SYNAPTIC PLASTICITY OF BASAL GANGLIA OUTPUT
NEURONS IN MOVEMENT DISORDERS

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy,

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Information storage in the central nervous system is believed to emerge from changes in the functional efficacy of synaptic connections, a property known as synaptic plasticity. Parkinson’s disease (PD) and dystonia are movement disorders that are the result of basal ganglia dysfunction. In PD, basal ganglia abnormalities are the result of the loss of dopamine, which leads to paucity of movements, rigidity of the limbs, and rest tremor. A unique characteristic of synaptic plasticity in the basal ganglia is that a certain level of endogenous dopamine and dopamine receptor activation seems to be required for its induction. In dystonia, a model has emerged in which a fundamental deficit within the basal ganglia generates impaired inhibition in the motor cortex that results in increased excitability, abnormal processing of sensory feedback and increased striatal, brainstem, and cortical plasticity. This thesis addresses the question of whether movement disorders such as PD and dystonia are associated with abnormal plasticity in the output nuclei of the basal ganglia, the SNr and the GPi. To do this, a pair of microelectrodes was used to simultaneously stimulate and record alterations in field evoked potentials (fEPs) from the substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi) using a variety of stimulation protocols in patients undergoing stereotactic neurosurgery for the implantation of deep brain stimulating (DBS) electrodes for treatment of PD or dystonia. Using these methods, we have previously shown that activity-dependent synaptic plasticity is measurable in the SNr of PD patients and that this plasticity is sensitive to low doses of L-dopa. Here we
demonstrate for the first time, that activity-dependent synaptic plasticity is also present in human GPi. Also, we provide additional evidence that the fEP is the result of primarily striatopallidal and striatonigral activation, and show that the fEP is useful in confirming microelectrode position and even in revealing functional deafferentiation. Furthermore, we demonstrate that short interval inhibition is reduced in basal ganglia output nuclei of dystonic patients and partially normalized following high frequency stimulation (HFS). In both generalized and cervical dystonia, the paired pulse ratio at low interstimulus intervals correlates with motor dysfunction, suggesting that abnormalities exist in synaptic function of striatopallidal and / or striatonigral terminals in dystonia patients and that such abnormalities may be contributing to the pathophysiology of dystonia, either independent of, or in addition to the increased excitability and plasticity observed in cortical areas in dystonia patients. Finally, we demonstrate a lack of a form of plasticity called depotentiation in basal ganglia output nuclei of PD patients with dyskinesia. The ability of a synapse to reverse previous potentiation may be crucial to prevent saturation of the storage capacity of motor information. Loss of this ability at the output nuclei could lead to the basal ganglia retaining unessential information that is normally erased, and may underlie, or at the very least contribute to the cellular basis for dyskinetic movements. Together, these findings suggest that abnormal plasticity in the basal ganglia output nuclei plays an important role in movement disorders such as PD and dystonia and that altered long-lasting changes in synaptic transmission represent an important mechanism by which basal ganglia function is impaired in movement disorders.
ACKNOWLEDGEMENTS

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And to Senya. Whatever I write here is insufficient. I will be forever thankful and remember the ways you helped me and the love and support you gave me during this entire process, parts planned and unplanned. And finally, to Owen, I promise to explore the world with you, explain the world to you, and expect the world of you.
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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine or 2,4,5-trihydroxyphenethylamine</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>AP</td>
<td>Action potential</td>
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<tr>
<td>BFMDRS</td>
<td>Burke-Fahn-Marsden dystonia rating scale</td>
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<tr>
<td>BG</td>
<td>Basal ganglia</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<tr>
<td>D1R</td>
<td>D1 subtype of dopamine receptor</td>
</tr>
<tr>
<td>D2R</td>
<td>D2 subtype of dopamine receptor</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DARPP32</td>
<td>Dopamine and cAMP-regulated phosphoprotein 32 kDa</td>
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<tr>
<td>DAT</td>
<td>Dopamine active transporter</td>
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<tr>
<td>DBS</td>
<td>Deep brain stimulation</td>
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<tr>
<td>ECB</td>
<td>Endocannabinoid</td>
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<tr>
<td>EPSP</td>
<td>Excitatory post synaptic potential</td>
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<td>fEP</td>
<td>Field evoked potential</td>
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<td>Gamma-aminobutyric acid</td>
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<td>Globus pallidus externus</td>
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<td>HFS</td>
<td>High frequency stimulation</td>
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<td>Inhibitory post synaptic potential</td>
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LFS – Low frequency stimulation
LTD – Long-term depression
LTP – Long-term potentiation
mGluR – Metabotropic glutamate receptor
MPTP - 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMDA - N-methyl-d-aspartate
NMDAR - N-methyl-d-aspartate receptor
PAS – Paired associative stimulation
PD – Parkinson’s disease
PP1 – Phosphoprotein phosphatase 1
PPN – Pedunculopontine nucleus
SNc – Substantia nigra pars compacta
SNr – Substantia nigra pars reticulata
STN – Subthalamic nucleus
TMS – Transcranial magnetic stimulation
TTX - Tetrodotoxin
TWSTRS – Toronto Western spasmodic torticollis rating scale
UPDRS – Unified Parkinson’s disease rating scale
Chapter 1 - INTRODUCTION

1.1 Basal Ganglia

The basal ganglia (BG) are a group of subcortical nuclei involved in motor and cognitive functions. The major components of the BG are the striatum, the subthalamic nucleus (STN), the substantia nigra, and the globus pallidus. The striatum is the primary input structure of the BG, receiving input from cortex, and is itself composed of the caudate and putamen. The STN is a secondary input structure, also receiving input from the cortex. The substantia nigra is composed of two segments, the pars compacta (SNc) and pars reticulata (SNr), with each region functionally discrete and composed of distinct neuron types. Similarly, the globus pallidus is composed of two segments, the external segment (GPe) and internal segment (GPi). Like the nigra, each segment is functionally discrete, receiving different inputs and projecting to different targets. The GPi and SNr are the major output nuclei of the BG and project to a number of structures. These include the thalamus, where GPi and SNr projections are relayed to the motor cortex via the ventral lateral and ventral anterior nucleus of the thalamus (Schell and Strick, 1984), and the superior colliculus, where SNr projections synapse on upper motor neurons that command eye movements (Hikosaka et al., 2000).

1.1.1 Striatum

The striatum is the major input structure of the basal ganglia and is composed of the caudate and putamen. Rostrally, these two nuclei appear as one structure but further caudally they are separated by the internal capsule (Utter and Basso, 2008).
Different cortical areas project to distinct regions of the striatum resulting in multiple parallel information processing circuits. For the purpose of this review, discussion will be limited to the motor circuit. In the motor circuit, excitatory cortical afferents to the basal ganglia arise from the primary motor cortex, the somatosensory cortex, the premotor cortex, and the supplementary motor cortex (Kemp and Powell, 1970). The motor circuit is a closed circuit since the cortical regions providing the input are also the target of basal ganglia output; the primary target of the basal ganglia, via the thalamus, is the supplementary motor cortex (Utter and Basso, 2008).

Medium spiny striatal projection neurons (MSNs) also receive input from dopaminergic neurons of the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). These striatal projection neurons have two types of dopamine (DA) receptors, D1 and D2. Both are metabotropic G-protein coupled receptors but their activation has opposite effects; DA binding to a D1 receptor results in depolarization of MSNs while DA binding to D2 receptors results in MSN hyperpolarization (Sealfon and Olanow, 2000).

Thus, the action of D1 receptors is to increase corticostriatal influence whereas the action of D2 receptors is to decrease corticostriatal influence and there is some evidence to suggest a differential distribution of DA receptor subtypes on the dendrites of striatal neurons. Striatal neurons projecting straight to the output nuclei of the basal ganglia have predominantly D1 receptors while those projecting to nuclei other than the output, or indirectly, have predominantly D2 receptors on their dendrites and as a result, should exert opposing influences on motor function. This dichotomy led to the classic
notion of the indirect pathway inhibiting movement and the direct pathway facilitating movement (Albin et al., 1989) which will be covered in more detail in a later section.

Interestingly, it seems that while D1 and D2 receptors are segregated in the majority of striatal neurons, a substantial number of striatal neurons coexpress both dopamine receptor mRNA subtypes (Lester et al., 1993), with some studies contending that almost all striatal neurons contain dopamine receptors of both classes (Aizman et al., 2000). However, the new technique of optogenetics has allowed direct testing of the original hypothesis and appears to validate the idea that activation of the ‘direct’ pathway facilitates movement and activation of the ‘indirect’ pathway inhibits movement. Optogenetic activation of direct and indirect pathway medium spiny projection neurons, achieved through the expression of a light activated protein under control of regulatory elements for the dopamine D1 or D2 receptor, lead to increased locomotion during direct pathway activation and induced a parkinsonian state during indirect pathway activation (Kravitz et al., 2010).

In addition to the projection neurons of the striatum, there are three primary types of interneurons within the striatum: cholinergic large aspiny neuron or tonically active neurons (Bolam et al., 1984; Kemp and Powell, 1970), GABAergic medium aspiny neurons (Kita and Kitai, 1988) and the somatostatin, neuropeptide Y, and NADPH diaphorase containing aspiny neurons (Wu and Parent, 2000).

**1.1.2 Subthalamic Nucleus**

The STN is a biconvex-shaped structure that lies ventral of the thalamus (Utter and Basso, 2008). Conventionally, the anatomical position of the STN in the BG is
within the indirect pathway, but it is also now considered a secondary input structure of the basal ganglia, because like the striatum it receives input directly from cortex (Nambu et al., 2002). Functionally, the STN is divided into a dorsolateral sensorimotor region and a ventromedial associative region (Hamani et al., 2004). In primates, cortical afferents to the motor region of STN arise from the primary motor cortex, the pre-motor cortices, the supplementary motor area (SMA), and the pre-supplementary motor area (Nambu et al., 2002).

The GPe sends afferent inhibitory GABAergic projections to the STN, while the STN sends reciprocal excitatory glutamatergic projections back to the GPe, and also sends excitatory glutamatergic projections to the output nuclei of the basal ganglia, the GPi and SNr. These glutamatergic projections from STN are the only excitatory projections within the BG (Kitai and Deniau, 1981). While the majority of afferent projections to the STN arise from the pallidum and motor cortex, many other nuclei send afferent projections to the STN. These include dopaminergic projections from the substantia nigra (François et al., 2000), glutamatergic projections from thalamus (Parent and Hazrati, 1995a) and frontal and supplementary eye fields (Matsumura et al., 1992), cholinergic projections from the PPN (Lavoie and Parent, 1994), and serotonergic projections from the dorsal raphe (Canteras et al., 1990). As such, many nuclei are contributing to tuning the STN’s contribution to the modulation of the output nuclei of the basal ganglia.

Brief motor cortical stimulation in vivo meant to approximate a descending motor command generates a triphasic response in STN neurons (Fujimoto and Kita, 1993; Maurice et al., 1998; Magill et al., 2004). The first is excitatory and mediated by the
direct cortico-subthalamic pathway. The second is a period of reduced activity, mediated by feedback inhibition from the GPe and lasts 5ms. The final response is a second phase of excitation, which lasts for 5 ms and is due to striatal-mediated inhibition of the GPe, which results in brief disinhibition of the STN.

STN neurons are capable of rhythmically discharging action potentials in the absence of any synaptic input, giving STN neurons autonomous pacemaker activity (Bevan et al., 2007). This activity is driven by voltage-dependent Na+ (Nav) channels, which underlie persistent and resurgent currents (Do and Bean, 2003). STN neurons “rest” at depolarized membrane potentials where Nav channels open. In the absence of opposing currents, progressive depolarization and action potential generation occurs, ultimately leading to the production of rhythmic, autonomous single-spike activity in the absence of synaptic input when the cycle repeats (Surmeier and Bevan, 2003).

Tonic spontaneous activity of pallidal neurons in slices retaining axonal connections to the STN reduces the frequency and regularity of autonomous STN activity, predominantly through the phasic activation of synaptic GABA_A R (Hallworth and Bevan, 2005). The end of hyperpolarization in STN neurons results in a rebound burst of APs (Beurrier et al., 1999) driven by CaV3 voltage-gated Ca2+ channels, which underlie a low-threshold Ca2+ spike upon which a burst of APs ride (Bevan et al., 2007; Hallworth et al., 2003).

1.1.3 Substantia Nigra

The substantia nigra is comprised of a compact and a diffuse clustering of neurons that lie dorsal to the cerebral peduncle in the ventral midbrain (Utter and
Basso, 2008). It consists of two anatomically and functionally distinct sections, the pars compacta (SNC) which contains large DA cells that provide the dopaminergic input to the striatum, and the pars reticulata (SNr), which together with the GPi, are GABAergic output nuclei of the basal ganglia.

1.1.3.1 Pars Compacta

The SNc is a thin, disk-shaped nucleus of densely packed cells dorsal and superior to and covering most of the dorsolateral and anteroposterior extent of the larger, more extensive pars reticulata (Hanaway et al., 1970). The dopaminergic cells of the SNc modulate the flow of information from the cortex through the basal ganglia.

The majority of afferent inputs to the pars compacta are GABAergic and arise from within the basal ganglia; the striatum (Bolam and Smith, 1990), the external segment of the globus pallidus (Grofová, 1975), and the SNr (Tepper et al., 1995) all send GABAergic projections to the dopaminergic SNc. Electrical stimulation of any of these structures induces inhibition of SNc dopaminergic neurons (Paladini et al., 1999).

The SNc sends dopaminergic projections throughout the basal ganglia. The primary projection is to the striatum, where dopamine modulates activity at the input of the basal ganglia (Graybiel, 1990), but direct dopaminergic axonal projections to the pallidum and STN exist (Cossette et al., 1999), as do ventrally projecting dopamine-releasing dendrites to the SNr (Cheramy et al., 1981; Geffen et al., 1976; Korf et al., 1976).

SNc dopaminergic neurons fire spontaneously in vivo at approximately 4 to 5 Hz (Dai and Tepper, 1998). These cells do not respond to active or passive movements
(DeLong et al., 1983) but do respond to reward and behavioural conditioned stimuli (Schultz, 1986).

1.1.3.2 Pars Reticulata

Compared to the overlying cell rich SNc, the SNr is characterized by a low neuronal density, the cells being interspaced within a dense neuropil of radially extending dendrites issued from both the SNr and SNc neurons (Deniau et al., 2007).

D1-containing striatal MSNs provide a major source of GABAergic input to SNr neurons (Parent and Hazrati, 1995b), with the GPe providing an additional source of GABAergic input (Bevan et al., 1996). Since the striatum also sends GABAergic projections to the GPe, the striatum can simultaneously influence the SNr via two circuits. This will be discussed in more detail in a later section, but briefly, functionally the circuits are expected to exert opposite effects on SNr cells; contrary to the inhibitory function of the direct striatonigral projection, the striato-pallido-nigral projection should exert an excitatory influence on SNr cells via a disinhibition (Deniau et al., 2007). STN neurons provide an additional route through which information is passed to basal ganglia output neurons; glutamatergic STN projections, themselves integrating information from GPe and directly from cortex, tonically excite SNr neurons (Féger and Robledo, 1991). The SNr also has a recurrent axon collateral network through which SNr cells themselves are engaged in mutual inhibitory interactions (Mailly et al., 2003).

The SNr sends GABAergic projections to 3 primary efferent targets: the ventral medial and parafascicular thalamic nuclei from which projections to the motor cortex arise (Deniau and Chevalier, 1992; Tsumori et al., 2003), the superior colliculus which is
implicated in orienting behavior and eye movement (Hikosaka et al., 2000), and to regions of the pontine tegmentum controlling postural tone and locomotion (Takakusaki et al., 2004). As mentioned in the preceding section, the SNr also sends inhibitory projections up to the SNc. GABAergic nigrothalamic, nigrocollicular, and nigrosegmental neurons are characterized by short-duration APs and a spontaneous repetitive firing of 40–80 Hz (Deniau et al., 1978; Wilson et al., 1977), thereby exerting a tonic inhibitory influence on the target structures.

The short-duration APs are the result of a strong voltage-dependent K+ conductance; this conductance prevents the membrane potential from reaching the Na+ inactivation level, leading to continuous high frequency discharge of SNr cells (Nakanishi et al., 1987). Inward currents that depolarize SNr cells up to the AP threshold drive the spontaneous pacemaker activity. These currents are caused by a slowly inactivating voltage-dependent tetrodotoxin (TTX)-sensitive Na+ current and a TTX-insensitive current mediated in part by Na+ (Atherton and Bevan, 2005). The precision of the spontaneous activity is controlled by a post-spike hyperpolarization caused by the small conductance calcium-activated potassium channel (SK), which activates following Ca2+ entry during an AP discharge (Deniau et al., 2007).

1.1.4 Globus Pallidus

Like the striatum, the globus pallidus is separated into two nuclei. Laterally is the external segment of the globus pallidus (GPe) and medially is the internal segment of the globus pallidus (GPI). Both nuclei share a similar morphology and both send inhibitory GABAergic projections to their respective targets (Smith et al., 1987) but they
are functionally distinct. Both the GPe and GPi receive inhibitory GABAergic input from the striatal MSNs, and a direct projection exists from the GPe to the GPi.

1.1.4.1 External Segment

The GPe is a relatively large nucleus located caudomedially to the striatum that receives glutamatergic excitatory afferent input from the STN and GABAergic inhibitory afferent input from D2-containing MSNs of the striatum (Kita, 2007). GABAergic projections from the GPe extend throughout the basal ganglia to the STN, SNc, SNr, GPi and striatum (Parent and Hazrati, 1995b). GPe neurons also send local GABAergic collateral projections to other GPe neurons and a small number of projections outside of the basal ganglia to the thalamus, inferior colliculus, and PPN (Kita, 2007). The GPe has substantial influence on the ultimate output of the basal ganglia, due to its direct inhibitory projections to the output nuclei and the fact that it has a primary inhibitory influence on the STN, which itself sends excitatory projections to the output nuclei.

The majority of GPe neurons fire at a relatively high mean frequency of 55 Hz with periods of spontaneous pauses, while the remainder are low frequency bursting neurons (Kita, 2007). The majority of both types of neurons respond to somatosensory input and passive limb movements (Filion et al., 1988).

1.1.4.2 Internal Segment

The GPi is located ventral to the GPe with the two structures being separated by a medullary lamina. The GPi is one of the output nuclei of the basal ganglia and must integrate all preceding information and relay the final signals to targets outside the basal
ganglia. It receives glutamatergic excitatory afferent input from the STN and GABAergic inhibitory afferent input from D1-containing MSNs of the striatum as well as the GPe.

GABAergic projections from GPi ascend from the basal ganglia and terminate in the ventral anterior/ventral lateral thalamic complex, primarily in the ventral lateral nucleus (VLo), the parvicellular part of the ventral anterior nucleus (VApc), and the centromedian nucleus (CM) (Kuo and Carpenter, 1973). GPi fibers entering the thalamus terminate primarily on the soma and proximal dendrites of thalamic projection neurons but also occasionally synapse with GABAergic inhibitory interneurons in the thalamus (Nambu, 2007). In addition to ascending projections to thalamus, the GPi also sends descending inhibitory projections to the PPN as well as a large projection to the lateral habenular nucleus (Parent et al., 2001).

The majority of GPi neurons fire spontaneously without pauses at a frequency of 60-80 Hz with this activity believed to be exerting a tonic inhibitory effect on its target nuclei (Nambu, 2007). These cells share the properties of SNr cells, namely spontaneous repetitive firing, rebound firing after a hyperpolarizing current pulse, and low-threshold calcium spikes (Nambu and Llinaś, 1994). A minority of cells found in the GPi, named “border cells” due to their location near the perimeter of pallidal segments, fire at a lower frequency (20–50 Hz) with a regular discharge pattern, and are thought to be cholinergic neurons of the nucleus basalis (DeLong, 1971), a structure laying just inferior to the globus pallidus.
1.1.5 Receptor Composition at Basal Ganglia Output Neurons

The GPi and SNr are considered to be very similar in morphology and physiology; they both receive excitatory inputs from the STN and inhibitory inputs from the striatum, GPe, and local axon collaterals. The vast majority of the synapses onto basal ganglia output neurons are GABAergic in nature (Ribak et al., 1981, 1979) (Figure 1, left side). Approximately 70% of synaptic terminals in output nuclei arise from GABAergic striatal spiny neurons (Shink and Smith, 1995), while GABAergic afferents from GPe make up approximately 15% of terminals. Finally, approximately 10% of inputs are glutamatergic afferents from the STN (Nambu, 2007), and both output nuclei also receive dopaminergic projections from the SNc (Smith and Kieval, 2000). They also both innervate motor thalamic and brainstem nuclei. As such, the GPi and SNr are the output nuclei of the basal ganglia and relay the final signals outside the basal ganglia. How the SNr and GPi integrate, balance, and choose between these final signals is due in large part to the pre and post-synaptic neurotransmitter receptor composition in the SNr and GPi.

The distribution of GABA_A and GABA_B receptors (GABA_ARs and GABA_BRs respectively) in the output nuclei of the basal ganglia is shown in Figure 1, right side. GABA_ARs are located primarily on the postsynaptic membrane at synapses of striatal and pallidal projections (Fujiyama et al., 2002). Extrasynaptic GABA_ARs are present but their density is much lower than at synapses (Boyes and Bolam, 2007). In contrast, GABA_BRs are more widely distributed than GABA_ARs and are mainly located extrasynaptically (Boyes and Bolam, 2003). They also colocalize with GABA_ARs postsynaptically at striatopallidal, striatonigral and pallidonigral synapses (Boyes and
Bolam, 2003). Additionally, they are located presynaptically at the same three synapses listed above, as well as on glutamatergic terminals from subthalamic projections (Galvan et al., 2004).

GABA<sub>A</sub>Rs are ionotropic and ligand-gated ion channels composed of five subunits, with a central Cl<sup>-</sup> permeable pore. Once two GABA molecules bind to the extracellular receptor domain, a conformational change is induced and Cl<sup>-</sup>, in most adult neurons, moves into the cell resulting in an inhibitory hyperpolarization (T. Goetz et al., 2007). The K<sup>+</sup>Cl<sup>-</sup> co-transporter (KCC2) extrudes intracellular chloride, generating an inwardly directed chloride gradient that makes increases in chloride conductance hyperpolarizing (Payne et al., 2003, 1996).

The GABA<sub>B</sub>R is a metabotropic G-protein coupled receptor functioning as a heterodimer in which the GABA<sub>B(1)</sub> subunit binds GABA and the GABA<sub>B(2)</sub> subunit is responsible for G-protein coupling and signaling (Emson, 2007). Activation of the presynaptic GABA<sub>B</sub>R results in a signaling cascade that inhibits the release of neurotransmitters through a decrease in membrane Ca<sup>2+</sup> conductance whereas activation of postsynaptic GABA<sub>B</sub>Rs results in a signaling cascade that increases membrane potassium conductance through G-protein coupling to inwardly rectifying potassium channels (Nicoll, 2004)
Figure 1 – Predominance of GABAergic synapses and distribution of GABA_A and GABA_B receptors in the GPi and SNr. Left side: ~ 70% synapses in SNr and GPi are with GABAergic striatal MSNs spiny neurons, while GABAergic afferents from GPe are ~ 15% of terminal and glutamatergic STN afferents are ~ 10%. Right side: GABA_A receptors are located primarily on the post synaptic membrane at synapses of striatal (Str) and pallidal (GPe) projections. Extrasynaptic GABA_A receptors are present but their density is much lower than at synapses. In contrast, GABA_B receptors are more widely distributed than GABA_A receptors and are mainly located extrasynaptically. They also colocalize with GABA_A receptors postsynaptically at striatopallidal, striatonigral and pallidonoigral synapses. They are also located presynaptically at the same three synapses listed above, as well as on glutamatergic terminals from subthalamic projections. Modified from (Shink and Smith 1995; Boyes and Bolam, 2007).
Put simply, fast inhibitory neurotransmission is mediated by synaptic GABA\(_A\)Rs while more complex modulatory activity takes place through GABABRs; they act as heteroreceptors and autoreceptors modulating the probability of release through presynaptic receptors and the excitability of neurons through postsynaptic receptors (Boyes and Bolam, 2007).

Additionally, binding studies show a prevalence of D1 receptors in the primate SNr and GPi (Richfield et al., 1987), with electron microscopy suggesting that these receptors are located on axons and terminals of direct striatal afferents in the output nuclei (Yung et al., 1995). Microdialysis releasing D1 agonist locally in the SNr or GPi results in increased GABA release (Trevitt et al., 2002) and decreased neuronal discharge of the SNr and GPi (Galvan et al., 2005). Microdialysis of a D1 antagonist locally in the GPi resulted in increased GPi firing (Kliem et al., 2007). It appears then, that dopamine acts at presynaptic sites in basal ganglia output nuclei to enhance GABA release from striatal projections. GABA can then act at all GABA\(_A\) and GABA\(_B\)Rs mentioned above to modulate the response of the output nuclei.

However, in addition to the prominent GABAergic projections to the GPi and SNr, the output nuclei also integrate excitatory transmission from glutamatergic projections from STN. Ionotropic AMPA and NMDA receptors are found in the postsynaptic densities of glutamatergic synapses on output nuclei neurons, while ionotrophic kainate (KA) receptors and group I metabotropic glutamate receptors (mGluRs) are found at peri- and extrasynaptic locations (Galvan et al., 2006).
Dopamine also plays a role at subthalamonigral / pallidal synapses. Activation of D1 enhances while activation of D2 depresses subthalamonigral excitatory postsynaptic currents (EPSCs). Interestingly, simultaneous blockade of both receptors induces larger evoked EPSCs, suggesting that D2-receptors have more influence than D1-receptors in the modulation of transmission (Ibanez-Sandoval et al., 2006).

To summarize, the inhibitory output of the GPi and SNr is modulated by input from other basal ganglia nuclei. The functional role of BG output nuclei in motor and cognitive functions is particularly exemplified by diseases such as Parkinson’s disease and dystonia, described in the following section, as models of basal ganglia activity discussed in later sections are primarily derived from studying the abnormal basal ganglia.

### 1.2 Parkinson’s Disease

First described by James Parkinson in 1817, Parkinson’s disease (PD) 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).

1.2.1 The Clinical Features of Parkinson’s Disease

Parkinson’s disease has classically been considered a pure motor disorder characterized by several cardinal motor features. These 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). In addition to these cardinal features, it is becoming increasingly recognized that PD is also characterized by non-motor manifestations (Gallagher et al., 2010). Whereas by definition, the cardinal motor signs are always present in PD patients, the non-motor signs, while prevalent, are not present in every patient.

The manifestation of PD motor 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).
1.2.1.1 Bradykinesia & Akinesia

Bradykinesia is characterized by reduced speed when initiating and executing a single movement, by a progressive reduction in the amplitude of movements during repetitive simple movements, and by a higher difficulty in executing sequential or simultaneous movements (Mazzoni et al., 2007). It is also the cardinal PD feature that appears to correlate best with degree of striatal dopamine deficiency (Vingerhoets et al., 1997).

It is hypothesized that bradykinesia results from a disruption in normal motor cortex activity mediated by reduced dopaminergic function in the basal ganglia (Jankovic, 2008); decreased cortical firing correlates with haloperidol induced bradykinesia in animal models (Parr-Brownlie and Hyland, 2005), while functional neuroimaging shows impaired recruitment of cortical systems responsible for regulating kinematic parameters of movement such as velocity (Turner et al., 2003).

Akinesia or poverty of movements refers to reduced frequency and amplitude of spontaneous automatic movements such as blinking, arm swinging or facial expression, and a lack of associated movements such as manual gesticulation. Such changes likely result from changes in dopaminergic modulation outside of basal ganglia circuits. It has been hypothesized that hypokinesia could be related to a lack of dopaminergic control of central pattern generators (Rodriguez-Oroz et al., 2009).

1.2.1.2 Rigidity

Rigidity is characterized by an increased muscular tone at rest and presents as a higher resistance to passive movements. It may occur proximally (e.g. neck, shoulders,
hips) and distally (e.g. wrists, ankles) and later in the disease may also occur axially, resulting in postural deformity (Jankovic, 2008). The underlying mechanism of rigidity in PD is poorly understood, and a direct relationship between dopamine deficiency and rigidity has not been found (Rodriguez-Oroz et al., 2009).

One potential mechanism may be increased excitability of long loop reflex pathways (Delwaide, 2001). Rapid stretching of a contracting muscle results in reflex responses of the homonymous muscle at different latencies, with the most rapid response being the familiar monosynaptic involuntary stretch reflex readily assessed by tapping a tendon with a reflex hammer. A longer latency response corresponds to transcortical involvement; it is hypothesized that if this transcortical loop is hyperactive, then an enhanced response to stretching may appear clinically as rigidity (Delwaide, 2001).

However, it is frequently observed that voluntary movement of the contralateral limb, a test known as Froment’s maneuver, can accentuate or even unmask latent rigidity (Broussolle et al., 2007). This implies that a systems-level, distributed brain network may contribute significantly to the mechanism of rigidity in PD. Indeed, a recent study supports the notion that rigidity is associated with widespread changes in the brain, as opposed to a single discrete locus. The study used functional imaging techniques to determine distributed brain connectivity patterns that predict clinical rigidity scores and found that a widely distributed cortical/subcortical network is associated with rigidity observed in PD patients (Baradaran et al., 2013).
1.2.1.3 Tremor

Parkinsonian tremor is a resting tremor of 4 to 6 Hz predominating in the distal parts of limbs that is suppressed by voluntary movements and is the most common and easily recognized symptom of PD. PD rest tremor can also involve the lips, chin, jaw and legs but, unlike essential tremor, rarely involves the neck and head or voice. Thus a patient who presents with head tremor most likely has essential tremor, cervical dystonia, or both, rather than PD (Jankovic, 2008).

Some patients with PD may display postural tremor, phenomenologically identical to essential tremor, which precedes the onset of parkinsonian tremor or other clinical features of PD for many years. Indeed, a growing body of evidence indicates that essential tremor is a risk factor for PD, with a subset of essential tremor patients predisposed to developing PD (Shahed and Jankovic, 2007).

The origin of this resting tremor is unknown and there is a lack of correlation between the severity of tremor and the level of striatal dopamine depletion. Moreover, striatal lesions can produce akinesia and rigidity but not resting tremor. There is some evidence for the involvement of the cerebellum in postural, but not rest tremor. A recent study found that motor cortical stimulation with transcranial magnetic stimulation reset the tremor rhythm for both rest and postural tremor while cerebellar transcranial magnetic stimulation reset postural but not rest tremor (Ni et al., 2010). Other studies seem to suggest the involvement of the cerebellum. New data using SPECT scanning and functional MRI suggests that rest tremor in PD patients correlates with dopamine depletion in the pallidum rather than in the striatum, and that the pallidum was active at
the onset of tremor, whereas the cerebellar circuit has activity correlating with the magnitude of on-going tremor (Helmich et al., 2011). Thus, there has to be basal ganglia pathology to have tremor, but the cerebellar pathways more directly produce the tremor. As such, it could be that resting tremor is due to changes in dopaminergic modulation of basal ganglia structures such as the GPi, which in turn could lead to a synchrony of the cerebellothalamocortical circuit (Helmich et al., 2011).

1.2.1.4 Postural Instability

Postural instability, or balance impairment, is a common problem in Parkinson’s disease, and represents one of the most disabling symptoms in the advanced stages of the disease, as it is associated with increased falls and loss of independence (Kim et al., 2013). The first large scale prospective study on postural instability showed that previous falls, disease duration, dementia, and loss of arm swing were independent predictors of falling (Wood, 2002). There were also significant associations between disease severity, balance impairment, depression, and falling (Wood, 2002).

Other factors influencing the occurrence of postural instability in patients with PD include other parkinsonian symptoms, orthostatic hypotension, age related sensory changes (Bloem, 1992) and the ability to integrate visual, vestibular and proprioceptive sensory input, with some suggesting that abnormal sensory organizational processes for postural control may be an inherent property of PD in the majority of patients (Bronte-Stewart, 2002).

L-Dopa treatment of postural instability is marginally effective (Marsden, 1994), and L-Dopa has even been shown to have a worsening effect in a study that assessed
sway amplitude as a measure of postural instability (Bronte-Stewart, 2002). Very similar results have been obtained with STN stimulation, with small improvements in axial stiffness, sway area, mean sway velocity, and sway frequency, as well as a similar worsening of sway amplitude (Maurer et al., 2003). These effects, both beneficial and adverse, became more pronounced in the combined (L-Dopa & stimulation) treatment condition (Maurer et al., 2003). Pallidotomy has also been shown to be effective in improving the axial signs of Parkinson's disease, but the beneficial effects begin to wane after 12 months (Roberts-Warrior, 2000).

### 1.2.1.5 Non Motor Features of Parkinson’s Disease

As mentioned above, in addition to the motor symptoms, PD is often associated with a myriad of non motor symptoms. Both motor and non motor symptoms increase with disease progression and this is particularly the case for cognitive deficits and psychiatric disturbances, which can be present at the time of diagnosis (Foltynie et al., 2004) but become increasingly relevant over time, particularly in regard to dementia (Hely et al., 2005).

The non motor abnormalities range from cognitive deficits and psychiatric disturbances to pain, hyposmia, sleep disturbances, and autonomic failure (Gallagher et al., 2010). The main cognitive deficit is executive dysfunction, or a difficulty in planning and problem solving, maintaining and shifting attention, and a loss of the ability to initiate, execute, inhibit and monitor a sequence of actions (Dirnberger et al., 2005). As PD progression continues, in addition to further dopaminergic depletion in the motor areas of the posterior putamen, dopaminergic depletion increases in anterior regions of
the putamen and caudate, disturbing the associative and limbic circuits of the basal ganglia (Rodriguez-Oroz et al., 2009). Although not well understood, this supplementary depletion may also account for the psychiatric disturbances often seen in PD, such as depression, anxiety and apathy (Rodriguez-Oroz et al., 2009).

To summarize, early dopamine depletion disturbs the motor loop and is responsible for cardinal motor signs of PD. As the disease progresses, additional dopaminergic deficit disturbs the associative and limbic circuits of the basal ganglia giving rise to non motor features, such as cognitive and psychiatric abnormalities.

1.2.2 Etiology

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

Initial characterization of PD pathology focused on the presence of Lewy bodies, eosinophilic hyaline inclusions suggested to be pathogenic due to their consistent observation in post-mortem tissue in selectively vulnerable neuronal populations (Lang and Lozano, 1998a). However, some 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.

Oxidative stress has long been associated with the death of dopaminergic neurons, and the susceptibility of these neurons to oxidative stress was emphasized
with the discovery of the ability of 6-OHDA to destroy dopaminergic cells following stereotaxic injections in the substantia nigra (Ungerstedt et al., 1974). The subsequent discovery of antioxidant enzyme system deficiencies (Saggu et al., 1989), together with evidence of oxidative damage to lipids (Dexter et al., 1994), proteins (Alam et al., 1997a), and DNA (Alam et al., 1997b), strengthened the notion that oxidative stress was contributing to PD pathogenesis.

Interestingly, oxidative stress may not be confined to the brain in PD, as alterations in a variety of markers of oxidative damage are found in the periphery, suggesting a more generalized disease process (Kikuchi et al., 2002).

Additionally, the source and nature of the reactive oxygen species remains unknown. Dopamine metabolism appears an unlikely candidate, as this leads to the formation of hydrogen peroxide rather than the more reactive superoxide or hydroxyl radicals. Also, changes in dopamine metabolism would not explain why some nigral dopaminergic neurons are spared or other dopaminergic areas of the brain are unaffected or how nondopaminergic neurons die in PD (Schapira, 2008).

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 (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).

1.2.2.1 Genetic

While the great majority of parkinsonian cases have long been considered sporadic, the study of familial instances of parkinsonism have made it clear that genetic factors predispose certain individuals to the disorder. Indeed, genome-wide association studies have indicated that the close resemblance between familial and sporadic forms on the clinical and pathologic level also has its correspondence in their genetic underpinning: common genetic variants in genes identified in monogenic forms or in genes belonging to the identified pathways have been found to confer a risk of developing the sporadic disease (Gasser et al., 2011).

Evidence supporting a genetic etiology for PD is derived from genotyping kindreds with rare inherited forms of the disease. Genetic analysis has now confirmed a multitude of gene mutations to be associated with PD in both an autosomal dominant (including α-synuclein (Polymeropoulos et al., 1997) and LRRK2 (Zimprich et al., 2004))
and recessive (including Parkin (Hattori et al., 1998), PINK1 (Valente et al., 2004), and DJ-1 (Bonifati et al., 2003)) manner.

Mutations in α-synuclein leads to aggregation of α-synuclein proteins which themselves comprise the fibrillar component of Lewy Bodies (Spillantini et al., 1997), the protein aggregate long acknowledged as the pathological landmark in both familial and sporadic PD. The amino acid changes in α-synuclein proteins are thought to lead to an increased tendency of the protein to form oligomers and fibrillar aggregates (Karpinar et al., 2009), eventually resulting in neuronal dysfunction and cell death, although the particular relationship between mutations, aggregate formation, and their toxicity is still poorly understood (Gasser et al., 2011).

Mutations in the LRRK2 gene are also inherited in an autosomal dominant manner, and are the most common cause of dominantly inherited PD discovered to date (Gasser et al., 2011). Multiple disease-causing mutations have been identified, along with common variants that appear to act as risk factors of varying degrees. For example, one mutation was found in 9% of Chinese PD patients and 3% of healthy individuals (Farrer et al., 2007). The clinical manifestation of LRRK2-related PD closely resembles typical sporadic PD and the pathological changes in LRRK2-related PD are consistent with typical Lewy body pathology (Healy et al., 2008). However, despite its known kinase activity, the actual substrate and disease-relevant function of LRRK2 are still unknown (Dächsel and Farrer, 2010).

The most common cause of recessively inherited PD is mutations in the Parkin gene. In addition to the classic PD phenotype, mutations in Parkin lead to a much
earlier age of onset, foot dystonia, and hyperactive deep-tendon reflexes (Kubo, 2006). The Parkin gene encodes an E3 ubiquitin ligase that transfers activated ubiquitin to lysine residues in proteins destined for degradation by proteasomes. As such, 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. However, while the loss of dopaminergic neurons in the substantia nigra is typical of this form of the disease, the relationship between Parkin and Lewy bodies is unclear, with some postmortem studies showing Lewy body pathology (Farrer et al., 2001), and others a notable absence of Lewy bodies (Ishikawa and Takahashi, 1998). More recent studies suggest that Parkin may also play an important role in mitochondrial function in a common genetic pathway that is shared by PINK1 (Tan and Dawson, 2006), another protein in which mutations lead to recessively inherited PD.

Other forms of recessively inherited PD are due to mutations in the gene for PINK1, which encodes a mitochondrial protein kinase, and DJ-1, which encodes a protein that may function as a sensor of oxidative stress. The gene product functions of DJ-1 and PINK remain unclear but both appear to protect against mitochondrial damage (Martella et al., 2009a). Mutations in DJ-1 or PINK would render dopamine neurons susceptible to mitochondrial damage, leading to cell death.
1.2.2.2 Epidemiological

Epidemiological studies have reported that living in a rural area, drinking well water, farming, and exposure to pesticides may be significant risk factors for developing PD, suggesting there are environmental factors found in rural environments that may cause PD (Priyadarshani et al., 2001). Although the risk of PD increased with increased duration of exposure to pesticides, a detailed meta-analysis found no significant dose-response relationship, and no specific type of pesticide was identified (Priyadarshani et al., 2000). More recently, a family-based case-control study found that when classifying pesticides by functional type, both insecticides and herbicides were found to significantly increase risk of PD (Hancock et al., 2008). Specifically, two insecticide classes, organochlorines and organophosphorus compounds, were significantly associated with 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. What is of particular interest, is that the biochemical abnormalities identified in postmortem sporadic PD brain have mapped to the biochemical consequences of the gene mutations identified as causes of familial PD (Schapira and Jenner, 2011). Thus, there is a convergence of pathways that seem to lead to dopaminergic, and possibly nondopaminergic, cell death in PD.
1.2.3 Current Parkinson's Disease Treatments

1.2.3.1 Dopamine Therapy

Dopamine replacement therapy can virtually abolish the motor symptoms of PD, and in this respect, after almost 50 years of clinical use, the dopamine precursor levodopa (L-Dopa) is still the gold standard of symptomatic efficacy.

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 or serotonergic projections from the midbrain raphe nuclei to the striatum, and converted to dopamine by aromatic L-amino-acid dopa-decarboxylase (Cenci and Lundblad, 2006). 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 original dopaminergic function. L-Dopa significantly improves bradykinesia and akinesia (Vingerhoets et al., 1997) and also improves, to varying degrees, rigidity and tremor, 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, 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). L-Dopa induced dyskinesias will be discussed in more detail in a later section.

One strategy to reduce the risk for motor complications is the use of dopamine receptor agonists that 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 oedema. Converging evidence from experimental and clinical studies suggests that intermittent drug delivery is a major factor for the development of levodopa-related response oscillations and dyskinesias, and that continuous delivery of a dopaminergic drug will prevent pulsatile stimulation and avoid motor complications (Olanow et al., 2006). As such, efforts to improve dopaminergic drug delivery have focused on continuous levodopa delivery.

**Intestinal Levodopa Delivery**

An infusible formulation of 2/0.5 g levodopa / carbidopa embedded in a 100 ml gel cassette (Duodopa, Abbott Healthcare, North Chicago, IL, USA) has been developed with continuous intrajejunal delivery in mind. The cassette attaches to a
portable infusion pump and the gel is pumped through a transabdominal tube connected to a percutaneous endoscopic gastrostomy tube with the tip positioned in the jejunum or duodenum (Hauser, 2011). The gel is typically infused during waking hours, although the system allows for 24 hour delivery with an adjustable delivery rate where required.

Efficacy in treating motor fluctuations and dyskinesias in this manner is high, but the incidence of technical problems, including tube leakage, obstruction, and disconnection is also high (Devos, 2009). For this reason, as well as the invasiveness of the installation procedure, the population in which intestinal levodopa infusion can be considered is similar to the deep brain stimulation (DBS) population (Hauser, 2011), that is, patients whose fluctuations and dyskinesias cannot be adequately managed with available oral medications. In particular, intestinal levodopa infusion appears to be a reasonable alternative to DBS in patients with mild dementia and hallucinations, a group that would not normally be acceptable for DBS.

*Transdermal levodopa delivery*

Transdermal patch delivery of levodopa has been attempted through the use of levodopa ethylester, but this approach was ultimately terminated due to application site reactions deemed unacceptable for a product intended for chronic use (Heldman and Kushnir, 2006). Preliminary observations of a subcutaneous patch delivering a levodopa/carbidopa solution is in clinical testing, with early results suggesting that the method, when combined with orally administered levodopa, increases the bioavailability and efficacy of orally administered levodopa (Caraco et al., 2013). If ultimately
successful, this delivery method has the potential to significantly improve the current standard of treatment of PD patients.

**Extended Release Oral Levodopa**

Several extended-release oral formulations of levodopa have recently been compared with standard levodopa/carbidopa. Using one such formulation, IPX066, patients experienced less OFF time per day in addition to less frequent administration (Hauser, 2011), indicating that IPX066 maintains therapeutic levodopa concentrations significantly longer than standard levodopa. Future clinical trials will need to examine whether IPX066 use in early disease can reduce the development of dyskinesia.

**Novel Dopaminergic Drugs**

Several dopaminergic drugs in development are focused on inhibiting enzymes involved with dopamine metabolism and degradation in order to enhance central dopaminergic transmission (Poewe et al., 2012). These include inhibitors of monoamine oxidase-B (MAO-B), an enzyme that metabolizes dopamine by removing an amine group, and catechol-O-methyl transferase (COMT), an enzyme that inactivates catecholamines like L-Dopa and dopamine by adding a methyl group.

**1.2.3.2 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 noninvasive 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 GPi. The GPi 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. The two foremost explanations that have been proposed for the effect of HFS are a) that it silences stimulated neurons and b) that it introduces a new activity to the network (Hammond et al., 2008).

The first theory stems from the observation that, functionally, high frequency stimulation produces the same effect as a lesion of the stimulated area. Several studies have reported inhibition of neuronal activity locally during HFS in the GPi (Dostrovsky et al., 2000), STN (Filali et al., 2004), and SNr (Lafreniere-Roula et al., 2009) of humans, and GPi in primates (Boraud et al., 1996). The second theory proposes that HFS injects an HFS-driven activity within the circuit that subsequently propagates and modifies the pathological spontaneous activity in many nuclei. Studies examining the effect of HFS on downstream targets have found 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). Other work has shown that, in addition to firing rates, HFS affects additional properties of neuronal activity, 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.

Electrical stimulation of a nucleus with short duration pulses of less than 1 ms preferentially activates axons (Nowak and Bullier, 1998a, 1998b). This results in the generation of axonal spikes and the consequent antidromic and orthodromic activation of subsets of distant neurons that send axons to the stimulated structure or are synaptically connected to it (Hammond et al., 2008).

So, the two foremost explanations of DBS mechanism, inhibition and activation, are nonexclusive and likely the result of current spreading out radially from the site of simulation. Antidromic spikes produced by the HFS collide with spontaneous orthodromic spikes, leading to the blockade of ongoing (pathological) activity in subpopulations of basal ganglia neurons, as long as the orthodromic spontaneous activity is slower than the HFS introduced to the system. Axons activated by HFS also propagate spikes in the orthodromic direction and give rise to sustained neurotransmitter release. The question that still needs to be addressed is how postsynaptic responses, both excitatory and inhibitory, follow a high frequency chronic stimulation such as DBS, as the electrophysiological studies performed to date have focused on relatively short-term stimulations (Hammond et al., 2008).

The following section discusses alternative treatments currently being explored, such as novel drugs and gene transfer. Whether these treatments will ultimately serve as an adjuvant or replacement to DBS remains to be seen.
1.2.3.3 Other Treatments

A number of nondopamine receptors are expressed in different regions of the basal ganglia motor circuits and have become targets of Parkinson’s disease drug development.

Adenosine antagonists

The A\textsubscript{2A} adenosine receptor is expressed throughout the basal ganglia; it colocalizes with and acts as a modulator of dopamine receptors, and in PD its activation contributes to overactivity of the indirect pathway (Poewe et al., 2012). As such, A\textsubscript{2A} antagonists may reduce such overactivity and ameliorate parkinsonism.

In nonhuman MPTP monkeys, A\textsubscript{2A} antagonists provide motor benefit with little or no development of dyskinesia, and in animals treated with levodopa, improve motor function without worsening dyskinesia (Hodgson et al., 2010; Kanda et al., 2000). Interestingly, in rodent models there is also evidence of improvement in some non-motor functions including memory, olfaction, and mood (Prediger, 2010). Several antagonist candidates have reached the stage of clinical development but the efficacy and safety of A\textsubscript{2A} antagonists is still being investigated.

Glutamatergic antagonists

As mentioned, prolonged levodopa use unfortunately leads to the development of dyskinesias. Much work suggests that the development of dyskinesia is closely tied to excessive glutamatergic transmission in the striatum. Ionotropic glutamate receptors show altered subcellular localization and subunit composition in animal models of
levodopa induced dyskinesia (Cenci and Konradi, 2010). Indeed, amantadine, a weak nonspecific NMDA receptor antagonist, remains the only drug used in clinical practice with demonstrated long term anti-dyskinetic effectiveness (Wolf et al., 2010). However, other NMDA antagonists have failed to replicate the antidyokinetic effects of amantadine and researchers have shifted their focus to antagonists of AMPA and metabotropic glutamate receptors (Poewe et al., 2012).

In MPTP-lesioned and L-Dopa-treated monkeys, dyskinetic animals exhibited a redistribution of AMPA receptors, with an increased relative abundance of AMPA receptor subunits in the post synaptic membrane (Silverdale et al., 2010). This provided a rational for the treatment of dyskinesia with AMPAR antagonists since such redistribution would presumably cause increased sensitivity of striatal neurons to glutamate. However, while the use of AMPAR antagonists has been found to be well tolerated and safe, they have so far failed to demonstrate any benefit to PD symptoms, dyskinesia, or reducing “off” time (Eggert et al., 2010; Lees et al., 2012).

Metabotropic glutamate receptor 5 (mGluR5) is highly expressed postsynaptically on striatal neurons (Testa et al., 1994) where it positively modulates NMDA-mediated currents (Pisani et al., 2001) and dopamine-dependent signaling (Mao 2008 import). These effects increased the excitability of striatal spiny neurons (Pisani et al., 2001) and suggested that drugs acting at mGluR5 might prove useful for the treatment of movement disorders. Preclinical studies found that mGluR5 antagonists had antidyokinetic and anti-akinetic effects in both MPTP and 6-OHDA models of PD (Johnston et al., 2010; Rylander et al., 2010). Phase 2 clinical studies are ongoing, with early results demonstrating tolerability and some efficacy against dyskinesia (Fox, 2013).
Serotonin Agonists

Serotonin is released by dorsal raphe fibers in the striatum and serotonin 5-HT<sub>1A</sub> receptors are expressed both pre- and post-synaptically in striatum, where they modulate glutamatergic and dopaminergic neurotransmission (Huot et al., 2011). Interestingly, expression of 5-HT<sub>1A</sub> receptors appears to be increased in dyskinetic animals, as are serotonin transporter levels in human brain tissue (D Rylander et al., 2010), suggesting that levodopa treatment induces sprouting of serotonin axon terminals, with an increased incidence of synaptic contacts and a larger activity-dependent potentiation of dopamine release in the dopamine-denervated striatum.

Use of 5-HT<sub>1A</sub> receptor agonists in clinical trials have provided mixed results, with some reporting a reduction in dyskinesias (Olanow et al., 2004), improvements in on time without dyskinesia and reductions in off time, and others reporting no demonstrated efficacy against dyskinesias compared with placebo in randomized controlled trials (C. G. Goetz et al., 2007).

Gene and cell based therapies

Early attempts of pioneering embryonic cell transplantation in Parkinson’s disease were extremely promising. Dopaminergic nigrostriatal innervation was seemingly restored through embryonic dopaminergic cell transplants and was accompanied by new dopamine synthesis and reductions in rigidity and bradykinesia (Lindvall et al., 1990).

Unfortunately, subsequent studies including sham-surgeries failed to provide evidence of the symptomatic efficacy of this approach on conventional endpoints like
off-period motor function (Olanow et al., 2003). Additionally, more recent studies have shown potential host-to-graft propagation of Lewy-body disease in PD patients who previously received embryonic nigral transplants (Kordower et al., 2008; Li et al., 2008). Such observations have key implications not only for cell-based therapies but also for mechanisms of PD pathogenesis.

Recent work has focused on an alternative approach to restorative treatments in neurodegeneration, namely the viral vector-based targeted delivery of therapeutic genes. For example, the targeted delivery of the neurotrophic factor neurturin has been shown to restore and protect dysfunctional dopaminergic neurons in animal models of PD (Bartus et al., 2011). However, clinical studies in human subjects have not shown the same benefit (Marks et al., 2010) and whether this form of treatment will prove to be effective remains unknown. Another potential form of gene-based therapy is the introduction of the gene for glutamic acid decarboxylase (GAD), an enzyme responsible for synthesis of the inhibitory neurotransmitter GABA, into the STN. GAD gene transfer in the STN modifies the phenotype of STN neurons from predominantly excitatory to predominantly inhibitory (Luo et al., 2002), thereby reducing the excessive excitatory drive from STN to the output nuclei, and subsequently, reducing the excessive inhibitory output. Clinical trials of GAD infusion have shown the procedure to be safe and to convey a small benefit to motor scores in the OFF state when compared to patients receiving sham surgery (LeWitt et al., 2011). Whether these or other gene and cell based therapies will provide clinically meaningful long term benefit remains to be seen.
1.2.4 Dyskinesia

As mentioned in the previous section discussing current treatments of PD, the motor symptoms of PD are effectively treated with the DA precursor L-Dopa. L-Dopa is converted to dopamine and can rescue striatal DA dependent neuronal activity. However, L-Dopa treatment does not arrest disease progression and, with time, neuronal degeneration advances and leads to the emergence of a complex pattern of alterations that involves other basal ganglia nuclei. In addition, the initial excellent antiparkinsonian effects of L-Dopa are lost as time, and the disease, progresses. The continuing use leads to a pulsatile stimulation of DA receptors that causes a broader neuronal destabilization. As a result, new motor complications in the form of choreic, dystonic and ballistic movements develop, collectively known as L-Dopa-induced dyskinesia (LID). LID adversely affects the quality of life and increases healthcare costs in patients with PD (Péchevis et al., 2005), sometimes being more disabling than PD itself (Fahn, 2000). These very disabling long-term side effects of L-Dopa therapy represent a serious limitation to the current pharmacotherapy of PD.

Dyskinesia occurs more frequently with progression of PD, corresponding with degeneration of the nigrostriatal dopaminergic pathway. On average, 50% of subjects will have dyskinesia after five years and almost 90% by 10-15 years (Hely et al., 2005). Dyskinesia also occurs earlier and is more severe on the worse affected side. Patients with earlier-onset PD tend to be more prone to develop LID than patients with later onset PD (Kumar et al., 2005). Animal studies have suggested that at least 50% loss of dopaminergic terminals is required before dyskinesias will occur (Di Monte et al., 2000).
A wide range of clinical presentations of dyskinesia have been described, including neck, trunk, facial, and limb chorea and dystonia (Luquin et al., 1992). Peak-dose dyskinesia occurs when plasma levels of L-Dopa are high and tends to be primarily chorea with some dystonia (Lees et al., 1977). Diphasic dyskinesia, or “onset and end-of-dose dyskinesia,” occurs when plasma levels of L-Dopa are either rising or falling and tend to be predominantly dystonic (Lhermitte et al., 1978). Further, dystonic postures, particularly affecting the feet, are often present in the "off" period (Luquin et al., 1992).

1.2.4.1 Pharmacokinetics of L-Dopa

The short half life of 1 to 2 hours of L-Dopa is a key component in the abnormal pulsatile stimulation of postsynaptic dopamine receptors thought to lead to LID (Nutt, 2008). Under normal conditions, dopamine release is both tonic and phasic, implying that baseline levels of dopamine never fall below a certain threshold (Goto et al., 2007) and dopamine is removed from the synapse via the dopamine active transporter (DAT) (Torres et al., 2003). As PD progresses, especially in late stages of the disease where the buffering capacity of DAT has disappeared (Sohn et al., 1994), tonic dopamine release also ceases and dopamine release becomes exclusively phasic, i.e., pulsatile, following each dose of L-Dopa (Huot et al., 2013).

However, other factors can cause variability in L-Dopa access to the brain and add to the loss of normal regulation of synaptic dopamine and compound the pulsatile stimulation of dopamine receptors. Such factors include competition with dietary proteins for aromatic amine transporters and other gastrointestinal factors such as
constipation (Barichella et al., 2009). Additionally, it has been reported that chronic L-Dopa treatment induces the up-regulation of vascular endothelial growth factor in basal ganglia nuclei in both 6-OHDA rats and patients with PD and a history of LID (Ohlin et al., 2011). This growth factor can directly augment the permeability of the blood brain barrier by regulating the expression of endothelial tight junction proteins (Hawkins et al., 2010). Thus, altered blood brain barrier permeability may play an important role in the development of dyskinesia and synaptic levels of levodopa, since increased blood brain barrier permeability results in higher brain levels of L-Dopa (Westin et al., 2006).

Finally, genetic factors may play a role in L-Dopa metabolism. Catechol-O-methyltransferase (COMT) is an enzyme that degrades catecholamines, including levodopa and dopamine. The human COMT gene on chromosome 22q11 contains a common functional polymorphism, producing a substitution of valine to methionine at codon 158; this substitution causes lower COMT enzymatic activity resulting in higher bioavailability of levodopa and higher synaptic dopamine levels (D'Souza and Craig, 2006). A recent prospective study of 219 patients who were free of dyskinesias at the start of the study and underwent annual examinations revealed that patients carrying the substitution had an increased risk of developing dyskinesia during follow up (de Lau et al., 2012).

1.2.4.2 Mechanism

As a consequence of abnormal dopamine stimulation, many changes occur within the classic corticostriatal basal ganglia loop. Key features implicated in LID include: abnormal corticostriatal overactivity and synaptic plasticity, increased D1-
mediated stimulation and resultant increased activity of the inhibitory striatopallidal and striatonigral direct pathway, and reduced STN activity by increased inhibitory input from GPe. Details of changes in synaptic plasticity in LID will be left for a later section after sufficient background of the process itself is detailed. Here, the pre and postsynaptic changes at the nigrostriatal synapse will be briefly discussed.

1.2.4.2.1 Presynaptic Changes

Normal DA turnover results in low DA levels within the synaptic cleft under normal conditions, due to a combination of regulated DA release via a presynaptic D2 autoreceptor and DA reuptake via the DAT. Progressive loss of presynaptic DA terminals combined with impaired dopamine storage thus leads to impaired DA levels and dyskinesia development (Troiano et al., 2009). Loss of dopaminergic terminals means L-Dopa is converted to DA in other aromatic acid-decarboxlyase containing cells, such as serotonergic and endothelial cells. These cells contain the necessary enzymes to convert L-Dopa to DA, but lack the ability to store DA and thus, non-regulated release of DA into the synaptic cleft occurs. In particular, aberrant DA release from raphe-striatal serotonergic terminals has been shown to be associated with dyskinesia in rodent models of PD (D Rylander et al., 2010). Indeed, using 5HT1A or 1B agonists on presynaptic autoreceptors to reduce 5HT release can reduce dyskinesia in these models (Carta and Bezard, 2011). Unfortunately, the same approach in humans has not been clinically effective due to co-existant reduction in DA release.
1.1.4.2.2 Postsynaptic Changes

Postsynaptic changes are a very important factor in the development of dyskinesia, with phasic stimulation of postsynaptic DA receptors playing a key role. Indeed, nigrostriatal degeneration is not a prerequisite for LIDs since long-term treatment with L-Dopa alone is sufficient to induce dyskinesia in normal monkeys (Pearce et al., 2001). As mentioned above, with disease progression comes a loss of the normal buffering capacity of DAT due to the loss of the presynaptic terminal and tonic DA release slowing markedly, eventually becoming exclusively phasic, or pulsatile, following each dose of L-Dopa. Consistent with phasic stimulation of postsynaptic DA receptors playing a key role in the development of LID are the observations that de novo treatment with agents providing long-acting or continuous DA receptor stimulation in animal models can reduce or stop the occurrence of dyskinesia (Jenner, 2008). This work led to the use of long-acting DA agonists in early PD patients to prevent the onset of LIDs. Many PD patients with existing LID have seen success with continuous infusion of dopaminergic therapies in reducing established LID (Garcia Ruiz et al., 2008), as discussed in the above section detailing PD treatments.

In the striatum of non-human primates treated with MPTP, postsynaptic D1R density at the plasma membrane is elevated and becomes further increased with the onset LID (Guigoni et al., 2007). Similarly, in 6-OHDA rats treated with L-Dopa, an increased postsynaptic D1R density at the cell membrane correlates with the severity of LIDs (Berthet et al., 2009). Additionally, a recent study has shown that postsynaptic D1R internalization in striatal neurons can be enhanced by over-expression of the G-protein-coupled receptor kinase GRK6, and that this intervention alleviates LID in both
rat and primate models of PD (Ahmed et al., 2010). Changes in receptor density and sensitivity are very likely adaptive mechanisms employed by cells to maintain stability and functionality of DA transmission from residual DA neurons (Cenci and Konradi, 2010).

In addition to changes in dopamine receptor density, both NMDAR and AMPAR subcellular localization is altered. For example, in untreated MPTP monkeys NR1 and NR2B subunits of the NMDAR were found to be decreased in synaptosomal membranes, while the abundance of NR2A was unaltered (Hallett et al., 2005). Following L-Dopa treatment and onset of dyskinesia, NR1 and NR2B expression was normalized and NR2A levels were increased significantly above unlesioned control values, suggesting that a relative enhancement in the synaptic NR2A is implicated in LID (Hallett et al., 2005). Alternatively, studies in 6-OHDA rats have shown that the receptor-trafficking alteration most critically associated with LID is a redistribution of NR2B subunits between synaptic and extrasynaptic membranes (Fiorentini et al., 2006; Gardoni et al., 2006). A redistribution of AMPAR subunits also has been associated with LID; in a study on MPTP-monkeys receiving L-Dopa, dyskinetic animals were found to exhibit a redistribution of the GluR2/3 subunit of AMPARs from the vesicular fraction into the postsynaptic membrane, rendering striatal neurons of LID animals more sensitive to glutamate (Silverdale et al., 2010).

Increased receptor expression at the plasma membrane contributes to, and at the same time is the result of, altered intracellular signal transduction mechanisms in the dopaminceptive neurons. This will be discussed in detail in a section dedicated to altered plasticity in dyskinesia.
1.3 Dystonia

Dystonia is a heterogeneous group of movement disorders characterized by involuntary sustained muscle contractions, repetitive twisting movements, and postural abnormalities of the trunk, neck, face, or arms and legs (Tarsy and Simon, 2006). Dystonia can manifest focally, affecting an isolated body part such as the neck (cervical dystonia), or generalized to affect the whole body.

Precise information on the prevalence of dystonia has been difficult to establish because the existing epidemiological studies of the condition have adopted different methodologies for case ascertainment, resulting in widely differing estimates of prevalence. A recent meta-analysis and systematic review of existing population-based and service-based studies of primary dystonia incidence calculated an overall prevalence of primary dystonia of 16.43 per 100,000 (Steeves et al., 2012) but notes that due to the methodological limitations of the existing studies, their own prevalence estimate of primary dystonia likely underestimates the true prevalence of the condition.

The classification of dystonia has been through many revisions over time, influenced by a better understanding of clinical phenotype, genetics, and pathophysiology (Phukan et al., 2011). Currently, classification is primarily based on age of onset, distribution of affected body parts, and ultimate cause (Fahn, 2011). A category of secondary dystonia is used to describe cases in which dystonia arises due to a preexisting condition or other identifiable cause such as drug exposure.

Dystonia can develop at any age but those that are classified as early onset (<26 years old) are more likely to be severe (Phukan et al., 2011). The division into early and
late (>26 years) onset is used on the basis of the age by which carriers of a mutant
DYT1 gene will have developed dystonia (Bressman et al., 2002). Distribution of
affected body parts describes if and where dystonia may be localized. If localized to a
single body region, it is described as focal. If it has spread to adjoining regions, it is
described as segmental. Spread to non-adjoining regions is described as multifocal.
Generalized dystonia describes a condition in which there is involvement of legs, trunk,
and another body part, whereas hemidystonia describes a condition that affects the
body and limbs unilaterally. The age at onset of dystonia and the distribution of affected
body parts are connected; onset in the legs is common during childhood, and with
increasing age the site of onset ascends to the arms and hands, neck, and then cranial
muscles (Phukan et al., 2011). Late onset dystonia involving craniocervical muscles
remains localized or segmental in the vast majority of cases and is usually not
progressive (Bressman, 2004).

1.3.1 The Clinical Features of Dystonia

Dystonia arises from the involuntary concomitant activation of agonist and
antagonist muscles, with overflow of unwanted muscle contractions into adjacent
muscles (Tarsy and Simon, 2006). Dystonic movements can be slow or rapid, can vary
during different activities or postures, can become fixed in severe cases, and tremor is
sometimes present (Tarsy and Simon, 2006). Action dystonia refers to abnormal
postures that occur during voluntary activity and are sometimes task-specific. Some
localized dystonias respond to simple sensory tricks such as lightly touching the
affected body part.
Primary torsion dystonia (PTD) is the most common form of dystonia, accounting for approximately 75% dystonia cases across a wide clinical spectrum, from very early-onset generalized dystonia to adult-onset focal dystonia (Albanese et al., 2011). Focal PTD is about ten times as common as generalized forms, has onset in adulthood, and usually involves craniocervical muscles or arms (Phukan et al., 2011). Focal dystonia affecting the craniocervical region is more common in women than in men while the opposite is true for focal limb dystonia (Chan et al., 1991). A brief description of the clinical features of different types of primary dystonia follows.

1.3.1.1 Primary Generalized Torsion Dystonia

Primary generalized torsion dystonia is a progressive, disabling disorder that usually begins in childhood and is linked to several genetic loci (Tarsy and Simon, 2006). Dystonia beginning in childhood usually starts with action dystonia in one leg and approximately 3 of every 4 children with torsion dystonia have the disease progress, usually over the first 5 to 10 years, to generalized dystonia and often to severe disability (Marsden and Harrison, 1974). Children with onset in arm, trunk, or neck also may progress to generalized involvement, however, the illness tends to remain segmental in a larger proportion of these individuals than in those with onset in the arms or legs and dystonia beginning in the neck may have the greatest likelihood of remaining focal even in the young (Robottom et al., 2011).

1.3.1.2 Cervical dystonia

Cervical dystonia, also known as spasmodic torticollis, is the most common form of focal dystonia with onset typically between 30 and 50 years of age (Tarsy and Simon,
It often begins with neck stiffness and restricted head mobility which progress to
the main clinical symptoms of abnormal head posture, head tremor, and neck pain
(Tarsy and Simon, 2006). The abnormal head posture and tremor is the result of the
variable involvement of sternocleidomastoid, trapezius, and posterior cervical muscles,
with those muscles involved in the dystonia seeing increased muscle tone and bulk
(Phukan et al., 2011). Cervical dystonia typically progresses within the first 5 years
before stabilizing to follow a static course, although it can occasionally spread to
adjacent muscle groups and become segmental. Spontaneous symptom remission
occurs in approximately 20% of patients but many of those who do have remission
subsequently relapse (Jayne et al., 1984).

1.3.1.3 Blepharospasm

Blepharospasm is a form of cranial dystonia and is the result of dystonic
contractions of the orbicularis oculi (Grandas et al., 1988). Onset is usually in the sixth
decade, with the dystonic activity causing an increased blink frequency, forced eye
closure, or difficulty opening the eyes (Tarsy and Simon, 2006). Bright light, reading, or
driving typically aggravates symptoms and symptoms worsen over years, ultimately
leading to sustained eye closure and possibly functional blindness (Phukan et al.,
2011).

1.3.1.4 Oromandibular dystonia

Oromandibular dystonia affects the jaw muscles and causes involuntary
clenching, opening, or deviation of the jaw (Albanese et al., 2011). Muscles of the
mouth, tongue, or neck are frequently also involved, with severe cases causing jaw
pain, dysarthria, difficulty chewing, dysphagia, and dental trauma (Tarsy and Simon, 2006). Severity can increase when eating or talking (Phukan et al., 2011). The differential diagnosis includes temporomandibular joint disorders, bruxism, edentulous mouth movements, and tardive dyskinesia (Tarsy and Simon, 2006).

1.3.1.5 Laryngeal dystonia

Laryngeal dystonia or spasmodic dysphonia is a rare focal task-specific dystonia that affects laryngeal muscle control during speech and occurs sporadically in middle age (Ludlow, 2011). The most common form, representing 80-90% of cases, affects adductor muscles of the vocal cord and results in their hyperadduction closing the vocal folds too tightly and cutting off the voice (Parnes et al., 1978). The result is a strained or strangled voice quality. In a less common form the abductor muscles are affected with hyperabduction resulting in a separation of the vocal cords and a breathy voice with breaks in speech (Tarsy and Simon, 2006). Voice tremor can occur with both types of laryngeal dystonia (Phukan et al., 2011).

1.3.1.6 Writer’s cramp and other limb dystonias

Limb dystonias are frequently task-specific, that is they occur when a patient performs a specific task. Task-specific dystonia is thought to arise from chronic and repetitive movements, typically requiring extreme motor precision (Pont-Sunyer et al., 2010). Writer’s cramp is the most common form of task-specific dystonia, with onset between the ages of 30 and 50 years (Phukan et al., 2011). Excessive flexion of the thumb and index finger, with pronation of the hand and ulnar deviation of the wrist often occurs, with arm tremor in less than 50% of patients (Phukan et al., 2011).
Other task-specific dystonias affect those performing specific repetitive actions found in a number of professions (typist, cigar makers, florists, money counters, and letter sorters), musicians (piano, banjo, harpsichords, cello, clarinet), and sports players (golfers, dart players). It has been also described affecting lower limbs in dancers, runners, knife sharpeners, or foot painters (Pont-Sunyer et al., 2010).

Unlike orthopedic overuse syndromes with which they are often confused, limb dystonia responds poorly to rest (Tarsy and Simon, 2006).

### 1.3.2 Etiology

The pathophysiology of primary dystonia remains unknown. Lesions of the putamen and thalamus may cause secondary dystonia but gross pathological abnormalities and neurodegeneration have not been identified in primary dystonia, suggesting that biochemical or neurophysiologic abnormalities may be responsible (Tarsy and Simon, 2006). Indeed, research has uncovered neurophysiological abnormalities at multiple levels of the central nervous system. The central theme in these abnormalities is impaired inhibition, plasticity, and sensorimotor integration.

Behaviourally, there has been a longstanding hypothesis that the overflow of movement seen in dystonia is caused by a loss of inhibition. Human studies exploring inhibition and plasticity in dystonia have largely relied on the ability of transcranial magnetic stimulation (TMS) of the motor cortex to evoke measureable responses in a target muscle (Thompson et al., 2011). Because TMS stimulates the axons of neurons that synapse on pyramidal efferents, the size of the response produced by a stimulus reflects the intrinsic excitability of the cortex and motor evoked potentials recorded via
electromyography are indirect indicators of plasticity (Thompson et al., 2011). Early TMS studies showed a shift in the balance between excitation and inhibition in local circuits of the motor cortex (Ridding et al., 1995) and impaired cortical inhibition during voluntary muscle activation (Chen et al., 1997) in patients with focal dystonia.

Later studies demonstrated a loss of “surround inhibition” in dystonia, a phenomenon where muscles not involved in the desired movement are actively inhibited at the onset of a movement. It was shown that TMS triggered by index finger flexion suppressed motor-evoked potential amplitudes from the little finger muscle in healthy subjects while the amplitudes were enhanced in dystonia patients (Sohn and Hallett, 2004). This result supports the idea that disturbed surround inhibition is a principal pathophysiological mechanism of dystonia.

As mentioned, abnormalities in synaptic plasticity also appear central to the pathophysiology of dystonia. An in-depth explanation of plasticity and its many forms will be covered in a later section, but to introduce the concept simply and briefly, synaptic plasticity is a change in neurotransmission that either increases / strengthens, as in long term potentiation (LTP) or decreases / weakens, as in long term depression (LTD), the ability of a synapse to transmit information. Animal models show increased LTP and decreased LTD at corticostriatal synapses in slice preparations from a mouse model of dystonia (Martella et al., 2009b). In human patients, paired associative simulation (PAS), which combines TMS with median nerve stimulation, has shown that cranial and cervical dystonia is associated with enhanced corticospinal excitability compared to healthy controls (Quartarone et al., 2008). Increased excitability has also
been shown in the primary motor cortex (Quartarone et al., 2003) and in the primary somatosensory cortex (Tamura et al., 2009) of patients with focal hand dystonia.

To date, experiments exploring abnormal plasticity and altered inhibition have been limited to sensorimotor cortex, but such abnormalities could be secondary to changes originating in the basal ganglia and/or in addition to changes in the basal ganglia. The basal ganglia may act upon the motor cortex to enhance surround inhibition (Hallett, 2009; Mink, 1996) and/or changes in basal ganglia may contribute or be a major direct cause of the motor symptoms.

1.3.2.1 Genetic

While the pathophysiology of dystonia remains unidentified, many types of primary dystonia have been found to have an underlying genetic cause with clear monogenic inheritance in severe forms and potential genetic susceptibility factors in primary focal dystonia (Phukan et al., 2011). The genetic loci associated with primary dystonia include DYT1, 2, 4, 6, 7, 13, and 17. A great number of additional loci have been associated with dystonia-plus syndromes, paroxysmal dyskinesias, and heterodegenerative syndromes (Phukan et al., 2011).

The most common genetic cause is a mutation in the DYT1 gene that results in early-onset primary dystonia (Ozelius et al., 1997). DYT1 encodes a protein called torsinA, an ATPase chaperone-like protein, a type of protein involved in protein trafficking, membrane fusion, protein refolding, and degradation (Phukan et al., 2011). The most common mutation is a trinucleotide deletion (ΔGAG), which causes a deletion of a glutamic acid residue in the C-terminal region of torsinA (Ozelius et al., 1997).
TorsinA is found only in neurons, and is found particularly enriched in basal ganglia structures including the striatum, substantia nigra, and globus pallidus (Augood et al., 2003). A recent study showed that torsinA interacts with snapin (Granata et al., 2008), a protein known to play an essential role in vesicle exocytosis by enhancing the interaction between the SNARE complex and the synaptic vesicle marker, synaptotagmin. Mutant torsinA results in sequestering of both torsinA and snapin (Granata et al., 2008), which could potentially destabilize the balance between the exo and endocytic cycle, and ultimately affect neurotransmitter release (Granata et al., 2009) in key motor structures of the basal ganglia.

1.3.2.2 Environmental

Most cases of late onset primary dystonias appear to be sporadic. The actual cause is thought to probably be multifactorial, with several genes, along with environmental factors, combining to reach the threshold for dystonia (Phukan et al., 2011). Exposures to several environmental factors have been found to be more frequent in patients with primary dystonia than in control subjects. Studies have found a significantly greater frequency of neck/trunk trauma in cervical dystonia (Defazio et al., 1998), of work or leisure activities requiring repetitive and accurate motor tasks, such as playing an instrument or writing, in focal dystonias such as in writer’s cramp (Roze et al., 2009), of eye diseases in blepharospasm (Martino et al., 2005), and of sore throat in laryngeal dystonia (Schweinfurth et al., 2002).

A causal link for the majority of primary late onset dystonias and environmental exposure is lacking. However, the risk of writer’s cramp was associated with an abrupt
increase in writing time the year before dystonia onset and also increased with greater
time spent writing each day (Roze et al., 2009).

1.3.2.3 Secondary Dystonia

As previously mentioned, secondary dystonia describes cases in which dystonia
arises due to a preexisting condition or other identifiable cause. Such secondary causes
can include drug use, toxins, neurological and peripheral tissue damage, or as a
symptom of another neurological disease.

Secondary dystonia related to drugs can be effects of pharmacotherapy targeting
the DA system, such as levodopa, dopamine agonists, antipsychotic drugs, anti-
convulsant agents, and serotonin-reuptake inhibitors (Tarsy & Simon, 2006). Tardive
dystonia may occur after prolonged use of dopamine-receptor–blocking antipsychotic
drugs and metoclopramide. Dystonia may also occur as a result of the toxic effects of
manganese, carbon monoxide, carbon disulfide, and other chemicals (Tarsy and Simon,
2006).

Tissue damage, in the form of trauma, infection, infarcts, tumors etc. can also
cause secondary dystonia. Damage to the striatum and thalamus in particular are
common in secondary dystonias (Marsden et al., 1985).

In dystonia-plus and heterodegenerative disorders, dystonia is associated with
another movement disorder. In dystonia-plus disorders, there is no evidence of
neurodegeneration. Examples include myoclonus dystonia, a rare genetic disorder
causing myoclonic jerks and dystonia in childhood and adolescence (Quinn, 1996), and
dopa-responsive dystonia, another rare genetic childhood disorder causing foot
dystonia, gait abnormality, hyperreflexia, and eventually progressive generalized dystonia (Nygaard et al., 1991). In heterodegenerative disorders, dystonia arises as a symptom of a disorder with neurodegeneration, usually of the basal ganglia, such as PD or Hungtinton’s disease (Tarsy and Simon, 2006).

1.3.3 Current Dystonia Treatments

A variety of treatment approaches exist for dystonia, including pharmacotherapy, botulinum toxin, physical therapy, and surgery. Sensory tricks can also temporary ameliorate dystonic symptoms. The tricks consist of light touch to areas nearby to the site of dystonic contraction, which provide temporary relief of contraction (Phukan et al., 2011). Sometimes physical devices are used to help trigger these tricks (Jankovic, 2006).

1.3.3.1 Pharmacotherapy

As the name suggests, the previously mentioned dopa-responsive dystonia can be treated quite well with levodopa. Modest improvements with levodopa have been reported in patients with other types of dystonia besides dopa-responsive dystonia (Jankovic, 2004).

Anticholinergics are a pharmacologic intervention for generalized and segmental dystonia that are partially effective in 40 to 50% of patients but have considerable side effects, especially in adults (Phukan et al., 2011). Muscle relaxants such as benzodiazepines could provide additional benefit to patients for patients not responding well to anticholinergics alone (Jankovic, 2006).
Many other pharmacological interventions have been attempted in open-label trials, including baclofen, diphenhydramine, gabapentin, dopamine agonists, and dopamine antagonists, but they have failed to provide consistent benefit and are often accompanied by significant side effects (Phukan et al., 2011).

1.3.3.2 Botulinum Toxin

Botulinum toxin is the first-line treatment for primary cervical and cranial dystonia and is generally considered highly effective in the treatment of focal dystonia. It may also be used in patients with generalized or multifocal dystonia to treat selected muscles (Tarsy and Simon, 2006).

When injected into a muscle, the toxin functions by cleaving proteins involved with synaptic vesicle targeting and fusion with the presynaptic membrane thereby halting neurotransmitter release and blocking the release of acetylcholine into the neuromuscular junction (Jankovic, 2006). The clinical result is temporary muscle weakness with effects lasting up to 4 months with the benefit of botulinum toxin being roughly proportional to the degree of muscle weakness achieved (Hallett, 2000).

Botulinum toxin injections may become ineffective with time as antibodies are produced by the body (Jankovic, 2006), but the risk of antibody formation is much lower with current toxin preparations that have a lower protein load per injection (Jankovic et al., 2003).
1.3.3.3 Physical Therapy

Muscle stretching and strengthening may be used to avoid contractures, and mechanical assistive devices may reduce disability. Indeed, case studies suggest substantial benefit in terms of lower disability scores, less pain, and greater functional independence from an approach combining active and resisted movements, stretching of neck and trunk muscles, abdominal taping, and sensory tricks (Voos et al., 2013). Sensory training has also proven beneficial in small case studies of patients with focal hand dystonia. Following a 12 week program of aerobics, postural exercises, stress free hand use, and supervised, attended, individualized, repetitive sensorimotor training, subjects had substantial improvement in a range of somatosensory responses such as hand representation, target-specific performance, fine motor skills, sensory discrimination, as well as greater functional independence (Byl et al., 2003). Such techniques still remain relatively unstudied but the case reports suggest the need for more rigorous experimentation, especially with the use of physical therapy as an adjuvant therapy.

1.3.3.4 Deep Brain Stimulation

Long-term electrical high frequency stimulation of the globus pallidus internus (GPI) is now established as an effective treatment for various types of dystonia. The advantages of DBS lie in its reversibility, its adjustability, and lack of destruction of any part of the brain. Currently, candidates for the use of pallidal DBS in dystonia are patients with primary (genetic or sporadic) generalized and segmental dystonia, and patients with complex cervical dystonia (Capelle and Krauss, 2009). In contrast to
Parkinson’s disease, the beneficial effects of DBS in dystonia are not immediate but progressive over weeks to months; the first prospective, multicenter study found that majority of movement subscores had decreased at month 3 and remained stable through month 12 (Vidailhet et al., 2005). The success in the treatment of medically refractive generalized dystonia led to the study of the benefits of DBS for other types of dystonia. Severe medically refractive cervical dystonia has also responded well to GPi DBS, with patients still having approximately 50% improvements in movement subscores several years after surgery (Hung et al., 2007; Skogseid et al., 2012).

Similar to PD, while the clinical benefits of DBS in dystonia are clear, the mechanism of action of DBS remains uncertain. Early studies conducted during surgery found decreased neuronal discharge rates in pallidal neurons and proposed that dystonia was a hyperkinetic movement disorder resulting from suppression of basal ganglia output (Vitek et al., 1999) suggesting that DBS might restore the suppressed output. However, subsequent studies attributed lowered GPi discharge rates to propofol anesthesia, and demonstrated that patients who underwent the procedure with only local anesthetic had discharge rates similar to PD patients, who had hypokinetic disease and overactive basal output in PD (Hutchison et al., 2003).

More recently it has been shown that during short duration GPi-HFS, 50 to 70% of thalamic Vop neurons undergo a reduction in their average discharge frequency with a delay of a few milliseconds in normal monkeys (Anderson et al., 2003) or dystonic patients (Montgomery, 2006) suggesting that HFS activates GPi efferent axons that are GABAergic and inhibitory onto thalamic neurons.
1.4 Models of Basal Ganglia Function

1.4.1 Rate Model

As briefly discussed in previous sections, 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 2). Originally proposed by Albin (Albin et al., 1989) and DeLong (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 excessive inhibition of thalamic and cortical motor regions, and impairing voluntary movements. 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. However, dopamine can have dramatic effects in regions of the basal ganglia other than the striatum. For example, 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).
Early metabolic studies suggested that dystonia might be associated with reduced drive in the indirect pathway along the striatal-GPe connection, and increased inhibition of STN and GPi by GPe efferents (Mitchell et al., 1990). Further, pharmacologic studies suggested that drug-induced dystonias such as tardive dyskinesia are associated with a shift in balance towards the direct pathway (Casey, 1992). Therefore, the rate model predicts that a hyperkinetic movement disorder such as dystonia will be associated with reduced activity of basal ganglia output neurons. Recent single-cell recording studies in patients undergoing functional neurosurgery have reported no difference in GPe but reduced discharge rates in GPi compared to PD patients (Tang et al., 2007) and normal monkeys (Starr et al., 2005), while other studies have shown firing rates to be as high as in PD patients (Hutchison et al., 2003).

However, the rate model appears incomplete as it fails to explain the paradoxical results of pallidotomy, which reduces the inhibitory basal ganglia output to the thalamus while improving symptoms for both hypokinetic and hyperkinetic movement disorders (Marsden and Obeso, 1994), suggesting that abnormalities of basal ganglia output other than rate must play an important role in the development of abnormal movements.
Figure 2 - 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 SNc. 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.
1.4.1.1 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 2). 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.

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, 1990) (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., 2008).

1.4.1.2 Indirect Pathway

As its name implies, the indirect pathway connects the input of the basal ganglia to the output via 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 2). 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 of striatopallidal neurons results in cessation of GABA release from terminals that synapse with GPe neurons. 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)

Therefore, 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).

As alluded to in the section above on the striatum, anatomical evidence points out that the intrinsic circuitry is far more complex than the current models. For example, single axons of striatal neurons terminate in all nuclei, GPe, GPi and SNr (Parent and Hazrati, 1995a) indicating that the direct and indirect pathway is at the very least, an oversimplification. Additionally, a direct projection exists from the GPe to the GPi (Parent and Hazrati, 1995b) rather than through the STN, further precluding a simple direct and indirect pathway dichotomy.

1.4.2 Centre Surround

Another hypothesis of basal ganglia function has been proposed in which the basal ganglia functions through a centre-surround mechanism, originally proposed by Mink (1996). This hypothesis incorporates neuroanatomical and physiological findings not addressed in the original formulation of the rate model, namely, the fast monosynaptic projection from the cerebral cortex to the STN, termed the hyperdirect pathway (Nambu et al., 2002).
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 direct striatal pathway is focused inhibition of BG output nuclei (center) and the role of the indirect pathway is to provide a widespread facilitation of the basal ganglia output (surround), ultimately leading to a focused facilitation of the selected motor programs and surround inhibition of unwanted motor programs in the thalamus, brainstem, and cortex (Figure 3).

The anatomical basis for this model is based on the observance that STN neurons have a broad and diffuse divergence onto many output nuclei neurons whereas striatal neurons project directly to the output nuclei and terminate in discrete and dense synapses on a particular neuron (Mink, 1996; Parent and Hazrati, 1995a, 1995b, 1993). 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). However, the central idea that for any given movement there are a number of competing movement programs is experimentally difficult to prove (Gale et al., 2008).
That said, a loss of center-surround selectivity is consistent with several studies examining PD patients. When given apomorphine, a nonselective D1- and D2 DA receptor agonist, PD patients had an increase in the number of STN neurons that responded to movements around individual rather than multiple joints (Levy et al., 2001), suggesting dopamine improves selectivity for particular movements. Further, a number of studies’ data suggest that the Parkinsonian state is associated with an increase in the ratio of excitatory to inhibitory cells in the STN and GPi compared to the ratio found in normal monkeys (Gale et al., 2008), effectively serving to suppress movement.

Loss of surround inhibition also appears to play a role in dystonia. If the surround inhibitory mechanism in the motor loop is disrupted the lack of inhibition of antagonist or surround muscles leads to excessive motor command and causes co-contraction and / or overflow into inappropriate muscles (Kaji et al., 2005). Evidence for the loss of center surround selectivity in dystonia comes from a study where TMS triggered by index finger flexion suppressed motor-evoked potential amplitudes from the little finger muscle in healthy subjects while the amplitudes were enhanced in dystonia patients (Sohn and Hallett, 2004).
Figure 3 - 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).
1.4.3 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.

1.4.4 Oscillatory Models

Converging evidence from animal studies and recordings from DBS electrodes implanted in PD or dystonic patients for therapeutic purposes support the existence of synchronized activities within different nuclei of the basal ganglia.

For PD, the oscillatory model postulates that excessive 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). Synchronization of a group of neurons can produce oscillations in local field potentials (LFPs). LFPs are generated by action potentials but also in large part by the cumulative pre- and post-synaptic potentials related to the synaptic activity of neurons (Buzsáki et al., 2012). This oscillatory model of PD 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., 2006; Weinberger et al., 2009, 2006). 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 (Eusebio and Brown, 2009).

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 quantitative importance and the means by which it might disturb motor processing remains unclear (Eusebio and Brown, 2009). One thing that does seem 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., 2008a, 2008b). Additionally, in a study conducted on healthy subjects, it has been suggested that beta synchronization favours the maintaining of posture at the expense of new movement (Gilbertson 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, the presence of oscillatory activity in the basal ganglia may not in itself be abnormal; one study reported the presence of widespread coherent beta oscillation in the striatum of normal behaving monkeys (Courtemanche et al., 2003). Another 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).

While oscillatory activity in the beta frequency band in PD has received the majority of attention in terms of oscillatory models of BG activity, dystonic symptoms have been linked to low LFP oscillations (4 - 10 Hz) in the GPi (Chen et al., 2006; Silberstein et al., 2003; Tang et al., 2007), as have levodopa-induced dyskinesias (Alonso-Frech et al., 2006). These low frequency oscillations in the GPi of patients with primary generalized dystonia appear to be associated with the mobile elements of dystonia and not the postural component of this condition (Liu et al., 2006). In this regard, low frequency oscillations may disturb movement by inducing involuntary hyperkinetic movements in the setting of normal or exaggerated levels of dopaminergic stimulation (primary dystonia and treated PD) (Eusebio and Brown, 2007).
1.5 Synaptic Plasticity

Neurons connect to one another via a synapse, which is the primary site of information transmission in the nervous system. Information storage, including memory and behavioural adaptation, is believed to emerge from changes in the functional efficacy of synaptic connections, a property known as synaptic plasticity. The associative and persistent properties of plasticity have led to its widespread use as an experimental model for learning and memory in the brain.

The earliest hypotheses on mechanisms of information storage date back to the late 19th century, where it was 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 involve post-translational modifications of synaptic proteins while being protein-synthesis independent (Raymond, 2007). Other forms can be dependent on protein synthesis but independent of gene transcription, while still 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.
1.5.1 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).

1.5.1.1 LTP Mechanisms

Classically, activity-dependent LTP occurs at glutamatergic synapses containing the NMDA receptor. This receptor is permeable to Ca\(^{2+}\), but is blocked by physiological concentrations of Mg\(^{2+}\). Depolarization expels Mg\(^{2+}\) from the NMDA receptor channel, which in turn allows Na\(^{2+}\) and Ca\(^{2+}\) ions to pass into the postsynaptic cell (Nicoll, 1998). The 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).

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 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).

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 adenylate 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 SNARE proteins (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 an important facilitatory role in triggering mossy fiber LTP (Contractor et al., 2001; Lauri et al., 2003; Schmitz et al., 2003).
1.5.2 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).

1.5.2.1 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 in to 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 LTD 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).

1.5.3 Depotentiation

Synapses that have recently undergone potentiation can undergo a reversal of this synaptic strengthening in a process called depotentiation. That is, depotentiation is the response reduction that affects only responses that have been increased by LTP, and not the baseline responses affected by LTD (Wagner and Alger, 1996). It is a plasticity process that has received far less attention than LTP and LTD and its role in behaviour is still not clear. It has been proposed that depotentiation erases non-essential information and normalizes synaptic efficiency in the process (Picconi et al., 2008, 2003). Behaviourally, a loss of this ability to “forget” irrelevant synaptic signals may lead to the generation of aberrant motor patterns, such as dyskinesia (Calabresi et al., 2010, 2008).

1.5.3.1 Depotentiation Mechanisms

The most widely studied depotentiation induction protocol involves delivering LFS to the pathway being studied, similar to LTD (Sanderson, 2012). LFS used to induce depotentiation is often delivered at a frequency that is shown to be ineffective at
inducing LTD, and thus, in some reports 2 Hz LFS is able to induce depotentiation but is unable to induce LTD (Bashir and Collingridge, 1994; Huang et al., 1999). Additionally, theta frequency stimulation delivered following (Stäubli and Chun, 1996) or during (Larson et al., 1993) LTP induction has been shown to induce depotentiation.

To date, depotentiation has been studied exclusively at excitatory glutamatergic synapses. Similar to LTD, depotentiation has been reported to be dependent on the activation of NMDARs in some experimental conditions and on mGluRs in others (Sanderson, 2012). However, there is some evidence that the NMDAR subunit NR2B is involved in LTD whereas NR2A is involved in depotentiation (Liu et al., 2004). Several signaling pathways have been shown to be involved in depotentiation, with the key processes including phosphatase activity, the regulation of PKA signaling, and receptor trafficking.

In both LTD and depotentiation it is suggested that protein phosphatases dephosphorylate key targets like protein kinases and membrane receptors. In depotentiation, this leads to the failure of LTP. Like LTD, antagonists of certain protein phosphatases have been shown block depotentiation (Huang et al., 1999). However, there are differences in the protein phosphatases involved in depotentiation and LTD. For example, the protein phosphatase Calcineurin Aα is specifically involved in depotentiation, since while LTP and LTD can be induced in Calcineurin Aα knock out mice, depotentiation is absent (Zhuo et al., 1999). Alternatively, if the protein phosphatase PP2A is selectively antagonized, LTD is blocked but depotentiation is not (Nicholls et al., 2008).
AMPAR trafficking is involved in the expression of depotentiation, similar to LTP and LTD. AMPA receptor subunits have either long cytoplasmic tails (GluR1 and GluR2L) or short cytoplasmic tails (GluR2 and GluR3), and on these cytoplasmic tails are many binding sites that enable them to interact with specific binding partners to control their movements (Sanderson, 2012). During depotentiation, AMPAR subunits that have long cytoplasmic tails have been found to be predominantly involved in trafficking (Zhu et al., 2005). Additionally, the physical targets of dephosphorylation on AMPARs in depotentiation are distinct from those in LTD. In LTD serine 845 of GluA1 is dephosphorylated, but not serine 831, while in depotentiation, serine 831 is dephosphorylated and serine 845 is not (Lee et al., 2000).

1.5.4 GABAergic Plasticity

Activity-dependent long term changes in synaptic strength also 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. Similarly, diverse forms of inhibitory plasticity have been reported, manifesting as changes in either presynaptic GABA release or the number, sensitivity, and / or responsiveness of postsynaptic GABA\textsubscript{A} receptors (Castillo et al., 2011).
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<sub>A</sub> 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<sup>2+</sup> concentrations.

**1.5.5 Synaptic Plasticity and the Basal Ganglia**

Activity-dependent synaptic plasticity occurs throughout the basal ganglia and increasingly it is thought that this form of plasticity underlies the acquisition, maintenance, and elimination of certain types of learning, including positive reinforcement, stimulus-reward association, and motor learning (Wickens et al., 2003). Similar to synaptic plasticity described elsewhere in the brain, long term potentiation and long term depression have been described and intracellular calcium signalling plays an important role in the induction of plasticity (Wickens, 2009). The key modulating factor is dopamine, which plays an important role in determining the direction and magnitude of plasticity, as well modulating the requirements for induction. Notably, dopaminergic modulation of plasticity has been described at both excitatory and inhibitory synapses in the basal ganglia. The endocannabinoid signalling system has also been implicated in basal ganglia synaptic plasticity, particularly in the induction and maintenance of LTD in the striatum.
1.5.5.1 Synaptic Plasticity in the Corticostriatal Pathway

As detailed in a previous section, the primarily GABAergic and inhibitory striatum represents one of the main input regions in the basal ganglia, receiving a large convergence of excitatory glutamatergic cortical afferents and dopaminergic afferents from the SNc. LTP and LTD, the two main forms of synaptic plasticity, are both present at corticostriatal synapses and strongly depend on the activation of DA receptors. Indeed, unique to the basal ganglia, a certain level of endogenous DA seems to be required for the induction of both LTP and LTD (Calabresi et al., 2007).

Corticostriatal LTD was first demonstrated when it was shown that HFS of corticostriatal fibres consistently depressed the amplitude of extracellular field potentials and intracellularly recorded EPSPs of glutamatergic synaptic transmission (Calabresi et al., 1992b). This LTD was blocked by both D1R and D2R antagonists, and was absent in slices from rats that underwent unilateral 6-OHDA DA depletion injections. Interestingly, LTD was restored in 6-OHDA rats following co-administration of D1R and D2R agonists (Calabresi et al., 1992b). D2Rs appear to be necessary for LTD induction since mice lacking D2 receptors failed to show LTD after HFS of corticostriatal fibres (Calabresi et al., 1997).

A crucial role in the induction of corticostriatal LTD has also been suggested for the postsynaptic synthesis and release of endocannabinoids (ECBs) (Gerdeman et al., 2002). D2R activation has been linked to ECB release (Giuffrida et al., 1999) and enhanced ECB release by DA seems to be essential for eliciting ECB-induced LTD specifically in indirect-pathway MSNs (Kreitzer and Malenka, 2005). ECBs act
presynaptically at CB1 receptors, G-protein-coupled receptors whose activation ultimately leads to decreased neurotransmitter release due to effects on the activity of presynaptic voltage-sensitive calcium channels (Mato et al., 2008).

Corticostriatal LTP was initially revealed by removing Mg2+ ions from the external slice medium, thereby deinactivating NMDARs by removing the voltage dependent Mg2+ block (Calabresi et al., 1992). Under these conditions, repetitive HFS of cortical afferents potentiated EPSPs. Moreover, in vivo intracellular recordings showed that repetitive HFS of cortical inputs induced striatal LTP if combined with postsynaptic depolarization (Charpier and Deniau, 1997). Similar to LTD, the induction of LTP at corticostriatal synapses was blocked by unilateral 6-OHDA DA denervation in rats (Centonze et al., 1999). Corticostriatal LTP was also blocked by D1R antagonists (Kerr and Wickens, 2001) and lost in mice lacking the D1 receptor (Centonze et al., 2003). In contrast, corticostriatal LTP was enhanced by the D2R antagonist L-sulpiride or in mice lacking D2 receptors, whereas the D2R agonist quinpirole blocks LTP and even induces LTD when corticostriatal HFS is applied in a Mg2+-free environment (Calabresi et al., 1997).

D1R and D2R activation has opposite effects on the levels of intracellular cAMP in the striatum, stimulating and inhibiting adenylyl cyclase activity, respectively (Greengard et al., 1999). The intracellular levels of cAMP modulate the activity of PKA. A major substrate for PKA in MSNs is the DA- and cAMP-regulated phosphoprotein 32 kDa (DARPP32), which, in turn, functions as an inhibitor of protein phosphatase 1 (PP1) (Calabresi et al., 2007). Inhibition of PP-1 suppresses dephosphorylation of several downstream targets of PKA, thereby amplifying behavioral responses produced by
activation of cAMP signaling (Borgkvist and Fisone, 2007). Both corticostriatal LTP and LTD are eliminated following disruption of DARPP32, suggesting that stimulation of the D1/D2R-PKA-DARPP32-PP1 pathway is required for the induction of these two opposing forms of synaptic plasticity (Calabresi et al., 2000).

A third form of synaptic plasticity in the corticostriatal pathway, depotentiation, reverses established corticostriatal LTP to baseline levels following the application of a low frequency stimulation to previously potentiated corticostriatal fibers (Picconi et al., 2008, 2003). While not entirely understood, again DA receptor signaling and protein phosphatase activity is central to the mechanism. Pretreatment of slices with inhibitors of protein phosphatase 1 and 2A blocks depotentiation produced by 10 minutes of LFS after the induction of LTP and these protein phosphatase inhibitors have no effect on either the basal synaptic transmission or the membrane potential and input resistance of the recorded neurons (Picconi et al., 2003). Additionally, bath application of D1R agonists prevents LFS-induced depotentiation, and these effects are abolished by subsequent bath application of D1R antagonists (Dunnett, 2003; Picconi et al., 2003).

1.5.5.2 Synaptic Plasticity in the Output Nuclei of the Basal Ganglia

As discussed previously, the SNr and the GPi are the output nuclei of the basal ganglia and receive converging projections from the direct (striatonigral / striatopallidal) and indirect (subthalamonigral/pallidal) basal ganglia pathways as well as a parallel projection from GPe. Studies on synaptic plasticity at these synapses are very limited, but some recent studies have begun to tackle the issue and results indicate that, once again, dopamine is critical to the process.
Dopamine is released from non-synaptic release sites and hence does not act in a strict point-to-point manner, but rather by diffusion through the extracellular space via volume transmission (Misgeld et al., 2007). Like at corticostriatal D1 synapses, DA has a facilitatory effect on depolarization-induced GABA release from striatal terminals in the output nuclei, leading to an enhanced inhibitory post synaptic potential (IPSP). This facilitation is mediated by the cAMP/PKA pathway and involves voltage gated Ca2+ channels; a diagram of the proposed mechanism is shown in Figure 4. Following DA binding the presynaptic D1R and subsequent cAMP production, PKA is activated and acts on voltage-gated Ca2+ channels, with phosphorylation boosting Ca2+ influx. Depolarization-induced GABA release from striatal terminals was greatly increased in the presence of the D1R agonist SKF or cAMP and inhibited by a PKA inhibitor (Arias-Montano et al., 2007). Furthermore, preincubation of striatal slices with an antagonist of P/Q-type Ca2+ channels results in a marked inhibition of D1 stimulated GABA release (Arias-Montano et al., 2007).

Additionally, PKA has been shown to phosphorylate a number of exocytosis related proteins, such as snapin, snapsin, syntaxin, and SNAP-25 (Evans and Morgan, 2003), with phosphorylation of snapin, for example, increasing vesicle release probability (Thakur et al., 2004). The cAMP/PKA pathway has also been shown to increase recruitment of synaptic vesicles from the reserve pool, thereby increasing the size of the readily releasable pool of vesicles (Seino and Shibasaki, 2005).
**Figure 4**: Proposed mechanism of D1R-mediated facilitation of GABA release at striatopallidal and striatonigral terminals. Following striatal depolarization and activation of presynaptic D1Rs, cAMP is formed, PKA is activated, and proteins involved in exocytosis are phosphorylated leading to increased GABA release. Presumed PKA substrates and possible effects of their phosphorylation are: (i) P/Q-type voltage-activated calcium channels (enhanced Ca2+ influx); (ii) synapsins (increased vesicle trafficking); (iii) SNARE proteins (vesicle docking, priming and fusion); and (iv) nonmembrane active zone proteins such as RIM1a/2a (vesicle docking, priming, and fusion). AC, adenylyl cyclase; DA, dopamine; D1R, dopamine D1 receptor; PKA, protein kinase A; P/Q, P/Q-type voltage-activated calcium channels; RIMs, Rab3-interacting molecules. Continuous lines indicate established actions, while dotted lines indicate presumed effects. Modified from Arias-Montano et al., 2007.
It has previously been shown that both D1-mediated IPSC facilitation and CB1-mediated depression can be expressed by the same striatonigral synapse in the SNr (Yanovsky et al., 2003). IPSC facilitation depends on presynaptic D1R activation at striatonigral synapses, as mentioned above, and can be rapidly eliminated by D1R antagonists or CB1-receptor agonists (Aceves et al., 2011). Although SNr neurons also express D1Rs postsynaptically, increased IPSC amplitude was accompanied by corresponding decreases in paired pulse ratios without a change in the input resistance of postsynaptic neurons, indicating that D1-dependent IPSC facilitation is predominantly due to afferent striatonigral modulation (Aceves et al., 2011; Radnikow and Misgeld, 1998). Paired pulse ratios are the ratio of the amplitude of two closely spaced stimuli and give an indication of the probability of neurotransmitter release and changes in paired pulse ratios can indicate plasticity in terms of changes in the number of release-ready vesicles, the release probability of the individual vesicles, and in the responsiveness of postsynaptic receptors (Hanse and Gustafsson, 2001).

Dopamine decreases paired pulse ratios at striatonigral synapses (Aceves et al., 2011; de Jesus et al., 2011), indicative of an increase in release probability of GABA. Paired pulse depression is a reliable indicator of an increased probability of neurotransmitter release (Thomson, 2000) since the larger the initial probability of release, the more pronounced the depression of two closely spaced stimuli (Zucker & Regehr, 2002) because transmitter tends to be depleted following the first stimulus.

Striatonigral IPSC depression depends on CB1-receptor activation (Aceves et al., 2011), which in turn depends on active NMDA receptors (Ohno-Shosaku et al., 2007), similar to ECB-dependent LTD at the corticostriatal synapse. The induction of
depression can be blocked with CB1-receptor antagonists or by intracellular Ca2+ chelation, even when NMDA receptors are active, suggesting that depression is triggered by endogenous endocannabinoid release (Ohno-Shosaku et al., 2007; Yanovsky et al., 2003).

Therefore, basal ganglia output neurons (or at least those in the SNr; studies have yet to be performed in GPi) appear to act as coincidence detectors or gates, with the expression of either facilitation or depression depending on NMDA-receptor activation of SNr neurons during synaptic activity, at least in slice. When the subthalamonigral indirect pathway is inactive, NMDA receptors are inactive; activation of the striatonigral projection during this time may be presynaptically facilitated by the activation of D1Rs (Figure 5a). When the indirect pathway is active, NMDARs allow Ca2+ entry in to SNr cells, promoting ECB synthesis. The ECBs then act as retrograde messengers and presynaptically depress GABA release from the direct pathway (Figure 5b). Working as gates, these output nuclei may serve to tune the motor system and establish a balance between the direct and indirect pathway enabling the system to theoretically facilitate or decrease movement (Aceves et al., 2011).
Figure 5: *Interaction of cannbinergic and dopaminergic modulation at striatonigral synapses.* (A) When the indirect subthalamonic projection is inactive, NMDA receptors are inactive. In such conditions, activation of the direct striatonigral projection can be presynaptically facilitated by dopamine via the activation of D1 receptors. (B) When the indirect pathway is active NMDA receptors allow calcium entry into SNr neurons, promoting the synthesis of endocannabinoids, which acting as retrograde messengers, presynaptically depress GABA release from the direct pathway. This latter action may become LTD. NMDA receptors may be activated by other afferents to SNr. Modified from Aceves *et al.*, 2011.
1.5.6 Synaptic Plasticity and Parkinson’s Disease

As mentioned above, a unique characteristic of synaptic plasticity in the basal ganglia is that a certain level of endogenous DA and DA receptor activation seems to be required for the induction of LTP and LTD. As to be expected then, disease progression in PD and the associated destruction of the dopaminergic SNc leads to drastic alterations in basal ganglia synaptic plasticity. Indeed, corticostriatal LTD is lost in animal models of PD following DA denervation both in 6-OHDA rats (Calabresi et al., 1992b) and MPTP-treated monkeys (Quik et al., 2006) due to the failed activation of DA receptors during the induction phase of this form of synaptic plasticity (Picconi et al., 2012). Application of exogenous DA or co-activation of D1 and D2Rs can rescue lost LTD (Calabresi et al., 2007; Calabresi et al., 1992a). Furthermore, corticostriatal LTP is also lost following DA denervation in 6-OHDA rats (Picconi et al., 2003). As mentioned above, both corticostriatal LTP and LTD are also eliminated following disruption of DARPP32, suggesting that the D1/D2R-PKA-DARPP32-PP1 signal transduction pathway is critical for the induction of synaptic plasticity at these synapses, and that nigrostriatal denervation in PD impairs activity in this pathway.

During my MSc research I began the process of describing synaptic plasticity at the output nuclei of the basal ganglia in PD patients during surgery to implant DBS electrodes. This research described characteristics of a positive field evoked potential (fEP) in the SNr of PD patients, both OFF and ON dopaminergic medication. It was unique in providing human data supporting DA regulation of synaptic plasticity in the human basal ganglia, and suggested an important role for activity-dependent synaptic plasticity in basal ganglia dysfunction in vivo. We characterized activity-dependent
synaptic plasticity in the SNr of 18 PD patients, all of whom experienced a significant improvement in motor function during their preoperatively measured ON state (Table 1).
### Table 1 - *Patient Characteristics of SNr Plasticity Study* (Prescott et al., 2009)

<table>
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<tr>
<th>Case</th>
<th>Age/Sex/Worst Side</th>
<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>11</td>
<td>61/M/L</td>
<td>11</td>
<td>L-DOPA 1100mg, Ropinirole 15mg, L-DOPA 1150mg, Tolcapone 300mg, Amantadine 200mg,</td>
<td>1100</td>
<td>40 / 10</td>
<td>75</td>
<td>OFF (2)</td>
</tr>
<tr>
<td>12</td>
<td>58/M/B</td>
<td>13</td>
<td>L-DOPA 1600mg, Entacapone 800mg, L-DOPA 1450mg, L-DOPA 500mg, Entacapone 800mg,</td>
<td>1920</td>
<td>32.5 / 24</td>
<td>26</td>
<td>OFF &amp; ON</td>
</tr>
<tr>
<td>13</td>
<td>62/M/R</td>
<td>13</td>
<td>L-DOPA 1950mg, Amantadine 200mg, L-DOPA 1450mg, L-DOPA 500mg, Entacapone 800mg,</td>
<td>1150</td>
<td>28.5 / 10</td>
<td>65</td>
<td>OFF</td>
</tr>
<tr>
<td>14</td>
<td>54/M/L</td>
<td>17</td>
<td>L-DOPA 1950mg, Amantadine 200mg, L-DOPA 1450mg, L-DOPA 500mg, Entacapone 800mg,</td>
<td>1300</td>
<td>19.5 / 7.5</td>
<td>62</td>
<td>OFF &amp; ON</td>
</tr>
<tr>
<td>15</td>
<td>62/M/R</td>
<td>9</td>
<td>L-DOPA 1950mg, Amantadine 200mg, L-DOPA 1450mg, L-DOPA 500mg, Entacapone 800mg,</td>
<td>1950</td>
<td>27.5 / 4.5</td>
<td>84</td>
<td>OFF &amp; ON</td>
</tr>
<tr>
<td>16</td>
<td>46/M/R</td>
<td>10</td>
<td>L-DOPA 1600mg, Entacapone 800mg, L-DOPA 1600mg, Entacapone 800mg,</td>
<td>1530</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td>17</td>
<td>57/M/L</td>
<td>15</td>
<td>L-DOPA 1600mg, Entacapone 800mg, L-DOPA 500mg, Entacapone 800mg,</td>
<td>1920</td>
<td>48 / 20.5</td>
<td>57</td>
<td>ON *</td>
</tr>
<tr>
<td>18</td>
<td>44/M/R</td>
<td>14</td>
<td>L-DOPA 2.25mg, Pramipexole 2.25mg, L-DOPA 2.25mg, Amantadine 300mg,</td>
<td>825</td>
<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** |

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HFS did not induce a lasting change in fEP amplitude in patients in the OFF state (Figure 6 a,c and 7b). 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 12h defined OFF state (Figure 7a). 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, 12h of anti-PD medication withdrawal induced a noticeable increase in UPDRS III motor scores in the patients included in this study (Table 1), 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 (Figure 6 b,c and Figure 7b). When including all patients categorized as ON, there was no correlation between patients’ clinical ON rating based on UPDRS III motor subscale and the maximum inducible activity-dependent synaptic plasticity (Figure 7a). The lack of correlation in the ON group is due to three outliers and likely the result of variability in intraoperative ON states. 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.
Figure 6 - *L-Dopa* treatment of a Parkinsonian patient restores plasticity. (Patient #1 in Table 1) 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. 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. (Prescott et al., 2009)
Figure 7 - Dopamine Enhances Synaptic Plasticity, Population Data. a) OFF UPDRS III motor subscore (open circles) correlates strongly ($r^2=0.81$, $p<.001$) with inducible activity-dependent synaptic plasticity patients OFF 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). When included, no correlation exists ($r^2=0.02$). b) Difference between fEP amplitude measures in ON (open circles) and OFF (closed circles) 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. A two-way ANOVA revealed 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)
Limitations of the SNr study prevented testing 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 in the SNr via D1R 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 Ca2+ channels (to enhance Ca2+ influx), synapsins (to enhance vesicle trafficking) and SNARE proteins (to enhance vesicle docking, priming, and fusion) (Arias-Montano et al., 2007). Nevertheless, rapid postsynaptic changes in the SNr may also affect GABAergic activity. Recent work has demonstrated that neuronal activity can directly regulate the number of cell surface GABAARs 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 GABAAR 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 DA. 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).

The results 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. 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 Parkinson symptomatology.

1.5.7 Synaptic Plasticity and Dyskinesia

As outlined in the section on dyskinesia, with PD disease progression comes a
loss of the normal buffering capacity of DAT due to the loss of the presynaptic terminal
and tonic DA release slowing markedly, eventually becoming exclusively phasic, or
pulsatile, following each dose of L-Dopa. The continuing use of L-Dopa and pulsatile
stimulation of DA receptors causes a broader neuronal destabilization, such as changes
in DA receptor density and sensitivity, as well as alterations in NMDAR and AMPAR
localization.

As expected then, a dyskinetic motor response to L-Dopa is associated with an
altered form of synaptic plasticity in the corticostriatal pathway. In the rat 6-OHDA
model, DA denervation impairs corticostriatal LTP and LTD. Chronic L-Dopa treatment,
at a therapeutic dosage similar to that used in PD patients, restores LTP in both
dyskinetic and non-dyskinetic rats (Picconi et al., 2008, 2003). However, in
corticostriatal slices of animals that did not develop dyskinesia, depotentiation reversed
LTP at corticostriatal synapses following LFS, whereas slices from dyskinetic rats
showed no capacity for depotentiation (Picconi et al., 2008, 2003). Interestingly, unlike for LTP, the loss of LTD produced by 6-OHDA persists even after chronic L-Dopa administration (Picconi et al., 2011). The presumed consequence of this lack of depotentiation at corticostriatal synapses is that in the dyskinetic state, corticostriatal synapses that have been potentiated in vivo by a previous burst of firing would continue to show an augmented response to cortical input, irrespective of their salience for the animal’s ongoing behavior.

As discussed in above sections, DA modulates striatal MSNs via cAMP/PKA-dependent phosphorylation of downstream target proteins, and also depends on a concomitant reduction of dephosphorylation of the target proteins. This parallel mechanism is based on the ability of PKA to phosphorylate and activate DARPP-32, which in turn inhibits protein phosphatase 1 (PP-1) (Feyder et al., 2011). Inhibition of PP-1 suppresses dephosphorylation of downstream targets of PKA, thereby strengthening behavioral responses produced by activation of cAMP signaling (Borgkvist and Fisone, 2007; Fienberg et al., 1998).

The sensitization of D1Rs following DA depletion is reflected by the large increase in DARPP-32 phosphorylation observed in response to the administration of L-Dopa in rodent models of PD (Picconi et al., 2003; Santini et al., 2007) and in MPTP-treated monkeys (Santini et al., 2010). In dyskinetic mice, this enhanced DARPP-32 phosphorylation leads to activation of the extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), which in turn phosphorylates mitogen- and stress-activated kinase-1 (MSK-1) and histone H3, two downstream targets of ERK involved in transcriptional regulation (Santini et al., 2007). Pharmacological blockade of ERK or attenuation of
cAMP signaling in striatal MSN via genetic inactivation of DARPP-32 both reduce LID in these mice (Santini et al., 2007). In MPTP-treated monkeys, increased phosphorylation of DARPP-32 was observed in monkeys treated for the first time with L-Dopa and in chronically-treated dyskinetic parkinsonian monkeys, suggesting DARPP-32 is involved in the development, maintenance and expression of LID (Santini et al., 2010).

The abnormal activation of PKA and the concomitant over-activity of DARPP-32 and under-activity of PP-1 observed in experimental models of LID lead to changes in the state of phosphorylation of target effector proteins, with one of the manifestations of these changes being a lack of depotentiation at corticostriatal synapses. Thus, L-Dopa may cause dyskinesia through stimulation of sensitized D1Rs, hyperactivation of PKA, increased phosphorylation of DARPP-32 and inhibition of PP-1, and loss of corticostriatal depotentiation.

One possible mechanism by which inhibition of PP-1 via DARPP-32 may prevent depotentiation at the corticostriatal synapse involves changes in the state of phosphorylation of the GluR1 subunit of the AMPAR. Phosphorylation of GluR1 at Ser845 promotes glutamatergic transmission (Banke et al., 2000), with the state of phosphorylation of GluR1 dependent on the activity of DARPP-32, and DARPP-32 knock-outs lacking phosphorylation of GluR1 (Santini et al., 2007). PP-1 inhibition as a result of DARPP-32 overactivity would have the added effect of being unable to dephosphorylate GluR1 and other potential targets leading to targets remaining phosphorylated and potentiated.
Pharmacological and genetic interventions intended to decrease abnormal signal transduction at the level of these various intracellular cascades have been shown to attenuate LID in different animal models, but their use for the treatment of dyskinesia in PD patients may present problems related to long-term side-effects at both the central and peripheral level (Feyder et al., 2011).

1.5.8 Synaptic Plasticity and Dystonia

As discussed in the section on the etiology of dystonia, the pathophysiology of dystonia is tied to abnormal plasticity and inhibition. PAS studies combining TMS and median nerve stimulation have shown increased corticospinal (Quartarone et al., 2008), primary motor cortex (Quartarone et al., 2003), and primary somatosensory cortex (Tamura et al., 2009) excitability in dystonia patients compared to healthy controls. Additionally, in a TMS study comparing age matched groups of DYT1 gene carriers with dystonia, DYT1 gene carriers without dystonia, cervical dystonia patients, and healthy controls, DYT1 gene carriers with dystonia and subjects with torticollis had a significantly enhanced corticospinal excitability in comparison with healthy subjects and DYT1 gene carriers without dystonia (Edwards et al., 2006), suggesting that the propensity to undergo plastic change may affect the development of symptoms in genetically susceptible individuals. Interestingly, while they demonstrate normal corticospinal excitability, clinically unaffected DYT1 carriers do show subtle abnormalities in motor behaviour with impaired sequence learning (Ghilardi et al., 2003).
To date, experiments exploring abnormal plasticity and altered inhibition have been limited to cortex, but such abnormalities could be secondary to changes originating in the basal ganglia and/or in addition to changes in the basal ganglia. The basal ganglia may act upon the motor cortex to enhance surround inhibition (Hallett, 2009; Mink, 1996) and/or changes in basal ganglia may contribute to or be a major direct cause of the motor symptoms. However, PAS and TMS cannot probe basal ganglia plasticity for two main reasons. First, the current limiting distance for TMS is 2 cm below the skull in humans, and the basal ganglia is deeper (Bohning et al., 1997). Second, there is no described behavioural response, such as changing MEP amplitudes following cortical TMS, to be used as a positive indication of successful remote basal ganglia stimulation (Siebner and Rothwell, 2003).

However, mouse models of dystonia do indicate abnormalities in synaptic plasticity within the basal ganglia. In a study using mouse model of DYT1, corticostriatal LTP, LTD, and depotentiation were compared in transgenic mice with the human form of normal torsinA, the mutant form of torsinA, and non-transgenic control mice. MSNs recorded from control and normal human torsinA mice exhibited normal LTD, whereas in mutant human torsinA mice, LTD was absent (Martella et al., 2009b). Furthermore, while LTP could be induced in all the mice, it was greater in magnitude in mutant human torsinA mice and could not be reversed via LFS-induced depotentiation in mutant human torsinA mice specifically (Martella et al., 2009b). The enhancement of LTP combined with the inability to revert synaptic strength to pre-LTP resting condition may result in a deficient inhibition at circuit level, and in turn explain the failure to select from motor patterns, giving rise to the “overflow” phenomenon in dystonia (Quartarone and
Pisani, 2011). Interestingly, the study in question suggested that unbalanced cholinergic transmission plays a pivotal role in these alterations, as they found enhanced striatal acetylcholine tone and acetylcholinesterase activity in mutant torsinA mice (Martella et al., 2009b), providing a reason for the ability of anticholinergic agents to restore motor deficits in dystonia.

In the striatum, acetylcholine is released from cholinergic interneurons (Bolam et al., 1984) and although these interneurons represent a small minority of the striatal neuronal population, their extensive dendritic arborization can exert a great effect on striatal synaptic activity and plasticity (Pisani et al., 2007). In normal conditions, these interneurons are under an inhibitory control exerted by muscarinic autoreceptors and D2Rs, while in mutant torsinA mice, an excitatory response to D2R activation was observed, which results in an enhanced activity of cholinergic interneurons (Pisani et al., 2006). Indeed, LTD and depotentiation were restored, and LTP was normalized in mutant torsinA mice by application of muscarinic M1 receptor antagonists (Martella et al., 2009b), supporting the notion that enhanced striatal cholinergic tone may represent a major pathogenic step in dystonia.

As mentioned in the section on the genetic etiology of dystonia, torsinA is thought to play a critical role in protein processing through the secretory pathway via interactions with vesicle release machinery such as snapin, and that mutant torsinA in DYT1 dystonia results in the sequestration of torsinA and snapin. This is thought to destabilize the balance between exocytosis and endocytosis resulting in altered neurotransmitter release in key basal ganglia motor structures (Granata et al., 2009). Interestingly, other brain regions also display altered neurotransmitter release and
plasticity in DYT1 mouse models of dystonia. In the Schaffer collateral hippocampal pathway, mice with a heterozygous DYT1 knock-in have significantly enhanced paired pulse ratios, suggesting an impaired synaptic vesicle release (Yokoi et al., 2013). Additionally, whole-cell CA1 recordings in these mice demonstrated normal action-potential independent spontaneous pre-synaptic release, but a significant decrease in the frequency of spontaneous excitatory post-synaptic currents suggesting that the action-potential dependent pre-synaptic release was impaired (Yokoi et al., 2013).

Unfortunately, there are no human data to support altered basal ganglia plasticity in dystonia, and the lack of a clear dystonic phenotype in the available animal models represents a limitation that requires caution in the interpretation of the data obtained. The human mutant torsinA transgenic mice and the heterozygous DYT1 knock-in mice discussed above are just two of several animal models of DYT1 dystonia, and none of these models exhibit overt dystonia (Quartarone and Pisani, 2011).
1.6 Objective and Hypotheses

1.6.1 Objective

As discussed above, during my MSc research I began the process of describing synaptic plasticity in the SNr, an output nucleus of the basal ganglia, in PD patients during surgery to implant DBS electrodes. That work provided direct electrophysiological proof of the inter-relation between basal ganglia synaptic plasticity, dopamine, and PD in human patients. My PhD work has built on this line of inquiry, addressing the question of whether movement disorders such as PD and dystonia are associated with abnormal plasticity in the output nuclei of the basal ganglia, the SNr and the GPi.

Questions regarding basal ganglia plasticity and neuronal properties are traditionally explored with the use of animal models because of the experimental difficulties investigating these processes in human subjects. However, due to known limitations in correspondence between animal models and human clinical phenotypes (Wichmann, 2008), studies exploring these processes in human subjects, however limited, are required to close the gap between positive results from preclinical animal studies and frustrating failures in clinical trials (Calabresi et al., 2010).

Studies assessing plasticity at the human basal ganglia output can yield critical new insight into the pathophysiology of normal and abnormal movements and may impact our understanding of the mechanisms underlying the effectiveness of deep brain stimulation in movement disorders, and on the design of new drug therapies. For
example, the success of therapeutic strategies in LIDs in the future will likely depend on the development of experimental animal models that will enable screening for therapeutic compounds. If we can establish that the changes in plasticity observed in animal models are also present in human subjects, then we are one step closer to bridging the gap between the bench and bedside, and enabling the success of the future therapeutic strategies.

Similarly, evidence of abnormal plasticity in dystonia comes from measures of sensorimotor cortical organization and physiology, despite the fact that basal ganglia dysfunction has long been implicated in dystonia, and the primary output structure of the basal ganglia, the GPi, remains the most common target for ablative and DBS treatment of dystonia. As such, cortical abnormalities associated with dystonia such as impaired short interval cortical inhibition and the resultant increased cortical excitability, may be modulated by, or even secondary to abnormal plasticity in the basal ganglia. Currently, there are no human data to support the hypothesis of altered basal ganglia plasticity in dystonia, and experimentally investigating plasticity in the basal ganglia has remained difficult, as mentioned above. Furthermore, while studies in animal models of dystonia indicate altered basal ganglia plasticity, the lack of a clear dystonic phenotype in the available animal models limit the conclusions able to be drawn from studies of that nature. Intraoperative measures using fEPs and paired stimulation allow us to examine intrapallidal and intranigral inhibition and plasticity in dystonia patients, which could yield direct evidence of altered GABAergic synaptic function in dystonia patients. Data suggesting that the primary site of origin of loss of cortical inhibition and altered plasticity in dystonia are at the level of the basal ganglia output would add to our
knowledge of etiological factors in the disease to the pathophysiology seen at the time of clinical presentation. An improved understanding of these factors and abnormal plasticity would ultimately advance the development of interventions that could ameliorate dystonia symptoms and perhaps even reverse or prevent them in the first place.

**1.6.2 Hypotheses**

- The GPi is very similar to the SNr in terms of inputs and pre and postsynaptic receptor composition. We hypothesize that similar to our previous finding in the SNr, the GPi can undergo activity-dependent synaptic plasticity and that dopamine will enhance this synaptic plasticity.

- The cortical deficits associated with the pathophysiology of dystonia may result from abnormalities arising in the basal ganglia. We hypothesize that an inhibitory abnormality exists in the basal ganglia output nuclei of dystonia patients and is measurable using paired stimulation and that the degree of this abnormality will scale with the severity of dystonic symptoms.

- A loss of depotentiation at the corticostriatal synapse is an alteration in synaptic plasticity associated with dyskinesia in animal models of PD. We hypothesize that similar alterations are present and measurable at the output of the basal and that depotentiation will be altered in patients who suffer more severe dyskinesias.
Chapter 2 – Methods

2.1 General Methods

This chapter describes the general methods used in the experiments detailed throughout the thesis. Specifics related to individual experiments will be described in a separate methods section in subsequent chapters.

2.1.1 Patients and Consent

All patients underwent stereotactic functional neurosurgery for the implantation of deep brain stimulating quadripolar electrodes (Medtronic Model 3387, Minneapolis, MN) into the STN or GPI for either Parkinson’s disease or dystonia. The vast majority of patients who had STN DBS electrodes implanted were PD patients being treated for cardinal motor signs of PD. In two cases, dystonia patients with existing GPi DBS electrodes had additional DBS electrodes implanted in the STN. These two patients are included in the paired pulse chapter. The majority of patients who had GPi DBS electrodes implanted were dystonia patients, except for several cases in which PD patients suffering from severe dyskinesia were selected for GPi surgery. Pre-operative clinical assessments were performed by movement disorder specialists at Toronto Western Hospital. PD patients were assessed according to the Unified Parkinson’s Disease Rating Scale (UPDRS) motor assessment scale (Fahn et al., 1987), before and after acute L-Dopa challenge. Cervical dystonia patients were assessed according to the Toronto Western Torticollis Rating Scale (TWSTRS) (Comella et al., 1997), and generalized, segmental, and hemidystonia patients were assessed according to the
Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) (Burke et al., 1985). All experiments were approved by the University Health Network Research Ethics Board. Patients provided written informed consent to participate in the study prior to the procedure.

2.1.2 Operative Procedure

All experiments were performed during the surgical procedure for stereotactic, microelectrode-guided localization and placement of DBS electrodes (Medtronic Model 3387, Minneapolis, MN) for PD or dystonia.

In Parkinson’s disease patients, antiparkinsonian medications were withheld a minimum of 12 hours before the surgery. In all patients, a Leksell stereotactic frame (Elekta Inc., Atlanta, GA), was affixed to the patient’s head after injection of local anesthetic was applied. Pre-operative MR images were obtained and axial images were used to determine the x-, y- and z co-ordinates of the anterior and posterior commissures with respect to the stereotactic frame. In procedures targeting the STN, the pre-operative target was 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). In procedures targeting the GPi, the preoperative target was chosen to be the ventral border of GPi. Coordinates of the tentative GPi target were 20 mm lateral to the midline, 3 – 6 mm below the AC-PC line and 1-2 mm anterior to the midcomissural point (Hutchison and Lozano, 2000).
Following initial targeting, patients are brought to the operating room and positioned in a supine position on the operating room table. 5 cm incisions are made 3 cm lateral to the midline. 25 mm 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 with tips 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.

2.1.3 Microelectrode Setup and Neuronal Recordings

As mentioned above, a dual microelectrode setup was used to allow for simultaneous recording and stimulation. This setup, shown in Figure 8, was designed to fit the Leksell stereotactic frame and consists of a platform holding two hydraulic microdrive units (Model 2670, David Kopf Instruments) and a detachable guide tube assembly (Levy et al., 2007). Each microdrive has a range of motion of 21 mm, with the platform allowing for 25 mm of clearance. The two guide tubes were constructed by soldering two 23- gauge, thin walled stainless steel tubes (HTX-23TW; Small Parts Inc., Miami Lakes, FL) side by side. These were then set in the guide tube assembly in a position mediolateral to each other relative to the patient. A gradual 30-degree curve in one of the guide tubes allowed for adequate separation of the microdrive units. The dual inner guide tubes fit easily into the standard stereotactic frame outer guide tube, which
itself was constructed from 17 gauge stainless steel tubing (HTX-17; Small Parts Inc.) (Levy et al., 2007). The distal ends of the inner guide tubes were flush with the outer guide tube and the microelectrodes extended past the distal end of the guide tubes when driven by the hydraulic microdrives.

The two microelectrodes were independently inserted into the individual 23-gauge steel guide tubes with the parallel tips separated by a mediolateral distance of 600 to 800 µm. Each microelectrode was then attached to a Microdrive unit and further connected to a dedicated Guideline 3000 system (Axon Instruments, Calver City, CA) for purposes of amplification, filtering, visual display, audio monitoring, microstimulation, and impedance monitoring. Each microelectrode was grounded to its dedicated GS3000 system and the entire head stage was further grounded to the Axon instrument chassis.

The entire assembly was then affixed to the Leksell frame, with the 23-gauge dual cannulas inserted into the 17-gauge guide tube of the stereotactic frame. Each microelectrode was then driven by submillimeter increments into the brain by the independent manual hydraulic microdrives.
Extracellular recordings were made with dual independently driven microelectrodes (FHC microTargeting Platinum Iridium coated tungsten microelectrodes (FHC, Bowdoin, ME)) during the electrophysiological mapping procedure used to obtain physiological data for localizing the target for DBS electrodes. The tip length of the electrodes was 25 µm and the tips were plated with gold and platinum prior to final sterilization to reduce impedance to 0.1 – 0.4 MΩ at 1000 Hz. Single unit activity recorded from the microelectrodes was amplified, high-pass filtered (300 Hz), and monitored on a loudspeaker and oscilloscope. Additionally, recordings were amplified
5,000 times and filtered at 10 to 5,000 Hz (analog Butterworth filters: high-pass, one pole; low-pass, two poles) and displayed using the two separate GS3000 amplifiers. 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 flexors and extensors was sampled at 500 Hz to monitor any response to passive or active wrist movements.

2.1.4 Physiological Targeting

2.1.4.1 GPi

Recording sites were localized to the GPi on the basis of physiological landmarks. Irregularly firing neurons in the 60 – 90 Hz range were identified, along with border cells at the margins of the nucleus. The optic tract was identified by microstimulation-induced phosphenes below the ventral border of the nucleus and muscle contractions at sites posterior to the nucleus. The GPi and SNr were further identified by their inhibitory responses to microstimulation through the recording electrode at low intensities (thresholds of 2 – 4 uA) (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2009).

2.1.4.2 STN

Parasagittal trajectories were oriented 12 mm from the midline. A typical STN microelectrode trajectory starts 10 mm above the preliminary target and passes through the thalamic reticular nucleus and/or anterior thalamus, the zona incerta, the STN, and the SNr. Neuronal activity is monitored throughout the entire dorsal / ventral extent of
the trajectory. The dorsal border of STN is typically noted by an increase in background activity, or “noise”, and high-frequency neuronal discharge. The motor portion of STN was localized by noting neurons with tremor-related activity as well as neurons that responded to passive / involuntary or active / voluntary limb movements. The ventral border of STN was characterized by a decrease in background activity or “noise”, and the ventral border of STN and dorsal border of SNr was delimited by a region with sparse neuronal activity and a reduced background noise compared to that observed within the STN and SNr. The dorsal border of SNr was identified by the presence of neurons firing at a high rate (60 – 90 Hz) and a regular discharge pattern. The SNr was further identified by its inhibitory responses to microstimulation through the recording electrode at low intensities (thresholds of 2 – 4 uA) (Lafreniere-Roula et al., 2009).

2.1.5 Stimulation and Field Evoked Potentials

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.

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.

2.1.6 Analysis of Neuronal Activity & Statistics

Neuronal recordings were analyzed offline using Spike2 software version 6 (CED, Cambridge, UK) and statistical comparisons were conducted using Sigmastat software (Systat Software Inc., San Jose, USA). Details of specific analysis used in different experiments will follow in subsequent chapters.

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 1 Hz stimulation was defined 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 where applicable (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 \( y_o \), and \( b \) describes the steepness of the curve. Population data were fit with a regression line if the fit had a significance value of \( p < 0.05 \).
Chapter 3 - Nature of Extracellular Field Evoked Potentials in Basal Ganglia Output Nuclei

3.1 Introduction

The SNr and GPi 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, we believe our stimulation protocols are 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 and GPi 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 intracellularly measured IPSP was the same as the positive fEP, and that the potential was blocked in its entirety by the GABA antagonist picrotoxin. In our experiments we also see a positive fEP with a time course 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.

Additionally, results from paired stimulation 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 our study examining synaptic plasticity in the SNr of PD patients ON and OFF dopaminergic medication, 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 (Prescott et al., 2009). 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
Taken together, these observations suggest that the positive fEP is inhibitory and GABAergic in nature and that dopamine plays a role in presynaptic regulation of GABA release in this region.

In addition to the points outlined above, the following case report of a patient who underwent bilateral GPi DBS implantation to treat secondary hemidystonia resulting from a unilateral striatal stroke, provides additional evidence for the inhibitory and GABAergic nature of the field evoked potential in basal ganglia output nuclei.

Primary dystonia is often hereditary with childhood presentation, such as the DYT1 phenotype (Ozelius et al., 1997). Slow, irregular neuronal firing in the internal segment of the globus pallidus (GPi) is a common correlate (Tang et al., 2007; Vitek et al., 1999). Pharmacologic intervention is usually attempted to resolve symptoms before surgery is considered. Among surgical options, bilateral GPi deep brain stimulation (DBS) has become the preferred surgical technique to restore basal ganglia (BG) function in idiopathic dystonia. Although GPi DBS has shown long-term, significant clinical efficacy for primary dystonia (Kupsch et al., 2006; Vidailhet et al., 2005), empirical support for its use has accumulated unaccompanied by an understanding of its mechanisms of action.

In contrast to the consistent benefit shown for primary dystonia, surgical outcomes in secondary dystonia have been less consistent (Andrews et al., 2010). Some studies have suggested that abnormal activity in striatal neurons projecting to the pallidum may be implicated in patients with primary dystonia (Berardelli et al., 1998). Several cases of secondary dystonia arising from striatal degeneration or stroke have
also been reported (Aniello et al., 2008; Krystkowiak et al., 1998).

Unilateral stroke of one or more of the BG nuclei presents a rare opportunity to compare function and electrophysiology between intact and degenerated sides in the same patient during DBS surgery. Here, we report the case of an adult patient with hemidystonia secondary to a left subcortical stroke who underwent implantation of bilateral DBS electrodes in GPi.

3.2 Methods

3.2.1 Patient History

The patient was a 42-year-old, right-handed male with no family history of movement disorders. At the age of 9 years, he suffered an intraparenchymal hemorrhagic stroke of the left cerebral hemisphere of unknown etiology. He recovered with mild spastic right hemiparesis. Around the age of 20 years, he developed spasms of the right side of his neck and right arm, which produced severe pain. He later presented with marked torticollis and right laterocollis. Subsequent brain MRI revealed residual encephalomalacia involving the caudate, anterior putamen, and anterior limb of the internal capsule, and eventually left cerebral peduncle atrophy. His dystonic features progressed to include contralateral involvement.

The patient underwent dorsal rhizotomy, with only mild improvement in symptoms. Intrathecal baclofen treatment was also attempted without success. Alcohol and stress worsened his dystonia. The patient was receiving botulinum toxin injections three times per year, which improved pain and dystonic symptoms by about 20%. 
Although anticholinergic drugs were helping with his dystonia, he was experiencing blurred vision and memory loss while on the medication.

The patient presented to clinic with predominantly right-sided dystonia (with some bilateral involvement). He demonstrated spasms in his arms and both shoulders, moderate retrocollis and left torticollis, moderate scoliosis, and diffuse dystonic tremor. He also had dystonia of the right and left legs, slight dystonia of the left arm, obvious dystonia of the right arm, moderate pulling of the neck and definite bending of the trunk, which were intermittent at rest. The patient suffered from moderate pain (6/10; 0 = no pain, 10 = worst imaginable pain) most of the time. Though independent, he struggled with fine motor tasks, including writing with his right hand. He was somewhat prone to clumsiness while performing daily tasks and had an abnormal gait. The preoperative Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) movement score was 30.5/120. MRI (1.5 T) revealed degeneration of the left caudate, anterior putamen, and anterior limb of the internal capsule (Figure 9a).

Given that the patient was markedly disabled by dystonic movements and pain despite standard medical therapy and botulinum toxin injections, bilateral GPi DBS was indicated.

3.2.2 Stereotactic Surgery and Microelectrode Recordings

GPi DBS surgery was performed as described in detail in the general methods section above. The patient gave free and informed written consent and all protocols were approved by the Research Ethics Board at the UHN. Briefly, the target in posteroverentral GPi was prospectively chosen through stereotactic brain MRI and
microelectrode recordings were used to help determine the final target based on characteristic patterns of neuronal activity and response to microstimulation. The tentative target based on imaging was chosen as 20 mm lateral to the midline, 4 mm below the AC-PC line, and 2 mm behind the midcommissural point. A DBS quadripolar electrode (Medtronic model 3387) was implanted in the final target on both sides, determined by the results of microelectrode mapping, placing the lowest contact near the site of the last recorded pallidal neuron and 2 mm above the most superior site that showed visual phosphenes upon microstimulation at 100 uA (1 s, 200 Hz). Postoperative MRI confirmed final electrode position to be in posteroverentral GPi (Figure 9 b,c).

During the procedure, dual independently driven microelectrodes (25 um tip length, axes 600 um apart, 0.2–0.4 Mohm impedance at 1,000 Hz) were used for extracellular recordings at various locations along the track. Recordings were amplified 5,000–10,000 times and filtered at 10–5,000 Hz (analog Butterworth filters: high-pass, one pole; low-pass, two poles) using two Guideline System GS3000 amplifiers as described in detail in the general methods section. Microelectrode data was sampled and digitized at 15 kHz with a CED micro 1401 (Cambridge Electronic Design). EMG and accelerometer monitored movement of the contralateral arm.

Field potentials (fEPs) were evoked in GPi by stimulating with single pulses at 100 uA and 0.3 ms biphasic pulse width from one electrode and were recorded from the second electrode, with electrodes separated mediolaterally by approximately 600 um. Different depths were examined in some cases by moving the stimulating electrode in
250 um increments above and below the recording site to confirm that misalignment of microelectrode tip position was not responsible for the absence of cell firing and evoked field potentials.

### 3.2.3 Data Analysis

Spike2 software version 7 (Cambridge Electronic Design) was used for offline analysis of microelectrode recordings. Single-unit firing was extracted from raw extracellular recordings using a spike matching template algorithm and confirmed by spike interval analysis. Templates that included spikes separated by an interval of less than 2.0 ms were excluded due to an absence of the absolute refractory period.

An in-house burst detection algorithm in MatLab was used to determine the burst index for single units and characterize their firing pattern into regular, random, or bursty, as described in (Kaneoke and Vitek, 1996). Firing rate (Hz) and burst index were averaged across 7–8 single units within each side of GPi. Mean firing rate and burst index were compared between sides by the Student t test. Statistical significance was accepted at p < 0.05.

Poststimulus time histograms (PSTH, bin width = 250 us, time course = 150 ms) were generated based on firing of a single unit, averaged across 9 or more poststimulus intervals. fEPs were averaged within each side across raw traces recorded from various depths along the track.
Figure 9 - Striatal Stroke and DBS Electrode Positioning. a) Preoperative magnetic resonance imaging showing loss of the left putamen (axial section, white arrow indicates lateral border of lesion). Postoperative magnetic resonance imaging showing final DBS electrode position in the left (b) and right (c) internal segment of the globus pallidus, respectively (sagittal sections, white arrows indicate tips of DBS electrodes). d) High-resolution axial section showing location of DBS electrodes bilaterally. (Fuller, Prescott et al., 2013)
3.3 Results

3.3.1 Spontaneous Activity

A high background noise and a high density of rapidly firing cells characterized GPi on both sides. Cells fired at a frequency between 70 and 100 Hz. There was no discernable difference in patterns of spontaneous activity among different dorsoventral levels in GPi. The mean firing rate of single units in the right side (S1; 87 Hz) was not significantly different compared to that measured in the left side (S2; 82 Hz; Figure 10a). However, the mean burst index was significantly higher (1.4-fold, p < 0.001) across neurons in S2, suggesting greater bursting in S2 compared to more stable firing in S1 (Figure 10b). The burst detection algorithm further characterized the firing pattern of all neurons analyzed in S1 as ‘regular’. Of neuronal firing patterns analyzed in S2, 42.9% were characterized as ‘regular’, 28.6% as ‘bursty’, and 28.6% as ‘random’.

Figure 10 - Neuronal Firing Rates and Bursting in the GPi. a) Single-unit firing rates averaged within S1 (intact putamen, n = 8) and S2 (degenerated putamen, n = 7). The mean firing rate did not differ significantly between sides. b) Burst index values for single units are averaged within S1(n=8) and S2(n=7). The average burst index was significantly greater within S2 (* p < 0.001), suggesting a greater occurrence of bursting neurons (Fuller, Prescott et al., 2013)
3.3.2 Field Evoked Potentials

Field potentials evoked by single pulse stimulation at multiple sites in S1 were positive extracellular potentials (Figure 11). The mean peak amplitude (122 mV) had a latency of 3.9 ms (Figure 11a, black trace). A PSTH showed an inhibition of cell firing during the peak of the fEP (Figure 11b, black bars). Cell firing resumed 20 ms poststimulus and the firing rate rapidly returned to a stable baseline. In comparison, no fEP could be evoked in S2, (Figure 11a, red trace). A PSTH did not show any pause in cell firing, and the firing rate showed greater fluctuations compared with S1 (Figure 11b, red bars).

3.3.3 Clinical Follow-Up

The patient developed right hemiparesis and speech impairment soon after surgery. This was most likely due to oedema around the electrodes although postoperative MRI did not show evidence of acute bleeding or ischemic stroke. At 1-month follow-up, near complete recovery from these impairments was observed, although speech impairment was still noticeable. Compared to preoperative assessment, the patient reported a substantial improvement in pain and 38% improvement in BFMDRS movement score (Figure 12). There was a marked improvement in his dystonic tremor and no trunk spasms were seen. He no longer had dystonia of the left arm or leg. He could now write with his right hand, with some difficulty. Stimulator parameters were optimized to 60 us pulse width and 130 Hz frequency on both sides, with amplitude 2.3 V in S1 (contact C+ 1–) and 1.6 V in S2 (contact C+ 5–).
Figure 11 – fEP and PSTH of GPi, Intact vs. Degenerated Striatum. a) mean fEP recorded in GPi with intact striatum shown in black (n = 160) and with degenerated striatum shown in red (n = 99). The stimulus artifact appears at t = 0 s on both fEP traces. b) PSTH of the GPi with intact striatum shown in black, and with degenerated striatum shown in red. The positive peak of the fEP in the intact side occurs during inhibition of neuronal firing. In the degenerated side, neither the fEP nor inhibition of neuronal firing were detected after stimulus.
A 38% improvement was seen in BFMDRS movement score 1 month post-surgery. A resolution of dystonia symptoms and BFMDRS score of 2 was seen at most recent examination 22 months post-surgery. (Fuller, Prescott et al., 2013)
The patient demonstrated further marked improvements at a 3-month follow-up, including complete amelioration of his trunk dystonia and improvement in severity of right arm dystonia and pulling of the neck. His speech was no longer affected. All subsequent follow-up examinations were made at Kitano Hospital in Osaka, Japan. During the subsequent 6 months, the patient’s right arm and leg dystonia were resolved. At most recent examination (22 months postoperative), only mild neck dystonia was seen to persist, accompanied by mild pain. The BFMDRS movement score was 2 (Figure 12). Although his gait was still abnormal, it was less exaggerated compared to preoperative assessment. He no longer experienced tremor, clumsiness, or trouble with fine motor tasks, and could write without difficulty. Stimulator parameters were adjusted several times since the surgery. Current parameters were 90 μs pulse width and 160 Hz frequency on both sides, with amplitude 3.8 V in S1 (contact C+ 1–) and 3.0 V in S2 (contact C+ 5–).

3.4 Discussion

This patient with hemidystonia and severe damage to the left BG markedly improved after bilateral GPi DBS, despite the delayed onset and delayed treatment. The clinical presentation of some generalized dystonic features was surprising considering that brain MRI revealed only unilateral lesions to BG. To our knowledge, the occurrence of generalized dystonic features in patients with unilateral structural lesions has not been reported, although inter-hemispheric connections between BG nuclei such as the STN have been proposed (Whelan et al., 2004). Alternatively, partial damage not
revealed by the 1.5-T resolution of the brain MRI could have also affected the right BG, inducing dystonic contractions of the contralateral muscles.

That the mean firing rate of GPi neurons was not significantly different between sides might also seem surprising given that GPi is an output nucleus of the BG and influences the excitability of motor cortical areas and the muscles that these areas program (Berardelli et al., 1998). In many previous studies, GPi neurons in patients with dystonia were found to discharge at a lower frequency compared with the healthy population (Tang et al., 2007; Vitek et al., 1999), however, those in the present study fired at a mean frequency of 82–87 Hz, which is typical for normal GPi (Hutchison et al., 2003). This suggests that a reduction in absolute firing rate of GPi neurons is not sufficient for the development of dystonia in all cases.

Instead, an alteration in the pattern of discharge of pallidal neurons may be ultimately responsible for dystonic symptoms in some cases. Consistent with this hypothesis, pallidal neurons in the left side with degenerated striatum showed a less regular firing pattern and more bursts compared with the intact side. Left GPi contains a topographic representation of the right side of the body (Baker et al., 2010), in which the patient’s dystonia was more pronounced and clinically relevant. Bursting in neurons of GPi has previously been associated with primary dystonia (Vitek et al., 1998), and may alter the activity of target sensorimotor neurons in the thalamus through temporal summation of inhibitory postsynaptic potentials.

While the spontaneous firing pattern describes tonic activity in GPi, the fEP represents the combined phasic activity of several neuronal elements. The absence of the fEP in left GPi compared with a robust fEP in the contralateral side suggests that
striatal projections terminating in GPi are primarily responsible for this inhibitory field. This is consistent with, and provides independent support for anatomical studies in the monkey, since approximately 70% of synaptic terminals in the GPi and SNr arise from GABAergic striatal spiny neurons (Shink and Smith, 1995), while GABAergic afferents from GPe make up approximately 15% of terminals. While previous results in a primate model of dystonia demonstrated an overactivity of the direct striatopallidal pathway (Mitchell et al., 1990), a complete loss of function of this pathway was seen here. Striatal projection neurons are not tonically active but discharge phasically (Nambu, 2007), and as such, their death represents the loss of the major phasic GABAergic input to GPi.

While transient inhibitory postsynaptic potentials mediated by GABA_A channels are thought responsible for the inhibition of neuronal firing in the intact side of GPi (Kita, 2001), neuronal firing was random and uninhibited in the partially deafferented side. A consequence of this loss of phasic control may be a less precise selection of intended muscles during voluntary movement, represented by GPi neurons inhibited by direct striatopallidal projections. A second consequence may be a less precise inhibition of unintended muscles, represented by GPi neurons released from inhibition by the indirect pathway (Mink, 1996). The result may be the co-contraction of opposing muscles during voluntary movements, one of the hallmarks of dystonia.

The benefits of DBS for secondary dystonia have been inconsistent, varying from overwhelmingly positive in some cases of tardive dystonia (Trottenberg et al., 2005), to less robust in other cases arising from various cerebral insults (Cif et al., 2003). One group has reported a 10-year follow-up showing 50% improvement of pain, dystonia
and tremor after unilateral GPi DBS in a case of hemidystonia secondary to post-traumatic injury at age 24 (Loher et al., 2008). Another group reported a case of post-traumatic hemidystonia with onset in the right foot moving to the ipsilateral arm 1 year after the incident (Kang et al., 2010). Unilateral GPi DBS improved the foot dystonia moderately from a BFMDRS movement score of six to two and the authors of the study suggested that benefit from DBS might be dependent on the lesion being small and discrete. Our results do not support this hypothesis, since we observed GPi DBS to produce marked improvement for hemidystonia secondary to a large lesion.

Although successful in treating many forms of dystonia, the mechanism of DBS is unclear. In this particular case of a patient with a degenerated striatum, the lack of an inhibitory field in left GPi suggests that repetitive high frequency stimulation by the active DBS contact could not be driving striatal GABA inhibitory input to GPi to normalize or silence abnormal neuronal activity. Nor is it likely that antidromic activation of striatum was involved in therapeutic effects, since much of it was degenerated therefore no antidromic spike would be present; more likely is a ‘downstream’ effect on motor thalamus and/or interaction with STN.

The early therapeutic effects on phasic dystonic movements and pain and further gradual improvements in dystonic symptoms experienced by our patient over the following months are consistent in pattern with improvements in other dystonia patients that underwent implantation of bilateral DBS electrodes (Vidailhet et al., 2005). Gradual improvements may be indicative of slow, plastic changes occurring in the BG or downstream targets. These may have compensated or corrected for the virtual lack of
phasic inhibitory input to GPi during voluntary movements, the presence of abnormal patterns of spontaneous activity in GPi neurons, or both phenomena.

Further results are needed to determine whether implantation of unilateral or bilateral DBS electrodes in posteroventral GPi is indeed indicated for patients with dystonia secondary to degeneration of the putamen or other BG nuclei. Intraoperative recordings from these procedures might reveal more regarding the common correlates of diverse cases of dystonia and assist towards a better understanding of the physiological basis and treatment of dystonia. However, clinical outcomes from pallidal and thalamic stimulation will likely remain variable among patients. Additionally, the absence of a fEP in the GPi of a patient with a degenerated striatum, compared with a robust fEP found on the side with an intact striatum provides additional evidence that striatal projections terminating in GPi are primarily responsible for the fEP.
Chapter 4 - Response to Microstimulation and Synaptic Plasticity in the GPi of Dystonia Patients

4.1 Introduction

As discussed in the section on the etiology of dystonia, the pathophysiology of dystonia is tied to abnormal plasticity and inhibition, and to date, experiments exploring abnormal plasticity and altered inhibition have been limited to cortex, but such abnormalities could be secondary to changes originating in the basal ganglia and/or in addition to changes in the basal ganglia. Indeed, as discussed, rodent models of dystonia show enhancement of striatal LTP and an inability to revert synaptic strength to pre-LTP levels (Martella et al., 2009b), possibly resulting in deficient inhibition at the circuit level and giving rise to the “overflow” phenomenon in dystonia (Quartarone and Pisani, 2011).

To date, there is no human data to support altered basal ganglia plasticity in dystonia, and the lack of a clear dystonic phenotype in the available animal models represents a limitation that requires caution in the interpretation of the data obtained. Additionally, to our knowledge, synaptic plasticity has never been measured in human GPi. In a previous study examining synaptic plasticity in the SNr of PD patients, we showed that HFS was capable of potentiating fEPs from pre HFS levels (Prescott et al., 2009). Here, in a similar experiment, we assessed whether baseline fEP measures were potentiated following HFS throughout the GPi. Additionally, since the distribution of abnormality in the GPi of dystonia patients has been suggested to be associated with
disease severity (Zhuang et al., 2004), with firing rates and patterns being particularly altered in ventral GPi (Tang et al., 2007), we examined whether changes in activity-dependent plasticity might be affected by GPi location.

In addition to measuring whether HFS can potentiate fEPs in GPi, we examined the effects of focal GPi stimulation at different frequencies on fEPs and pallidal activity in an attempt to make some inferences regarding DBS mechanisms. As discussed in previous sections, DBS involves long-term electrical HFS, and when used in the GPi, has been established as an effective treatment for various types of dystonia. A clinical study in cervical dystonia has shown low-frequency stimulation of 5 Hz to be ineffective, while frequencies above 60 Hz produced improvement (Moro et al., 2009). Such work demonstrates a clear frequency-dependent effect of GPi DBS on therapeutic outcome, but the effects of different frequencies of electrical stimulation on pallidal neurophysiology are not well understood.

The mechanisms of DBS action are seemingly varied and complex, and the specific actions underlying therapeutic benefit remain unclear. Our group has previously reported that focal microstimulation leads to inhibition of neuronal firing, and the hypothesized mechanism was synaptic GABA release from the afferent terminals (Dostrovsky et al., 2000; Filali et al., 2004; Lafreniere-Roula et al., 2010; Wu et al., 2001). However, experimental and modeling studies indicate that DBS may also excite local neuronal cell bodies and/or their efferent fiber outputs, as well as fibers of passage (Bar-Gad et al., 2004; Hashimoto et al., 2003; Johnson and McIntyre, 2008). Such excitatory effects could lead to a more regular pattern of activity, with efferent fibers
driven at the stimulation frequency, which could reduce or even replace the irregular pathological neuronal activity (Garcia et al., 2005; Kringelbach et al., 2007).

Recording neuronal activity during stimulation with DBS macroelectrodes is difficult due to the presence of large stimulation artifacts and amplifier saturation. Methods have been proposed to overcome these technical issues using artifact removal and data substitution which can recover spiking data during stimulation (Hashimoto et al., 2002; Montgomery et al., 2005), but evoked potentials have not been recovered using these methods. We used open filter recordings and detected both spikes and fEPs using stimulation trains of biphasic pulses at different frequencies through a nearby microelectrode, which produced narrow artifacts that were removed and substituted with data sampled from within the train. Overall, the data support previous studies of DBS action in showing silencing of spike firing for short periods (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2010; Wu et al., 2001). These previous studies examined the effects of focal stimulation through the same electrode used for recording, and the duration of inhibition was measured after the end of the mostly short (0.5 s), high frequency (200 Hz) trains. We expanded on these studies to examine activity during the stimulation trains and the effects over a range of different stimulation frequencies. We compared the decrease in firing rate with fEPs. Stimulation-response plots were compared before and after short periods of tetanizing HFS, which can produce potentiation of the fEP (Prescott et al., 2009), and found direct evidence of potentiation of the inhibitory synaptic field potentials by HFS, which may be involved in the poststimulation changes in pallidal firing observed here and previously (Bugaysen et al., 2011; Erez et al., 2009).
4.2 Methods

4.2.1 Stereotactic Surgery and Microelectrode Recordings

GPi DBS surgery was performed as described in detail in the general methods section and the case report above. Patients gave free and informed written consent and all protocols were approved by the Research Ethics Board at the UHN. Similarly, dual independently driven microelectrodes (25 μm tip length, axes 600 μm apart, 0.2–0.4 Mohm impedance at 1,000 Hz) were used for extracellular recording and stimulation at various locations along the track as described previously. Furthermore, recordings were amplified and filtered using GS3000 amplifiers, and digitized with a CED 1401 data acquisition system, as described previously. Neuronal signals were monitored continuously during acquisition by computer display, and the spike signals were bandpass filtered at 300 Hz-5 kHz and monitored on an oscilloscope and loudspeaker.

4.2.2 Recording and Stimulation Protocol.

Dual microelectrode recording began 10 or 15 mm above target so that the recordings from both electrodes often began within the GPe and continued into the GPi. Between the GPe and GPi is the internal medullary lamina, an area of white matter, which is identified by an overall decrease in neuronal activity. Neurons of the GPi were identified by their characteristic high firing rate and spiking pattern, with or without short pauses. The ventral border of the GPi was determined based on decreased noise in recordings, as the electrodes passed into white matter, and identification of the optic tract (OT). The OT was identified by microstimulation to elicit visual percepts and/or by
recording responses evoked by turning the room lights off and on or by flashing a strobe light (Hutchison et al., 1994; Lozano et al., 1996).

In one set of experiments, we examined the effects of HFS on baseline fEP amplitudes at different depths throughout the GPi in 10 patients. The clinical characteristics of the patients and their daily doses of medications are shown in Table 2. The group, consisting of 3 men and 7 women, had a mean age (± SD) of 54 ± 16 years and mean disease duration (± SD) of 19 ± 10 years. fEPs were recorded from one electrode while stimulating at 1 Hz (100 μA, 0.3 ms biphasic pulse width) from the second electrode separated mediolaterally by 600 um at the same dorsoventral level. After obtaining a stable baseline of peak fEP amplitudes at 1 Hz, HFS (four 100 Hz trains, each lasting 2 s, spaced 10 s apart) was given to test for potentiation. Blocks of 10 pulses were tested every 30 sec for at least 2 minutes following HFS (Figure 13a). fEP amplitude was compared before and after HFS and sorted by depth from the OT.

In a second set of experiments, a well-isolated spike was recorded initially on one electrode, and stimulation was made with the second electrode with trains separated by 3 s at increasing frequencies (1, 2, 5, 10, 20, 30, 50, and 100 Hz). 10s trains were used for 1, 2, and 5 Hz, and 50 – 60 pulses/train for the remainder (Figure 13b). That is, the total electrical energy delivered during the train was matched by altering the train length. Baseline firing rate data were collected during an initial 20 to 30 s prestimulation period, followed by the stimulation trains from lowest to highest frequency, before and then again following HFS (again, four 100 Hz trains, each lasting 2 s, spaced 10 s apart). We chose the ascending series to avoid possible hysteresis from higher frequency stimulation trains above 20–50 Hz, since stimulation above 20–
50 Hz produced fEP potentiation (see Results). In two cases, the order of stimulation train frequency presentation post-HFS was reversed from highest to lowest, and no significant differences in fEP response patterns were seen.
### Table 2 - Clinical Characteristics of Patients Tested for fEP Potentiation in GPI

<table>
<thead>
<tr>
<th>Class of Dystonia (Patient #)</th>
<th>Age (years), sex</th>
<th>Disease duration (years)</th>
<th>Pre-op motor scores (Subscores): TWSTRS (s/d/p) BFMDRS (m/d)</th>
<th>Medication pre-op (daily dose in mg)</th>
<th># of sites tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical (1)</td>
<td>73, M</td>
<td>10</td>
<td>TWSTRS (21/20/11.5) BFMDRS (30/10)</td>
<td>Gabapentin 300mg TID, Clonazepam 1mg TID</td>
<td>2</td>
</tr>
<tr>
<td>Cervical (2)</td>
<td>69, F</td>
<td>13</td>
<td>TWSTRS (24/26/19) BFMDRS (30/6)</td>
<td>None</td>
<td>2</td>
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<tr>
<td>Sec. dystonia (3)</td>
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<td>33</td>
<td>BFMDRS (30/6)</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>Generalized (4)</td>
<td>61, F</td>
<td>15</td>
<td>TWSTRS (23/22/17.25) BFMDRS (30/6)</td>
<td>Clonazepam 1mg BID</td>
<td>3</td>
</tr>
<tr>
<td>Generalized (5)</td>
<td>53, M</td>
<td>35</td>
<td>TWSTRS (23/12/11.25) BFMDRS (19/6)</td>
<td>Baclofen 40mg/day, clonazepam 0.25TID, Artane 2mg TID, Nitoman 25mg AD</td>
<td>3</td>
</tr>
<tr>
<td>Cervical (6)</td>
<td>73, F</td>
<td>N/A</td>
<td>TWSTRS (16/12/15) BFMDRS (7.5/1)</td>
<td>Artane 2mg QD, clonazepam 2mg QHS, Effexor 225mg QD</td>
<td>2</td>
</tr>
<tr>
<td>Cervical (7)</td>
<td>52, F</td>
<td>17</td>
<td>N/A</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>Cranial (8)</td>
<td>62, F</td>
<td>6</td>
<td>TWSTRS (16/19/14.5) BFMDRS (38.5/7)</td>
<td>Lorazepam (4), Tylenol #1 (2 tabs PRN tid-qid), Esomeprazole, Botox</td>
<td>3</td>
</tr>
<tr>
<td>Sec. dystonia (9)</td>
<td>26, F</td>
<td>26</td>
<td>BFMDRS (38.5/7)</td>
<td>Artane 6mg/day</td>
<td>1</td>
</tr>
<tr>
<td>Sec. dystonia (10)</td>
<td>29, F</td>
<td>13</td>
<td>BFMDRS (14/4)</td>
<td>Topiramate 50mg BID</td>
<td>1</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>54(16)</td>
<td>19(10)</td>
<td>TWSTRS: 54(9) BFMDRS: 23(11)</td>
<td>Total:20</td>
<td></td>
</tr>
</tbody>
</table>

M = male; F = female; BFMDRS = Burke-Fahn-Marsden Dystonia Rating Scale; TWSTRS = Toronto Western Spasmodic Torticollis Rating Scale; N/A = not available. Subscales and scoring: s = severity; d = disability; p = pain; m = movement; t = total score.
Table 3 – Clinical Characteristics of Patients Tested for Frequency-Dependent Effects of Stimulation

<table>
<thead>
<tr>
<th>Class of Dystonia</th>
<th>Age</th>
<th>Disease Duration</th>
<th>Pre-op Motor Scores (Subscores)</th>
<th>Medication Pre-op (Daily Dose in mg)</th>
<th>Number of Sites Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Patient #)</td>
<td></td>
<td>(Years), Sex</td>
<td>TWSTRS (s/d/p)</td>
<td>BFMDRS (m/d)</td>
<td></td>
</tr>
<tr>
<td>Generalized, tremor (1)</td>
<td>53, M</td>
<td>35</td>
<td>TWSTRS (23/12/11.25)</td>
<td>BFMDRS (19/6)</td>
<td>3</td>
</tr>
<tr>
<td>Generalized, ataxia (2)</td>
<td>70, M</td>
<td>15</td>
<td>TWSTRS (18/18/13.5)</td>
<td>BFMDRS (23/6)</td>
<td>5</td>
</tr>
<tr>
<td>Cervical (3)</td>
<td>63, F</td>
<td>44</td>
<td>TWSTRS (18/18/13.5)</td>
<td>BFMDRS (23/6)</td>
<td>8</td>
</tr>
<tr>
<td>Segmental, tardive (4)</td>
<td>57, F</td>
<td>12</td>
<td>TWSTRS (18/18/13.5)</td>
<td>BFMDRS (23/6)</td>
<td>6</td>
</tr>
<tr>
<td>Cervical (5)</td>
<td>48, M</td>
<td>6</td>
<td>TWSTRS (23/25/16)</td>
<td>BFMDRS (87/20)</td>
<td>4</td>
</tr>
<tr>
<td>Cervical (6)</td>
<td>73, F</td>
<td>N/A</td>
<td>TWSTRS (22/16/16.25)</td>
<td>BFMDRS (11/10)</td>
<td>6</td>
</tr>
<tr>
<td>Cervical (7)</td>
<td>77, F</td>
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<td>TWSTRS (22/16/16.25)</td>
<td>BFMDRS (11/10)</td>
<td>2</td>
</tr>
<tr>
<td>Generalized (Lubag; 8)</td>
<td>40, M</td>
<td>Genetic</td>
<td>TWSTRS (16/12/15)</td>
<td>BFMDRS (7.5/1)</td>
<td>8</td>
</tr>
<tr>
<td>Cervical (9)</td>
<td>52, F</td>
<td>17</td>
<td>TWSTRS (16/12/15)</td>
<td>BFMDRS (7.5/1)</td>
<td>7</td>
</tr>
<tr>
<td>Neuroacanthocytosis (10)</td>
<td>32, M</td>
<td>10</td>
<td>TWSTRS (24/18/15.25)</td>
<td>BFMDRS (9/17)</td>
<td>3</td>
</tr>
<tr>
<td>Cervical (11)</td>
<td>50, F</td>
<td>8</td>
<td>TWSTRS (24/18/15.25)</td>
<td>BFMDRS (9/17)</td>
<td>4</td>
</tr>
<tr>
<td>Generalized (12)</td>
<td>62, F</td>
<td>6</td>
<td>TWSTRS (24/18/15.25)</td>
<td>BFMDRS (9/17)</td>
<td>2</td>
</tr>
<tr>
<td>Cervical (13)</td>
<td>43, F</td>
<td>4</td>
<td>TWSTRS (14/18/9.25)</td>
<td>BFMDRS (15.5/4)</td>
<td>2</td>
</tr>
<tr>
<td>Generalized Dyt 1 (14)</td>
<td>25, M</td>
<td>Genetic</td>
<td>TWSTRS (14/18/9.25)</td>
<td>BFMDRS (15.5/4)</td>
<td>2</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>53 (15)</td>
<td>22 (19)</td>
<td>TWSTRS: 47 (12)</td>
<td>BFMDRS m: 27 (27)</td>
<td>Total: 62</td>
</tr>
</tbody>
</table>
**Figure 13** – *Gpi Stimulation Protocols.* a) Baseline stimulation consisting of 10 s of 1 Hz stimulation was repeated twice and followed by HFS consisting of 2 s of 100 Hz stimulation applied and repeated every 10 s a total of 4 times. Following HFS, 10 s 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. B) The effects of 1, 2, 5, 10, 20, 30, 50, and 100 Hz stimulation on fEP amplitude and Gpi firing rate was tested. 10 s trains were used for 1, 2, and 5 Hz, and 50 – 60 pulses/train for the remainder so that the total electrical energy used was similar in each train. HFS was then delivered and the stimulation trains were then tested a second time.
4.2.3 Analysis of Neuronal Activity

Spikes were sorted offline by template-matching software (Spike2, Cambridge Electronic Design) and were accepted for further analysis if they met the following criteria: i) consistent, distinct shape that could be separated with a high degree of certainty from the spike waveforms of other neurons and background noise, and ii) inter-spike intervals of a minimum duration of 1.5 ms to rule out activity from another unit. Recordings at sites of identified border cells were included in the analysis and showed no difference in their response pattern to stimulation compared with the typical high-frequency discharge cells of the GPi. In this study, the artifacts produced by microstimulation were short (0.6 ms) lasting and were removed from the signal, starting from the onset of the artifact to the end. The removed section was replaced with an equivalent period of neural data immediately prior to the stimulus artifacts but in a reversed order, producing a smooth transition in the signal at the site where the artifact was removed. The “cleaned” neuronal recordings were then used for spike sorting with the template-matching algorithm, since spike band-pass filtering without removal of the artifact will increase the duration of the artifacts (Bar-Gad et al. 2004).

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 1 Hz stimulation was defined as the “event”. In the experiment examining the effects of HFS, fEP amplitudes were normalized to a percent scale, with the average of baseline measures in each patient considered as 100%, and sorted by depth from the optic tract. The ventral region was categorized as 1-3mm from OT, the
mid-ventral as 4-6mm from OT, the mid-dorsal as 7-9mm from OT, and the dorsal region as region 10-14mm from OT. Potentiation was evaluated at each site 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 $$y_o$$, and $$b$$ describes the steepness of the curve. Population data were fit with a regression line if the fit had a significance value of $$p < 0.05$$.

In the experiment examining the frequency-dependent effects of stimulation on fEP and pallidal activity, the average amplitude of all potentials during the stimulation frequency trains as well as the first and last fEP amplitudes were measured. The average, first, and last fEP amplitudes were normalized to the baseline fEP measure at 1 Hz pre HFS. A further measure of spike inhibition, termed “silent period”, was defined as the time from the beginning of the last stimulus artifact in the train to the time of occurrence of the first spike. The average firing rate during the train was measured for each stimulation frequency and normalized to the baseline firing rate during the prestimulation control period. The results of average fEP amplitude and firing rate were plotted against stimulation frequency before and after HFS. Statistical analysis was performed using SigmaStat software. Changes in normalized firing rate, fEP amplitude, and silent period in the GPi sites, due to HFS and stimulation frequency, were analyzed by two-way ANOVA with frequency as a repeated measure, followed by Tukey’s post hoc t-tests for all pairwise comparisons. Significance was set at $$p < 0.05$$. 
4.3 Results

4.3.1 Potentiation of fEP in GPi Following HFS

Potentiation of baseline fEP amplitudes following HFS was measured in 10 patients at 20 sites (Table 2). Interestingly, there was an observable difference in the amount of potentiation induced, depending on the depth from which the test was performed (Figure 14).

A two way ANOVA of fEP amplitude across multiple depths reveals a significant difference between depths (DF=3,F=153,p<0.001) with pairwise comparisons revealing a significant difference (p<0.001) between all depth groups except 4-6mm and 7-9mm (p=1). The ANOVA also showed a significant difference between time points (DF=4,F=9.55,p<0.001).

Regression analysis was used to approximate a plateau value \(y_0\) for the multiple depth regions; the ventral region (1-3mm from OT) displayed little or no activity-dependent plasticity following HFS. The mid-ventral (4-6mm from OT) displayed more (but not significant) change \((y_0=105\%, p=0.2)\), than the ventral region but much less than the mid-dorsal (7-9mm from OT) region \((y_0=128\%, p=0.01)\). The dorsal region (10-14mm from OT) underwent the most change in fEP amplitude \((y_0=146\%, p=0.02)\).
**Figure 14** – *Spatial Distribution of Synaptic Plasticity in GPi of Dystonia Patients.* A) Trajectory through GPi showing depth categories as distance from optic track. Dorsal (D), ventral (V), and the intercommissural (AC-PC) line are labeled. Relative positions of the optic track (OT), the internal (GPi), and external (GPe) segments of the globus pallidus are shown. B) Population data of fEP amplitude measures at different depths displays marked differences between groups. The ventral most region (n=4) of GPi has reduced activity-dependent plasticity compared to the middle (n=10) and dorsal (n=6) regions in patients with dystonia (but not sorted by type). This profile is similar to SNr in OFF state in PD patients (see Prescott *et al.*, 2009) and may explain why OFF-period dystonia can occur in this patient group.
4.3.2 Baseline Neuronal Firing Rates

Recordings of well-isolated single units, which met our criteria for location in pallidum, stability, and complete data recorded before, during, and after stimulation of the GP, were obtained at a total of 25 different sites. Of these, 19 neurons were recorded both pre- and post-HFS. Six neurons were lost before post-HFS trains could be applied and were tested with stimulation trains pre-HFS only. The mean baseline firing rate of all neurons before stimulation was 51.2 ± 26.2 (SD) Hz. The average for the six border cells was 45.2 Hz, the five under propofol were 39.9 Hz, and GP neurons not under propofol had an average firing rate of 61.5 Hz. Neuronal response patterns were similar in all sites tested and in patients with various classes of dystonia shown in Table 3. Therefore, the data were pooled together.

4.3.3 Firing Rate and fEP During Stimulation

The frequency-dependent effects of stimulation on the firing rate of cells and the fEP amplitudes at the same sites were tested. Figure 15 illustrates the change in the firing rate (Figure 15a) of the neuronal population, plotted with the simultaneously recorded average fEP amplitude (Figure 15b) at the same GP sites pre-HFS (n = 25) and post-HFS (n=19) during stimulation trains at different frequencies. During stimulation below 5 Hz, no change in the average neuronal firing rate was observed when compared to the prestimulus control period. Stimulation above 5 Hz led to significantly lower firing rates (p < 0.05) until the cell firing was virtually silenced at 50 and 100 Hz. However, the pre-HFS average fEP amplitude showed a different response from the firing rate and increased significantly at above 10 Hz compared with the 1 Hz
baseline (p < 0.05) until a maximum was reached at 20 Hz, after which it declined. Note that during stimulation at the highest frequency of 100 Hz, the average fEP amplitude was reduced to only 27% of baseline. Post-HFS, the average neuronal firing rate was decreased significantly compared with baseline but showed the same pattern of decrease and silencing at higher frequencies of 50 and 100 Hz. The average fEP amplitude at 1 and 2 Hz was significantly potentiated following HFS, and no further increase was seen at 20 Hz, after which it declined to a value of 21% of baseline at 100 Hz.

The GP cells showed frequency-dependent decreases in firing rate and were silenced at stimulation frequencies above 50 Hz both pre- and post-HFS. Analysis of firing rate data in Figure 15a revealed that there was a significant main effect of HFS \([F(1,335) = 21.066, p < 0.001]\) and frequency \([F(7, 335) = 190.137, p < 0.001]\) but no significant interaction \([F(7, 335) = 1.903, p = 0.068]\). Significant pairwise comparisons revealed decreased firing rates post-HFS at stimulation frequencies 20 Hz and below, (1–20 Hz; \(p < 0.05\)) and no difference at frequencies above 20 Hz.

For the fEP amplitude at the same sites, there was no significant main effect of HFS \([F(1, 335) = 1.052, p < 0.306]\). There was, however, a significant main effect of frequency \([F(7, 335) = 69.736, p < 0.001]\) and a significant interaction of HFS and frequency \([F(7, 335) = 6.685, p < 0.001]\). Significant pairwise comparisons between pre- and post-HFS revealed an increased average fEP amplitude at 1 and 2 Hz post-HFS and decreased amplitude at 20 and 30 Hz post-HFS (\(p < 0.05\)).
Figure 15 - Frequency-dependent Response of Firing Rate and fEP pre- & post-HFS. A) Pre-HFS, firing rate decreases as stimulation rate increases until it is silenced at 50 Hz. Post-HFS, overall firing rate is lower and again decreases with higher frequencies. B) Initial increase in fEP amplitude during stimulation train is seen until 20 Hz, after which it sharply declines. Post-HFS, the fEP was potentiated at 1 and 2 Hz, but no further increase was seen up to 20 Hz. Significant pairwise comparisons from pre- and post-HFS revealed decreased firing rate from 1 to 20 Hz, increased fEP amplitude post-HFS at 1 and 2 Hz, and decreased amplitude at 20 and 30 Hz.
4.3.4 Potentiation and Attenuation of fEP During Stimulation

A rebound bursting-like response after single stimuli of low-frequency stimulation, was seen in 8 of 25 cells. This led to an increase in the firing rate during 2 and 5 Hz stimulation in five of these cases, as illustrated in Figure 16a. At stimulation of 20 Hz and below, the fEP amplitude remained relatively constant during the stimulation train (Figure 16b). However, at 30 Hz and above, the fEP amplitude was progressively attenuated during the train, resulting in lower values of average fEP amplitude (Figures 15b and 16b). Furthermore, Figure 16b shows that although the fEP amplitudes were strongly attenuated at higher frequencies, the fEP still followed the frequency of each stimulation train, at least for the initial period.
To quantify the temporal evolution of fEP response amplitudes, the population average of the first and last fEP amplitude was determined for each stimulation train (Figure 17 a and b) for the 25 sites tested pre-HFS and the 19 sites tested post-HFS. ANOVA of the average first fEP amplitudes revealed that there was a main effect of HFS $[F_{(1, 335)} = 11.455, p < 0.001]$, indicating that overall, the effect of HFS was to potentiate the first fEP amplitude. There was also a main effect of frequency $[F_{(7, 335)} = 4.717, p < 0.001]$ and their interaction $[F_{(7, 335)} = 3.680, p < 0.001]$, due to the fact that pre-HFS, a significant increase in the first fEP amplitude compared with 1 Hz baseline was seen with stimulation frequencies above 30 Hz (Figure 17a; $p < 0.05$), after which it appeared that the potentiation saturated. Post-hoc pairwise comparison $t$-tests between pre- and post-HFS revealed significant increases in the post-HFS first fEP amplitude at stimulation frequencies of 1, 2, and 5 Hz ($p < 0.05$).
The population average of the last fEP amplitude in the trains pre- and post-HFS is shown in Figure 17b. Similar to the average fEP amplitude plots shown in Figure 15, there was a significant frequency-dependent decrease in fEP amplitude during the train compared with 1 Hz baseline at stimulation frequencies above 50 Hz both pre- and post-HFS (p < 0.05). There was no main effect of HFS [$F(1, 319) = 0.136, p = 0.712$] but a significant main effect of frequency [$F(7, 319) = 42.808, p < 0.001$] and their interaction [$F(7, 319) = 4.824, p < 0.001$], as fEP amplitude was attenuated within short stimulation trains at frequencies above 30 Hz. Post hoc pairwise comparisons between pre- and post-HFS revealed an increased last fEP amplitude post-HFS at 1 and 2 Hz and decreased amplitude at 20 and 30 Hz post-HFS (p < 0.05).
Figure 17 – Stimulation Above 20 Hz Potentiates the fEP, and Above 30 Hz Attenuates the fEP During the Train. a) Average 1st fEP amplitude of the train at each stim freq. Significant differences from baseline were seen following 20-Hz and higher stim pre-HFS. *pairwise comparisons from pre- and post-HFS revealed significant increased amplitudes post-HFS at 1, 2, and 5 Hz. b) Average last fEP amplitude of the train at each stim freq. *pairwise comparisons from pre- and post-HFS showed increased fEP amplitude at 1 and 2 Hz and decreased amplitude at 20 and 30 Hz. c) Silent period is prolonged by HFS. Silent period was defined as the latency between the last pulse of the stimulation train and the onset of the 1st spike. Higher stimulation frequencies significantly increased the silent period of the GPi neurons compared with the lower frequencies.
4.3.5 Inhibition of cell firing after the end of the train: silent period.

We measured the duration of inhibition in cell firing after the end of the stimulation trains (Figure 17c), similar to that reported in another recent study by our group (Lafreniere-Roula et al., 2010). The average silent period pre-HFS (n=25) was ~0.05 s for frequencies up to 10 Hz and then increased significantly at 50 and 100 Hz, as revealed by the pairwise post hoc t-test (p < 0.001). HFS increased the average silent period at all stimulation frequencies but not to a significant extent (n=19). There were main effects of HFS \( F(1, 319) = 4.233, p < 0.05 \) and frequency \( F(7, 319) = 22.011, p < 0.001 \) but no interaction effect \( F(7, 319) = 0.168, p = 0.991 \), and post hoc tests did not reveal significant increases at specific frequencies.

4.4 Discussion

The use of dual microelectrodes to microstimulate with one and record from the other allowed assessment of the frequency dependence, as well as the effects of conditioning HFS trains on local somatic spikes and evoked synaptic potentials, in the human GPi.

The potentiation of baseline fEP amplitudes following HFS is, to our knowledge, the first demonstration of activity-dependent synaptic plasticity in human GPi. We have previously shown that a similar form of activity-dependent synaptic plasticity was measurable in the SNr of PD patients and that this plasticity was sensitive to low doses of L-dopa. In the absence of dopaminergic medication, HFS did not induce potentiation of fEP amplitudes following HFS. Conversely, following administration of dopaminergic medication, synaptic plasticity was facilitated in the SNr by HFS (Prescott et al., 2009).
In the present study, the same HFS protocol increased fEP amplitudes in the GPi. Additionally, Chapter 6 shows that following administration of dopaminergic medication, synaptic plasticity is facilitated in the GPi of PD patients by HFS. Interestingly, the results appear to show differences in inducible activity-dependent synaptic plasticity across the dorsoventral extent of GPi, with dorsal sites undergoing greater potentiation than more ventral sites. Indeed, the most ventral sites appeared analogous to the PD OFF population in our SNr study that underwent no potentiation following HFS.

Such a result seems to go against the prevailing theory that dystonia is associated with increased cortical and basal ganglia plasticity. For example, human PAS studies indicate increased corticospinal (Quartarone et al., 2008), primary motor cortex (Quartarone et al., 2003), and primary somatosensory cortex (Tamura et al., 2009) excitability in dystonia patients compared to healthy controls. Furthermore, in mouse models of dystonia, corticostriatal LTP is of greater magnitude in torsinA mice compared to controls (Martella et al., 2009b). Alternatively, the lack of plasticity at inhibitory synapses in ventral GPi could be an inhibitory deficit contributing to exaggerated excitation and plasticity in cortical circuits in dystonia.

Unfortunately, there could be confounding effects from previous stimulation that may be altering the fEP at the more ventral test sites. In 7 of 10 patients, 2 or more HFS tests were performed in a single trajectory. As such, HFS at more dorsal sites could be influencing ventral sites via current spread and / or activation of striatal fibers of passage terminating in ventral GPi, and subsequent tests at more ventral sites might have potentiation masked as the baseline fEP might already be in a potentiated state.
However, in such a scenario it would be reasonable to expect that if ventral fEPs were affected by previous dorsal HFS then the baseline fEP amplitudes would be higher in the more dorsal sites prior to normalization to the percent scale. This was not the case, with non-normalized baseline fEP amplitudes being variable across sites. Future experimental design will need to take the above caveats into account in order to provide stronger evidence that there are differences in activity-dependent synaptic plasticity across the dorsoventral extent of GPi, but here we provide preliminary evidence that like changes in firing rates across the dorsoventral extent of GPi (Tang et al., 2007), there might exist differences in plasticity across the dorsoventral extent of GPi.

A key finding of this study is that synapses in the GPi do undergo potentiation following HFS, as seen in measures of fEP amplitudes at dorsal sites. The mechanisms of this potentiation were not directly tested but a strong case for dopamine-dependence can be made. In rat striatal slices, like at corticostriatal D1 synapses, DA facilitates depolarization-induced GABA release from striatum onto output nuclei, leading to an enhanced IPSP. Depolarization-induced GABA release from striatal slices is greatly increased in the presence of the D1R agonist SKF or cAMP and inhibited by a PKA inhibitor (Arias-Montano et al., 2007). Furthermore, preincubation of striatal slices with an antagonist of P/Q-type Ca2+ channels results in a marked inhibition of D1 stimulated GABA release (Arias-Montano et al., 2007). Following DA binding the presynaptic D1R and subsequent cAMP production, PKA is activated and acts on a variety of targets involved with GABA exocytosis, such as P/Q-type voltage-activated Ca2+ channels (to enhance Ca2+ influx), synapsins (to enhance vesicle trafficking), and SNARE proteins (to enhance vesicle docking, priming, and fusion) (Arias-Montano et al., 2007). Here,
tests were conducted in dystonia patients, and as such, we didn’t test in the ON and OFF dopaminergic medication state so we can’t state definitively whether DA was a necessary component of the activity-dependent changes we measured. The study in the next chapter attempts to increase the understanding of the mechanism of dysfunction of basal ganglia output neurons in dystonia by assessing putative GABAergic synaptic transmission in the basal ganglia output nuclei of dystonia patients using paired stimulation. Future work, perhaps in the MPTP primate model, can examine in vivo basal ganglia plasticity using different agonists and antagonists delivered via intracerebral microinjection to gain a better understanding of the mechanisms involved.

The key findings from the frequency-dependence experiments support several therapeutic mechanisms of GPi DBS. Consistent with earlier reports (Dostrovsky et al., 2000; Wu et al., 2001), we found that short HFS trains in the GPi can produce an inhibitory effect on local neuronal spiking, but also that longer trains produced synaptic fatigue or depression at frequencies above 50 Hz. We also observed ortho- and antidromic-like driving of action potentials (not shown here, but see (Liu et al., 2012), as well as rebound bursting and short-term plasticity of inhibitory synaptic components. It has been suggested that rebound bursting might play a role in synaptic plasticity (Aizenman and Linden, 1999; McElvain et al., 2010); however, we observed evidence of synaptic plasticity at all sites, including the sites without bursting.

With stimulation frequencies from 1 Hz up to 30 Hz, the GPi firing rate decreased, whereas the fEP amplitudes progressively increased pre-HFS, which supports the involvement of presynaptic GABA release. The inhibition of spontaneous
firing is consistent with earlier studies from our group (Dostrovsky et al., 2000; Wu et al., 2001). The increase in size of the fEP with increasing stimulation frequency peaking at 20–30 Hz suggests that there was a maximal amount of GABA in the synapse in this range, perhaps due to saturation of re-uptake from the synapse. Since we occasionally saw rebound bursting at low frequencies, which sometimes increased average firing rates, another possible contribution might be progressive membrane depolarization from an excitatory transmitter released at lower frequencies and resultant increase in the driving force for the inward chloride flux mediating the field IPSP (Eccles, 1964). With stimulation frequencies above 30 Hz, a significant decrease in fEP amplitude was observed at all sites during the stimulation trains. This is likely due to synaptic fatigue, since in hippocampal in vitro preparations, GABAergic IPSPs are “labile” and attenuate after a period of repetitive stimulation due to reduction of driving force (Huguenard and Alger, 1986; McCarren and Alger, 1985) and decrease in synaptic release (Thompson and Gähwiler, 1989).

At frequencies of 50 Hz and above, the neuronal firing was silenced, but the fEP was still present during the train, albeit at a much reduced amplitude. This is consistent with recent findings from our group showing a maximal post-HFS inhibition of firing (here termed silent period) of 1–2 s and decreased inhibition duration with increasing, longer train lengths (Lafreniere-Roula et al., 2010). Synaptic fatigue would lead to both low fEP amplitudes and loss of decreased inhibition of spiking with increasing, longer train lengths. During long train stimulation of 10–20 s, most of the cells returned to firing after 0.5–10.6 s, and during this latter period, the fEP amplitude was reduced further (not shown here but see (Liu et al., 2012)). We did not examine the response after
many minutes of continuous HFS due to time restriction in the OR, but it is likely that the fEP amplitudes would have remained at a minimal level. These results are similar to those of Urbano et al., (2002), who used high-resolution calcium imaging and field potential recordings to examine effects of stimulation of thalamocortical fibers on cortical activity. They found a similar sigmoid-shaped, frequency-response curve with marked reduction of synaptic activity above 60 Hz, similar to our firing-rate curve. In addition, microdialysis studies measuring the GABA release/unit time in patients during continuous HFS have found a high initial release of ~1 uM GABA over 10 min, which is followed by a continuous, stable GABA release of ~0.3 uM in the fractions after 30 min of DBS (Kilpatrick et al., 2010). This finding is limited to the temporal resolution of ~10 minute collection intervals but suggests that even if some synapses were depressed at the site of stimulation, the efferent fibers surrounding this region, driven by the high frequency, might sustain a postsynaptic inhibition of downstream targets via continuous GABA release. A sustained, regular high frequency discharge firing is physiological for basal ganglia output neurons.

The fEP amplitude and silent period were both potentiated by HFS. These responses suggest the involvement of short-term synaptic plasticity at inhibitory synapses in human GPi. In slice experiments, inhibitory GABAergic synaptic plasticity has been demonstrated in the rat GP (Hanson and Jaeger, 2002; Sims et al., 2008), which is the homologue of primate GPe. Sims et al., (2008) showed that the synaptic plasticity at GABAergic synapses was characteristic only of the striatopallidal pathway and not present on the axonal terminals from other GP neurons. Primate GPi differs from rat GP and primate GPe in not having significant, recurrent collaterals (Hazrati and
Parent, 1992). Also, the GPe-to-GPi projection is sparse and predominately located on the soma, whereas the striatopallidal direct pathway projections are much more numerous but with a tendency to be located on the dendrites (Shink and Smith, 1995; Smith et al., 1994). These anatomical features support the notion that the inhibitory synaptic plasticity reported here is predominantly in the direct striatopallidal pathway.

This study demonstrates inhibition of neuronal firing of GP neurons during stimulation, as well as a return in spontaneous firing with longer trains. Importantly, the findings also show that during higher frequency stimulation, the fEP is depressed, indicating that there would be a loss of transmission of information (including pathological signals) to GPi neurons. The data support the notion that GPi DBS has complex and varied mechanisms, including increased long-term inhibitory synaptic efficacy.

Previous studies using microelectrodes to infer DBS mechanisms have noted that a DBS electrode would stimulate a much larger population of neurons than a microelectrode, since it occupies a much larger volume (Erez et al., 2009). The result would be a regular synchronized, high frequency output to premotor structures in brain stem and thalamus, which lacks any “pattern-sculpting” by basal ganglia input structures. Given that pauses in GP neuron firing are thought to be related to dystonia (Hutchison et al., 2003; Sani et al., 2009), the elimination of pathological inhibitory phasic movement-related signals may help to restore more “normal” voluntary movements and the function of reciprocal inhibition to eliminate overflow. These spatial/volume constraints may explain why microstimulation is usually ineffective,
whereas macro-stimulation is effective on the target premotor structures. Low basal firing rates in GPi, purported as “the cause” of dystonia (Nambu et al., 2011), may only serve to prolong pauses, producing an excessive sculpting of GPi output.

One major and important limitation of the frequency-dependent study is that the effects of HFS could only be examined for short-duration trains, compared with durations of days and longer in clinical applications. Although some of the clinical effects of DBS are already apparent after such short trains, others may take much longer, especially when considering GPi stimulation for dystonia. Clearly, the findings of this study cannot directly explain the long latency effects of DBS, although they may well be involved in an initial step(s), which leads over time to long onset plasticity. Thus it is important to determine and understand what are the short-term effects of stimulation, as they may be responsible not only for mediating short-onset DBS effects but also be important in initiating and maintaining long-term changes. Another possible limitation is that the microstimulation electrode tip is much smaller than the DBS electrode contacts, meaning that even with the lower stimulus intensities used, the charge density near the tip of the microelectrode is higher than the current densities near the tip of the DBS contacts. However, the charge density at our recording distance is comparable with the charge density produced clinically from DBS electrodes (Wu et al., 2001), and thus our findings should be applicable to understanding the mechanisms that might be involved in DBS stimulation. Limitations compared with studies in animal models are the inability to use various drugs to help elucidate the mechanisms and the fact that the patients in our study were not homogenous in terms of their underlying pathophysiology (Table 3), although it is not clear to what extent this heterogeneity
might affect the mechanisms responsible for the therapeutic effects of the DBS. On the other hand, these studies have the advantage over animal studies in that it is not known how well the animal models correspond to the human conditions, and furthermore, we know that the HFS stimulation is therapeutically effective in these patients.

As mentioned above, the next chapter attempts to address mechanisms of dysfunction of basal ganglia output neurons in dystonia. The effects of focal HFS on GABA release are further examined at the striatopallidal and striatonigral synapse using paired stimulation.
Chapter 5 - Paired Stimulation in Basal Ganglia Output Nuclei

5.1 Introduction

To examine the possibility that impaired inhibition and synaptic plasticity within the basal ganglia play a role in dystonia, the present study used a pair of microelectrodes to test paired pulse inhibition in the globus pallidus interna (GPi) and substantia nigra pars reticulata (SNr) of dystonia and PD patients undergoing implantation of deep brain stimulating (DBS) electrodes.

Dystonia is a movement disorder characterized by involuntary muscle contractions resulting in spasms and abnormal postures. Dystonia can manifest focally, affecting an isolated body part such as the neck (cervical dystonia), or generalized to affect the whole body. Medical therapy for dystonia involves anticholinergics and GABA-B agonists such as baclofen, but many side effects limit their therapeutic efficacy. The primary treatment for focal dystonia is botulinum toxin, but the muscle paralysis only lasts a few months and patients can develop antibodies that neutralize its efficacy. Bilateral deep brain stimulation (DBS) of the globus pallidus internal segment (GPi) has proven efficacious in medically refractory cervical (Hung et al., 2007) segmental and generalized dystonia (Krauss, 2010; Ostrem and Starr, 2008), but its mechanism of action is poorly understood.

The pathophysiology of dystonia appears to be associated with abnormal plasticity and reduced inhibition at cortical, brainstem, and spinal levels. Converging evidence from animal (Martella et al., 2009b; Napolitano et al., 2010) and human
studies (Chen and Udupa, 2009; Quartarone et al., 2006) suggests that a key element in the pathophysiology of dystonia is impaired synaptic function leading to abnormal plasticity and motor dysfunction (Peterson et al., 2010).

Most experimental evidence of abnormal plasticity in dystonia comes from measures of sensorimotor cortical organization and physiology. However, basal ganglia dysfunction has long been implicated in dystonia, and its primary output structure, the GPi, remains the most common target for ablative and DBS treatment of dystonia. As such, abnormal plasticity in reflexes and cortical areas may be modulated by, or even secondary to abnormal plasticity in the basal ganglia, but experimentally investigating plasticity has remained difficult in basal ganglia structures. With this in mind, a model of dystonia has emerged in which a fundamental deficit within the basal ganglia generates impaired inhibition in motor cortex that results in increased excitability, abnormal processing of sensory feedback and increased striatal, brainstem, and cortical plasticity. The result is a maladaptive neural reorganization of motor circuits (Quartarone and Pisani, 2011).

Impaired short interval cortical inhibition and increased excitability have been described for various forms of dystonia using paired pulse methods with transcranial magnetic stimulation (TMS) of motor cortex. For example, pairing an initial subthreshold pulse with a suprathreshold TMS pulse at different intervals (1 – 15 ms) induced less inhibition in both hemispheres compared to controls (Ridding et al., 1995). A similar result was obtained using paired suprathreshold TMS pulses (110%, 20 – 200 ms intervals) and measuring surface EMG in the affected limb (Chen et al., 1997). Intracortical inhibition was reduced at all intervals below 100 ms in dystonia patients in
the hemisphere contralateral to an isometric contraction of the affected limb. The origin of this cortical impairment is unknown but may share a common pathology with the basal ganglia. The internal segment of the globus pallidus (GPI) and substantia nigra pars reticulata (SNr) are the major output nuclei of the basal ganglia. They project to thalamus and brainstem where they produce inhibition by releasing GABA (Parent and Hazrati, 1995a, 1995b). Their main inputs are also GABAergic, originating in the striatum and the external segment of the globus pallidus (GPe); they also receive an excitatory glutamatergic input from the STN (Parent and Hazrati, 1995a, 1995b). Previous studies have shown that stimulating within the GPI and SNr preferentially activates the GABAergic axon terminals of striatal and/or external pallidal neurons, thereby causing the release of GABA and inhibition of the firing of GPI and SNr neurons (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2009; Prescott et al., 2009).

To gain a better understanding of the mechanism of dysfunction of basal ganglia output neurons in dystonia we assessed putative GABAergic synaptic transmission in the basal ganglia output nuclei of dystonia patients and PD patients on dopaminergic medication undergoing implantation of deep brain stimulating (DBS) electrodes. Short-term plasticity and synaptic function were assessed in the GPI and SNr using a paired-pulse protocol by stimulating through one electrode and recording the evoked response from the other electrode. A significant factor in regulating the activity of basal ganglia output neurons is the extent of presynaptic neurotransmitter release at the striatopallidal and striatonigral synapses which can be monitored using measurements of paired pulse facilitation and depression (de Jesus et al., 2011; Dobrunz and Stevens, 1997; Warre et al., 2011). Short-term plasticity involves the facilitation or depression of synaptic
responses dependent on preceding activity, and therefore can modify the ability of a synapse to provide sustained inhibition or excitation. Facilitating synapses generally display high paired pulse ratios (PPR) and exhibit a low probability of neurotransmitter release whereas non facilitating synapses display lower PPRs indicative of a higher probability of release (Zucker and Regehr, 2002; Zucker, 1989).

We show that basal ganglia output nuclei of dystonia patients undergo less paired pulse depression of local field evoked potentials in the basal ganglia output nuclei compared to PD patients on dopaminergic medication, and that paired pulse depression could be restored in dystonia patients following focal HFS. These findings suggest that abnormalities exist in synaptic function of striatopallidal and/or striatonigral terminals in dystonia patients and that these abnormalities may contribute to the pathophysiology of dystonia, either independent of, or in addition to the increased excitability and plasticity observed in cortical areas in dystonia patients. These findings also suggest that HFS is capable of enhancing striatopallidal and striatonigral GABA release in basal ganglia output nuclei, indicating a possible mechanism for the therapeutic benefits of DBS in the GPi of dystonia patients.

5.2 Methods:

5.2.1 Patients

Experiments were performed during stereotactic functional surgery for implantation of DBS electrodes in the subthalamic nucleus or GPi of Parkinson’s disease and dystonia patients. Recording and stimulation was performed in the GPi of 5
dystonia patients (9 sites), the SNr of 2 dystonia patients (3 sites), the SNr of 8 PD patients (9 sites), and the GPi of 5 PD patients (10 sites). The clinical characteristics of the dystonia patients and Parkinson’s disease patients are shown in Table 4 and Table 5 respectively. All experiments were performed while the patients were awake and under local anesthesia only. All PD patients were tested while ON dopaminergic medication approximately 30 minutes after oral administration of 100 mg of L-Dopa (Sinemet 100/25®), except in one case for each structure, where measurements were taken in the OFF medication state in the right GPi/SNr and in the ON dopaminergic medication state in the left GPi/SNr, approximately 30 minutes after L-Dopa administration. 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.

### 5.2.2 Surgical Procedure

Extracellular recordings were made with dual independently driven microelectrodes (25 μm tip length, axes 600 - 800 μm apart, 0.2 - 0.4 MΩ impedance at 1,000 Hz) during the electrophysiological mapping procedure used to obtain physiological data for localizing the target for DBS quadripolar electrodes, as described several times previously.
**Table 4**

Dystonia patient information.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Disease duration (years)</th>
<th>Medication (+ daily dose where available)</th>
<th>Dystonia type</th>
<th>TWSTRS (S/D/P)</th>
<th>BFMDRS (M/D)</th>
<th>Structure tested (# sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45/M</td>
<td>10</td>
<td>Domperidone, Clonazepam, Clonazapine, Risperidone</td>
<td>Cervical</td>
<td>18/17/14.75</td>
<td></td>
<td>GPI (3)</td>
</tr>
<tr>
<td>2</td>
<td>57/F</td>
<td>35</td>
<td>Botox</td>
<td>Cervical</td>
<td>25/20/15.5</td>
<td></td>
<td>GPI (3)</td>
</tr>
<tr>
<td>3</td>
<td>62/F</td>
<td>21</td>
<td>Lorazepam 2–3 mg</td>
<td>Segmental</td>
<td>–</td>
<td>21.5/5</td>
<td>GPI (1)</td>
</tr>
<tr>
<td>4</td>
<td>66/M</td>
<td>10</td>
<td>Lorazepam 1 mg</td>
<td>Segmental</td>
<td>–</td>
<td>17/5</td>
<td>GPI (1)</td>
</tr>
<tr>
<td>5</td>
<td>69/F</td>
<td>40</td>
<td>Clonazepam 0.5 mg QID, Percocet 1 tab</td>
<td>Cervical</td>
<td>28/19/16.75</td>
<td>–</td>
<td>GPI (1)</td>
</tr>
<tr>
<td>6</td>
<td>24/M</td>
<td>24</td>
<td>NA</td>
<td>DYT1 Generalized</td>
<td>–</td>
<td>63/10</td>
<td>SNr (2)</td>
</tr>
<tr>
<td>7</td>
<td>44/F</td>
<td>10</td>
<td>Artane 2 mg, Clonazepam 1 mg, Baclofen 15 mg</td>
<td>Segmental</td>
<td>–</td>
<td>21/11</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>Mean</td>
<td>52.4 ± 15.8</td>
<td>21.4 ± 12.4</td>
<td>–</td>
<td>–</td>
<td>(S) 23.7 ± 5.1</td>
<td>(M) 30.6 ± 21.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(D) 18.7 ± 1.5</td>
<td>(D) 7.8 ± 3.2</td>
<td>(P) 15.7 ± 1.0</td>
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</tbody>
</table>
**Table 5**

Parkinson's disease patient information.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Disease duration (years)</th>
<th>Medication (daily dose)</th>
<th>L-Dopa equivalence (mg/day)</th>
<th>UPDRS III (OFF/ON)</th>
<th>Structure tested (# sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44/F</td>
<td>14</td>
<td>Sinemet 100/25 5/day, Mirapex 2.25 mg/day, Amantidine 300 mg/day</td>
<td>825</td>
<td>52.5/18.5</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>2</td>
<td>51/M</td>
<td>12</td>
<td>Sinemet CR 100/25 6/day, Comtan 200 mg 5×/day, Azilect 1 mg QD, Requip 12 mg/day</td>
<td>740</td>
<td>35.5/15.5</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>3</td>
<td>63/M</td>
<td>10</td>
<td>Sinemet 100/25 16/day, Comtan 200 mg 8/day, Mirapex ×4 mg/day</td>
<td>2320</td>
<td>37/8</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>4</td>
<td>57/F</td>
<td>9</td>
<td>Sinemet 100/25 3 tabs/day, Sinemet CR 100/25 4 tabs/day, Sinemet CR 200/50 1/day Comtan 200 mg 5×/day, Mirapex 1.5 mg BID, Amantidine 100 mg TID</td>
<td>1200</td>
<td>37/14.5</td>
<td>SNr (2)</td>
</tr>
<tr>
<td>5</td>
<td>56/F</td>
<td>12</td>
<td>Sinemet 100/25 TID, Sinemet CR 100/25 TID, Requip 18 mg/day, Rasigline 0.5 mg BID</td>
<td>825</td>
<td>31/9</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>6</td>
<td>65/M</td>
<td>18</td>
<td>Sinemet 100/25 5.5 tabs/day, Requip 16 mg/day</td>
<td>816</td>
<td>41/14</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>7</td>
<td>56/M</td>
<td>10</td>
<td>Sinemet 7tabs/day, Requip 3.75 mg/day, Amantidine 100 mg TID</td>
<td>762.5</td>
<td>26/6</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>8</td>
<td>57/M</td>
<td>13</td>
<td>Sinemet 100/25 15tabs/day, Comtan 200 mg 8×/day, Mirapex 1.5 mg/day</td>
<td>1950</td>
<td>48.5/23.5</td>
<td>SNr (1)</td>
</tr>
<tr>
<td>9</td>
<td>58/F</td>
<td>15</td>
<td>Sinemet 100/25 11tabs/day, Mirapex 1.5 mg/day, Amantidine 100 mg BID</td>
<td>1250</td>
<td>39/23</td>
<td>Gpi (2)</td>
</tr>
<tr>
<td>10</td>
<td>62/M</td>
<td>17</td>
<td>Sinemet 100/25 10 tabs/day, Comtan 200 mg 5×/day, Sinemet CR 100/25 1 tab QHS</td>
<td>1290</td>
<td>41/19.5</td>
<td>Gpi (2)</td>
</tr>
<tr>
<td>11</td>
<td>61/M</td>
<td>16</td>
<td>Sinemet 100/25 1.5 tabs/day, Liquid Sinemet 500 mg, Amantidine 50 mg TID</td>
<td>650</td>
<td>68/35</td>
<td>Gpi (2)</td>
</tr>
<tr>
<td>12</td>
<td>69/F</td>
<td>27</td>
<td>Sinemet 100/25 20 tabs/day, Comtan 200 mg 6×/day</td>
<td>2400</td>
<td>35.5/31</td>
<td>Gpi (2)</td>
</tr>
<tr>
<td>13</td>
<td>73/M</td>
<td>17</td>
<td>Sinemet 100/25 9 tabs/day, Sinemet CR 100/25 QHS, Comtan 200 mg 4×/day</td>
<td>1155</td>
<td>59/24</td>
<td>Gpi (2)</td>
</tr>
<tr>
<td>Mean</td>
<td>59.4±7.5</td>
<td>14.6±4.7</td>
<td>-</td>
<td>1244.9±603.9</td>
<td>42.2±11.8/18.6±8.7</td>
<td></td>
</tr>
</tbody>
</table>
5.2.3 Recordings from GPi and SNr

Pre-surgery, the tentative STN or GPi target was identified by brain imaging (MRI) on the basis of the stereotactic coordinates and direct imaging of the structures, as described previously. Briefly, coordinates of the tentative STN target were 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) and coordinates of the tentative GPi target were 20 mm lateral to the midline, 3 – 6 mm below the ACPC line and 1-2 mm anterior to the midcommissural point (Hutchison and Lozano, 2000). Recordings started 10-15 mm above tentative targets and target nuclei were then localized via characteristic neuronal discharge patterns described elsewhere in detail (Hutchison et al., 1998, 1994; Lozano et al., 1995). The SNr was identified by the presence of neurons firing at a high rate (60 – 90 Hz) and a regular discharge pattern. Similarly, GPi neurons firing in the 60 – 90 Hz range were identified, along with border cells at the margins of the nucleus. The GPi and SNr were further identified by their inhibitory responses to microstimulation through the recording electrode at low intensities (thresholds of 2 – 4 uA) (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2009). Finally, in GPi trajectories, the optic tract was identified by microstimulation-induced phosphenes below the ventral border of the nucleus and muscle contractions at sites posterior to the nucleus.

5.2.4 Stimulation protocols

Evoked field potentials (fEPs) were recorded from one electrode while stimulating with single pulses (100 uA, 0.3 ms biphasic pulse width) from a second electrode at the
same dorsoventral level but separated by 600 – 800 um, as described elsewhere in detail (Prescott et al., 2009). Paired pulse studies were conducted in 20 patients at 31 sites using a customized Spike2 script deployed to run a randomized set of inter-stimulus intervals (ISIs: 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300 ms, each interval repeated 3 times) at 100 ua and 0.3 ms biphasic pulse width both pre and post high frequency stimulation (HFS – 4 trains of 2s at 100Hz, with each train separated by 10s). In some of the initial cases, fEP amplitudes were measured every 5th interval throughout the 120 s total run time with a single pulse to confirm a stable baseline. These stimuli in GPi and SNr did not elicit movements and were not perceived by the patients.

5.2.5 Data Analysis and Statistics

Paired pulse ratios (PPR) were calculated by dividing the peak amplitude of the 2nd fEP by the peak of the 1st fEP. These peaks were determined by measuring the difference between baseline amplitude immediately before the first or second stimulus and peak amplitude of the fEP. These values from a single experiment were the average for each interval presented 3 times in random order and were used to calculate the PPR for a given interval at a given site. Paired pulse ratios were compared for each ISI before and after HFS, across disease type, and output nuclei using a multiple ANOVA for main effects and interactions with significance levels set to p < 0.05. Further, post-hoc Bonferroni t-tests tested all pairwise comparisons between pre and post HFS within groups (GPi PD, GPi Dystonia, SNr PD and SNr Dystonia, respectively) with significance levels set at p < 0.05.
5.3 Results

5.3.1 Paired Pulse Test Site Location

All tested sites were located in the basal ganglia output structures, the SNr or GPi. We tested 9 sites in the GPi of 6 dystonia patients, 3 sites in the SNr of 2 dystonia patients, 10 sites in the GPi of 5 PD patients, and 9 sites in the SNR of 8 PD patients. The approximate location of test sites included in the study is shown in Figure 18. For the GPi sites, recording and stimulation took place throughout a depth of approximately 10 mm along the axis of the electrode track (Figure 18, left side). For the SNr sites, recording and stimulation took place in dorsolateral SNr in both dystonia and PD patients (Figure 18, right side). There was no significant correlation in paired pulse response across multiple depths in either the GPi or SNr. For example, at 20ms in both dystonia and PD cases, the distance from the optic tract in GPi cases and distance from the dorsal border of SNr in STN cases had no effect on the PPR response (Figure 19a-d).
Figure 18 - Paired Pulse Test Locations in the GPi and SNr. Composite figure showing the location of groups of neurons tested for a field evoked response using paired pulses in the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr). Recording and stimulation was performed with 2 microelectrodes along dorsoventral trajectories of the GPi (left) and STN (right) of dystonic patients (closed circles) and PD patients (open circles). Dorsal (D), ventral (V), anterior (A) and posterior (P) are labeled. Positions of the optic tract (OT), the internal (GPi), and external (GPe) segments of the globus pallidus are shown at left. Positions of the thalamus (Thal), subthalamic nucleus (STN), and substantia nigra pars reticulata (SNr) are shown at right. (Prescott et al., 2013)
**Figure 19 - Paired Pulse Response Across Multiple Depths in the GPi and SNr of Parkinson’s and Dystonia Patients.** Examined possible correlation between depth and paired pulse response at 20 ms ISI. The distance from the optic tract in the GPi of dystonia patients (a) and Parkinson’s diseases patients (b) had no effect on the PPR response. Similarly, the distance distance from the dorsal border of SNr in dystonia patients (c) and Parkinson’s disease patients (d) had no effect on the PPR response.
5.3.2 ON vs. OFF Dopaminergic Medication Paired Pulse Response

For the majority of experiments in PD patients, paired pulse experiments in the SNr and GPi were performed while the patient was ON dopaminergic medication since our previous results have indicated that extrastriatal dopamine modulates activity dependent synaptic plasticity at basal ganglia output neurons (Prescott et al., 2009). However, we did perform one comparison of the paired pulse response in the ON vs OFF dopaminergic medication state in both basal ganglia output structures. In the OFF medication state, while there was measured paired pulse depression at low interstimulus intervals, there was no change in the paired pulse response in either the GPi or SNr when comparing before and after HFS (Figure 20a & 20b, open and closed circles). However, in the ON medication state, while the initial (pre HFS) paired pulse response in both the SNr and GPi was similar to the response in the OFF medication state, there was an increase in the amount of paired pulse depression following HFS at low interstimulus intervals (Figure 20a & 20b, open and closed inverted triangles).
PPR SNr ON vs OFF PD

PPR GPi ON vs OFF PD
Figure 20 - Paired Pulse Response in the ON vs. OFF Dopaminergic Medication State in Both Basal Ganglia Output Structures in PD Patients. A) In the OFF medication state in GPi, there was no change in the paired pulse response when comparing pre (closed circle) and post (open circle) HFS. There was paired pulse depression at low interstimulus intervals both pre and post HFS. In the ON medication state in GPi, the initial paired pulse response pre HFS (closed inverted triangle) was similar to the response in the OFF medication state. However, following HFS (open inverted triangle), there was an increased paired pulse depression at low interstimulus intervals. B) In the OFF medication state in SNr, while there was paired pulse depression at low interstimulus intervals both pre and post HFS, there was no change in the paired pulse response when comparing pre (closed circle) and post (open circle) HFS. In the ON medication state in SNr, the initial paired pulse response pre HFS (closed inverted triangle) was similar to the response in the OFF medication state. However, similar to the GPi, following HFS (open inverted triangle), there was an increased paired pulse depression at low interstimulus intervals.
5.3.3 Disease Type, Stimulation State, and Interstimulus Interval

Basal ganglia output neurons undergo less paired pulse depression at low ISI in the dystonic state. A multiple ANOVA of paired pulse ratios revealed significant interactions between interstimulus interval, high frequency stimulation, output nuclei, and disease type. Additionally, significant interactions were detected between disease type and interstimulus interval (F=36.8, p<0.0001), and high frequency stimulation and interstimulus interval (F=22.5, p<0.0001).

5.3.4 Paired Pulse Response in GPi of Dystonia and PD Patients

Paired pulse response curves were constructed for the GPi in both disease types, before and after high frequency stimulation. Figure 21 shows an example from a dystonia patient of the raw data used to construct these curves. As can be seen in the example (Figure 21) and in the population comparisons (Figure 22a, closed circles, and Table 6), there is no paired pulse depression in the GPi of dystonia patients at any ISI before HFS. However, following HFS, the paired pulse ratio was significantly depressed from the pre HFS condition at interstimulus intervals less than 35 ms (Figure 22a, open circles) with the largest depression evident at the shortest 20 ms ISI (see Figure 22a inset for 20 ms raw trace). Bonferroni pairwise comparisons revealed a significant (p<0.01) reduction in PPR at 20 ms (0.57 +/- 0.12), 25 ms (0.74 +/- 0.15), and 30 ms (0.86 +/- 0.15). The example in Figure 21 shows a reduction in PPR at 50 ms following HFS but the reduction was not significant across the population. HFS increased the amplitude of peak 1 and peak 2 across most intervals in the GPi of dystonia patients, as
seen in Figure 21 (50ms and 200ms), with a greater magnitude of change of peak 1 accounting for reductions in PPR. Peak 1 increased a minimum of 1.14x (300ms) and a maximum of 1.31x (50ms) while peak 2 increased a maximum of 1.23x (also at 50ms).

At the smallest 20ms interval, the amplitude of peak 2 decreased slightly (0.78x) following HFS as seen in Figure 21 (20ms).

<table>
<thead>
<tr>
<th>Interval (ms)</th>
<th>PRE HFS PPR GPI Dys +/- SD</th>
<th>PRE HFS PPR GPI PD +/- SD</th>
<th>PRE HFS PPR SNr Dys +/- SD</th>
<th>PRE HFS PPR SNr PD +/- SD</th>
<th>POST HFS PPR GPI Dys +/- SD</th>
<th>POST HFS PPR GPI PD +/- SD</th>
<th>POST HFS PPR SNr Dys +/- SD</th>
<th>POST HFS PPR SNr PD +/- SD</th>
</tr>
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<td>20</td>
<td>0.94 +/-</td>
<td>0.58 +/-</td>
<td>0.95 +/-</td>
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<td>0.08</td>
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<td>0.11</td>
<td>0.12</td>
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**Table 6- Mean Paired Pulse Ratios in the SNr and GPI of Dystonia and PD Patients Pre and Post High Frequency Stimulation.** No paired pulse depression in the GPI and SNr of dystonia patients at any ISI before HFS.
Figure 21: Paired Pulse Response in the GPi of a Dystonic Patient Before and After HFS. Top traces show paired pulse responses before HFS at 20, 50, and 200 ms. Bottom traces show the paired pulse responses at the same ISIs following HFS. Notice the large reduction in amplitude of 2nd peak at 20 ms following HFS (slight reduction can also be seen at 50 ms following HFS). Ratio remains unchanged at 200 ms following HFS. Note also inhibition of spiking after single and paired pulses.
In contrast to the results in the GPi of dystonia patients, paired pulse stimulation before HFS with an ISI of < 50 ms caused paired pulse depression in the GPi of Parkinson’s patients ON L-Dopa (Figure 22b, closed circles and Table 6), similar to the ON vs. OFF example discussed above. Greater depression was evident as the ISI was reduced. Following HFS, paired stimulation at ISIs < 50 ms induced additional depression of the paired pulse ratio in the GPi of Parkinson’s patients ON medication (Figure 22b, open circles) with Bonferroni pairwise comparisons revealing a significant (p<0.05) reduction occurring at 20 ms (0.44 +/- 0.05), 30 ms (0.66 +/- 0.07), 35 ms (0.76 +/- 0.05), and 40 ms (0.82 +/- 0.07). Again, the largest depression was seen at the shortest 20 ms ISI (Figure 22b, inset). HFS increased the amplitude of peak 1 and peak 2 at all intervals in the GPi of PD patients, with a greater magnitude of change of peak 1 accounting for reductions in PPR. Peak 1 increased a minimum of 1.32x (200ms) and a maximum of 1.79x (25ms) while peak 2 increased a minimum of 1.02x (20ms) and a maximum of 1.37x (40ms).
Figure 22: Paired Pulse Response Curve in the GPi of Dystonia and Parkinson’s Disease Patients. Shown is the paired pulse ratio as a function of PPI before and after high frequency stimulation in the GPi. A) In the GPi of dystonia patients (n=9), there is no significant paired pulse depression at any ISI before HFS (closed circles). Following HFS (open circles) there is a marked increase in paired pulse depression at ISIs < 40ms. Inset shows representative example trace of paired response at 20ms ISI in the GPi of a dystonia patient before (black) and after (grey) HFS. B) In the GPi of PD patients on dopaminergic medication (n=10), paired stimulation before HFS with an ISI of < 50 ms revealed paired pulse depression (closed circles). Following HFS (open circles), additional depression of the paired pulse ratio occurred at ISIs < 50ms. Inset shows representative example trace of paired response at 20ms ISI in the GPi of a PD patient before (black) and after (grey) HFS. Significant (p < 0.05) Bonferroni pairwise comparisons between pre and post HFS within groups are indicated by *.
5.3.5 Paired Pulse Response in SNr of Dystonia and PD Patients

Paired pulse response curves were also constructed for the SNr in both disease types, before and after high frequency stimulation. Figure 23a (closed circles) and Table 6 show that, like the GPi of dystonia patients, there is no paired pulse depression in the SNr of dystonia patients at any ISI before HFS. However, again similar to the GPi of dystonia patients, following HFS there was significant paired pulse depression evident in the SNr of dystonia patients at shorter interstimulus intervals when compared to the pre HFS condition (Figure 23a, open circles) with the largest depression again occurring at 20ms, the shortest interval tested (Figure 23a inset). Bonferroni pairwise comparisons showed there was significant reduction (p<0.01) in PPR in the SNr of dystonia patients post HFS at 20 ms (0.48 +/- 0.05), 25 ms (0.62 +/- 0.10), 30 ms (0.76 +/- 0.11), 40 ms (0.84 +/- 0.07), and 75 ms (0.98 +/- 0.02). HFS increased the amplitude of peak 1 and peak 2 across most intervals in the SNr of dystonia patients, with a greater magnitude of change of peak 1 again accounting for reductions in PPR. Peak 1 increased a minimum of 1.44 (300ms) and a maximum of 1.96x (25ms) while peak 2 increased a maximum of 1.44x (also at 40ms). At the smallest 20ms interval, the amplitude of peak 2 decreased slightly (0.79x) following HFS.

In contrast to the results in the SNr of dystonia patients (and similar to the results in the GPi of PD patients), paired stimulation before HFS with an ISI of < 75 ms induced paired pulse depression in the SNr of Parkinson’s patients ON L-Dopa (Figure 23b, closed circles and Table 6). Again, greater depression was evident as the ISI was decreased. Notably, following HFS, paired stimulation at all but 50 ms and the longest 300 ms ISI further depressed the PPR in the SNr of Parkinson’s patients ON medication.
(Figure 23b, open circles). Bonferroni pairwise comparisons showed there was significant reduction in PPR at 20 ms (0.31 +/- 0.14), 25 ms (0.49 +/- 0.11), 30 ms (0.62 +/- 0.06), 35 ms (0.71 +/- 0.07), 40 ms (0.74 +/- 0.03), 75 ms (0.92 +/- 0.02), 100 ms (0.92 +/- 0.06), 150 ms (0.93 +/- 0.03), and 200 ms (0.93 +/- 0.04) with the largest depression again seen at the shortest 20 ms ISI (Figure 23b, inset). HFS increased the amplitude of peak 1 and peak 2 at all intervals in the SNr of PD patients, with a greater magnitude of change of peak 1 accounting for reductions in PPR. Peak 1 increased a minimum of 1.33x (200ms) and a maximum of 1.56x (75ms) while peak 2 increased a minimum of 1.06x (25ms) and a maximum of 1.34x (100ms).
**Figure 23: Paired Pulse Response Curve in the SNr of Dystonia and PD Patients.** Shown is the paired pulse ratio before and after high frequency stimulation across a range of interstimulus intervals in the SNr. A) In the SNr of dystonia patients (n=3), there is no measurable paired pulse depression at any ISI before HFS (closed circles), similar to the GPi of dystonia patients. Following HFS in these patients, paired pulse depression occurs at intervals < 75ms (open circles). Inset shows representative example trace of paired response at 20ms ISI in the SNr of a dystonia patient before (black) and after (grey) HFS. B) In the SNr of PD patients on dopaminergic medication (n=9), paired stimulation before HFS with an ISI of < 75 ms induced paired pulse depression (closed circles). Following HFS, further paired pulse depression was seen at all but the longest 300ms interval (open circles). Inset shows representative example trace of paired response at 20ms ISI in the SNr of a PD patient before (black) and after (grey) HFS. Significant (p < 0.05) Bonferroni pairwise comparisons between pre and post HFS within groups are indicated by *. 
5.3.6 PPR and Motor Score Correlations

The paired pulse ratio before HFS at the 20ms interval for each site tested in each patient was correlated to the available motor scores in an attempt to correlate motor dysfunction and measures of neurotransmitter release. For PD patients, PPR before HFS was tested for correlation to the UPDRS III motor sub scores in the ON ($R^2 = 0.072$) and OFF ($R^2 = 0.00057$) medication states, respectively (Figure 24a). Two tailed t-tests revealed that neither correlation was significant. For cervical dystonia patients, PPR before HFS was tested for correlation to the TWSTRS severity ($R^2 = 0.63$), disability ($R^2 = 0.53$), and pain scores ($R^2 = 0.51$) respectively (Figure 24b). Two tailed t-tests revealed a significant correlation between PPR and TWSTRS severity scores ($p < 0.05$). Correlations for disability ($p = 0.062$) and pain ($p = 0.074$) suggested a trend but were not significant. Finally, for generalized dystonia patients, PPR before HFS was tested for correlation to the BFMDRS movement ($R^2 = 0.76$) and disability ($R^2 = 0.37$) scores respectively (Figure 24c). There was a significant correlation between PPR and BFMDRS movement scores ($p < 0.05$), but not disability scores ($p = 0.27$). These results suggest that in both generalized and cervical dystonia, the paired pulse ratio at low interstimulus intervals correlates with several measures of motor dysfunction.
The pathophysiology of dystonia is increasingly thought to be tied to alterations in plasticity and abnormal inhibitory processes. Abnormalities of short latency inhibitory mechanisms in both sensory and motor systems are thought to be a hallmark of dystonia. To date, experiments exploring abnormal plasticity and altered inhibition have been limited to sensorimotor cortex, but such abnormalities could be secondary to changes originating in the basal ganglia and/or in addition to changes in the basal ganglia. The basal ganglia may act upon the motor cortex to enhance surround inhibition (Hallett, 2009; Mink, 1996) and/or changes in basal ganglia may contribute or be a major direct cause of the motor symptoms. Using microelectrode-evoked paired pulse stimulation, we show here that an inhibitory abnormality exists in two major basal ganglia output nuclei, the GPi and SNr, of dystonia patients and furthermore that the

**Figure 24: Paired Pulse Ratio Correlations with UPDRS, TWSTRS, and BFMDRS.** PPRs from baseline conditions (pre HFS) at the lowest 20 ms ISI were correlated to a given patient’s respective motor rating scale. A) PPR before HFS was correlated to the OFF (closed circles) and ON (open circles) UPDRS III motor sub scores of PD patients. PPRs include measures from both SNr and GPi. B) For cervical dystonia patients, PPR before HFS was correlated to the Toronto Western Spasmodic Torticollis Rating Scale. Correlations were performed for the individual measures of Severity (closed circles, max score of 35), Disability (open circles, max score of 30), and Pain (closed inverse triangles, max score of 20). Correlation includes PPR measures from both SNr and GPi. C) For generalized dystonia patients, PPR before HFS was correlated to the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS). Correlations were performed for the individual measures of Movement (closed circles, max score of 120) and Disability (open circles, max score of 30). Again, the correlation includes PPR measures from both the SNr and GPi.

**5.4 Discussion**

The pathophysiology of dystonia is increasingly thought to be tied to alterations in plasticity and abnormal inhibitory processes. Abnormalities of short latency inhibitory mechanisms in both sensory and motor systems are thought to be a hallmark of dystonia. To date, experiments exploring abnormal plasticity and altered inhibition have been limited to sensorimotor cortex, but such abnormalities could be secondary to changes originating in the basal ganglia and/or in addition to changes in the basal ganglia. The basal ganglia may act upon the motor cortex to enhance surround inhibition (Hallett, 2009; Mink, 1996) and/or changes in basal ganglia may contribute or be a major direct cause of the motor symptoms. Using microelectrode-evoked paired pulse stimulation, we show here that an inhibitory abnormality exists in two major basal ganglia output nuclei, the GPi and SNr, of dystonia patients and furthermore that the
effects of high frequency microstimulation in GPi/SNr on local field evoked potentials can restore paired pulse depression in dystonia patients.

The present study describes the response of the basal ganglia output neurons, the SNr and GPi, in both dystonia patients and Parkinson’s disease patients to intra nuclei paired pulse stimulation. We found that paired pulse depression was present at interstimulus intervals below 75 ms in the basal ganglia of PD patients on dopaminergic medication after low dose L-Dopa. The paired pulse ratio was decreased following a HFS protocol. The change in ratio was due primarily to an increased response to the first pulse of the pair, and to a lesser extent, a decrease in the second pulse. A change in paired pulse depression of GABAergic inhibitory potentials is an example of synaptic plasticity. The possible functional significance of such a change is that this might increase the ability of basal ganglia output neurons to convey phasic inhibitory signals driven by sensory inputs. We have previously reported that extrastriatal dopamine can modulate activity-dependent synaptic plasticity in the SNr of PD patients (Prescott et al., 2009). The current study indicates that the same is true for the GPi of PD patients.

In contrast, paired pulse depression was absent in both basal ganglia output nuclei of dystonia patients at all interstimulus intervals but restored to baseline levels seen in PD patients without dystonia following high frequency stimulation. Paired pulse depression is a reliable indicator of an increased probability of neurotransmitter release (Thomson, 2000); the larger the initial probability of release, the more pronounced is depression for two closely spaced stimuli (Zucker & Regehr, 2002) since transmitter tends to be depleted during the first stimulus. These results indicate that the probability of neurotransmitter release in both the SNR and GPi appears to be lowered in dystonia...
patients when compared to PD patients on dopaminergic medication. However, following HFS, paired pulse depression in basal ganglia output nuclei of dystonia patients is restored to levels seen in the PD patients on dopaminergic medication. As mentioned, changes in paired pulse ratios following HFS were due to increases in the field evoked inhibitory potential. These changes might function to enhance the ability of basal ganglia output neurons to convey phasic inhibitory signals driven by sensory inputs. Interestingly, the degree of this change is larger in dystonia patients than in PD patients on dopaminergic medication, supporting the notion of enhanced inhibitory plasticity in basal ganglia output sites.

It is also of note that the paired pulse results appear to be consistent across a heterogeneous group of generalized and cervical dystonia patients. This suggests an underlying neuronal dysfunction causing an impaired inhibitory neurotransmitter release, common to multiple forms of dystonia. Although not tested here, this commonality may extend to other task specific dystonias as well. Indeed, it has previously been shown using magnetic resonance spectroscopy that in patients with focal task specific dystonia, brain GABA levels are selectively reduced in cortical and subcortical regions contralateral to the dystonic limb (Levy and Hallett, 2002). Our findings of reduced paired pulse depression, suggestive of reduced probability of neurotransmitter release, in the GABAergic SNr and GPi of dystonic patients may account for the findings of Levy and Hallett. Consistent with a role for deficient GABAergic activity in dystonia, drugs that potentiate GABAergic activity, such as benzodiazepines and baclofen, are modestly effective in suppressing dystonic movements (Tanabe et al., 2009). Moreover, application of bicuculline to the motor
cortex of monkeys causes abnormal co-contraction of agonist and antagonist muscle groups during activities requiring fine motor skills, and produces abnormal movements reminiscent of task specific dystonia (Matsumura et al., 1991).

Previous observations suggest that micro stimulation in the GPi and SNr primarily activates inhibitory GABAergic projections from the striatum (Dostrovsky et al., 2000; Prescott et al., 2009) and that GABA release from terminals of medium spiny neurons is enhanced by activation of presynaptic receptors of the dopamine D1 family and reduced by activation of presynaptic receptors of the dopamine D2 family (Guzman et al., 2003). As a consequence, these receptors have a strong impact on short-term plasticity. Additionally, it has been reported that dopaminergic D1 agonists have an enhancing effect on striatonigral IPSC amplitude and paired pulse depression with a presynaptic site of modulation (de Jesus et al., 2011). Indeed, when measuring the paired pulse response in the off dopaminergic medication state, while there was measured paired pulse depression at low interstimulus intervals, there was no change in the paired pulse response in either the GPi or SNr when comparing before and after HFS. It would appear then, that changes in paired pulse depression in these nuclei are a measure of changes in the probability of GABA release from the terminals of medium spiny striatal neurons in the GPi and SNr and that this release is regulated by the presence of dopamine, and is impaired in dystonia patients. It would be interesting to note whether there exist alterations in release probability in other basal ganglia nuclei or at the thalamic targets of the SNr and GPi of dystonia patients.

Additionally, both the SNr and GPi also receive GABAergic projections from local axon collaterals and the external segment of the globus pallidus, and changes in
release probability as measured in dystonia patients may be indicative of functional deficits in the local neuronal population. Interestingly, when compared to striatal inputs, the local axon collaterals are known to display a significantly lower PPR, and it has been proposed that this local inhibitory connectivity may be the predominant inhibitory regulator during resting behaviour and may control timing of action potential firing and synchronization of functionally relevant neuronal pools (Sims et al., 2008).

Further evidence for an impaired neurotransmitter release mechanism in dystonia arises from studies on genetic forms of dystonia. Indeed, the idea that abnormal excitability and plasticity can provide a link between genetic factors and phenotype may be central to dystonia (Peterson et al., 2010). Torsin is thought to possibly facilitate processing of proteins in transit through the secretory pathway to the cell surface. A recent study showed that TorsinA interacts with snapin (Granata et al., 2008), a protein known to play an essential role in vesicle exocytosis by enhancing the interaction between the SNARE complex and the synaptic vesicle marker, synaptotagmin. Snapin appears to be the link between TorsinA and the synaptic release machinery. Mutant TorsinA results in sequestering of both TorsinA and snapin (Granata et al., 2008), which could potentially destabilize the balance between the exo and endocytic cycle, and ultimately affect neurotransmitter release (Granata et al., 2009). It remains to be determined if these proteins are defective in GABA terminals in the GPi and SNr of dystonic patients.

Questions regarding basal ganglia plasticity and neuronal properties are traditionally explored with the use of animal models because of the experimental difficulties of investigating these processes in human subjects. However, due to known
limitations in correspondence between animal models and human clinical phenotypes (Wichmann, 2008), studies such as the current one, while limited in their own sense, most notably by lack of healthy controls, provide a direct window into the pathophysiology of these deep brain structures in the diseased state.

Here we demonstrate a deficit in inhibition in the basal ganglia output nuclei of dystonia patients compared to Parkinson’s disease patients on dopaminergic medication. These findings suggest that abnormalities exist in presynaptic function of GABAergic striatopallidal and/or striatonigral terminals in dystonia patients and that these abnormalities may result in increased excitability and plasticity observed in the cortex of dystonia patients. High frequency stimulation seems to “normalize” pre-synaptic function to a degree, suggesting that one mechanism of action of deep brain stimulation is enhancement of GABA release.
Chapter 6 - Lack of Depotentiation in Basal Ganglia Output Neurons of PD Patients with Levodopa-Induced Dyskinesia

6.1 Introduction

We have previously reported that in PD patients undergoing implantation of DBS electrodes in the STN, application of the dopamine precursor levodopa potentiates activity-dependent synaptic plasticity in the SNr, a major output structure of the basal ganglia (Prescott et al., 2009). A review article of that work suggested that the results opened the way to a new experimental approach in the field of PD research, strengthening the view of PD as a ‘synaptopathy’ that leads to neuronal network destabilization and can be rapidly counteracted by the manipulation of a neurotransmitter system (Calabresi et al., 2009). The review authors’ hope was that, in the future, the study of human synaptic plasticity might shed light on the complex mechanisms underlying symptoms of the disease and the disabling long-term side effects of treatment with levodopa. Here, we further explore alterations in human synaptic plasticity in basal ganglia output nuclei with a focus on changes to depotentiation in patients who develop levodopa-induced dyskinesia. In order to avoid neuronal network destabilization, the mechanisms underlying synaptic plasticity need to be tightly controlled and, in experimental models of PD (Picconi et al., 2008, 2003), one critical form of synaptic plasticity, depotentiation, is selectively lost during dyskinesias.

As previously covered, PD is a movement disorder characterized by the degeneration of dopamine neurons in the SNc. The SNc supplies dopamine to the basal
ganglia, a group of nuclei involved in the control and regulation of volitional movements. When the dopaminergic input is lost, patients present with symptoms of akinesia/bradykinesia, rigidity and resting tremor. The dopamine precursor levodopa effectively treats these motor symptoms, but many patients become resistant to the treatment and/or develop debilitating levodopa-induced dyskinesias (LID) over time.

In the 6-OHDA experimental animal model of PD, a dyskinetic motor response to L-Dopa is associated with an altered form of synaptic plasticity in the corticostriatal pathway. In 6-OHDA rats, DA denervation impairs corticostriatal LTP and LTD. Chronic L-Dopa treatment, at a therapeutic dosage similar to that used in PD patients, restores LTP in both dyskinetic and non-dyskinetic rats (Picconi et al., 2008, 2003). However, in corticostriatal slices of animals that did not develop dyskinesia, depotentiation reversed LTP at corticostriatal synapses following LFS, whereas slices from dyskinetic rats showed no capacity for depotentiation (Picconi et al., 2008, 2003). The presumed consequence of this lack of depotentiation at corticostriatal synapses is that in the dyskinetic state, corticostriatal synapses that have been potentiated in vivo by a previous burst of firing would continue to show an augmented response to cortical input, irrespective of their salience for the animal’s ongoing behavior.

Our previous work determined that, in PD patients, changes in synaptic plasticity in the ON and OFF dopaminergic medication state in the SNr, one of the primary basal ganglia output nuclei, mirrored changes described in corticostriatal slice work of the 6-OHDA model. Corticostriatal slice work in the 6-OHDA model also suggests that abnormal involuntary movements such as dyskinesia are the result of alterations to synaptic plasticity at the basal ganglia input. The aim of this study was to determine
whether altered synaptic plasticity was also measurable in basal ganglia output neurons of PD patients who had developed L-Dopa-induced dyskinesia. We investigated the effects of LFS on synapses in the GPi and SNr that had already undergone HFS-mediated potentiation and found that the plasticity abnormalities observed in experimental models of L-Dopa-induced dyskinesia also appear to be present in basal ganglia output neurons of PD patients with dyskinesia. That is, GPi and SNr synapses in patients with less severe dyskinesia underwent greater depotentiation following LFS than patients with more severe dyskinesia.

6.2 Methods

6.2.1 Patients

To examine the relationship between dopamine, depotentiation and dyskinesia in the basal ganglia output nuclei of PD patients, experiments were performed during bilateral STN or GPi DBS procedures, with the first hemisphere typically performed following 12 hours of dopaminergic medication withdrawal, and where possible, the second hemisphere 20-30 minutes after 100 - 200 mg L-Dopa/carbidopa. 24 patients underwent STN DBS, 3 patients underwent GPi DBS and 1 patient underwent a bilateral pallidotomy, all for PD. All experiments were performed while the patients were awake with local anesthesia only. Patients’ clinical information is shown in Table 7. The group was comprised of 22 men and 6 women and had a mean age and disease duration of 59.8 +/- 7.2 and 13.1 +/- 4.6, respectively. All 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 7 - Clinical Characteristics of Patients Tested for fEP Depotentiation in SNr and GPi

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<td>GPI (2)</td>
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<td>1310</td>
<td>32 / 12.5</td>
<td>1 &amp; 2</td>
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</table>

Mean 59.8 +/- 7.2 13.1 +/- 4.6 ------------------------------ 1284 +/- 500 40 +/- 12 / 19 +/- 9 1.67 +/- 1.00 & 1.15 +/- 0.99
6.2.2 **Surgical Procedure**

Extracellular recordings were made with dual independently driven microelectrodes (25 µm tip length, axes 600 - 800 µm apart, 0.2 - 0.4 MΩ impedance at 1,000 Hz) during the electrophysiological mapping procedure used to obtain physiological data for localizing the target for DBS quadripolar electrodes, as described several times previously.

6.2.3 **Stimulation Protocols**

Field-evoked potentials (fEPs) were recorded from one electrode in the SNr or GPi while stimulating with single pulses (100 µA, 0.3 ms biphasic pulse width) from a second electrode separated by 600 – 800 um in the same structure, as described previously. The ability of LFS to depotentiate previous HFS-mediated potentiation was tested in the SNr of 13 patients at 17 sites in the OFF medication state and 14 patients at 18 sites in the ON medication state. LFS was tested in the GPi of 2 patients at 3 sites in the OFF medication state and 2 patients at 3 sites in the ON medication state. 12 patients were tested in both the OFF and ON L-Dopa states, while 1 and 2 patients were tested in the OFF and ON state only, respectively.

After obtaining a stable baseline of fEP amplitudes at 1 Hz, HFS was delivered, consisting of 4 trains of 2 seconds at 100 Hz, with each train separated by 10 seconds. Following HFS, blocks of 10 pulses at 1Hz were tested every 30 seconds for 90 seconds to measure the extent of potentiation of fEP amplitudes. Next, low frequency
stimulation was delivered at 2 Hz for 60 seconds at 20ua through the proximal electrode (Figure 25a). This LFS protocol was based on a paradigm previously shown to depotentiate HFS-mediated potentiation at the corticostriatal synapse in normal rats and 6-OHDA rats receiving levodopa (Picconi et al., 2008, 2003). Blocks of 5 pulses at 1Hz measuring any changes in fEP amplitude were tested at 20 and 40 seconds during the 60 second LFS, followed by a block of 10 pulses at 1 Hz given immediately after the LFS train. A final block of 10 test pulses at 1 Hz was given 30 seconds later. The HFS protocol was then repeated followed by a block of 10 1 Hz test pulses to measure any metaplastic changes in fEP response following LFS compared to the initial HFS.

Potentiation and depotentiation was measured using fEP amplitudes in both OFF and ON dopaminergic medication states; where possible patients were tested in both states with the first hemisphere being tested following 12 hours of dopaminergic medication withdrawal and the second side following administration of Sinemet 100/25®. In several instances, patients were tested in either the ON or OFF state exclusively, based on the surgeon’s recommendation several hours before or at the time of the procedure.

Several trials were performed using a baseline stimulation of less than 1Hz pulses to verify the suitability of 1 Hz stimulation as a baseline (Figure 25b). In these trials, tests pulses were delivered manually (since the lowest train is 1Hz on our stimulator) at approximately 0.2 – 0.3 Hz in several conditions to see if there was a difference compared to our 1 Hz baseline stimulation. These tests were performed to examine whether 1 Hz might be fast enough to induce some form of plastic change on its own and would therefore be unsuitable as baseline stimulation. All tests took place in
the SNr. Continuous 0.2 - 0.3 Hz stimulation before and after HFS was performed at 5 sites in 4 patients. 4 sites were tested in the ON medication state and 1 site was tested in the OFF medications state. Additionally, continuous 0.2-0.3 Hz stimulation was performed before and after LFS at 1 site in the ON medication state.

Additionally, plasticity experiments were performed in 4 patients at 8 sites with the order of HFS and LFS reversed (Figure 25c). That is, LFS was given first to test whether it would cause depression of fEP amplitudes in a LTD-like manner. 5 sites were tested in the OFF medication state and 3 sites were tested in the ON medication state.
Figure 25 – Potentiation & Depotentiation Stimulation Protocols. a) Baseline 1 Hz stim was potentiated with HFS as described previously. Further 1 Hz stim following HFS measured potentiation. LFS was then applied to measure for possible depotentiation. 1 Hz stim followed LFS to measure depotentiation. A final HFS was given in some cases. b) Manual 0.2-0.3 Hz stimulation was applied to test suitability of 1 Hz as baseline stimulation. C) HFS and LFS presentation was reversed to test for LFS effects on baseline fEP amplitudes.
6.2.4 Analysis of fEP Amplitudes

The recordings were analyzed offline using Spike2 software version 5 (CED, Cambridge, UK). fEP amplitudes were 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 following HFS and depotentiation following LFS was evaluated at each site in both the ON and OFF 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 \( y_o \), and \( b \) describes the steepness of the curve. Population data were fit with a regression line if the fit had a significance value of \( p < 0.05 \).

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.

To assess the relationship between dopamine, depotentiation and dyskinesia, the amount of depotentiation induced with LFS was correlated to the sum of the dyskinesia scores of UPDRS items 32 + 33.
6.3 Results

6.3.1 Sub 1Hz Baseline Stim

Continuous 0.2 - 0.3 Hz stimulation before and after HFS was performed in the OFF (Figure 26a) and ON (Figure 26b) medication state, with no marked difference compared to the 1 Hz stimulation we use for baseline measures (ie Figures 27 & 28). Additionally, 0.2 - 0.3 Hz stimulation following LFS (Figure 26c) induced no change from baseline measures, just like in the LFS results discussed below and shown in Figure 32. 1 Hz stimulation is a suitable baseline stimulation frequency.
Figure 26: Slow Baseline and Test Pulses. a) Sub 1 Hz baseline and test stimulation following HFS in the OFF state produces a fEP response very similar to results using 1 Hz stimulation. b) Similarly, sub 1Hz baseline and test stimulation in the ON state produces results similar to those seen using 1 Hz stimulation. c) Sub 1 Hz baseline and test stimulation following 2Hz LFS also produced a result analogous to results seen using 1 Hz stimulation (see Figure 32).
6.3.2 HFS, LFS and Potentiation in the SNr of PD Patients

Similar to results from Prescott et al., 09 (See Figure 7), we observed dopamine-dependent potentiation of fEP amplitudes following HFS in the SNr of PD patients. In the absence of dopaminergic medication, HFS did not induce a lasting change in fEP amplitudes in the SNr. The effects of HFS on fEP amplitudes in the SNr was measured at 17 sites in 14 patients in the OFF state. The population data for the OFF group shown in Figure 27a (closed circles) reveal a significant initial 132.6 ± 3.9 % increase in fEP amplitude following HFS that then decayed by 180s to baseline. Regression analysis on population data from the OFF group revealed a $y_o$ fEP amplitude plateau value of 103.4 +/- 0.95.

The effects of HFS on fEP amplitudes in the SNr were examined at 18 sites in 13 patients in the ON state (Figure 27a; open circles). The largest fEP amplitude measures occurred immediately following the conditioning stimuli (177.9 ± 7.8% 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 $y_o$ plateau value of 134.2 ± 0.9 % above baseline, indicating that HFS potentiates fEP amplitudes in the presence of dopamine producing an LTP-like effect. The regression function for the OFF and ON groups was highly significant with plateau values at p < 0.001.

Interestingly, LFS did not appear to reverse this LTP-like effect either in the OFF state or ON state in the population curve. The decay in fEP amplitude in the ON group...
from our previous study without LFS (129.3 ± 5.2%, Prescott et al., 2009) was similar to the fEP amplitude decay following LFS in the current study (134.2 ± 0.9%) (Figure 27b).

**Figure 27 - High and Low Frequency Stimulation in the SNr of PD Patients.** a) In the OFF state (closed circles), HFS failed to induce a lasting change in fEP amplitude measures in the SNr, with amplitudes returning to baseline by 180s. Following application of dopaminergic medication (open circles) HFS potentiated fEP amplitudes significantly above baseline and OFF measures. Significant difference between fEP amplitude measures in OFF and ON populations following HFS and subsequent LFS in the SNr (* denotes p <0.01). However, when the results were overlaid (b) with our previously published results with HFS only, it appears that LFS had no effect on the population results.
6.3.3 LFS and Depotentiation in SNr of PD Patients

While the population results initially indicated that there was no difference in fEP amplitudes following LFS compared to our previous study using HFS only (Figure 27b above), we noted that individual examples showed a marked difference to the population results. fEPs in some patients ON medication with no dyskinesias underwent some depotentiation following LFS (example shown in Figure 28a), whereas fEPs in other patients, some who developed mild dyskinesia during testing, did not undergo any change following LFS (example shown in Figure 28b).

Figure 28 – LFS Responder vs. Non-Responder Example. a) LFS is capable of depotentiating previous HFS-mediated potentiation in individual patients. Example from patient ON medication with no dyskinesias at time of test (Patient 3 in Table 7). b) LFS is not capable of depotentiating previous HFS-mediated potentiation in patient who experienced mild dyskinesia at time of ON testing (Patient 7 from Table 7).
As such, we sorted the patients by their combined UPDRS 32 and 33 scores. These scores measure dyskinesia duration (divides waking day into 4 segments, 1 point per segment, max of 4) and severity (0-4, 0 = normal, 4 = severe) respectively. Patients were divided into two groups; i) patients with combined UPDRS 32 and 33 scores of less than or equal to 2, indicative of milder dyskinesia, and ii) patients with combined UPDRS 32 and 33 scores of 3 or more, indicative of more severe dyskinesia. Of the 18 test sites measured in the ON medication state, 9 came from patients with UPDRS 32 & 33 scores ≤ 2, and 9 came from patients with UPDRS 32 & 33 scores ≥ 3. Sorting by dyskinesia severity revealed a very interesting result across the population: those patients categorized as having less severe dyskinesia (UPDRS 32 & 33 ≤ 2) responded to LFS with marked depotentiation, with HFS-mediated potentiation being reversed to pre-HFS baseline levels following LFS when patients were ON dopaminergic medication (Figure 29, closed triangles). Meanwhile, those patients categorized as having more severe dyskinesia (UPDRS 33 & 32 ≥ 3), did not respond to LFS and underwent no depotentiation with HFS-mediated potentiation remaining potentiated (Figure 29, open triangles).

Regression analysis on population data from these groups’ fEP amplitudes revealed that the ON group with UPDRS items 32 & 33 ≤ 2 that responded to LFS had a y₀ plateau value of 106.90 +/- 1.67 (p < 0.01) following LFS. This was a value close to baseline and very close to the OFF y₀ plateau value of 103.39 +/- 0.94 (p < 0.001), indicating that LFS reverses HFS-mediated potentiation within this patient group. Regression analysis of the population data from ON group with UPDRS items 32 & 33 ≥
3 that did not respond to LFS had a $y_o$ plateau value of 148.36 +/- 1.38 (p < 0.01) following LFS, a value significantly higher than our previously published 129.3 +/- 5.2 in patients ON dopaminergic medication following HFS alone.

![Graph showing response to LFS Sorted by Clinical Dyskinesia Scores.](image)

**Figure 29** – *Response to LFS Sorted by Clinical Dyskinesia Scores.* Sorting population by sum of items UPDRS 32 & 33 for dyskinesia severity and duration reveals 2 groups within the ON data; one group, with a UPDRS 32 & 33 score ≤ 2 that responded to LFS and underwent depotentiation (closed triangles), and one group, with a UPDRS 32 and 33 score ≥ 3 that did not respond to LFS and underwent no depotentiation (open triangles). These groups are plotted above beginning during LFS and denoted with triangles. ** denotes p < 0.01, * denotes p < 0.05.

### 6.3.4 HFS and Potentiation / LFS and Depotentiation in GPi of PD Patients

We also observed dopamine-dependent potentiation of fEP amplitudes following HFS in the GPi of PD patients; the HFS and LFS experiments were performed in several PD patients undergoing pallidotomy or implantation of GPi DBS electrodes. While the number of patients is much smaller, the outcome is analogous to the results...
of sites tested in the SNr. 2 patients were tested at 3 sites in both the OFF and ON medication state.

The effects of HFS on fEP amplitudes in the GPi in the OFF state are shown in Figure 30a (closed circles). An initial increase of 131.2 +/- 8.2% was seen in the OFF state in the GPi, similar to the initial 132.6 ± 3.9% increase in fEP amplitudes in the SNr in the OFF state. Regression analysis was not performed in the GPi in the OFF state due to the low number of cases. The effects of HFS on fEP amplitudes in the GPi in the ON state is shown in Figure 30a (open circles). Following HFS, fEP amplitudes were significantly increased above amplitudes from the OFF group (p < 0.05). The initial increase in fEP amplitudes following HFS in the ON state in GPi was 177.1 +/- 14.4%, again measuring very similar to the 177.9 ± 7.8% seen in the SNr in the ON state.

Additionally, there appears to be a similar outcome in terms of LFS responders and non-responders for depotentiation of HFS-mediated potentiation (Figure 30b), though the number of samples leaves this as an observation only. In one example (Figure 30b, left), LFS appears to reverse some of the HFS-mediated potentiation. This patient (#19 from Table 7) had UPDRS 32 & 33 scores of 1 and 1. A second example (Figure 30b, right) taken from a patient with scores of 2 and 1 (#24 in Table 7) shows no depotentiation following LFS. The patient with a higher sum dyskinesia underwent less depotentiation.
Figure 30: High and Low Frequency Stimulation in the GPi of PD Patients. A small number of PD patients were tested while undergoing pallidotomy or GPi DBS procedures. A) Significant difference between fEP amplitude measures in OFF (black, n=2) and ON (white, n=2) populations following GPi HFS and subsequent LFS (* denotes p < 0.05). B) While data in this patient set is limited, there appears to be both LFS responders and non-responders in tests conducted in the GPi of patients ON dopaminergic medication.
6.3.5 Clinical Correlation

The percentage of change in fEP amplitude following LFS at each site measured in SNr and GPi was correlated to the patients' clinical dyskinesia scores. The GPi and SNr were grouped due to the low number of GPi trials and the similarities in morphology and physiology of GPi and SNr. A significant correlation was found between the amount of depotentiation of fEP amplitudes following LFS in individual patients and their clinical dyskinesia scores in both the ON and OFF state, with the magnitude of change being greater in the ON state. In the ON state, we found a close inverse linear relationship ($r^2 = 0.591, p < .001$) between the patients' clinical dyskinesia scores (higher values indicating worse severity and duration of dyskinesia) and the percentage reduction in fEP amplitudes following LFS (Figure 31 closed circles). Similarly, in the OFF state, we found a close inverse linear relationship $r^2 = 0.503, p < .001$ between the patients’ clinical dyskinesia scores and the percentage reduction in fEP amplitudes following LFS (Figure 31 open circles).

That is, in both the ON and OFF medication state, patients with less dyskinesia (lower scores of UPDRS items 32+33) undergo greater depotentiation following LFS than patients with more severe dyskinesias (higher scores of UPDRS items 32+33).
Plasticity experiments were performed in 4 patients at 8 sites (all in the SNr) with the order of HFS and LFS reversed. That is, LFS was given first to test whether it would cause depression of fEP amplitudes in a LTD-like manner in the absence of other stimuli. This did not appear to be the case, with LFS having no effect on baseline measurements when given in either the OFF (Figure 32 closed circles) or ON (Figure 32 open circles) medication state.

**6.3.6 LFS Preceding HFS**

Figure 31 – Correlation of Clinical Dyskinesia Scores and Depotentiation Following LFS in Basal Ganglia Output Neurons. The amount of depotentiation induced with LFS in basal ganglia output neurons correlates to patients’ clinical dyskinesias scores (UPDRS items 32+33) in both the ON (closed circles) and OFF (open circles) medication state. Patients with less dyskinesia (lower scores) undergo greater depotentiation following LFS in basal ganglia output neurons than patients with more severe dyskinesias (higher scores).
open circles) medication state. No change in fEP amplitude was measured in either case. Furthermore, HFS-mediated potentiation did not appear to be altered when HFS was given following LFS; when HFS followed LFS in the ON medication state (n=3), fEP amplitudes increased to 182.7 +/- 17.5 % of baseline, similar to the measure of 177.9 ± 7.8% baseline when HFS preceded LFS in the ON medication state in the SNr (see Figure 32). When HFS followed LFS in the OFF medication state (n=5), fEP amplitudes increased to 120.5 +/- 12.7% of baseline, again similar to increases (132.6 ± 3.9 % of baseline) seen when HFS preceded LFS in the OFF medication state in the SNr.

![Graph](image)

**Figure 32 – LFS Preceding HFS.** When the order of the stimulation is reversed, LFS had no effect on measures of fEP amplitudes in the ON (open circles) or OFF (closed circles) medication state. When HFS followed LFS, fEP amplitudes increased by a magnitude similar to measures taken when HFS preceded LFS, in both the ON and OFF medication state (see Figure 26).
6.4 Discussion

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 D1R activation in the direct pathway and inhibitory inputs mediated by D2R activation in the indirect pathway (Albin et al., 1989; DeLong, 1990). However, dopamine can also have dramatic effects in other regions of the basal ganglia. For example, 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). Furthermore, we have previously found that application of the dopaminergic medication levodopa leads to potentiation of fEP amplitudes following HFS in the SNr of PD patients, whereas in the absence of dopaminergic medication, fEP amplitudes remained unchanged in the SNr following HFS (Prescott et al., 2009). This finding indicated that dopaminergic anti-parkinsonian drugs can mediate the induction / restoration of a form of synaptic plasticity in the SNr, one of the primary output nuclei of the basal ganglia.

Another form of synaptic plasticity, depotentiation, which is the reversal of previously established LTP by a low frequency stimulation protocol, has been found to be dependent on dopaminergic signaling in the basal ganglia, and to be selectively lost in an experimental model of L-Dopa-induced dyskinesia (Picconi et al., 2008, 2003). Here, we build on these findings, demonstrating for the first time that the dopaminergic
signalling and plasticity abnormalities observed in experimental models of L-Dopa-induced dyskinesia also appear to be present in basal ganglia output neurons of PD patients with dyskinesia.

We recorded fEPs from one electrode while stimulating with single pulses from a second electrode in both the SNr and GPi in the ON and OFF dopaminergic medications states. Potentiation was induced via the same HFS protocol previously shown capable of potentiating fEP amplitudes in the SNr of PD patients. Following HFS-mediated potentiation, depotentiation was tested with a LFS protocol. We found that in some patients on medication, HFS-mediated potentiation was reversed to baseline conditions following LFS, whereas in other patients on medication, some of whom developed mild dyskinesia during testing, HFS-mediated potentiation underwent much less or no change following LFS. Furthermore, we found a strong correlation between the depotentiation of fEP amplitudes following LFS and patients’ clinical dyskinesia scores; patients with less severe dyskinesia (lower scores of UPDRS items 32+33) underwent greater depotentiation following LFS than patients with more severe dyskinesia (higher scores of UPDRS items 32+33). This correlation between clinical scores and depotentiation suggests that the ability of basal ganglia output nuclei to undergo depotentiation might be selectively lost in patients who develop levodopa-induced dyskinesia. Such a dramatic alteration may have a profound effect on how the SNr and GPi effectively integrate and choose synaptic information to be relayed outside the basal ganglia. Dysregulated DA-dependent signalling and sustained potentiation of striatonigral / striatopallidal synapses could be causing abnormal gating of upstream motor commands, leading to an inability to suppress unwarranted, aimless movements.
It has been proposed that depotentiation erases non-essential information and normalizes synaptic efficiency in the process (Picconi et al., 2008, 2003), allowing synapses to “forget” irrelevant synaptic signals. If output nuclei synapses are congested with irrelevant signals, a lack of depotentiation may alter the capacity for experience-dependent modifications of action selection. Behaviourally, a loss of this ability to “forget” irrelevant synaptic signals may lead to the generation of aberrant motor patterns, such as dyskinesia (Calabresi et al., 2010, 2008).

What is particularly notable about this result is that it was found at GABAergic synapses of basal ganglia output nuclei. To our knowledge, studies of depotentiation have been conducted exclusively at glutamatergic synapses. The GPi and SNr are considered very similar in morphology and physiology; they both receive excitatory inputs from the STN and inhibitory inputs from the striatum, GPe, and local axon collaterals. The vast majority of the synapses at basal ganglia output neurons are GABAergic in nature (Ribak et al., 1981, 1979), with 70% of synaptic terminals in output nuclei arising from GABAergic striatal spiny neurons, and 15% of terminals arising from GABAergic afferents from GPe (Shink and Smith, 1995). Additionally, approximately 10% of inputs are glutamatergic afferents from the STN (Nambu, 2007), and both output nuclei also receive dopaminergic projections from the SNc (Smith and Kieval, 2000). However, as described previously, the fEP we use for measures of synaptic plasticity in the GPi and SNr appears to result primarily from the activation of the direct striatonigral/pallidal connection. Additionally, binding studies show a prevalence of D1 receptors in the primate SNr and GPi (Richfield et al., 1987), with electron microscopy
suggesting that these receptors are located on axons and terminals of direct striatal afferents in the output nuclei (Yung et al., 1995).

Previous studies have shown that enhanced D1R-mediated GABA release, which is what we believe potentiation of fEP amplitudes in the ON state is measuring, involves the cAMP/PKA pathway. D1-mediated enhancement of post synaptic currents in the SNr was found to be coupled to the formation of cAMP in the pre synaptic terminal (Jaber et al., 1996). cAMP formation leads to PKA activation, which itself then phosphorylates key targets involved with GABA exocytosis, such as P/Q-type voltage-activated Ca2+ channels (to enhance pre synaptic Ca2+ influx), synapsins (to enhance vesicle trafficking) and SNARE proteins (to enhance vesicle docking, priming, and fusion) (Arias-Montano et al., 2007). The presynaptic cAMP/PKA pathway has also been shown to increase recruitment of synaptic vesicles from the reserve pool, thereby increasing the size of the readily releasable pool of vesicles (Seino and Shibasaki, 2005). It is also possible that rapid postsynaptic changes in the GPi and SNr may also affect GABAergic activity. Neuronal activity has been shown to directly regulate the number of cell surface GABAARs 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 GABAAR insertion and subsequent post-synaptic accumulation has yet to be established.

Several signaling pathways, including the cAMP/PKA pathway, have been shown to be involved in depotentiation, albeit not previously at GABAergic synapses. To reverse LTP, phosphorylated targets need to be dephosphorylated by protein phosphatases, with the regulation of PKA signaling controlling phosphatase activity, and
antagonists of phosphatases capable of blocking depotentiation (Huang et al., 1999; Nicholls et al., 2008; Zhuo et al., 1999). If the presynaptic D1Rs at striatonigral and striatopallidal synapses have become sensitized in the dopamine depleted state, like their more prominent and often-studied nigrostriatal D1R cousins, it is possible that presynaptic PKA is abnormally active and causing a concomitant state of hyperphosphorylation of its target effector proteins, in turn suppressing phosphatase activity and blocking depotentiation in basal ganglia output nuclei of PD patients with LID. Hopefully, the findings detailed here show the need for further basic studies of the neuropharmacology of synaptic plasticity in GPi and SNr in slice, and that groups that specialize in such work will attempt to test this proposed mechanism.

One caveat is that the convergence of inputs in the SNr and GPi and the inability to see whether L-Dopa is directly acting at this level of the basal ganglia means that it is possible that the effects we are observing represent the indirect downstream effect of modulation occurring in the striatum or STN rather than representing a direct effect of L-Dopa on the output nuclei. In vivo primate recordings could offer the opportunity to simultaneously monitor activity and measure changes in plasticity in multiple basal ganglia structures at the same time. It seems likely that changes will be occurring wherever dopamine receptors are normally active in the basal ganglia.

The results of this study reaffirm 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. Furthermore, we show for the first time in human patients that abnormalities in plasticity at basal ganglia output nuclei are associated with L-Dopa-induced dyskinesia. Particularly, we found that dyskinesia was associated with changes
in depotentiation, or the reversibility of previously potentiation, corroborating the findings from animal models of PD and L-Dopa induced dyskinesia.

Chapter 7 – General Summary and Significance

The results of the studies presented in this thesis suggest that abnormal plasticity in the basal ganglia output nuclei, the GPi and SNr, plays an important role in movement disorders such as PD and dystonia. Altered long-lasting changes in synaptic transmission may represent an important mechanism by which basal ganglia functions are impaired in movement disorders.

We measured changes in synaptic transmission using a fEP, and it appears that the changes we are measuring via the fEP are the result of primarily striatopallidal and striatonigral activation. Further, the fEP itself has proven itself to be an effective clinical tool in the operating room; its presence further confirms the identification of subcortical structures like the SNr and GPi, particularly useful in confirming the boundary between the SNr and STN and optimal DBS electrode placement. Additionally, absence of the fEP may reveal functional deafferentiation, as in the case of striatal lesion.

Using fEP measures, we have previously shown that a form of activity-dependent synaptic plasticity was measurable in the SNr of PD patients and that this plasticity was sensitive to low doses of L-dopa. Here we show, to our knowledge, the first demonstration of activity-dependent synaptic plasticity in human GPi, the other basal ganglia output nucleus. Furthermore, we demonstrate a difference in the amount of activity-dependent synaptic plasticity inducible across the dorsoventral extent of GPi in dystonia patients, with ventral sites undergoing much less change than dorsal sites.
This lack of plasticity at inhibitory synapses in ventral GPi could be an inhibitory deficit contributing to exaggerated excitation and plasticity in cortical circuits in dystonia.

Using paired stimulation and fEP measures of changes in paired pulse depression, we showed that the probability of GABA release from the terminals of medium spiny striatal neurons in the GPi and SNr is regulated by the presence of dopamine, and is impaired in dystonia patients. Further, we demonstrated that in both generalized and cervical dystonia patients, the paired pulse ratio at low interstimulus intervals correlates with motor dysfunction. Notably, these results were consistent across a heterogeneous group of generalized and cervical dystonia patients, possibly suggesting an underlying neuronal dysfunction causing an impaired inhibitory neurotransmitter release, common to multiple forms of dystonia.

Finally, the demonstration of the lack of depotentiation in both basal ganglia output nuclei in PD patients with dyskinesia is an important validation of animal models of levodopa-induced dyskinesia. This is an important and relevant finding for the design of novel anti-dyskinetic therapies. Further, these results were found at inhibitory GABAergic synapses and found during in vivo recording in patients, both firsts in the study of depotentiation to our knowledge. The ability of a synapse to reverse previous potentiation may be crucial to prevent saturation of the storage capacity of motor information. Loss of this ability at the output nuclei would lead to the basal ganglia retaining unessential information that is normally erased, and may underlie, or at the very least contribute to the cellular basis for dyskinetic movements.
Questions regarding basal ganglia plasticity and neuronal properties are traditionally explored with the use of animal models because of the experimental difficulties investigating these processes in human subjects. However, due to known limitations in correspondence between animal models and human clinical phenotypes (Wichmann, 2008), studies such as the current one, while limited in their own sense, most notably by lack of healthy controls, provide a direct window into the pathophysiology of these deep brain structures in the diseased state. These experiments assessing plasticity at the basal ganglia output are yielding critical new insight into the pathophysiology of normal and abnormal movements and may impact our understanding of the mechanisms underlying the effectiveness of deep brain stimulation in movement disorders, and on the design of new drug therapies.
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