-synaptic mechanisms as a result of changes in gene expression. Of these mechanisms, the first two have also been characterized at inhibitory GABAergic synapses and will be covered below.

Changes in the probability of transmitter release have been reported at GABAergic synapses (Caillard et al., 1999a; Caillard et al., 1999b), with modifications
occurring in the number of functional releasing sites. One study showed that ~30% of postsynaptic GABA_A receptors are associated with non-functional presynaptic terminals in hippocampal cultures (Kannenberg et al., 1999), suggesting the existence of presynaptically silent GABAergic synapses. Such a scenario might indicate a presynaptic mechanism of switching on or off neurotransmitter release (Gaiarsa et al., 2002).

Changes in the properties of receptors via alterations in postsynaptic intracellular Ca^{2+} levels (and subsequent signal transduction cascades) have also been reported at GABAergic synapses (Gaiarsa et al., 2002). In the CA1 region of the adult hippocampus, HFS induces NMDA-dependent LTP of GABAergic synapses (Wang and Stelzer, 1996). The suggested mechanism involves an increase in the efficacy of postsynaptic GABA_A receptors, induced by activation of the Ca^{2+}-sensitive phosphatase calcineurin (Lu et al., 2000).

Similarly, changes in the number of available GABA receptors have been shown to affect the synaptic efficacy at inhibitory synapses. In an experimental model of temporal lobe epilepsy, a direct relationship between the number of synaptic GABA_A receptors and the quantal size at potentiated GABAergic synapses has been found in the adult dentate gyrus (Nusser et al., 1998). Insertion of new GABA_A receptors is thought to underlie the increase in amplitude of IPSCs. Additional evidence of this mechanism occurring at GABAergic synapses comes from cultured hippocampal cells. Blocking clathrin-dependent endocytosis of GABA_A receptors in these cells causes a large increase in quantal size (Kittler et al., 2000).

In summary, several mechanisms for the induction and maintenance of long-term plasticity have been reported at inhibitory synapses in different brain regions. Not
surprisingly, all of these forms of plasticity, like their excitatory counterparts, are triggered by changes in intracellular Ca\textsuperscript{2+} concentrations.

1.4.4 Synaptic Plasticity in the Basal Ganglia

While the hippocampus has long been the focus of plasticity studies, many other brain regions have been shown to undergo plastic changes, including the basal ganglia. To this point, the majority of work on synaptic plasticity in the basal ganglia has been directed at the glutamatergic corticostriatal synapse, primarily because of the positioning of the striatum as a major input structure of the basal ganglia. Notably, this synapse is also under the neuromodulatory control of dopamine. Resultantly, much work has been focused on the hypothesis that dopamine plays a key role in long-lasting changes in neural responses occurring in this region (see review by (Wickens, 2009)).

Indeed, evidence from the Calabresi laboratory strongly supports a role of synaptic plasticity in PD pathology and/or symptomatology, brought about by changes in striatal dopamine levels as a result of the disease’s progression. This group reported that corticostriatal LTD could not be induced in slices prepared from 6-OHDA dopamine-depleted rats, but could be restored by bath application of exogenous dopamine, or co-application of both D\textsubscript{1} and D\textsubscript{2} receptor agonists (Calabresi et al., 1992; Calabresi et al., 1994). Furthermore, LTD could be prevented from occurring at the corticostriatal synapse in normal slices by pretreatment with either D\textsubscript{1} or D\textsubscript{2} antagonists (Calabresi et al., 1992; Calabresi et al., 1994). These long-lasting reductions in synaptic strength are thought to be initiated postsynaptically but expressed presynaptically via a 2\textsuperscript{nd} messenger,
here an endocannabinoid that travels from the postsynaptic cell and activates presynaptic
CB1 receptors (Kreitzer and Malenka, 2008).

The basic model that has emerged is that HFS in or near the dorsolateral striatum
stimulates both glutamatergic and dopaminergic fibers while also activating L-type Ca2+
channels. HFS-induced elevations of glutamate activate postsynaptic mGluRs, while
increases in dopamine activate D2 receptors. Activation of mGluRs and L-type Ca2+
channels leads to endocannabinoid production and release (Hashimotodani et al., 2005)
while D2 receptor activation serves to enhance the production of endocannabinoids
(Giuffrida et al., 1999). Endocannabinoids are then released from striatal MSNs and
activate CB1 receptors of the excitatory presynaptic cell leading to the induction of
presynaptic inhibition of neurotransmitter release and LTD (Ronesi et al., 2004).

A study using intrastriatal microstimulation obtained results that indicated HFS
could induce robust LTD at indirect-pathway MSNs, but not at direct-pathway MSNs
(Kreitzer and Malenka, 2007). Further, the same study found that direct activation of
mGluRs gave rise to an endocannabinoid-mediated inhibition in indirect pathway but not
direct pathway MSNs, suggesting that indirect-pathway MSNs more readily release
endocannabinoids. Furthermore, the study found that LTD at this synapse is not induced
by depolarization or application of mGluR agonist alone. However, application of a
mGluR agonist in the presence of a D2 agonist is sufficient to induce LTD (Kreitzer and
Malenka, 2007). Thus, dopamine mediates a form of long-lasting inhibition at indirect-
pathway synapses, consistent with i) dopamine as an inhibitor of indirect-pathway
function (Albin et al., 1989), and ii) dopamine as a signal that gates synaptic plasticity
(Schultz, 2002).
In contrast, LTD at direct-pathway MSNs can be blocked by increased dopamine (Shen et al., 2008), consistent with its role in potentiation of direct-pathway function (Albin et al., 1989). In accordance with such potentiation, it has also been shown that Dopamine D1 receptors are involved in striatal LTP (Calabresi et al., 2000), while dopamine depletion has been shown to block striatal LTP (Centonze et al., 1999). Whereas D1 and D2 receptors appear to act synergistically to enable LTD, they seem to operate in opposition during induction of LTP (Centonze et al., 1999); corticostriatal LTP is blocked by D1 receptor antagonists (Kerr and Wickens, 2001) and lost in mice lacking the D1 receptor (Centonze et al., 2003), whereas LTP can be blocked using a D2 receptor agonist and enhanced using a D2 receptor antagonist (Calabresi et al., 1997).

As mentioned above, since PD is primarily characterized by the degeneration of nigral dopaminergic projections to the striatum, much work has focused on characterizing the changes induced on striatal neuronal plasticity caused by such disruptions. Notably, corticostriatal LTP is lost in nigral lesioned 6-OHDA rats but can be restored by chronic (Picconi et al., 2003) or long-term low-dose (Picconi et al., 2008) L-Dopa treatment. L-Dopa has long been on the frontline of treatment for PD sufferers, but an unfortunate consequence of its use is the development of dyskinesias, severe involuntary movements, in the vast majority of these patients (Obeso et al., 2000a). Interestingly, after the induction of LTP at corticostriatal synapses, LFS can depotentiate LTP. This depotentiation is selectively lost in rats that developed a dyskinetic response to L-Dopa treatment (Picconi et al., 2003; Picconi et al., 2008). Hence, it is conceivable that pharmacological modulation of corticostriatal synaptic plasticity might prove useful in the treatment of motor symptoms observed in PD (Picconi et al., 2005).
1.4.5 Measuring Plasticity in Human Subjects

To this point, one experimental paradigm, paired associative stimulation (PAS), has proven successful in inducing LTP-like changes in human subjects. Developed by Stefan, PAS measures changes in motor cortex excitability by using low-frequency median nerve stimulation paired with transcranial magnetic stimulation of the sensorimotor system of the motor cortex (Stefan et al., 2000). LFS with an interstimulus interval of at 10 ms decreases motor cortex excitability while at intervals above 25 ms enhances motor cortex excitability (Wolters et al., 2003). These changes in excitability are long lasting, region specific, and are blocked by NMDAR or L-type voltage-gated channel antagonist (Stefan et al., 2002).

This same protocol has been used to demonstrate that PD patients, both dyskinetic and non-dyskinetic alike, have deficient LTP-like effects in the human motor cortex (Morgante et al., 2006). Interestingly, this study also found that treatment with L-Dopa restores potentiation of motor evoked potentials in non-dyskinetic patients but not in dyskinetic patients. Such results further indicate that PD is associated with aberrant plasticity and that changes in dopamine levels are intricately tied to changes in plasticity.
1.4.6 LTP and LTD as Models for Behaviour

It should be noted that both LTP and LTD are experimental phenomena which are used to demonstrate the repertoire of long-lasting changes of which individual synapses are capable. It has been near impossible for researchers to demonstrate identical synaptic modifications due to the same mechanisms underlying some form of LTP or LTD occurring in vivo in response to experience (Malenka and Bear, 2004). However, given the ubiquity of the assorted forms of LTP and LTD at both excitatory and inhibitory synapses throughout the brain, and the computational advantage they afford, it seems certain that the brain takes advantage of its circuits’ capability to express long-lasting activity-dependent modifications as at least one of the fundamental mechanisms by which experiences modify neuronal behaviour.
2 OBJECTIVE & HYPOTHESIS

2.1 Objectives

The progressive loss of SNc neurons that characterizes PD pathology leads to impaired levels of dopamine at the basal ganglia input. However, as touched on in the opening paragraphs of the Introduction, the SNc also sends ventrally projecting dendrites to the SNr (Cheramy et al., 1981; Geffen et al., 1976; Korf et al., 1976; Robertson et al., 1991), and little is known of the effects of dopamine released from these ventral SNc projections. Alterations in dopamine levels have been shown to be coupled to changes in synaptic plasticity at the corticostriatal synapse; this study set out to determine if similar processes were ongoing at basal ganglia output structures, specifically, the extent to which dopamine modulates synaptic plasticity in the SNr.

During the course of initial intra-operative mapping sessions it was determined that stimulation in the SNr evoked a positive extracellular field potential in SNr. Since the SNr receives projections from a variety of structures, both excitatory and inhibitory, it became clear that determining the nature of this extracellular field would be important, since it would be alterations in this field’s amplitude which would tell us something about the plastic properties of basal ganglia output neurons.

To summarize, the two objectives of this study were: i) to determine the nature of the positive field evoked potential (fEP) we observe in substantia nigra pars reticulata (SNr) recordings, and ii) to determine the relationship between dopamine and synaptic plasticity by measuring changes in fEP amplitude following stimulation in PD patients ON and OFF dopaminergic medication.
2.2 Hypotheses

1 The positive field (fEP) we observe in our recordings of basal ganglia output neurons is a GABA-mediated field IPSP.

2 Dopamine enhances synaptic plasticity in basal ganglia output neurons.
3 METHODS

3.1 Patients

Using intraoperative microelectrode recordings, we studied 18 patients undergoing stereotactic surgery for implantation of bilateral STN-DBS electrodes. Patients undergo STN DBS surgery for the treatment of the cardinal signs of PD: akinesia and bradykinesia, rigidity and tremor. PD patients are selected for surgery based on clinical evaluations of each patient’s response to levodopa and the degree to which they suffer from levodopa-induced dyskinesias (Lang and Lozano, 1998a). The clinical characteristics of the patients and their daily doses of anti-PD medications are shown in Table 1. The group, consisting of 14 men and 4 women, had a mean age (± SD) of 58.9 ± 6.8 years and mean disease duration (± SD) of 13.3 ± 4.4 years. Six patients were most affected on their right side, while nine patients were most affected on their left side. Three patients were severely affected bilaterally. Patients normally underwent a minimum of 12 hours of anti-PD medication withdrawal before microelectrode mapping for DBS implantation, and were awake with local anesthesia for measures of synaptic plasticity in SNr following completion of the electrophysiological mapping of the STN. The UPDRS III ON and OFF motor scores given in Table 1 were obtained during patient work-up for DBS and were taken at an earlier time point. The OFF measures were taken following 12 hours of anti-PD medication withdrawal while the ON measures were performed following a dose 20% greater than patients’ normal morning dose. The normal morning dose is typically 15-25% of the daily dose. Six patients were studied first in the “OFF” state following 12 hour withdrawal and then in the “ON” state after oral
administration of 100 mg of levodopa (Sinemet 100/25®) in the contralateral hemisphere. An additional 6 patients were studied only in the OFF state in order to avoid the occurrence of severe dyskinesia during surgery. In 4 cases the patient was given one tablet of Sinemet 100/25® immediately before the procedure as it was deemed medically necessary for the patient (Patients 4, 6, 8, and 9 in Table 1). UPDRS motor scores indicate that all patients had some degree of motor improvement when ON L-Dopa with an average improvement (± SD) of 61.5 ± 13.0 % in the ON state. The experiments were approved by the University Health Network and University of Toronto Research Ethics Boards. Patients provided written informed consent prior to the procedure.
<table>
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<th>Disease duration (years)</th>
<th>Medication (Daily Dose)</th>
<th>L-DOPA equivalence (mg/day)</th>
<th>UPDRS III (OFF/ON)</th>
<th>% Imprv</th>
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<td>46 / 16.5</td>
<td>64</td>
<td>ON (2)</td>
</tr>
<tr>
<td>5</td>
<td>57/M/L</td>
<td>10</td>
<td>L-DOPA 1500mg</td>
<td>1500</td>
<td>33 / 6</td>
<td>82</td>
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</tr>
<tr>
<td>6</td>
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<td>41 / 18</td>
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</tr>
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<td>7</td>
<td>57/F/B</td>
<td>9</td>
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<td>1450</td>
<td>48 / 18.5</td>
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<td>712.5</td>
<td>43.5 / 20.5</td>
<td>53</td>
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<tr>
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<td>61/M/L</td>
<td>11</td>
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<td>32.5 / 24</td>
<td>26</td>
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</tr>
<tr>
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<td>28.5 / 10</td>
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<td>1300</td>
<td>19.5 / 7.5</td>
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<td>52.5 / 18.5</td>
<td>65</td>
<td>ON **</td>
</tr>
</tbody>
</table>

| Mean | 58.9 ± 6.8 | 13.3 ± 4.4 | ========= | 1301 ± 409 | 39.0 ± 8.9 / 15.1 ± 5.5 | 61.5 ± 13.0 |

**Table 1** Patient Characteristics
3.2 Surgery

The surgical procedure for stereotactic, microelectrode-guided STN localization and placement of DBS electrodes (Medtronic Model 3387, Minneapolis, MN) for PD has been described elsewhere in detail (Hutchison et al., 1998) and will be reviewed briefly here. Patients’ antiparkinson medications are withheld a minimum of 12 hours before the surgery. A stereotactic frame is affixed to the patient’s head after local anesthetic is applied. Pre-operative MR images are obtained and axial images are used to determine the x-, y- and z co-ordinates of the anterior and posterior commissures with respect to the stereotactic frame. The pre-operative target is chosen to be the ventral border of STN. Coordinates of the tentative target are 12 mm lateral to the midline, 2 to 4 mm posterior to the mid-commissural point and 3 mm below the AC-PC line (Hutchison and Lozano, 2000). Patients lie in a supine position on the operating room table and 5 cm incisions are made 3 cm lateral to the midline. Burr holes are then drilled at the coronal suture and the underlying dura mater is opened to allow the microelectrodes access to the brain. Surgical fibrin glue (Tisseel, Baxter) is used to cover the dural opening and prevent cerebrospinal fluid loss during the surgery. A Leksell arc is attached to the head frame and set to the coordinates of the target. A cannula is inserted into the brain to a depth of 10 mm above target and the inner stylet is removed. Two microelectrodes, enclosed in individual steel guide tubes and spaced 600 to 800 μm apart, are then inserted into the cannula and driven by submillimeter increments into the brain by independent manual hydraulic microdrives.
3.3 Intraoperative Microelectrode Field Evoked Potentials & Neuronal Recordings

Dual gold and platinum plated parylene-C insulated tungsten microelectrodes were used during surgery. Microelectrode tip length was approximately 25 μm and impedances ranged from 0.2-0.4 MΩ at 1000 Hz. As mentioned above and shown in Figure 5, microelectrodes were spaced 600-800 μm apart and were driven by submillimeter increments through a steel tube guide tube into the brain by independent manual hydraulic microdrives (Figure 5). Recordings were amplified 5,000-10,000 times and filtered at 10 to 5,000 Hz (analog Butterworth filters: high-pass, one pole; low-pass, two poles) using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, CA). Microelectrode data were sampled and digitized at 12 kHz with a CED 1401 (Cambridge Electronic Design [CED], Cambridge, UK) and EMG of ipsi- and contralateral wrist and foot flexor and extensor muscles was sampled at 500 Hz to monitor any dyskinetic movements.

Neuronal activity was continuously recorded along linear tracks from ventral thalamus, through zona incerta and STN, to dorsal SNr (example traces are shown in Figure 6a). Target nuclei were localized via characteristic neuronal discharge patterns described elsewhere in detail (Hutchison et al., 1998). Briefly, after passing through thalamus, typically consisting of bursting cells, and the quieter zona incerta, entry into STN was marked by an increase in background activity and large amplitude, irregularly firing spikes. Exit from STN was noted by a decrease in noticeable background activity. If the length of the track within STN was longer than 5 mm the track was considered to run through the middle of the STN and was sufficient for a site of implantation.
Otherwise, further tracks were recorded until the middle of the nucleus was found. The SNr was identified by the presence of neurons with a significantly higher discharge rate and more regular firing pattern (vs. STN). The SNr neurons also displayed characteristically low thresholds (2–4 µA) for microstimulation-induced inhibition of firing (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2009). An example of a typical trajectory is shown in Figure 8. All recording sites were deemed to be near the region of the soma and the spike amplitude was continuously monitored in order to confirm stability of electrode position.

Since the primary goal of the microelectrode recordings was to confirm the location of STN, in order to improve clinical benefit to PD patients from DBS implantation, the number of SNr recording sites that could be tested for plasticity responses per patient and the length of time spent at each recording site was limited by the clinical aim of the surgery.
Figure 5 Microelectrode Apparatus. Schematic of the two microelectrodes that are inserted into the brain of patients with PD during DBS surgery (A). Each microelectrode is encased in its own guide tube (B), allowing for independent manipulation of microelectrode depth (C). Microelectrodes are separated by approximately 600 um. One microelectrode can be replaced with a macroelectrode (bottom C) for stimulation purposes. (modified from Levy et al J Neurosurg 60: 277, 2007)
Figure 6 Example of neuronal traces. A) Raw traces showing examples of typical cell firing found in different basal ganglia structures. Top trace shows a thalamic bursting cell. Each burst typically contains two to six spikes in quick succession. Bursts can occur at various intervals but typically occur between 3-6 Hz during recordings. The 2nd trace shows an STN cell. This cell fires fast (20-40 Hz) and slightly irregular. Third trace shows a SNr cell. This cell fires very fast (typically > 50Hz) and in a highly regular manner. Adapted from (Hutchison et al., 1998). B) An example of an SNr cell recorded during stimulation at 1 Hz from the 2nd electrode (B, top trace). The stimulus artefact (3 large vertical lines) is visible, as are the fEPs (arrow). The bottom plot is an example of wavemark data. The spikes were extracted from the raw recording and assigned to a template corresponding to the shape of the action potential. All of the spikes in this example were classified as belonging to a single template.
3.4 Stimulation

fEPs were recorded from one electrode while stimulating with single pulses (100 uA, 0.3 ms biphasic pulse width) from a second electrode separated mediolaterally by 0.5 – 1.0 mm at the same dorsoventral level within the SNr. Depth profiles were examined in some cases by moving the stimulating electrode in 250 um increments above and below the recording site for up to a 3 mm separation. Paired pulse response (PPR) curves were constructed in 6 patients using a variety of paired pulse interstimulus intervals (20, 30, 50, 100, & 200ms) by comparing the ratio of the peak amplitude of the 2nd fEP to the 1st fEP.

After obtaining a stable baseline of peak fEP amplitudes at 1 Hz, high frequency stimulation (HFS) was given, consisting of four 100 Hz trains, 2 seconds in length, repeated 4 times every 10 seconds (100 uA, 0.3ms pulse width). Blocks of 10 pulses were tested every 30 sec for at least 2 minutes, or until a stable plateau had occurred.

Plasticity was quantified using fEP amplitudes in both OFF and ON dopaminergic medication states, with the first side being done after 12 hours off medication and the second side following administration of Sinemet 100/25®. Typically, 25 to 30 minutes had elapsed between the time of administration (Sinemet 100/25® was given as recording began on the “ON” track) and SNr testing. The sites where synaptic plasticity was tested are shown in Figure 8 and were determined by track reconstruction using neurophysiological landmarks and a customized brain atlas-based program.
**Figure 7** HFS Stimulation Protocol. Baseline stimulation consists of 10 seconds of 1 Hz stimulation repeated twice. High frequency stimulation is then applied. 2 seconds of 100 Hz stimulation is applied and repeated every 10 seconds a total of 4 times. Following HFS, 10 second trains of 1 Hz stimulation are resumed and repeated every 30 seconds for at least 2 minutes to test for a change in fEP amplitude.
3.5 Analysis of Neuronal Activity

Neuronal recordings were analyzed offline using Spike2 software version 6 (CED, Cambridge, UK). Post stimulus time histograms (PSTHs, 250 us bin width, time base 150 ms normalized to firing rate in Hz) were constructed of the high frequency spiking of putative GABAergic output neurons of SNr in 2 patients. PSTHs show the likelihood of an event (spike) falling at a given period after an event (test pulse) on a different channel. The histograms simply show the number of events that fell in a particular time bin. Spike analysis was performed using a spike matching template algorithm in Spike2 (See Figure 6b for an example of a template with a single type of spike). Briefly, spikes were extracted from a waveform channel and assigned to a template corresponding to the shape (amplitude, slope, latency, etc.) of a given spike. For PSTHs, only those spikes identified as belonging to a single template were included, i.e. a single unit was used for analysis.

fEP amplitudes were evaluated using the monophasic W_FP script in Spike 2. This script detects the amplitude and latency of peaks in the neuronal recordings at user defined events. Here, the beginning of the 1 Hz stimulation artefact was used as the “event”. These measurements were then normalized to a percent scale, with the average of baseline measures in each patient considered as 100%, and sorted by medication state (OFF vs ON). Synaptic potentiation was evaluated in each patient in all medication states by fitting an exponential function to the fEP amplitudes using Sigma Plot software (SPSS, Chicago, USA): \( y = y_o + ae^{-bx} \) where \( y_o \) is the plateau value (relative to baseline fEP amplitude) to which the function decays, \( a \) is the difference of the maximum (first)
value of the exponential curve to $y_0$, and $b$ describes the steepness of the curve.

Population data was fit with a regression line if the fit had a significance value of $p < 0.05$.

### 3.6 Statistics

All statistical comparisons were conducted using Sigmastat software (Systat Software Inc., San Jose, USA). A 2-way ANOVA was performed on the normalized data testing the main effects of DRUG (ON vs OFF) and TIME following HFS. A post-hoc Bonferroni t-test tested all pairwise comparisons between ON and OFF at each time point. A $p$ value of 0.05 was taken as significant.
4 RESULTS

4.1 fEP Test Sites

All sites tested for a field evoked response were located in the SNr. We tested a total of 24 SNr sites in 18 patients. The approximate locations of test sites included in the study are shown in Figure 8. Recordings took place in dorsolateral SNr and test locations were independent of medication state. fEPs could not be evoked in the STN region using the stimulation protocols described above.
Figure 8 Field Amplitude Test Locations. Composite figure showing the location of sites tested for a field evoked response in the substantia nigra pars reticulata (SNr). Sites tested following application of dopaminergic medication are shown as closed circles. Neurons tested following 12 hours of dopaminergic medication withdrawal are shown as open circles. The locations of sites tested for synaptic plasticity were determined by track reconstruction using neurophysiological landmarks (shown in the example trajectory from a patient in the study; in this case, the mapping was performed while the patient was OFF) found using microelectrode recordings, and a customized brain atlas program. * denotes a SNr site on the trajectory that was included in the OFF sample. Dorsal (D), ventral (V), anterior (A), and posterior (P) axes are labeled. Relative positions of the thalamus (Thal), hypothalamus (Hpth), and subthalamic nucleus (STN) are shown ((Prescott et al., 2009) used with permission from Brain).
4.2 Field Potential Characteristics

Blocks of 1 Hz test pulses at incrementally increased stimulation distance were conducted in 2 patients and revealed a positive field persisting for 2.5 mm dorsoventrally through the SNr with the peak field amplitude having a latency (± SD) of 5.5 ± 0.8 ms (An example is shown in Figure 9). Post stimulus time histograms of the cell firing were analyzed in 2 cases and both revealed that the positive peak of the fEP occurred during inhibition of firing (Figure 10). Additionally, the enhancement of fEP amplitude in the ON state post HFS was associated with a slower recovery of the spontaneous firing rate (Figure 10). This effect was examined in more depth in another patient; a large reduction in firing rate was observed in the ON state at 5 seconds following HFS and an ANOVA revealed an overall reduction in firing rate that persisted for up to 35 seconds following HFS in the ON state (p<0.001) (Figure 11). In the same patient in the OFF state, no significant reduction in firing rate was observed following HFS (p=0.787) (Figure 11).
Figure 9 Example of depth profile of fEP in patient #12 SNr while ON medication. Upon entering the SNr, the stimulating electrode is kept in a fixed position while the recording electrode was moved down in 250 um increments. fEPs were recorded at increasing distances from a fixed stimulation point (-1.75 mm along track in this example), and a (+) field persisted for approximately 2.5 mm ventrally through the SNr.
Figure 10 Post stimulus time histograms of SNr neuronal firing in Parkinson’s disease (Patient #1). Traces show the average of 10 raw fEPs overlaid on a PSTH of the same time course (150ms). Traces on the top are from a patient in the OFF state, before (left trace) and following (right trace) high frequency stimulation. Traces on the bottom are from the other side on the same patient following administration of one tablet of Sinemet 100/25®. The positive peak of the field evoked potential occurs during inhibition of SNr cell firing in both the OFF and ON states. Notice that lower firing rate is associated with a larger field ((Prescott et al., 2009) used with permission from Brain).
Figure 11 Firing Rate of a SNr cell during fEP amplitude measures. (Patient #8). Top traces show firing rate in Hz of SNr cells during fEP amplitude measures, before and after HFS, both ON (red) and OFF (black) L-Dopa in the same patient. Bar graph on bottom depicts the percentage change in firing rate from baseline at each measurement in time. Following HFS there is a reduction in firing rate lasting for ~ 30s with the effect being highly significant in the ON state. Interestingly, baseline firing rates were higher while the patient was ON L-Dopa, although the measures were made at different sites on the left (ON) and right (OFF) side of the brain. * denotes a significant (p<0.001) reduction in firing rate from baseline measures in the ON state.
4.3 Paired Pulse Response

A paired pulse response curve was constructed for six patients by comparing the paired pulse ratio before and after high frequency stimulation at a range of interstimulus intervals. The results have been averaged across patients in Figure 12. Paired pulse depression is most apparent at short (20 and 30 ms) interstimulus intervals, both before and after HFS, evidenced by small paired pulse ratios (PPR). Before HFS, the PPRs at 20 and 30 ms intervals were 0.48 ± 0.018 and 0.76 ± 0.014 respectively. Following HFS, paired pulse depression was similar in magnitude at short interstimulus intervals (20 ms = 0.31 ± 0.11; 30 ms = 0.74 ± 0.19). However, as the interstimulus interval increased, there was a marked increase in PPR. Before HFS, the PPRs for intervals of 50, 100, and 200 ms were 1.10 ± 0.050, 1.12 ± 0.051, and 1.07 ± 0.072 respectively. Following HFS the PPRs at the same intervals were 0.88 ± 0.037, 0.94 ± 0.065, and 0.94 ± 0.072. A 2-way ANOVA revealed a significant decrease in paired pulse ratio following HFS (p=0.015) and a highly significant reduction in paired pulse ratio both before and after HFS at 20 ms (p<0.001). Interstimulus intervals causing maximal paired pulse depression (20 and 30 ms) are similar to those when inhibition of SNr firing occurred following single pulses as shown in Figure 10.
Figure 12 Paired Pulse Measures. A) Raw traces of SNr neuronal activity and fEPs during paired pulse measurements following high frequency stimulation (Patient # 18). Traces, from top to bottom, are taken during paired pulse measurements with interstimulus intervals of 100, 50, and 30 ms respectively. Greater paired pulse depression is seen at smaller interstimulus intervals, as denoted by the arrow on the bottom trace. B) Paired Pulse Response Curve. Shown is the paired pulse ratio before and after high frequency stimulation average across six patients at increasing interstimulus intervals (20, 30, 50, 100, and 200ms). A 2-way ANOVA revealed a reduction in PPR following HFS (p=0.015) with a highly significant reduction (denoted by *) in PPR at 20 ms both before and after HFS ((Prescott et al., 2009) used with permission from Brain).
4.4 DA Modulation of Synaptic Plasticity in the SNr in PD Patients

Effects of HFS (four 2s 100Hz trains) on fEP amplitudes were examined in 13 patients in the OFF state. In these patients (see Table 1 for daily medication use and L-Dopa equivalence), HFS did not induce a lasting change in fEP amplitude (see example). A typical example is shown in Figure 13a and c (open circles), where a modest increase in fEP amplitude returned to baseline by ~ 50 – 100 s. However, in some patients a larger initial increase in fEP amplitudes was seen in the OFF state, with a subsequent rapid decay toward baseline (example in Figure 14). In this case, the patient reported that he was only about 50% of his worst OFF state. We found a close inverse linear relation ($r^2 = 0.81$, $p< .001$) between the patients’ clinical OFF rating based on UPDRS III motor subscale (high values indicate worse motor symptoms) and the peak of activity-dependent synaptic plasticity induced by HFS (Figure 15a open circles). Patients with a higher UPDRS OFF score underwent less change in fEP amplitudes following HFS. The population data for the OFF group shown in Figure 15b (open circles) reveals a significant initial $28.9 \pm 4.9 \%$ increase in fEP amplitude following HFS that then decayed by 100s to baseline. Regression analysis on population data from the OFF group revealed a $y_o$ fEP amplitude plateau value no different than baseline ($2.3 \pm 3.8 \%$ above baseline).
Figure 13 L-DOPA treatment of a Parkinsonian patient (Patient #1) restores plasticity. a) Averaged fEP measures pre (black) and immediately post (grey) HFS (10 sweeps per trace) in a patient in the OFF state. b) Averaged fEP measures pre (black) and immediately post (grey) HFS (10 sweeps per trace) in the same patient following administration of 100mg L-Dopa. Note the large increase above baseline measures in the ON state. c) Open circles are individual fEP peak amplitudes before L-Dopa treatment and closed circles are ~20 minutes following L-Dopa administration. High frequency stimulation (HFS) does not induce a change in fEP amplitude in the SNr of a patient 12 hours removed from L-Dopa treatment. Following administration of L-Dopa, HFS induced an increased fEP amplitude response in the SNr. Note higher plateau reached in the ON L-Dopa state by 2 min post HFS ((Prescott et al., 2009) used with permission from Brain).
Figure 14 L-DOPA treatment of a different Parkinsonian patient (Patient # 3) enhances activity-dependent synaptic plasticity in SNr. Open circles are individual fEP peak amplitudes before L-Dopa treatment and closed circles are ~20 minutes following L-Dopa administration. High frequency stimulation (HFS) induces a marked initial increase and subsequent decline to baseline in fEP amplitude in the SNr of a patient 12 hours removed from L-Dopa treatment. Patient self-reported feeling only “50% OFF”. Following administration of L-Dopa, HFS induced an increased and sustained fEP amplitude response in the SNr of this patient.

L-Dopa treatment of PD patients markedly improved motor UPDRS in all patients preoperatively (Table 1). Note that the total daily L-Dopa equivalences are approximately 10x greater than the dose administered intraoperatively. Following administration of L-Dopa, the same HFS protocol induced a much larger increase in fEP amplitudes (Fig 13b). Such fEP amplitude increases persisted over several minutes of testing (Figure 13c; closed circles). There was also a significant correlation between the patients’ clinical ON rating based on the UPDRS III motor subscale and the maximum value of activity-dependent synaptic plasticity induced in the ON group ($r^2 = 0.80$, $p<0.001$) if three
outliers are excluded (Figure 15a closed circles – outliers shown in grey – see Discussion section 5.4).

Effects of HFS on fEP amplitudes were examined in 12 patients in the ON state (Figure 15b; closed circles). The largest fEP amplitude measures occurred immediately following the conditioning stimuli (200.3 ± 19.5 % above baseline) with subsequent measures showing a decrease in fEP amplitude at each time point with an exponential decay function. Regression analysis on population data from the ON group’s fEP amplitudes revealed a $y_0$ plateau value of 29.3 ± 5.2% above baseline. The regression function for the OFF and ON groups was highly significant with plateau values at $p < 0.001$. Additionally, for the ON group, a $b$ value describing the steepness of the curve was determined to be 0.019 ± 0.0036 ($p < 0.05$), which corresponds to a half life ($1/0.019$) of 52.6 s for the decay function. The OFF group’s $b$ value was slightly higher at 0.026 ± 0.015 (not significant), corresponding to shorter half life of 38.8 s.

A two-way ANOVA of population data revealed a highly significant difference between ON and OFF groups (DF=1, $f=799$, $p<0.001$) and a significant difference between time points (DF=5, $f=69$, $p<0.001$). It also revealed an interaction between medication state and time, i.e. the ON / OFF amplitude is also dependent on the time of measurement (DF=5, $f=17$, $p<0.001$).
Figure 15 Dopamine Enhances Synaptic Plasticity, Population Data. a) OFF UPDRS III motor subscore (open circles) correlates strongly ($r^2 = 0.81, p< .001$) with degree of activity-dependent synaptic plasticity inducible in patients 12 hours removed from anti-PD medication. ON UPDRS III motor subscore (black closed circles) correlates strongly ($r^2 = 0.80, p< .001$) if three outliers are excluded (grey closed circles). If the three outliers are included, no correlation exists ($r^2 = 0.02$). b) Clear difference between fEP amplitude measures in ON (Red) and OFF (Black) populations following HFS, with the ON group experiencing an increase in amplitude of 29.3% (SEM ± 5.2) above baseline measures following plateau, while the OFF group undergoes a transient increase and subsequent decline back to baseline by 160s. Curves were fit using exponential decay function $y = y_0 + ae^{-bx}$, where $y_0$ is the plateau value to which the function decays, $a$ is the difference of the maximum (first) value of the exponential curve to $y_0$, and $b$ describes the steepness of the curve. A two-way ANOVA reveals that the difference in the mean values among between ON and OFF after allowing for effects of differences in TIME is significant (DF=1, $f=799, p<0.001$). Likewise, the difference in the mean values between time points after allowing for effects of differences in medication state is significant (DF=5, $f=69, p<0.001$); the test also reveals an interaction between DOPA state and time i.e. the ON / OFF amplitude also depended on the time point (DF=5, $f=17, p<0.001$) ((Prescott et al., 2009) used with permission from Brain).
5 DISCUSSION

This study describes the characteristics of the positive fEP in the SNr of PD patients, both OFF and ON dopaminergic medication. It is unique in providing human data supporting dopamine regulation of synaptic plasticity in the human basal ganglia, and suggests an important role for activity-dependent synaptic plasticity in basal ganglia dysfunction.

5.1 Inhibitory Nature of the Field

The SNr receives numerous projections from a multitude of sources, chief among them the inhibitory GABAergic projection from medium spiny neurons of the striatum (Bolam et al., 2000; Parent and Hazrati, 1995a; Parent and Hazrati, 1995b). The external segment of the globus pallidus (GPe) also sends a small, but significant, GABAergic contribution to the SNr (Smith and Bolam, 1989). Additionally, the STN sends excitatory projections to the SNr. These glutamatergic projections from the STN to the output structures of the basal ganglia have been shown to form asymmetric synapses (Ribak et al., 1981), primarily on the dendrites and shafts, but with a very small number of boutons terminating on the somata (Kita and Kitai, 1987). The vast majority of the terminals in the region form symmetric synapses with the somata and are GABAergic in nature (Ribak et al., 1979; Ribak et al., 1981). The rapid inhibitory responses characteristic of GABAergic transmission in basal ganglia structures are mediated by the activation of
GABA_A receptors, which are found exclusively at symmetric synapses (Galvan et al., 2006).

Based on several observations, our stimulation protocol is primarily activating the inhibitory GABAergic projections, either from the striatum or the GPe. During our field recording measurements, all of the field potential measurements in the SNr are positive. Precht and Yoshida demonstrated the inhibitory nature of a positive field in the SNr by observing that spontaneous activity of neurons located in the SNr was strongly suppressed conjointly with the occurrence of the caudate-evoked (GABAergic) positivity (Precht and Yoshida, 1971; Yoshida and Precht, 1971). They also demonstrated that the time course of the intracellular IPSP was the same as the positive fEP, and that the potential was blocked in its entirety by the GABA antagonist picrotoxin. In the present study, we also saw a positive fEP and its time course was the same as the inhibition of SNr activity, suggesting that the observed stimulation-evoked positive fEP is associated with an inhibitory event, most likely local GABA release.

5.2 Dopamine and GABA Release

Our paired pulse studies also point to activation of the GABAergic projections. In the SNr, dopamine D1 receptors are present at the terminals of the GABAergic striatonigral projection (Altar and Hauser, 1987; Barone et al., 1987). Previous striatal studies have shown that paired pulse depression predominates at synapses under the influence of D1 receptors, whereas paired pulse facilitation predominates at synapses at which D2 receptors are active (Guzman et al., 2003). In this study, paired pulse
depression was evident at short interstimulus intervals prior to HFS and at all interstimulus intervals following HFS, suggesting that the stimulation evokes effects involving the presynaptic D1 receptors. Slice studies indicate that stimulation of D1 receptors found in the SNr increases extracellular GABA (Aceves et al., 1991; Aceves et al., 1995; Floran et al., 1990; Timmerman and Abercrombie, 1996) and that this facilitated GABA release in turn enhances GABA_A IPSCs in nondopaminergic neurons of the SNr (Radnikow and Misgeld, 1998). Taken together, these observations suggest that our positive fEP is inhibitory and GABAergic in nature and that dopamine plays a role in presynaptic regulation of GABA release in this region.

5.3 Dopamine and Plasticity at the Basal Ganglia Output

Dopamine action in the basal ganglia is usually considered in terms of its modulation (or lack thereof in PD) of indirect and direct striatal output via the dopaminergic nigrostriatal projection. In this region, dopamine concomitantly provides excitatory inputs mediated by D1 receptor activation in the direct pathway and inhibitory inputs mediated by D2 receptor activation in the indirect pathway (Albin et al., 1989; DeLong, 1990) (Figure 16a). However, dopamine can also have dramatic effects in other regions of the basal ganglia. Indeed, nigral dopamine depletion has been shown to impair motor performance independent of striatal dopamine neurotransmission, while increased nigral dopamine release can counteract striatal dopamine impairments (Andersson et al., 2006). Here, we posit that dopamine can also act directly in the SNr by influencing synaptic plasticity at striatonigral synapses (Figure 16c). As previously
mentioned, GABAergic and glutamatergic signals converge in the SNr. Based on the results discussion outlined above, it appears that the field evoked potentials are GABAergic in nature. In Parkinson’s disease, activity of the GABAergic neurons of the output nuclei is thought to be enhanced and to cause excessive inhibition of the thalamic neurons. This inhibition of thalamic activity might thus act as a ‘brake’ on activity of the supplementary motor cortex resulting in the onset and lasting effects of the parkinsonian syndrome (Bezard et al., 2001) (Fig. 16b). If this is the case, potentiation of GABAergic signals onto substantia nigra pars reticulata, by lowering neuronal firing rate, could (at least in part) mediate the beneficial symptomatic effects of L-Dopa (Figure 16c).
Figure 16 Plasticity at Basal Ganglia Output. The implications of LTP-like plasticity of GABAergic signals onto SNr neurons is shown. A) During physiological conditions the tightly regulated activity of the direct and indirect pathways controls the activity of the output nuclei. B) During Parkinson’s disease, in the absence of a pharmacological treatment, dopamine deficiency causes overactivity of the indirect pathway and reduced activity of the inhibitory GABAergic direct pathway, disinhibiting the output nuclei and thus causing excessive inhibition of the motor thalamus. In this condition, high frequency stimulation does not induce a lasting change in the GABAergic field potential amplitude in the SNr. C) After the administration of L-Dopa, the same high-frequency stimulation protocol induces potentiation of the GABAergic field potential amplitudes, which is associated with a reduction of the excessive inhibitory activity that the output nuclei exert on the motor thalamus. Inhibitory GABAergic connections are represented in red, excitatory glutamatergic connections in green and modulatory dopaminergic connections in black. Modified from Calabresi, 2009.
5.4 Plasticity and Motor Behaviour

Previous studies have suggested a link between LTP and dopamine at the corticostriatal synapse. Indeed, LTP is absent in dopamine lesioned (6-OHDA) rats, but can be restored with chronic L-Dopa treatment (Picconi et al., 2003). Here, we sought to characterize activity-dependent synaptic plasticity in basal ganglia output neurons in 18 PD patients, all of whom experienced a significant improvement in motor function during their preoperatively measured ON state (Table 1). HFS did not induce a lasting change in fEP amplitude in patients in the OFF state (Figure 16b). However, there was a strong correlation between the patients’ clinical OFF rating based on the UPDRS (high values indicate worse motor symptoms) and the initial degree of activity-dependent synaptic plasticity that could be induced in the same 12 hour defined OFF state. Although the long-duration response to L-Dopa (Nutt et al., 1995) and the variable half-life of some dopamine agonists (Rinne et al., 1997) could have interfered with the severity of the OFF state, 12 hours of anti-PD medication withdrawal induced a noticeable increase in UPDRS III motor scores in the patients included in this study, and all measures of fEP amplitudes were done following a similar period of anti-PD medication withdrawal.

Comparatively, during the intraoperative ON state, L-Dopa intake coupled with HFS caused an increased fEP amplitude response in a manner reminiscent of LTP-like changes, in addition to decreased SNr firing rates. When including all patients categorized as being ON, there was no correlation between patients’ clinical ON rating based on UPDRS III motor subscale and the maximum inducible activity-dependent
The lack of correlation in the ON group is likely the result of variability in intraoperative ON states. As shown in Figure 15b, by excluding three outliers we see a very strong correlation. Such variability could be derived from a number of sources including, but not limited to, ineffectiveness of a single dose of L-dopa in patients taking high doses, the timing of the transient ON period of a single dose, and when the measurements were performed. For example, the patient whose ON UPDRS outlier is furthest to left in Figure 15b (UPDRS score 4.5, Patient #15) normally received 1950 mg of L-Dopa equivalence daily. So, for the ON rating measurements done in clinic (score of 4.5), this patient would have received a dose 20% greater than his normal morning dose (already 15-25% of daily dose), or approximately 350-575 mg of L-Dopa. This is much more than the 100 mg received intraoperatively and as such, increases variability into the ON correlation. Future studies of this nature will attempt to reduce such variability where possible by matching intraoperative ON doses to those given in clinic.

5.5 Possible Mechanism for Dopaminergic Modulation of Plasticity in SNr

The rate model predicts that the administration of L-Dopa reduces the elevated firing rates of basal ganglia output neurons in the OFF state (Hutchison et al., 1997). Dopamine is thought to have diverse and complex actions on the physiological activity of the basal ganglia. It can both inhibit and enhance neuronal activity, depending on the level of membrane depolarization and physiological state of the neuron (Calabresi et al., 2007). Our observations of lowered SNr firing rates, coupled with enhanced inhibitory
synaptic plasticity are consistent with the rate model, and give further hints as to how the loss of dopamine can directly affect GABAergic striatonigral synapses.

Limitations of the current study prevented the testing of whether dopaminergic regulation of GABAergic activity was achieved by a pre or postsynaptic mechanism, but previous work suggests that such actions are likely presynaptic. Enhancement in miniature inhibitory post synaptic currents (mIPSCs) in the SNr via D1 receptor activity has previously been shown to be coupled with the formation of cAMP in the pre synaptic terminal (Jaber et al., 1996). The enhancing effects of D1 receptor stimulation on mIPSC activity in the SNr can be mimicked by forskolin, which is known to activate adenylate cyclase (Radnikow and Misgeld, 1998). A more recent study has proposed that D1-receptor mediated GABA release involves the cAMP/PKA pathway, with PKA ultimately phosphorylating key targets involved with GABA exocytosis, such as P/Q-type voltage-activated Ca\textsuperscript{2+} channels (to enhance Ca\textsuperscript{2+} influx), synapsins (to enhance vesicle trafficking), and SNARE proteins (to enhance vesicle docking, priming, and fusion) (Arias-Montano et al., 2007).

Nevertheless, rapid post synaptic changes in the SNr may also affect GABAergic activity. Recent work has demonstrated that neuronal activity can directly regulate the number of cell surface GABA\textsubscript{A}Rs by modulating their ubiquitination and consequent proteosomal degradation in the secretory pathway (Saliba et al., 2007). However, a link between dopamine and the level of GABA\textsubscript{A}R insertion and subsequent post synaptic accumulation has not been established to date, but demonstration of such a link would support a post-synaptic action of dopamine.
Given the evidence from the present study and the earlier studies detailed above (paired pulse depression, lack of postsynaptic dopamine receptors, no current evidence of a link between dopamine and GABA<sub>A</sub>R levels), it appears that the most likely site of action the dopamine mediated plastic change is presynaptic. However, further basic studies of the neuropharmacology of synaptic plasticity in SNr slice would be required to definitively conclude a presynaptic site of action and further elucidate mechanisms.

5.6 Applicability of Findings to the GPi

The work presented here has focused on basal ganglia output neurons in the SNr, but the other major output structure of the basal ganglia is the GPi, which is the main projection to the thalamus in the somatomotor loop to cortex. The GPi is anatomically similar to the SNr in having a major GABAergic input from striatum and minor glutamatergic input from STN. Like the SNr, it has a relatively homogeneous population of tonically active GABAergic output neurons. Preliminary work has recorded a similar positive fEP using single pulse stimulation in the pallidum of patients undergoing the implantation of DBS in the GPi for dystonia, a movement disorder in which sustained muscle contractions cause twisting and repetitive movements or abnormal postures. HFS produces a long lasting enhancement in the amplitude of the fEP in the GPi of dystonia patients who have never been exposed to chronic L-Dopa therapy. The magnitude of this enhancement of amplitude is much greater than the plasticity we observed in the OFF state in the SNr of PD patients indicating that synaptic plasticity is indeed lacking in the absence of dopamine. In addition, the marked enhancement of plasticity we have
reported in the ON state is greater than seen following HFS in the dystonia “quasi-control” group, suggesting an enhanced plastic response to small doses of L-Dopa in PD patients exposed to chronic L-Dopa therapy. This may have bearings on mechanisms of L-Dopa-induced dyskinesias. A more rigorous investigation of these finding will be continued in future studies.
6 CONCLUSION

The results of this study indicate that synaptic plasticity can be measured in basal ganglia output neurons of PD patients and that the presence of plasticity is sensitive to low doses of L-Dopa. This study is unique in being the first of its kind to measure plasticity at the level of the basal ganglia output neurons in humans. The in vivo nature of the study provided us with clinically relevant data about the nature of plasticity in pathological conditions of PD as well as the opportunity to compare quantified electrophysiological measures in basal ganglia output with actual motor improvements in patients with PD.

In the absence of dopaminergic medication, plasticity is lacking following HFS. Conversely, following administration of dopaminergic medication, synaptic plasticity is facilitated in the SNr by HFS. The close correlation between motor behaviour and the potential of nigral synapses to undergo activity-dependent changes suggests that dysfunction of direct dopaminergic action at the basal ganglia output plays an important role in PD symptomatology.

7 FUTURE STUDIES

As mentioned above, ongoing studies are currently exploring the applicability of the findings described here to the GPi, the other major output structure of the basal ganglia. Additionally, one crucial question that arises from the results outlined in this study and the ongoing GPi studies is whether it is possible that plasticity also mediates
the long-term complications of L-Dopa therapy and, in particular, dyskinesias. It is well accepted that, in order to avoid neuronal network destabilization, the mechanisms underlying synaptic plasticity need to be finely regulated and, in experimental models of L-Dopa-induced-dyskinesia, a crucial form of homeostatic synaptic plasticity, depotentiation, is selectively lost (Picconi et al., 2003; Picconi et al., 2005; Picconi et al., 2008). Thus, it remains possible that L-Dopa, via the continuous and uncontrolled increase of the strength of GABAergic synapses onto output nuclei neurons, may lead to progressive destabilization of postsynaptic firing rates, virtually reducing these to zero and thus leading to pathological disinhibition of thalamic nuclei and the onset of abnormal involuntary movements (Calabresi et al., 2009) (Figure 17).
Aberrant Plasticity in L-Dopa-induced dyskinesia. Following chronic administration of L-Dopa, uncontrolled potentiation of the GABA-mediated inhibition of the output nuclei might result in long-term suppression of firing rates, which in turn might lead to pathological disinhibition of the thalamus and the subsequent onset of pathological hyperkinetic behaviours, such as L-Dopa-induced dyskinesias. Inhibitory GABAergic connections are represented in red, excitatory glutamatergic connections in green and modulatory dopaminergic connections in black.

Studies assessing synaptic plasticity at the basal ganglia output are poised to yield critical new insight into the pathophysiology of abnormal movements and may impact on resolving the mechanisms underlying the effectiveness of deep brain stimulation in movement disorders. Ultimately, the hope is that studies of human synaptic plasticity might shed light on the complex mechanisms underlying symptoms of Parkinson’s
disease and the disabling long-term side effects of treatment with L-Dopa, as well as
beginning to elucidate the means by which DBS acts to normalize a pathological basal
ganglia.
Reference List


