OSCILLATORY ACTIVITY IN THE BASAL GANGLIA OF PATIENTS WITH PARKINSON’S DISEASE

by

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A thesis submitted in conformity with the requirements for the Degree of Doctor of Philosophy
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ABSTRACT

Parkinson’s disease (PD) is a movement disorder that is of basal ganglia origin. It is characterized by a severe loss of dopaminergic input to the striatum and symptoms such as bradykinesia, rigidity and tremor. There is growing evidence that PD is associated with pathological synchronous oscillatory activity in the basal ganglia, which primarily occurs in the 11-30 Hz range, the so-called beta band. The aim of this project was to better understand the oscillatory activity recorded from the basal ganglia of PD patients and to elucidate the significance of this activity in PD. To do this, neuronal firing and local field potentials (LFPs) were recorded from the subthalamic nucleus (STN) and globus pallidus internus (GPi) of PD patients undergoing stereotactic neurosurgery for implantation of therapeutic deep brain stimulation electrodes. Beta oscillatory LFP activity in the STN and GPi was found to be coherent with, and reflect to a certain degree, rhythmic activity in a population of local neurons. I have demonstrated for the first time that the degree of beta oscillatory firing in the STN, which is maximal in the motor portion, correlates with the patients’ benefit from dopaminergic medications, but not with baseline motor deficits. My study has also established that beta oscillatory firing in the STN does not positively correlate with the patients’ tremor scores and that during periods of tremor patients tend to have less beta oscillatory firing and increased neuronal oscillatory firing at the tremor frequency. Temporal examination of the LFPs recorded during periods of intermittent resting tremor revealed that stronger tremor is associated with increased LFP power in the low gamma range (35-55 Hz) and there is a decrease in the ratio of beta to gamma coherence. Similarly, a change in balance between oscillatory activities was observed during levodopa-induced dyskinesias. Finally, when the oscillatory activity in the GPi of PD patients was compared to that in dystonia I found that in dystonia, oscillatory LFP activity is less likely to reflect the neuronal firing. These findings indicate that beta oscillatory activity in the basal ganglia might reflect the degree of dopamine deficiency in
the striatum and that the relative strength of oscillatory rhythms may play an important role in mediating the pathological features in PD.
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<td>Toronto Western Spasmodic Torticollis Rating Scale</td>
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<td>TMS</td>
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<tr>
<td>Ventral tegmenta area</td>
<td>VTA</td>
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<tr>
<td>Unified Parkinson’s Disease Rating Scale</td>
<td>UPDRS</td>
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CHAPTER 1 – GENERAL INTRODUCTION

The main focus of the work presented in this thesis is a study of the oscillatory activity recorded with microelectrodes from the basal ganglia in patients with Parkinson’s disease (PD) and its significance in mediating the motor symptoms of the disease. The data is mostly descriptive, aiming to characterize the nature of synchronized oscillations in PD and to relate it to some clinical aspects. The data from microelectrode recordings in human enable to examine both cell firing and local field potentials (LFPs) and can contributes critical new insight into this area of research, which is almost entirely derived from LFP recordings taken from the much larger contacts used for deep brain stimulation.

The general introduction, which follows, is intended to provide an extensive literature overview of Parkinson’s disease (including etiology, clinical features and treatments) and the anatomy and physiology of the basal ganglia. Some models of the basal ganglia function in relation of PD will be presented, followed by an overview of electrophysiological and behavioral findings in PD patients and animal models of PD. The specific aims of the studies presented in the thesis will be described at the end of this chapter.

Chapter 2 provides a detailed description of the methods used in this research work. Each study is then presented in a separate chapter:

Chapter 3: Beta oscillatory activity in the subthalamic nucleus and its relation to dopaminergic response.
Chapter 4: The relationship between subthalamic oscillatory activity and tremor.
Chapter 5: Oscillatory activity in the globus pallidus: comparison between Parkinson’s disease and dystonia.

A general discussion on the results and their significance is given in Chapter 6.

An additional study describing the physiological properties of the pedunculopontine nucleus (as well as a literature review about this structure) is included in the Appendix.
1.1 - Parkinson’s disease

Parkinson’s disease is a progressive age-related neurodegenerative movement disorder that was first described by James Parkinson in 1817. The pathologic hallmark of the disease is progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) that project to the striatum (Hornykiewicz, 1966). The dopaminergic loss in the striatum leads to alterations in the activity of the neural circuits within the basal ganglia that regulate movement (Lang and Lozano, 1998) resulting in a difficulty to perform both voluntary and involuntary movements. Parkinson’s disease also involves non-motor symptoms such as depression, dementia and sleep disturbances (Ziemssen and Reichmann, 2007; Poewe, 2008) and is therefore associated with significant disability and substantially decreased quality of life.

PD is the second most common neurodegenerative disorder after Alzheimer’s disease (Lew, 2007) affecting approximately 0.3% of the world’s general population and 3% of the people over the age 65 (Zhang and Roman, 1993). In North America alone, over one million people have been diagnosed with PD (Lang and Lozano, 1998). Prevalence of PD increases with age. The mean age of onset is approximately 60 years of age although 5 to 10% of patients are diagnosed before the age of 40 and are considered to have “young-onset Parkinson’s disease” (Bennett et al., 1996; Lang and Lozano, 1998). The mortality among affected individuals is two to five times higher than among aged matched controls (Louis et al., 1997).

1.1.1 - Etiology of Parkinson’s disease

A severe loss (~90%) of dopamine-secreting neurons in the SNc of the basal ganglia is the pathological hallmark of PD (Hornykiewicz and Kish, 1987; Hornykiewicz, 1966). Noradrenergic, serotonergic and cholinergic neuron loss also occurs in other brain structures (Jellinger, 1991). The exact cause of neural degeneration remains unclear, but may be due to a combination of factors including excitotoxic cell death (Przedborski and Jackson-Lewis, 1998) genetics (Gasser, 2007) and environmental factors (Priyadarshi et
Lewy bodies
Lewy body formation is suggested to be pathogenic in PD. Lewy bodies are eosinophilic hyaline inclusions that are consistently found in vulnerable neuronal populations in parkinsonian brains (Lang and Lozano, 1998). In particular, they can be found in the brain stem, basal forebrain and cortex. Lewy bodies are not specific to Parkinson's disease and are found in other neurodegenerative disorders (McKeith, 2000) as well as in elderly people without clinically evident PD (Gibb and Lees, 1988), suggesting they are unrelated to the symptoms of PD.

Mitochondrial failure and oxidative stress
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent neurotoxin with selective effects on nigral dopaminergic neurons (Marsden and Jenner, 1987). In the early 1980’s, severe parkinsonism developed in some subjects exposed to MPTP (Langston et al., 1983). As a lipophilic compound, MPTP can cross the blood brain barrier. Once in the brain, MPTP is converted within serotonergic neurons and astrocytes by monoamine oxidase into the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) (Chiba et al., 1984; Brooks et al., 1989). Thereafter MPP+ is released into the extracellular space and is then taken up into dopaminergic neurons by the plasma membrane dopamine transporter (Mayer et al., 1986). Subsequently, MPP+ rapidly accumulates in the mitochondrial matrix and inhibits Complex 1 of the electron transport chain, leading to energy failure and cell death (Chan et al., 1991). In addition, the energy failure of the cell further promotes other toxic and genetic insults, thereby increasing its susceptibility to apoptosis (Rossetti et al., 1988). MPTP is widely used today to reproduce parkinsonian symptoms in nonhuman primate models of PD.

It has been suggested that in Parkinson's disease, there is an excess of reactive oxygen species and increased oxidative stress (Lang and Lozano, 1998). Elevation of iron levels detected in the SNc of PD patients is believed to be an important factor in causing oxidative stress (Hirsch and Faucheux, 1998).
Excitotoxic cell death

Excitotoxic mechanisms are implicated to play a role in neuronal degeneration in the SNc. Over-excitation of the glutamatergic N-methyl-d-aspartate (NMDA) receptor leads to an increase in intracellular calcium levels that could potentially lead to mitochondrial damage and cell death (Nicholls and Budd, 1998). The subthalamic nucleus (STN), which sends glutamatergic projections to the SNc, is known to be hyperactive in PD. Therefore, the excessive glutamatergic drive from STN could be the source of excitotoxic death of dopaminergic neurons in SNc (Lang and Lozano, 1998).

Genetics

There is increasing evidence that genetic factors have an important role in Parkinson's disease. Studies of families with inherited forms of PD suggest a few genes that are associated with the disease. Mutations in the gene alpha-synuclein in families with a rare autosomal inherited form of dominant PD lead to aggregation of alpha-synuclein protein, a major constituent of Lewy body fibrils (Wakabayashi et al., 2007). Another cause for dominant PD is mutations in the gene for LRRK2 that might be acting as a primer for neurodegeneration (Singleton, 2005). Mutations in the parkin gene, DJ-1 and PINK1 all cause autosomal recessive parkinsonism of early onset. Parkin, DJ-1 and PINK1 proteins protect against mitochondrial damage and oxidative stress. Loss of function mutations in one of those genes render dopamine neurons more susceptible to mitochondrial dysfunction and oxidative stress (Dodson and Guo, 2007; Gasser, 2007). It should be noted, however, that known genetic forms of PD can account for only a small percentage of all cases of sporadic PD, suggesting further genetic etiology awaits discovery (Marras and Lang, 2008).

Epidemiology

The discovery of the selective ability of various exogenous toxins (e.g. MPTP) to induce nigral cell death attracted great interest in potential environmental factors capable of causing Parkinson's disease. Epidemiological studies have reported that people living in a rural area are at significantly increased risk of getting PD. This might be related to farming and the exposure to potential neurotoxins present in pesticides (Priyadarshi et al.,
1.1.2 - The clinical features of Parkinson’s disease

The cardinal motor symptoms of PD include bradykinesia (a reduction in amplitude and velocity of voluntary movement), often with akinesia (initiation difficulty and absence of spontaneous movement), rigidity (increased resistance to passive movement), tremor at rest and postural instability (Jankovic, 2008). PD symptoms worsen with time (Calne and Langston, 1983; Koller and Montgomery, 1997) with one side of the body initially affected and bilateral involvement as the disease progresses (Poewe and Wenning, 1998). The initial diagnosis is made on the basis of the presence and asymmetry of these symptoms and a good response to levodopa. Levodopa (also called L-Dopa) is an oral dopamine precursor and is currently the most effective therapy in the management of PD (Lang and Lozano, 1998). A number of rating scales are used for the evaluation of impairment and disability in patients with PD although the most well established is the Unified Parkinson’s Disease Rating scale (UPDRS) (Ramaker et al., 2002). The UPDRS has four components: part I, mentation, behavior and mood; part II, activities of daily living; part III, motor; part IV, therapy complications.

Investigation of the clinical symptoms expressed by PD patients is important because it provides valuable clues regarding the physiology and pathophysiology of the brain regions involved, thereby helping in the design of care for PD patients today and leading to new directions in future research.

1.1.2.a - Akinesia and bradykinesia

Akinesia refers to the lack or paucity of spontaneous movement whereas bradykinesia refers to slowness of movement. It has been proposed that akinesia might result from an inability to integrate cognitive and motor processes (Brown and Jahanshahi, 1996) while bradykinesia results from an inability to generate the appropriate amount of muscle activity (to provide enough force) as measured with electromyography (EMG) (Hallett and Khoshbin, 1980). Because patients with PD have decreased electromyographic
activity (Berardelli et al., 2001), they need a series of multiple agonist bursts to accomplish larger movements. Bradykinesia can be also measured by testing reaction time or movement speed. Reaction times are significantly delayed in PD patients (Heilman et al., 1976; Evarts et al., 1981) and are even slower with increasing task complexity (Kutukcu et al., 1999). Similarly, increasing task complexity slows movement speed and increases the length of pauses between the elements in sequential task (Benecke et al., 1986; Benecke et al., 1987).

Bradykinesia is considered the most characteristic clinical feature of PD (Marsden, 1984) and it appears to correlate best with the degree of dopamine deficiency in the striatum (Vingerhoets et al., 1997). It has been hypothesized that bradykinesia results from altered motor cortex activity that is mediated by dopamine depletion. Reduced firing rates of cortical neurons following dopamine receptor blockade in the rat have been shown to correlate with bradykinesia (Parr-Brownlie and Hyland, 2005). Functional neuroimaging studies also suggest impairment in the recruitment of cortical and subcortical structures that regulate kinematic movement parameters such as velocity (Turner et al., 2003). The deficit appears to be localized in the putamen and globus pallidus (Lozza et al., 2002), resulting in the reduction in the muscle force produced at the initiation of movement.

Interestingly, bradykinesia is dependent on the cognitive state of the patient. A patient who becomes excited, for example, may be able to make quick movements such as catching a ball or may be able to suddenly run in case of a danger. This phenomenon (kinesia paradoxica) suggests that PD patients have intact motor programs but have difficulties accessing them without an external trigger, such as a loud noise or a visual cue (Jankovic, 2008).

1.1.2.b - Rigidity
Rigidity in PD manifests as an increase in resistance of a joint to passive movement. The subjective correlate of rigidity is a feeling of stiffness and a reduced ability to relax limb muscles (Klockgether, 2004). Rigidity is the consequence of an enhanced response of muscles to stretch (Lee, 1989). However, it cannot be explained by an exaggeration of the
short-latency spinal component of the stretch reflex, which is mediated by a monosynaptic spinal reflex (Klockgether, 2004). Since surgical interventions, such as lesion or deep brain stimulation, on central structures (e.g. basal ganglia) improve rigidity (Krack et al., 2003; Fine et al., 2000; Lang et al., 1999), it is likely that central mechanisms are involved. Indeed, the stretch reflex has a long-latency component which, at least in part, represents the output of a transcortical reflex loop (Marsden et al., 1983; Deuschl and Lucking, 1990). Several studies have shown that the amplitude and duration of the long-latency response of the stretch reflex are increased in PD patients (Lee and Tatton, 1975; Tatton and Lee, 1975; Cody et al., 1986; Rothwell et al., 1983; Berardelli et al., 1983). Since the cerebral cortex is not primarily affected in PD, it is plausible that the over-excitability of the transcortical reflex loop is due to an abnormal output of the basal ganglia (Klockgether, 2004). This view is supported by the fact that the long-latency response of the stretch reflex is also abnormal in another disorder affecting the basal ganglia (e.g. Huntington’s disease) (Noth et al., 1985).

Another mechanism involved in rigidity is an abnormal response to the shortening in the antagonist muscle. In normal subjects, antagonist muscle activity is suppressed during stretching. In PD patients, an exaggerated EMG response can be recorded in antagonist muscles resulting in an almost constant joint torque independent of joint position (Xia and Rymer, 2004). All these mechanisms appear to be under the control of the dopaminergic system, as dopamine replacement medications effectively reduce rigidity and normalize the abnormal stretch responses (Klockgether, 2004).

1.1.2.c - Tremor

Tremor is the most easily recognized symptom of PD. It occurs in approximately 75% of patients with PD (Hughes et al., 1993), typically during rest (Deuschl et al., 1998). Parkinson’s tremor is defined as 4-6 Hz rhythmic activation of antagonist muscles, usually in the distal portions of limbs (Shahani and Young, 1976). Resting tremor is diminished during active movements of the limb and may be enhanced during periods of mental stress (Deuschl et al., 1998). Unlike rigidity and bradykinesia, tremor does not necessarily get worse with disease progression and the severity of tremor does not
correlate with dopamine deficiency in the striatum (Stebbins et al., 1999; Deuschl et al., 2000). Post-mortem and imaging studies suggest that the pathophysiology of rest tremor may be distinct from that of rigidity and bradykinesia (Jellinger, 1999; Pavese et al., 2006). A more detailed review on the pathophysiology of resting tremor in PD will be given in sections 1.3.4 and 1.4.5.

Low amplitude 4-10 Hz postural and action tremors are also expressed in some PD patients (Findley et al., 1981; Hadar and Rose, 1993; Palmer and Hutton, 1995). Postural tremor appears when patients hold a posture while action tremor is expressed during voluntary movement of the affected limb (Findley et al., 1981).

1.1.2.d - Postural instability
Postural instability is generally a manifestation of the late stages of PD (Jankovic, 2008) and can become the most debilitating symptom to a patient’s quality of life (Bloem, 1992). Postural instability is one of the most common causes of falls and contributes significantly to the risk of hip fractures (Williams et al., 2006). The balance impairment is due to abnormal modulation of postural reflexes in the lower extremities (Beckley et al., 1991). Although the timing of associated postural adjustments is normal in PD, their size may decrease (Dick et al., 1986) due to inability to modify the size of posturally stabilizing long-latency reflexes (Beckley et al., 1993). Postural instability may also be due to an increase in muscle tone and tremor in the lower extremities (Burleigh et al., 1995), orthostatic hypotension (a sudden fall in blood pressure) and age related sensory changes (Bloem, 1992).

1.1.2.e - Other motor abnormalities
Patients with PD may exhibit a number of secondary motor symptoms that may impact on their functioning (Jankovic, 2008). Some patients display a re-emergence of primitive reflexes such as the glabellar reflex (subjects blink in response to repetitive tapping on their forehead) due to a breakdown of the frontal lobe inhibitory control (Vreeling et al., 1993; Thomas, 1994). Speech disorders are also frequently observed in PD patients and are characterized by monotonous, soft and breathy speech with variable rate and word
finding difficulties (Critchley, 1981). A number of neuro-ophthalmological abnormalities may be seen in patients with PD including decreased blink rate, ocular surface irritation, altered tear film, visual hallucinations and decreased convergence (Bioussé et al., 2004). In addition, abnormalities in ocular pursuit and saccades (including antisaccades) are also observed (Rascol et al., 1989). PD patients may also suffer from respiratory disturbances. Upper airway obstruction and chest wall restriction are both common (Shill and Stacy, 2002; Sabate et al., 1996) and are associated with significant morbidity and mortality. The obstructive pattern may be related to rigidity, cervical arthrosis or restricted range of motion in the neck, and the restrictive pattern may be related to chest wall rigidity (Shill and Stacy, 1998).

1.1.2.f - Non-motor features

PD patients also suffer from non-motor symptoms which impair their quality of life quite considerably. Non-motor symptoms consist of autonomic dysfunction, cognitive/neurobehavioral disorders, sleep disturbances and sensory abnormalities (Ziemssen and Reichmann, 2007; Poewe, 2008). Autonomic failures include cardiovascular dysfunction (e.g. orthostatic hypotension), as well as gastrointestinal, urogenital and thermoregulatory dysfunctions (Jankovic, 2008).

Greater than 80% of patients at 15 years after their initial assessment are reported to show cognitive decline and ~50% meet the diagnostic criteria for dementia (Hely et al., 2005). PD related dementia is also associated with a number of other neuropsychiatric conditions. Among patients with dementia, nearly 60% suffer from depression, and many suffer from apathy (54%), anxiety (49%) or hallucinations (44%) (Aarsland et al., 2007). The prevalence rates of depression in patients with PD have been reported to be as high as 40% (Cummings, 1992; Cummings and Masterman, 1999). Depression is predominantly caused by fronto-cortical dysfunction and by degeneration of monoaminergic neurotransmitter systems (Ziemssen and Reichmann, 2007). Cognitive impairment in PD might also result from the dysfunction of non-dopaminergic neuronal systems (Pillon et al., 1989).
Sleep disturbances such as insomnia, daytime sleepiness and rapid eye movement sleep behavior disorder (RBD) are very frequent in PD (Comella, 2003). RBD occurs in approximately one-third of PD patients and is characterized by an increase in violent dream content accompanied by dramatic, violent and potentially injurious motor activities (e.g. yelling, swearing, grabbing, punching, kicking, jumping and other) which may also involve the bed partner (Gagnon et al., 2006). Insomnia and daytime sleepiness are also frequent with about 50% prevalence (Gjerstad et al., 2007; Arnulf et al., 2002). The sleep abnormalities observed in PD patients are possibly the result of a severe loss of hypocretin (orexin) neurons (Fronczek et al., 2007; Thannickal et al., 2007).

Sensory symptoms such as olfactory dysfunction (Doty et al., 1992) and pain (Djaldetti et al., 2004; Tinazzi et al., 2006) are also frequent but are often not recognized as PD symptoms (Jankovic, 2008).

1.1.3 - Dopamine therapy in Parkinson’s disease

As mentioned above, by the time PD is diagnosed most (~90%) of the dopaminergic SNc neurons have degenerated (Hornykiewicz, 1966; Hornykiewicz and Kish, 1987). Positron emission tomography (PET) with the levodopa analogue 6-fluorodopa has demonstrated that in humans exposed to MPTP, dopaminergic loss can exist in the absence of clinical signs (Calne et al., 1985). The late appearance of the clinical symptoms is due to compensatory mechanisms (Bezard et al., 1997b). Previous to the appearance of symptoms, remaining SNc neurons produce larger amounts of dopamine and striatal neurons become more sensitive to dopamine. There is also increased glutamatergic inputs to the SNc during the presymptomatic period (Bezard et al., 1997b; Bezard et al., 1997a; Bezard et al., 1999). When enough SNc neurons degenerate, compensatory mechanisms can no longer balance the loss of dopamine and PD symptoms appear (Bezard and Gross, 1998).

The dopamine precursor levodopa was discovered in the 1960’s (Cotzias et al., 1967) and is the most potent drug for controlling PD symptoms. When administered orally,
levodopa is absorbed mainly in the duodenum and the proximal bowel. Unlike dopamine, levodopa is able to cross the blood brain barrier. Following absorption into brain, levodopa is taken up by residual dopaminergic neurons in striatal terminals and is converted into dopamine by the enzyme aromatic L-amino-acid decarboxylase dopamine. Dopamine is then stored into vesicles of the presynaptic neuron from which it is released into the synaptic cleft to stimulate dopamine receptors on post-synaptic striatal cells (Thanvi and Lo, 2004). At the pre-synaptic level, dopamine acts to increase dopamine synthesis and transmission. Early studies suggested that the relatively intact serotonergic input to the basal ganglia from the dorsal raphe nucleus may help conversion of levodopa to dopamine (Tanaka et al., 1999).

Metabolism of dopamine involves its reuptake by the presynaptic neuron and the enzymatic conversion to 3, 4, dihydroxyphenyl acetic acid by monoamine oxidase inhibitor and to 3-methoxytyramine by the enzyme catecholamine-o-methyl transferase. Levodopa can be broken down in the periphery as well. Thus, it is routinely administered in combination with peripheral decarboxylase inhibitor (such as benserazide or carbidopa) to reduce its conversion to dopamine at the periphery (and associated side effects such as nausea and vomiting) and to increase dopamine availability in the central nervous system.

Levodopa significantly improves bradykinesia and akinesia. PET studies have demonstrated that the degree of nigrostriatal dopamine deficiency in PD correlates most with bradykinesia compared to other symptoms (Vingerhoets et al., 1997). Levodopa also improves rigidity, tremor (Yuill, 1976), hypometria (small amplitude movements) (Beckley et al., 1995), the performance of complex tasks (Benecke et al., 1987), and the generation of internally cued movements (Burleigh-Jacobs et al., 1997). However, not all symptoms of PD are responsive to dopaminergic medication. For instance, dopaminergic medications have no significant effect on postural instability, suggesting that nondopaminergic mechanisms are involved in this symptom (Bloem et al., 1996).

Unfortunately, patients with longstanding levodopa treatment often develop motor fluctuations, where the effects of levodopa switch rapidly and unpredictably from no
effect ("off"), to a positive effect ("on"), to uncontrollable involuntary movements (dyskinesias) (Obeso et al., 2000a; Obeso et al., 2000b; Thanvi and Lo, 2004). Levodopa-induced dyskinesias are observed in the majority of patients who have been treated for 5–10 years with levodopa (Schrag and Quinn, 2000). These medication-related motor fluctuations are difficult to treat and become a major contributor to disability in some patients. The neurological basis of levodopa-induced dyskinesias is unclear but might be related to the discontinuous stimulation of dopamine receptors that accompanies intermittent levodopa dosing, leading to dopamine receptor hypersensitivity (Chase, 1998; Bezard et al., 2001). A recent PET study found reduced dopamine transporter expression on presynaptic sites in PD patients with dyskinesias (Troiano et al., 2009). Decrease in metabolism and/or decrease in the mean firing rate of basal ganglia neurons might also be involved in levodopa-induced dyskinesias as well as overactivity of cortical motor areas (Bezard et al., 2001). An interesting fact is that the emergence of dyskinesias in PD patients correlates with the levodopa-induced reduction in bradykinesia, but not rigidity (Caligiuri and Peterson, 1993).

One strategy to reduce the risk for motor complications is the use of dopamine receptor agonists which have been proved safe and effective as initial therapy in early stages of Parkinson's disease. However, it is still controversial whether they must be started early, as opposed to initiation only after the levodopa complications develop (Ahlskog, 2003). Dopamine agonists such as bromocriptine, pergolide and apomorphine bind at postsynaptic receptor sites independently of the dopamine terminal. They have a longer half life than levodopa (half life of levodopa is approximately 90 minutes), thereby reducing receptor sensitivity and the development of motor complications (Junghanns et al., 2004). Unfortunately, dopamine agonists have adverse effects on their own including nausea, hypotension, hallucinations, daytime sleepiness, pathological gambling, cardiac valvulopathy and edema.

1.1.4 - Deep brain stimulation for Parkinson’s disease

Stereotactic neurosurgery to treat PD in humans started with the initial work of Cooper
who showed that chemical lesions of the pallidum in patients with PD could effectively treat parkinsonian symptoms (COOPER, 1954). Starting in the 1950s, thalamotomy (surgical destruction of the motor thalamus) and pallidotomy (surgical destruction of the internal segment of the globus pallidus) were regularly performed to alleviate the symptoms of PD. In these procedures, an electrode is placed in the target area of the brain, using stereotactic neurosurgical techniques, and the exposed electrode tip is heated with radiofrequency current to create the lesion. In 1969, with the introduction of levodopa, the number of lesioning operations declined dramatically (Speelman and Bosch, 1998; Wichmann and DeLong, 2006b). But in the mid 1970’s, as a result of the shortcomings of the levodopa therapy in the long-term treatment, thalamotomy gradually regained its place (Siegfried, 1980). Later in the early 1990’s, with the better understanding of the important role of the globus pallidus and the subthalamic nucleus in the organization of normal motor control and PD (Alexander et al., 1990), there was a resurgence of pallidotomy, which was also effective in alleviating levodopa-induced dyskinesias (Laitinen et al., 1992a; Laitinen et al., 1992b; Lozano et al., 1995; Baron et al., 1996; Kishore et al., 1997; Narabayashi et al., 1997).

The modern era of deep brain stimulation (DBS) to treat PD symptoms began in 1987 when Benabid and colleagues reported their experience with high-frequency (>100 Hz) stimulation in the ventralis intermedius (Vim) nucleus of the thalamus for treating PD tremor (Benabid et al., 1991; Benabid et al., 1987). Subsequently, DBS was applied to basal ganglia structures, namely the internal segment of the globus pallidus (GPI) and the subthalamic nucleus (STN), to treat all symptoms of PD (Guridi et al., 1993; Siegfried and Lippitz, 1994; Limousin et al., 1995; Obeso et al., 1997). This procedure involves implantation of an electrode into the target region using stereotactic neurosurgical techniques (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 using noninvasive radio-telemetry to achieve maximal clinical benefit. The clear advantage of DBS over lesioning is that there is minimal destruction of brain tissue and the electrode can be potentially removed without creating permanent damage (Lozano and Mahant, 2004).
DBS is recommended to PD patients who are levodopa-responsive but suffer from levodopa-related complications and motor fluctuations that cause significant disability (Lozano and Mahant, 2004). The choice of target is largely dependent on the neurosurgeon. Although thalamic stimulation has been well established as an effective treatment for PD tremor (Benabid et al., 1996), it is rarely indicated since it does not alleviate the other major features of PD (Lozano, 2000). Stimulation of the GPi or STN, on the other hand, have been shown to exert beneficial effects for most of the main symptoms of PD (Pahwa et al., 1997; Gross et al., 1997; Tronnier et al., 1997; Krack et al., 1997; Ghika et al., 1998; Krack et al., 1998; Volkmann et al., 1998; Kumar et al., 1998; Limousin et al., 1998; Kumar et al., 1999c; Moro et al., 1999). The most remarkable effect of both G Pi and STN surgery is a marked reduction in levodopa-induced dyskinesias. While G Pi stimulation directly suppresses levodopa-induced dyskinesias, STN DBS allows patients to reduce their levodopa intake, leading to a reduction in dyskinesias (Ghika et al., 1998; Krack et al., 1998; Moro et al., 1999). Since STN allows the reduction of anti-parkinsonian medication, most centers prefer the STN to G Pi surgery (Lozano and Mahant, 2004). G Pi, on the other hand, has became the favored target in the treatment of patients with dystonia (Kumar et al., 1999b; Yianni et al., 2003; Vidailhet et al., 2005), a syndrome characterized by sustained concurrent contractions of the agonist and antagonist muscles, thereby producing abnormal, and sometimes painful, movements or postures. It includes a vast array of diseases with different etiologies and presentations.

Nowadays, DBS in different brain regions is remarkably effective in some brain areas (i.e. STN, thalamus and G Pi) to treat a range of neurological disorders, and the efficacy for neuropsychiatric disease is awaiting blinded trials. It should be emphasized, however, that despite the therapeutic benefits, the mechanisms of action of DBS are still unclear. Whether the therapeutic effects are local or system-wide or whether the effects are related to inhibition or excitation are still a matter of debate. Differences in techniques, anatomy, cell type, and experimental setting limit the ability to make direct comparisons across the different studies (Perlmutter and Mink, 2006). Multiple reports have documented the
modulation of both discharge rates and firing patterns during and following high-frequency stimulation throughout the cortico-basal ganglia loop. Early studies reported inhibition of neuronal activity within the stimulated nucleus during high-frequency stimulation in the GPi (Dostrovsky et al., 2000) and STN (Filali et al., 2004) of humans, and GPi in primates (Boraud et al., 1996). Although these findings are in line with the similar clinical effects of lesions and DBS, there are some evidence for only minor effects of DBS on mean firing rates (McCairn and Turner, 2009). In studies that have examined the effect of high-frequency stimulation on downstream targets, the findings suggest an activation of efferent axons either directly or through activation of local cell bodies to axon initial segments (Anderson et al., 2003; Hashimoto et al., 2003). Continuous high-frequency stimulation in the STN (Hashimoto et al., 2003) or pallidum (Bar-Gad et al., 2004; McCairn and Turner, 2009) resulted in time-locked changes in firing in the primate globus pallidus. Time-locked changes in firing were also found in the STN following cortical (Nambu et al., 2000) and pallidal (Nambu et al., 2000; Kita et al., 2005) stimulation, and in the thalamus following STN stimulation (Xu et al., 2008). High-frequency stimulation was also shown to affect other properties of firing patterns such as oscillatory and burst activity (Brown et al., 2004; Wingeier et al., 2006; Meissner et al., 2005; Kuhn et al., 2008; Montgomery, 2006; Hahn et al., 2008; Dorval et al., 2008; Xu et al., 2008; McCairn and Turner, 2009) suggesting that it suppresses the pathological patterns of activity in the basal ganglia outputs. A recent study in MPTP-treated monkeys has suggested that short-term depression of synaptic transmission may contribute to the mechanism underlying the effects of high-frequency stimulation (Erez et al., 2009). Importantly, cortical high-frequency stimulation may also have antiparkinsonian effects as shown in MPTP monkeys (Drouot et al., 2004).

1.2 - The basal ganglia: anatomy and physiology

The basal ganglia (BG) are a group of subcortical nuclei that are involved in motor and cognitive functions. The principle components of the BG are the striatum, the STN, the globus pallidus (GP) and the substantia nigra (SN). The striatum is the main input structure and is divided into the caudate and putamen which are separated by the internal
capsule. The STN can also be considered an input nucleus because, like the striatum, it receives direct input from the cerebral cortex (Kitai and Deniau, 1981; Monakow et al., 1978). The GP is divided by the internal medullary lamina into the internal segment (GPi) and external segment (GPe). The two divisions of the GP have different inputs and outputs and are functionally distinct. Similarly, the SN is divided into the pars compacta (SNc) and pars reticulata (SNr). These two parts of the substantia nigra share similar inputs but have different outputs and are composed of neurochemically distinct neuron types. In post-mortem tissue the SNc is defined by the black staining of neuromelanin which is present in dopaminergic neurons. The GPi and SNr are the major output nuclei of the BG through which the BG project to the thalamus, the superior colliculus and the pedunculopontine nucleus (PPN) (Tepper et al., 2007; Herrero et al., 2002). There are no direct outputs from the basal ganglia to spinal or brainstem motor neurons.

Through the thalamus, the BG influence motor, sensory, and cognitive cortical information processing (Middleton and Strick, 1994; Middleton and Strick, 1996; Middleton and Strick, 2002; Hoover and Strick, 1993). The BG also influence movements of the head and eyes through the superior colliculus (Hikosaka et al., 2000), and influence spinal cord processing and aspects of locomotion and postural control through the PPN (Takakusaki et al., 2004; Garcia-Rill et al., 1983).

Basal ganglia dysfunction is associated with a range of debilitating clinical conditions whose most obvious manifestations are disturbances in movement. These movement abnormalities can be found not only in well defined movement disorders such as Parkinson’s disease, Huntington’s disease and dystonia but also in diseases such as obsessive-compulsive disorder, schizophrenia and various addictive behaviors. Many of these disorders, including Parkinson's disease, have associated cognitive and affective components as well.

1.2.1 - Functional organization and circuitry

1.2.1.a - Connectional architecture
The vast majority of neurons in the BG release gamma-aminobutyric acid (GABA) and most are projection neurons. The striatum, both segments of the GP and the SNr are each composed of GABAergic projection neurons, whereas the STN contains glutamatergic projection neurons and the SNC contains mainly dopaminergic projection neurons. The striatum also contains clearly defined populations of interneurons, all but one (the cholinergic interneurons) of which are GABAergic (Tepper et al., 2007).

The principle pathways of information flow throughout the basal ganglia and their associated regions are illustrated in Figure 1.F1. The BG receive major excitatory input from the cerebral cortex (particularly supplementary motor area, premotor cortex, precentral motor, and postcentral sensory areas) and the intralaminar thalamic cell nuclei (e.g. centromedian-parafascicular complex). These afferents are glutamatergic and terminate in the striatum and STN. The BG (GPi and SNr) send output back to the cortex via the ventrolateral thalamus. For the last two decades, basal ganglia circuitry has been considered to be dominated by two parallel pathways by which cortical information is transmitted to the output nuclei of the basal ganglia, the GPi and SNr (Alexander and Crutcher, 1990; Smith et al., 1998; Albin et al., 1989; DeLong, 1990). In the so-called direct pathway, striatal GABAergic neurons monosynaptically inhibit the GABAergic output neurons in GPi and SNr. In the indirect pathway, striatal neurons inhibit the GABAergic neurons of the GPe. GPe neurons innervate GPi/SNr via direct projections and indirectly by innervating the glutamatergic neurons of the STN that excite GPi/SNr. As opposed to the direct pathway, the indirect pathway excites GPi/SNr. It is important to note, however, that the separation between these two pathways is not absolute. Anatomical evidence indicate that individual striatal neurons can be involved in both the direct and indirect pathways (Kawaguchi et al., 1990; Parent et al., 1995; Parent et al., 2000; Wu et al., 2000; Levesque and Parent, 2005b). In addition to these two pathways, a cortical glutamatergic ‘hyperdirect’ pathway to STN also exists (Monakow et al., 1978; Kitai and Deniau, 1981). Activation of this pathway excites GPi/SNr neurons.
1.2.1.b - Differential effects of dopamine

The balance between direct and indirect pathways is regulated by the differential actions of dopamine, from the SNC, on striatal neurons. The striatal neurons giving rise to the direct and indirect pathways are similar in their electrophysiological and morphological properties, but are distinguished in their neurochemical features. The direct pathway neurons express the dopamine D\textsubscript{1} receptor together with substance P and dynorphin whereas the indirect pathway neurons express the dopamine D\textsubscript{2} receptor and enkephalin (Aubert et al., 2000; Le Moine and Bloch, 1995; Gerfen et al., 1995). D\textsubscript{1} receptors are

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**Figure 1.F1.** Simplified schematic representation of basal ganglia connections. Excitatory (black) and inhibitory (gray) projections are shown. GPe: globus pallidus externus; GPi: globus pallidus internus; SNC: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; SC: superior colliculus; PPN: pedunculopontine nucleus; D1 and D2: dopamine receptor subtypes.
coupled to G proteins that activate adenylate cyclase to raise cyclic adenosine monophosphate (cAMP) levels, thereby producing an excitatory effect. $D_2$ receptors are coupled to different types of G proteins that activate phosphodiesterase which break down cAMP, thereby producing an inhibitory effect (Girault and Greengard, 2004). Thus, striatal dopamine enhances transmission along the direct pathway (acting on $D_1$ receptors) and reduces transmission along the indirect pathway (acting on $D_2$ receptors). Together these actions result in a net reduction in GPi and SNr activity (DeLong and Wichmann, 2007).

1.2.1.c - Parallel organization

In addition to the serial connections between basal ganglia nuclei, there are many distinct circuits which connect the BG, thalamus, and vast regions of the cortex. Electrophysiological and anatomical studies suggest that the main circuits passing through the BG remain “segregated” under normal conditions and that the functional architecture of the BG is essentially parallel in nature (Alexander et al., 1986; Hoover and Strick, 1993). It has been suggested that there are five closed loops within the basal ganglia-thalamocortical architecture, each of which centered upon a separate part of the frontal lobe (Alexander et al., 1986). The “motor” circuit connects the primary motor cortex, supplementary motor area and ventral premotor cortex. The “oculomotor” circuit connects the frontal and supplementary eye fields. There are two “associative” circuits, one involving the dorsolateral prefrontal cortex and the other involving the lateral orbitofrontal cortex. Finally, a “limbic” circuit connects to the anterior cingulate and the medial orbitofrontal cortex (Alexander et al., 1986; Alexander et al., 1990). More recently, however, it has been proposed that these circuits are rather interconnected (at the level of their output to different cortical regions) (Joel and Weiner, 1994). This organization may allow the BG to concomitantly participate in both the motor and complex non-motor functions of the cerebral cortex (Alexander et al., 1990; DeLong et al., 1984b; Middleton and Strick, 1994).

1.2.2 - The striatum
Subdivisions of the striatum

The striatum, the major input structure of the BG, is comprised of two main nuclei, the caudate and putamen. At the rostral end of the striatum, the two nuclei appear as one structure. Further caudally, the caudate and putamen are separated by the internal capsule (Utter and Basso, 2008). In primates, the putamen receives cortical projections mainly from somatosensory and motor areas, and has been therefore related to pure motor aspects such as the execution of movements or the processing of proprioceptive information (Crutcher and DeLong, 1984a; Crutcher and DeLong, 1984b; Kimura, 1986; Alexander et al., 1986). Putaminal neurons are somatotopically organized throughout the rostrocaudal extent of the nucleus (Alexander and DeLong, 1985b). Microstimulation in the putamen results in movements of individual body parts, perhaps due to the activation of grouped putamen output neurons known as striatal microexcitable zones (Alexander and DeLong, 1985a). It has been shown that single putamen neurons change their firing in response to a conditioned flexion movement in cats (Cheruel et al., 1994) and, in rats, have been shown to have integrative receptive fields for combined somatosensory and auditory stimuli, which may be used to facilitate motor responses (Chudler et al., 1995).

The caudate nucleus, on the other hand, receives extensive input from prefrontal cortex and parietal association area and is therefore believed to play a role in associative functions such as planning and anticipation of upcoming events (Apicella et al., 1991). Neurons in the caudate nucleus of monkeys integrate sensory, motor, and non-motor responses driven by the frontal cortex to produce coordinated behavior (Aldridge et al., 1980; Nishino et al., 1984). In freely moving cats, caudate neurons show changes of activity related to conditioned stimuli, to initiation of movement or to reinforcement during a reaction time task (Amalric et al., 1984).

Finally, the ventral extension of the striatum (also called the nucleus accumbens) serves limbic (emotional aspects) functions and receives input from the anterior cingulate and medial orbitofrontal cortex (Alexander et al., 1986).
**Projection neurons of the striatum**

The major output neurons of the striatum are GABAergic, so-called medium spiny neurons, that account for more than 90% of striatal neurons (Bolam et al., 1981). These neurons project primarily to the globus pallidus and SNr but also send local axon collaterals to other striatal MSNs (Gerfen, 1988). Although all medium spiny neurons are GABAergic, they can be further classified on the basis of their projection targets and other neurotransmitters they contain. Medium spiny neurons projecting directly to the SNr and GPi comprise the ‘direct pathway’ and express GABA, substance P and dynorphin (an opioid peptide), whereas medium spiny neurons that project to the GPe and comprise the ‘indirect pathway’ express GABA and enkephalin (Albin et al., 1989).

Anatomical studies demonstrate that the striatopallidal projection system in primates is highly ordered and displays a high degree of specificity with respect to its target sites in the pallidum (Hazrati and Parent, 1992c). Electrical stimulation of the striatum produces a short latency inhibition of both GPi and GPe neurons and is followed by a longer period of excitation, especially in the GPe (Tremblay and Filion, 1989). The early inhibition followed by excitation was always displayed by neurons located in the center of the pallidal zone of influence of each striatal stimulation site. At the periphery of the zone, however, only excitation was observed. This topological arrangement suggests that excitation is used, temporally, to control the magnitude of the central striatopallidal inhibitory signal and, spatially, to focus and contrast it onto a restricted number of pallidal neurons (Tremblay and Filion, 1989).

**Interneurons of the striatum**

In addition to the projection neurons of the striatum, there are many types of interneurons within the striatum. The striatal interneurons are classified into three groups. The first are the cholinergic large non-spiny neurons (Kemp and Powell, 1971;Bolam et al., 1984), also known as tonically active neurons (TANs) because of their physiological phenotype. The two other groups are the parvalbumin/GABAergic medium non-spiny neurons, and the somatostatin, neuropeptide Y, and NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) diaphorase containing non-spiny neurons (Gerfen and Wilson, 1996).
TANs are the most well studied interneurons of the striatum (Pisani et al., 2001). They are activated by presentation of sensory stimuli of behavioural significant or stimuli linked to reward, as they become synchronized in firing or develop pauses (Aosaki et al., 1995; Raz et al., 1996). Recently, it has been shown that TANs respond to instruction stimuli associated with motivational outcomes but not to unassociated ones in monkeys (Kimura et al., 2003; Yamada et al., 2004). In addition, TANs in the caudate nucleus tend to respond more to stimuli associated with motivational outcomes, whereas in the putamen, TANs tend to respond to ‘go’ signals especially for an action anticipating a reward. These findings suggest a distinct, pivotal role played by TANs in the caudate nucleus and putamen for motivation and reward (Kimura et al., 2003; Yamada et al., 2004).

Afferent projections of the striatum
The striatum receives excitatory input from many cortical areas (Kemp and Powell, 1970) and thalamic nuclei (Cowan and Powell, 1956). Anatomical and physiological studies have shown that different cortical areas project to distinct regions of the caudate and putamen. Cortical areas send somatotopically organized glutamatergic projections onto the dendritic tips of medium spiny neurons (Kemp and Powell, 1970). Although cortico-striatal inputs are distributed, there is also massive convergence of inputs to individual striatal neurons (Flaherty and Graybiel, 1991; Flaherty and Graybiel, 1993; Flaherty and Graybiel, 1994). This convergence of corticostriatal projections might play a role in the integration of cortical information from diverse regions of the cortex (Graybiel et al., 1994). Electrical stimulation of motor cortex, together with intracellular recordings in the ipsilateral striatal projection field in rats, demonstrated long-term potentiation of cortico-striatal synaptic transmission (Charpier and Deniau, 1997). This suggests the existence of activity-dependent plasticity at glutamatergic cortico-striatal synapses. The physiological long-term potentiation at corticostriatal synapses can be also induced by low frequency (5 Hz) synchronization of cortical afferents (Charpier et al., 1999), suggesting that the striatal output neuron may operate as a coincidence detector of converging cortical information.
In addition to the cerebral cortex, the striatum receives excitatory inputs from the thalamus. The centromedian (CM) and parafascicular (Pf) nuclei are an important source of thalamostriatal projections. High resolution anterograde tracer studies have shown that the CM and Pf provide distinct inputs to different parts of the striatum (Sadikot et al., 1992a; Sadikot et al., 1992b). The CM projects to the entire sensorimotor territory of the striatum, whereas the Pf provides complementary input to the entire associative region (Sadikot and Rymar, 2008).

The striatum also receives a major input from dopaminergic neurons located in the ventral midbrain. Dopaminergic innervation of the striatum arises from two nuclei, the SNC and the ventral tegmental area (VTA) (Beckstead et al., 1979). Dopamine acts on post-synaptic dopamine receptors that are located at the base of the dendritic spines of medium spiny neurons, and is therefore in a position to modulate the effect of the corticostriatal excitatory input to these neurons (Gerfen, 1988). In addition to dopamine receptors on medium spiny neurons, presynaptic dopamine receptors are also present on corticostriatal terminals (Arbuthnott et al., 1998), where they act to dampen striatal excitation (Bamford et al., 2004). Dopamine also modulates striatal aspiny neurons (cholinergic interneurons) (Aosaki et al., 1998). It has been shown that long-term depression of synaptic transmission in the corticostriatal pathway is blocked by dopamine receptor antagonists or lesions of the nigrostriatal dopaminergic pathway and can be restored by the application of exogenous dopamine (Calabresi et al., 1992).

\[ D_1 \] and \[ D_2 \] dopamine receptors

The \( D_1 \) and \( D_2 \) dopamine receptors are the major subtypes expressed in the striatum. Both \( D_1 \) and \( D_2 \) receptors are G-protein coupled receptors. Binding of dopamine to the \( D_1 \) receptor results in depolarization of striatal neurons whereas binding to the \( D_2 \) receptor cause neuronal hyperpolarization (Sealfon and Olanow, 2000). Thus, the action of \( D_1 \) receptor is to enhance corticostriatal influence whereas the action of \( D_2 \) receptor is to reduced corticostriatal influence. It was originally suggested that \( D_1 \) and \( D_2 \) receptors are differentially expressed on the dendrites of striatal neurons. Striatal neurons of the direct pathway express \( D_1 \) receptors whereas those neurons projecting indirectly express \( D_2 \).
receptors. Later studies have argued that a substantial subpopulation of medium spiny neurons co-express D1 and D2 receptors (Surmeier et al., 1996; Aizman et al., 2000). This controversy was recently resolved due to the availability of transgenic mice expressing enhanced green fluorescent protein selectively for D1 and D2 receptors. It was confirmed that dopamine receptor subtypes are differentially expressed on separate populations of spiny neurons. These studies also show that the role of dopamine in synaptic plasticity is different for D1 and D2 MSNs (activation of D1/D2 leads to spike-timing dependent LTP/LTD respectively) (Shen et al., 2008), and that D1 and D2 MSNs have different morphology (D2 MSNs have smaller dendritic threes than D1 MSNs) (Gertler et al., 2008) and electrophysiological properties (D2 MSNs dendrites are more excitable) (Day et al., 2008).

The original dichotomy between the two population of MSNs led to the classic notion of the indirect pathway inhibition of movement and the direct pathway facilitation of movement (Gerfen et al., 1990). Although this classification has had tremendous value, anatomical studies indicate that it is oversimplified and that several modifications need to be made. For example, neurons projecting to the GPi and SNr can also send axon collaterals to the GPe, arguing that direct and indirect pathways are not completely segregated (Kawaguchi et al., 1990).

*Mosaic organization of the striatum*

The second level of functional compartmental organization in the striatum is the segregation of medium spiny neurons into striosomes and matrix compartments, which differ in several cytochemical markers, input-output connections, and time of neurogenesis. The striosomes are identified by patches of dense opiate receptor binding, and are enriched in enkephalin- and substance P-like immunoreactivity. The matrix has a high acetylcholinesterase activity, and a dense plexus of fibres displaying somatostatin-like immunoreactivity (Pert et al., 1976; Graybiel and Ragsdale, Jr., 1978; Gerfen, 1985; Herkenham and Pert, 1981; Graybiel and Chesselet, 1984; Chesselet and Graybiel, 1986). The matrix comprises the majority of the striatum and receives input from somatosensory and associational areas of the cerebral cortex, while the striosomes receive
input from limbic and the prefrontal cortex. Striosomes project mostly to dopaminergic neurons of the SNc, whereas the matrix gives rise to the parallel striatonigral and striatopallidal pathways (Gerfen, 1984). These anatomical features suggest that striosomes and matrix play different roles in the processing of cortical inputs. Indeed, during relatively neutral behavioral conditions, the highest metabolic activity in the striatum was observed in the matrix rather than in the striosomes (Brown et al., 2002). On the other hand, in vivo electrical stimulation through electrodes centered in or around the striosomes, but not the matrix, led to rapid acquisition and maintenance of bar-pressing, self-stimulation behaviors associated with reward (White and Hiroi, 1998). It is therefore currently believed that reward-related, limbic forebrain circuits are centered in the striosomes, while sensorimotor and associative circuits are present in the matrix.

1.2.3 - Subthalamic nucleus

The subthalamic nucleus is a biconvex-shaped structure (Yelnik and Percheron, 1979) that is densely populated by glutamatergic projection neurons (Rafols and Fox, 1976; Iwahori, 1978; Afsharpour, 1985). In the STN there are no intra-nuclear interactions as a result of recurrent collaterals (Kita et al., 1983b), but there are some evidence for the existence of interneurons (Rafols and Fox, 1976; Yelnik and Percheron, 1979; van der Kooy and Hattori, 1980; Levesque and Parent, 2005a). The STN plays a central role in BG circuitry since it receives input from, and projects to, many diverse nuclei (Alexander and Crutcher, 1990).

Intrinsic organization of the STN

The STN in primates can be separated into three distinct functional subdivisions. It is subdivided into two rostral thirds and a caudal third. The rostral two-thirds can be further divided into medial third and lateral two-thirds (Figure 1.F2). The medial potion of the rostral two-thirds is thought to comprise the limbic and part of the associative territories. The ventral aspect of the lateral portion of the rostral two-thirds comprises the additional portion of the associative territory. The dorsal part of the lateral portion of the rostral two-thirds and the caudal third are related to motor circuits (Parent and Hazrati,
In more general terms, the STN is largely divided into a dorsolateral sensorimotor portion and a ventromedial associative portion. In primates, the dorsolateral region was shown to be somatotopically organized. Within this region, the lateral fraction contains neurons that respond to arm movements and the more medial fraction contains neurons that respond to leg movement (DeLong et al., 1985; Wichmann et al., 1994a; Rodriguez-Oroz et al., 2001). The ventromedial portion of the STN is involved in oculomotor and associative aspects of motor behaviour and the neurons are activated during visual and oculomotor tasks (Matsumura et al., 1992).

Subthalamic nucleus afferents

The STN receives excitatory glutamatergic projections from diverse regions of the ipsilateral cortex. In primates, most of the cortical afferents to the STN arise from the primary motor cortex, supplementary motor area (SMA), pre-SMA, and the dorsal and ventral premotor cortices (Hamani et al., 2004). These projections, as part of the motor

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**Figure 1.F2.** Schematic representation of the intrinsic organization of the subthalamic nucleus (STN) according to the tripartite functional subdivision of the basal ganglia. Modified from Hamani et al., Brain, 2004.
loop, innervate predominantly the dorsal aspect of the STN and are somatotopically
organized. Primary motor cortex fibres related to the leg, arm and face are represented
from medial to lateral in the lateral portion of the STN. The medial potion receives fibers
from the SMA and premotor cortex in an inverse somatotopic distribution with leg, arm
and face represented from lateral to medial respectively (Nambu et al., 1996;Nambu et al.,
1997;Nambu et al., 2002). The ventromedial STN, that is involved in circuits related to
eye movements, receives afferents from the frontal eye field and the supplementary
frontal eye field (Monakow et al., 1978). In rodents, prelimbic-medial orbital areas of the
prefrontal cortex project to the medial STN as part of the limbic loop (Groenewegen and
Berendse, 1990). Cortical afferents from the primary motor cortex to the STN in rodents
and cats originate mainly in layer V and are composed of collaterals of the pyramidal
tract or cortical fibres that also innervate the striatum (Kitai and Deniau, 1981;Giuffrida
et al., 1985). The cortico-subthalamic projections terminate primarily on distal dendrites
and spines of STN neurons (Moriizumi et al., 1987).

The STN receives major GABAergic inhibitory input from the GPe. The topographic
distribution of these afferents varies from species to species. In rodents, the lateral
portion of the pallidum innervates the lateral STN, while the medial and ventral portions
of the pallidum innervate the medial STN (Parent and Hazrati, 1995a). In primates the
picture is more complex. Generally, motor and limbic portions of the GPe innervate their
corresponding territories in the STN. However, the associative GPe afferents innervate
not only the associative portion of the STN but also the motor territory (Carpenter et al.,
1981;Shink et al., 1996;Joel and Weiner, 1997). Pallidal afferents have been shown to
terminate more commonly on proximal dendrites and cell bodies of STN neurons (Parent
and Hazrati, 1995a). The inhibitory drive of the GPe on the STN is supported by
electrophysiological studies in monkeys showing that muscimol (a GABA receptor
agonist) injections into the STN decrease the activity of STN neurons, while bicuculline
(a GABA receptor antagonist) injections slightly increase the activity of STN neurons
(Wichmann et al., 1994b). It seems possible that pallidal inputs interact with cortical
inputs to shape STN responses (Ryan and Clark, 1991). Cortical stimulation results in a
short latency excitation of the STN followed by an inhibitory period that is likely
mediated by the GPe (Fujimoto and Kita, 1993).

The STN also receives direct projections from the thalamus and these originate mainly from the CM and Pf nuclei (Sugimoto et al., 1983; Sugimoto et al., 1983; Sadikot et al., 1992a). In primates, the Pf nucleus is the predominant thalamic source of input to the STN, innervating mainly the associative and limbic STN territories. On the other hand, there are only small number of fibres from the CM nucleus, which projects mainly to the sensorimotor STN (Sadikot et al., 1992a; Parent and Hazrati, 1995a). These afferents are glutamatergic and terminate mainly on dendrites of STN cells (Mouroux and Feger, 1993).

Brainstem afferents to the STN have also been described. The STN receive direct dopaminergic projections from the SNc (Brown et al., 1979; Lavoie et al., 1989; Francois et al., 2000). These projections are known to modulate the cortical and pallidal inputs to the STN. In addition, the pedunculopontine and laterodorsal tegmental nuclei send cholinergic inputs to the STN (Jackson and Crossman, 1983; Scarnati et al., 1987; Lee et al., 1988; Lavoie and Parent, 1994b), although non-cholinergic inputs from the pedunculopontine nucleus also exist (Rye et al., 1987). Another source of afferent innervation to the STN in rodents is the dorsal raphe nucleus (Woolf and Butcher, 1986; Canteras et al., 1990). This pathway is serotoninergic and may also be involved in the modulation of STN activity.

**Subthalamic nucleus efferents**

The STN is the only nucleus of the BG that exerts an excitatory glutamatergic drive to its target nuclei. Its major efferent projections are directed to both segments of the globus pallidus. These projections arborize uniformly and affect an extensive number of cells (Smith et al., 1990; Hazrati and Parent, 1992b; Parent and Hazrati, 1995a). The excitatory STN drive to the globus pallidus is supported by electrophysiological studies in intact monkeys in which a reduced firing rate in the globus pallidus is observed following STN lesion (Hamada and DeLong, 1992). In addition to the pallidum, the STN projects to both component of the substantia nigra, the SNC and the SNr. Although most of these
projections innervate the SNr, some axons innervate the SNc perhaps regulating dopamine release (Groenewegen and Berendse, 1990; Smith et al., 1990; Parent and Hazrati, 1995a). The striatum receives scant projections from the STN in non-human primates (Kita and Kitai, 1987; Smith et al., 1990). These projections provide en passant excitatory influence over striatal cells (Parent and Hazrati, 1995a). In summary, the ventromedial associative and limbic regions of the STN innervate mostly the caudate nucleus and the SNr, while the dorsolateral sensorimotor portion innervate mostly the putamen and the GP (Parent and Smith, 1987; Parent, 1990).

Aside from the main efferent projections described above, the STN also projects to the pedunculopontine nucleus and ventral tegmental area (Jackson and Crossman, 1981; Granata and Kitai, 1989; Smith et al., 1990).

**Pharmacological properties of STN cells**

GABA plays a major role in STN physiology, modulating the firing rates and patterns of its neurons (Bevan et al., 2007). The GABAergic activity within the STN occurs mainly through the activation of post-synaptic GABA_A receptors (Bevan et al., 2000). Recent evidence, however, has shown that GABA_B receptors also play a role, but their effects are mostly pre-synaptic (Shen and Johnson, 2001). STN neurons also express glutamate receptors. Both N-methyl-D-aspartate (NMDA) and 2-aminomethyl-phenylacetic acid (AMPA) have been described in STN neurons, although AMPA receptors are more prevalent (Klockgether et al., 1991; Shen and Johnson, 2000).

STN neurons express dopamine D_5 receptors, and also D_1 and D_2 (Baufreton et al., 2005b), which are also present in the pre-synaptic afferent terminals (Mansour et al., 1991; Svenningsson and Le Moine, 2002). While some studies suggest that dopamine agonists have an excitatory effect in STN (Mintz et al., 1986; Kreiss et al., 1996), others imply that it reduces STN activity (Campbell et al., 1985; Hassani and Feger, 1999). In STN slices, dopamine application suppresses both glutamatergic EPSPs (excitatory postsynaptic potentials) and GABAergic IPSPs (inhibitory postsynaptic potentials) (Hassani and Feger, 1999; Shen and Johnson, 2000). In rodents, although the systemic
effects of selective D₁ and D₂ agonists are not consistent, it has been shown that systemic administration of apomorphine, a non-selective dopamine agonist, increases STN activity. Generally, it seems that co-activation of D₁ and D₂ receptors excite STN neurons (Kreiss et al., 1997; Ni et al., 2001). It is important to consider, however, that most of the structures that innervate the STN are modulated by dopamine. Thus, systemic application of dopaminergic agents might produce a complex cascade of responses (Hamani et al., 2004).

Cholinergic agonists applied topically in rodents, increase the activity of STN neurons (Feger et al., 1979). Application of muscarinic agonists in slices reduce both EPSPs and IPSPs in the STN, perhaps through M3 receptors (Flores et al., 1996; Shen and Johnson, 2000). Since the effects of the later potentials are greater, the end result is excitation of STN neurons (Rosales et al., 1994; Shen and Johnson, 2000). Serotonin also increases the spontaneous activity of STN neurons in slice preparations (Flores et al., 1995). Opioids have been shown to inhibit both GABAergic and glutamatergic synaptic inputs to the STN through pre-synaptic μ and δ receptors (Shen and Johnson, 2002).

**Physiological properties of STN cells**

In vitro, STN neurons are capable of firing independently of synaptic input due to their pacemaker voltage-gated sodium channels. In the absence of GABAergic and glutamatergic synaptic inputs, STN neurons rhythmically fire action potentials at 5-15 Hz (Bevan and Wilson, 1999). This property may, in part, underlie the tonic activity of STN neurons in vivo. STN neurons fire 13-18 spikes/sec in normal rodents, and 18-25 spikes/sec in non-human primates (Georgopoulos et al., 1983; DeLong et al., 1985; Matsumura et al., 1992; Wichmann et al., 1994a; Overton and Greenfield, 1995; Hassani et al., 1996; Bergman et al., 1994).

Most of the neurons in the STN respond to cortical stimulation, usually with a triphasic response. This response consists of initial excitation, followed by inhibition and a second excitation (Kitai and Deniau, 1981; Fujimoto and Kita, 1993; Nambu et al., 2000). The first excitation occurs ~2 ms following cortical stimulation and is believed to be directly
related to the activation of cortico-subthalamic pathways. The following inhibition is thought to be the result of activation of GPe neurons. The second excitatory peak, which takes place ~15 ms after cortical stimulation, might be due to the activation of the cortico-striatal pathway and the subsequent inhibition of GPe neurons, resulting in disinhibition of the STN (Fujimoto and Kita, 1993; Feger et al., 1997; Nambu et al., 2000). In agreement with this notion, lesions of the globus pallidus do not interfere with the first excitation of the STN, but substantially decrease the inhibitory component of the response and increase the second excitatory peak. Striatal lesions, on the other hand, induce the opposite effects (Ryan and Clark, 1992).

Nearly all STN neurons respond to pallidal stimulation (Kita et al., 1983a). The pattern of response depends on the amount and/or amplitude of the IPSPs that are generated following pallidal stimulation. For instance, large IPSPs are strong enough to completely reset the autonomous activity of STN neurons and can promote synchronization because they completely deactivate the voltage-gated sodium channels that underlie autonomous activity (Bevan et al., 2002a; Bevan et al., 2002b; Baufreton et al., 2005a; Bevan et al., 2007). On the other hand, individual IPSPs with small amplitudes promote only partial resetting of STN neurons, which results in phase-dependent delays between spikes, leading to desynchronization among STN neurons. Importantly, multiple IPSPs do not only reset STN firing but occasionally promote a rebound depolarization and a burst of action potentials by bringing the membrane potential closer to the equilibrium potential of GABA current (to a more hyperpolarized state) (Bevan et al., 2002a; Bevan et al., 2002b; Bevan et al., 2000). Therefore, synchronous activity of GPe neurons has the potential to synchronize the activity of STN neurons (Bevan et al., 2000). It has been suggested that during normal movement, GABAergic activation is asynchronous and provides only a small contribution to the synchronization of STN. It is possible that asynchronous feedback inhibition by the GPe acts to limit STN synchronization and provide the basis for de-correlated, functionally segregated, activity (Bevan et al., 2000).

In normal monkeys, between 30 and 50% of the neurons in STN are related to passive and/or active movement, and their majority are activated solely by manipulation around a
single contralateral joint (DeLong et al., 1985; Wichmann et al., 1994a). As mentioned above, most of the movement-related neurons are localized in the dorsal portion of the STN and are somatotopically organized (Wichmann et al., 1994a). In addition, about 20% of STN neurons are responsive to eye fixation, saccadic eye movements or visual stimuli. These neurons are located primarily in the ventral portion of the STN and participate in the visuooculomotor circuits (Matsumura et al., 1992).

1.2.4 - *Globus pallidus*

In primates, the GP is divided by the internal medullary lamina into the internal (GPi) and external (GPe) segments. In rodents, the homologue of the GPi is the entopeduncular nucleus (EPN) that is surrounded by the fibres of the internal capsule, and the GPe is referred to as the GP (Nambu, 2007). Both GPi and GPe share similar morphology and their neurons use GABA as their neurotransmitter (Smith et al., 1987). The GPe has strong interconnections with the STN, while the GPi projects outside the basal ganglia (Nambu, 2007).

*Globus pallidus afferents*

The GABAergic striatal afferents are the main input to the GP and they terminate along the dendrites (Shink and Smith, 1995). As mentioned above, the substance P-containing MSN neurons mainly project to the GPi, and the enkephalin-containing MSN neurons mainly project to the GPe. In addition to inhibitory striatal input, the STN sends excitatory glutamatergic input to both segments of the GP (Carpenter et al., 1981; Joel and Weiner, 1997). Anatomical tracing techniques demonstrate that subthalamic and neostriatal axonal terminals make convergent synaptic contact with individual GPi neurons that project to the thalamus (Bevan et al., 1994b). It was suggested that the STN uniformly excites a large number of GPi neurons, whereas the striatum exerts a more specific inhibitory control upon selected subsets of STN-driven pallidal neurons to give a more localized effect (Parent and Hazrati, 1993). The GPi also receives afferent input from the GPe that terminates close to the cell body (Shink and Smith, 1995). Other inputs to the GP include glutamatergic inputs from the intralaminar thalamic nuclei.
serotonergic inputs from the dorsal raphe nucleus, glutamatergic and cholinergic inputs from the pedunculopontine nucleus, and dopaminergic inputs from the SNC. These axons arborize more profusely in the Gpi than in the GPe (Lavoie et al., 1989; Lavoie and Parent, 1990; Lavoie and Parent, 1994b).

1.2.4.a - Globus pallidus externus

Globus pallidus externus efferents

The GPe sends inhibitory GABAergic projections to many BG nuclei including the STN Gpi, SNr and striatum (Carpenter et al., 1981; Bolam and Smith, 1992; Parent and Hazrati, 1995a). GPe axons in STN, GP and SNr terminate on the soma and proximal dendrites. In the striatum, GPe axons terminate on aspiny interneurons and the dendritic shafts of spiny projection neurons (Sato et al., 2000). In addition, GPe local collateral axons form synapses on the somata and proximal dendrites of other GPe neurons (Kita, 2007). A small number of GPe neurons also projects to the dorsal thalamus, reticular thalamic nucleus, inferior colliculus and the PPN (Kita, 2007).

Physiology of the globus pallidus externus

The GPe is considered to be an intrinsic nucleus of the BG because it receives most of its input from structures of the BG (i.e. the striatum and STN) and sends output to other BG nuclei (Parent and Hazrati, 1995a). As mentioned above, the GPe receives input from the enkephalin-containing MSN neurons that primarily express the D2 receptor, and is viewed to be part of the indirect pathway. Electrophysiological studies demonstrate that the majority of GPe neurons exhibit high frequency firing interspersed with spontaneous pauses and have a mean firing rate of about 55 Hz. The rest of the neurons exhibit low-frequency firing and bursts (DeLong, 1971; Gardiner and Kitai, 1992; Ni et al., 2000a). It has been reported that 11% of the cells recorded in the GPe display an oscillatory correlogram in the 5-20 Hz range, and fewer than 5% of recorded pairs are correlated (Raz et al., 2000). GPe neurons respond to somatosensory input and are involved in the control of movement parameters (DeLong, 1971; Georgopoulos et al., 1983; Dormont et al., 1997). Ninety-one percent of GPe neurons have been shown to respond to the passive movement of only one joint (Filion et al., 1988). The GPe can strongly inhibit the
neurons of the output structures of the BG directly through its connections to GPi/SNr and indirectly via the STN (Alexander and Crutcher, 1990). It has been shown that injection of bicuculline into the GPe of intact monkeys, causes increased GPe activity and elicits dyskinesias (Crossman et al., 1988; Matsumura et al., 1995). Electrical stimulation of the GPe has been shown to increase movement times of the contralateral arm (Horak and Anderson, 1984a).

1.2.4.b - Globus pallidus internus

*Globus pallidus internus efferents*

The GPi projects to the ventral anterior/ventral lateral thalamic complex (i.e. parvicellular part of the ventral anterior nucleus and oral part of the ventral lateral nucleus), and gives off axon collaterals to the centromedian nucleus (Kuo and Carpenter, 1973; Kim et al., 1976). These fibres terminate predominantly on the soma and proximal dendrites of thalamic projection neurons. Pallidal fibres also contact thalamic GABAergic inhibitory interneurons, suggesting that pallidal inputs not only directly inhibit thalamic projection neurons but also disinhibit projection neurons via interneurons (Ilinsky et al., 1997). GPi neurons also project to the habenular nucleus and PPN (Parent et al., 1981; Parent and De Bellefeuille, 1983).

*Physiology of the globus pallidus internus*

The GPi plays a key role in the BG network as it integrates information from the striatum, GPe and STN. The GPi is part of the indirect pathway since it receives excitatory input from the STN, as well as part of the direct pathway since it receives inhibitory input from the substance P-containing striatal neurons (Parent and Hazrati, 1995b). Unlike GPe neurons that exhibit pauses of activity, GPi neurons fire spontaneously with a mean rate of 60-80 Hz without pauses (DeLong, 1971; DeLong et al., 1985). This activity is believed to exert a tonic inhibitory effect on the target nuclei of the GPi (i.e. the thalamus and brainstem). Little oscillatory firing or synchronized pairing has been demonstrated in the GPi of normal animals (Nini et al., 1995; Raz et al., 2000). Neurons of the GPi are relatively large (20-60 μm) (Yelnik et al., 1984) and have impressive dendritic trees that are believed to play a role in the convergence of incoming information from the striatum.
In addition to the main group of GABAergic GPi neurons, there is another group of neurons named “border cells” that are located near the perimeter of pallidal segments, and adjacent to or in the external or internal medullary lamina. These cells exhibit a regular discharge pattern with a mean frequency of 20-50 Hz, and are considered to be cholinergic (DeLong, 1971).

Through the hyperdirect, direct and indirect pathways, the GPi receives topographical inputs from the cerebral cortex. In the motor territories of the GPi (ventral two-thirds of caudal GPi), the hindlimb, forelimb, and facial regions are somatotopically represented from the dorsal part to the ventral part (DeLong, 1971; DeLong et al., 1985; Yoshida et al., 1993; Strick et al., 1995). In normal animals, sensory-motor fields have been shown to correlate mainly with one joint of the contralateral limb (Filion et al., 1988; Boraud et al., 2000a). About 70% of responding neurons are activated and 30% inhibited in response to movement of a corresponding body part (Anderson and Horak, 1985; Mink and Thach, 1991a; Turner and Anderson, 1997; Boraud et al., 2000a). It is important to note that during voluntary limb/arm movement, a strong majority of GPi neurons display an increase rather than decrease in firing rate (Georgopoulos et al., 1983; Anderson and Horak, 1985; DeLong et al., 1985; Mitchell et al., 1987a; Hamada et al., 1990; Nambu et al., 1990; Mink and Thach, 1991a; Turner and Anderson, 1997). Therefore, it was suggested that excitatory inputs from the STN to the GPi contribute to voluntary movement (Nambu, 2007). Border cells show similar response pattern as GPi and GPe neurons, suggesting that they share similar inputs (Mitchell et al., 1987b).

Importantly, movement-related activity of neurons in the GPi occurs not only later than motor cortex (DeLong et al., 1984a) but also about 80-100 ms after the onset of muscle contraction, indicating that they are not involved in the preparation or the initiation of movement (DeLong, 1971; Anderson and Horak, 1985; Mink and Thach, 1987). Studies on parameter encoding are more divergent. Some studies show a certain correlation between GPi firing rate and kinematic parameters such as velocity, direction or strength (Georgopoulos et al., 1983; Anderson and Turner, 1991), while in other studies this correlation depends on the context (Mink and Thach, 1991a; Mink and Thach,
In monkeys performing sequential wrist movements, pallidal neurons code more prominently for predictable and easy movements and signal the end of a movement sequence (Brotchie et al., 1991b), suggesting that BG function is more related to the performance of automatic than voluntary movements. In addition, pallidal neurons encode the serial position of an event in remembered sequential pointing movements in monkeys, suggesting that the GPi may be involved in encoding the spatiotemporal characteristics of a sequence of actions (Mushiake and Strick, 1995).

Injections of bicuculline into the GPi of intact monkeys induces dose-dependent hypokinesia with dystonic attitudes in contralateral limbs, whereas muscimol injections elicit choreiform movements (Burbaud et al., 1998). Neurotoxic blockade or lesions of the GPi lead to poor braking of movement (Horak and Anderson, 1984b; Mink and Thach, 1991b) and inaccuracy of control of the final adjustment of the limb to the target (Kato and Kimura, 1992). It has been shown that electrical stimulation in the ventrolateral GPi reduces movement times for the contralateral arm of normal monkeys, whereas stimulation in the dorsal GPi increases movement times (Horak and Anderson, 1984a).

1.2.5 - Substantia nigra

The substantia nigra is located dorsal to the cerebral peduncle in the ventral midbrain, and plays an important role in reward, addiction and movement. Although the substantia nigra appears as a continuous band in brain sections, it consists of two anatomically distinct parts with very different connections and functions, the pars compacta and pars reticulata (Rinvik and Grofova, 1970; Gulley and Wood, 1971; Juraska et al., 1977; Yelnik et al., 1987; Braak and Braak, 1986).

1.2.5.a - Substantia nigra pars compacta

The pars compacta of the substantia nigra receives GABAergic input from striatal neurons and sends dopaminergic input back to the striatum (Graybiel, 1990). In addition, collaterals of the nigrostriatal axons provide extrastriatal dopaminergic innervation of the pallidum and STN (Cossette et al., 1999). The dopaminergic neurons of the SNc
modulate the flow of information from the cortex through the BG (Bevan et al., 1996). In intact monkeys, dopaminergic neurons have a relatively low discharge rate (0.5-8 Hz) and long impulse duration (2.05 ms on average) (Aebischer and Schultz, 1984). It would appear that these neurons are not related to movement itself, but change their activity in response to rewards, during procedural learning and in response to conditioned stimuli (DeLong et al., 1983; Schultz, 1986b). Their activity can also be depressed by systemic injection of the dopamine agonist apomorphine (Aebischer and Schultz, 1984).

In addition to striatal input, SNC dopaminergic neurons are innervated by several other BG nuclei. In the rat, SNC neurons receive multiple GABAergic inputs from neurons in the globus pallidus and from axon collaterals of SNr projection neurons (Smith and Bolam, 1990; Celada et al., 1999). Electrical stimulation of GABAergic pathways originating in the striatum, GP or SNr produces inhibition of SNC dopaminergic neurons in vivo (Paladini et al., 1999). This inhibition appears to be mediated, at least in part, by the GABA_A receptor subtype. Interestingly, it has been shown that the discharge pattern of SNC dopaminergic neurons in vivo can be differentially modulated by local application of GABA_A and GABA_B receptor antagonists. The GABA_A antagonists (i.e. bicuculline, gabazine and picrotoxin) strongly induce burst firing in dopaminergic neurons, whereas GABA_B antagonists cause a modest shift to a more regular firing in 50% of the cases (Paladini and Tepper, 1999). This suggests that dopaminergic neurons are under tonic GABAergic inhibition mediated by GABA_A receptors. On the other hand, STN stimulation has been shown to promote burst discharge in a subpopulation of SNC neurons, most likely via NMDA receptors (Chergui et al., 1993; Chergui et al., 1994), suggesting that excitatory inputs to midbrain dopaminergic neurons may participate in the control of the dopamine release in target areas (Chergui et al., 1993).

1.2.5.b - Substantia nigra pars reticulata
Together with the GPi, the pars reticulata of the substantia nigra provides the main output nucleus of the BG. The SNr receives GABAergic inputs from the striatum (Hedreen and DeLong, 1991; Mendez et al., 1993; Lynd-Balta and Haber, 1994) and GPe (Mendez et al., 1993; Bevan et al., 1996), and glutamatergic inputs from the STN (van der Kooy and
Hattori, 1980; Smith et al., 1990). These inputs from striatum, GPe and STN converge onto single SNr neurons (Smith and Bolam, 1991; Bolam et al., 1993; Bevan et al., 1994a). The lateral half of the SNr is innervated by striatal subterritories that process inputs from sensory and motor cortical areas, whereas the more medial region is innervated by striatal subterritories that are related to prefrontal and limbic cortical areas (Deniau et al., 2007). The SNr innervates the ventral medial and parafascicular thalamic nuclei, the superior colliculus and the PPN (Beckstead et al., 1979; Di et al., 1979; Gerfen et al., 1982; Appell and Behan, 1990; Bickford and Hall, 1992).

Compared to the overlying cell-rich pars compacta, the SNr is characterized by a low neuronal density with smaller cell bodies (Francois et al., 1985; Yelnik et al., 1987). Although the SNr is mainly composed of GABAergic projection neurons (Oertel and Mugnaini, 1984; Yelnik et al., 1987; Smith et al., 1987), dopaminergic and cholinergic neurons also exist (Hattori, 1993; Deutch et al., 1986; Decavel et al., 1987; Campbell and Takada, 1989; Gould and Butcher, 1986; Martinez-Murillo et al., 1989). In rats, dopaminergic neurons are clustered in the caudal and ventral SNr (Deutch et al., 1986), while the cholinergic neurons are mainly located caudally (Gould and Butcher, 1986; Martinez-Murillo et al., 1989). The dopaminergic neurons are distinct from and not considered part of the SNc. In addition to projection neurons, putative local circuit neurons have been also described in the rat (Gulley and Wood, 1971; Juraska et al., 1977) and monkey SNr (Schwyn and Fox, 1974). These neurons, however, appear to be very few in number and their functional role is still unknown (Deniau et al., 2007).

Functional properties of the SNr

As opposed to the dopaminergic nigrostriatal neurons, the GABAergic output neurons of the SNr are characterized by short-duration action potentials and a spontaneous repetitive high-frequency firing rate reaching 40-80 spikes/sec in vivo (Wilson et al., 1977; Deniau et al., 1978). These electrophysiological properties of SNr neurons are attributed to their strong voltage-dependent K+ conductance, which allows for a short duration action potential and prevents the membrane potential from reaching the Na+ inactivation level (Nakanishi et al., 1987). No synchronized oscillations have been shown in the SNr of
normal animals (Wichmann and DeLong, 1999). SNr neurons show phasic responses during motor tasks and with somatosensory examination in monkeys (Mora et al., 1977; DeLong et al., 1983; Schultz, 1986a; Lestienne and Caillier, 1986), cats (Joseph et al., 1985) and rats (Gulley et al., 1999; Gulley et al., 2002). The SNr is also involved in oculomotor functions (Hikosaka and Wurtz, 1983c; Hikosaka and Wurtz, 1983a; Joseph and Boussaoud, 1985). For example, it has been shown that prior to rapid eye movements, SNr neurons display a pause in activity, while superior colliculus neurons show a burst in activity (Hikosaka and Wurtz, 1989). Almost all task-related SNr neurons are inhibited during saccadic eye movement, suggesting that the pause in SNr provides a transient disinhibition of the superior colliculus, leading to an eye movement command (Hikosaka et al., 2000). It has been also shown that an injection of the GABA agonist muscimol into the SNr disrupts guided and reflex eye movements in the cat (Boussaoud and Joseph, 1985). From more recent work, it is becoming clear that the SNr is involved in higher motor functions and cognitive processes in addition to its role in movement (Hikosaka and Wurtz, 1983b; Basso and Wurtz, 2002; Bayer et al., 2004; Wichmann and Kliem, 2004).

1.3 - Models of basal ganglia function in relation to Parkinson’s disease

Models of basal ganglia circuitry have provided some clues into the pathophysiological mechanisms of movement disorders and the reasons that disruption of parts of the circuit by lesion or deep brain stimulation might improve motor function. The main three models will be discussed here: the classical rate model proposed over 15 years ago, the centre-surround model and the relatively recent oscillatory model. A more comprehensive review of the electrophysiological findings in PD patients and animal models of PD will be given in section 1.4.

1.3.1 - The rate model

The rate model was first proposed by Albin (Albin et al., 1989) and Delong (DeLong, 1990). According to this model, the basal ganglia facilitate and inhibit movement through
changes in firing rates in its nuclei. This model, which is largely based on the serial connections between BG nuclei (see Figure 1.F1), provides a coherent explanation of opposing movement disorders (i.e. hypo- and hyperkinetic) (DeLong, 1990). The rate model proposes that activation of the direct pathway causes decreased activity in GPi/SNr and facilitation of movements through a process of disinhibition of thalamocortical relay neurons (Chevalier and Deniau, 1990). On the other hand, activation of the indirect pathway disinhibits the STN and causes increased activity in GPi/SNr, therefore leading to suppression of movements. In other words, the rate model predicts that decreased GPi/SNr activity would result in movements that are fast and large and that increased GPi/SNr activity would result in movements that are slow and small (Alexander and Crutcher, 1990). A balance between the direct and indirect pathways is believed to exist at the level of the output nuclei of the BG whereby the inhibitory effect of the direct striatal output counteracts the excitatory effect of the STN in the indirect pathway.

The rate model in relation to Parkinson’s disease

As mentioned earlier, the neurotransmitter dopamine acts in the striatum on D₁ receptors to stimulate cells of the direct pathway, and on D₂ receptors to inhibit cells of the indirect pathway. In Parkinson’s disease, the result of the dopamine deficiency is reduced activity in the direct pathway from striatum to GPi, and increased activity in the indirect pathway via GPe and STN. Together, these changes give rise to increased activity in the GPi/SNr, inhibiting thalamic and cortical activation and impairing voluntary movement.

The rate model of the basal ganglia predicted that ablation or inactivation of GPi or STN would lead to the alleviation of parkinsonian symptoms. Indeed, STN lesions have been shown to reverse parkinsonian symptoms in MPTP monkeys (Bergman et al., 1990; Aziz et al., 1991; Guridi et al., 1994) and set the stage for the introduction of the STN as a neurosurgical target to treat PD patients (Benabid et al., 1994; Gill and Heywood, 1997; Krack et al., 2003). Even pallidotomy which had been largely abandoned since the advent of levodopa therapy, was revisited and proven to be very successful at alleviating PD symptoms in patients (Laitinen et al., 1992b; Lozano et al., 1995; Vitek and Bakay, 1997).
Shortcomings of the rate model

If the rate model is correct, one would expect that BG-related hypo- and hyperkinetic disorders would show the opposite pathological changes in BG. Indeed, increased indirect pathway activity has been reported in PD patients (Hutchison et al., 1994; Hutchison et al., 1998) and in MPTP-treated monkeys (a model of PD) (Bergman et al., 1994). Increased activity in the direct pathway has also been demonstrated in hyperkinetic pathophysiology such as in dystonia (Vitek, 2002; Starr et al., 2005).

However, a lesion in the Gpi (pallidotomy), which reduces the inhibitory BG output to the thalamus, improves symptoms for both hypokinetic and hyperkinetic disorders (including levodopa-induced dyskinesias in PD) (Marsden and Obeso, 1994a). This is inconsistent with the rate model that holds that the mechanisms underlying hypo- and hyperkinesia are reciprocal. Moreover, recent studies of firing rates in Gpi of patients with various movement disorders fail to confirm the predictions of the rate model (Hutchison et al., 2003; Tang et al., 2005). In addition, several studies in dopamine depleted primates have shown no change in spontaneous pallidal (Boraud et al., 2002), thalamic (Pessiglione et al., 2005) or motor cortical firing rates (Doudet et al., 1990; Watts and Mandir, 1992; Goldberg et al., 2002).

The evolving picture of the basal ganglia anatomy is much more complex than the simplified view of the direct and indirect pathways. As previously mentioned in section 1.2.2, striatal neurons projecting to the Gpi and SNr can also send axon collaterals to the GPe, suggesting that the direct and indirect pathways are not completely segregated (Kawaguchi et al., 1990; Parent et al., 1995; Parent et al., 2000; Wu et al., 2000; Levesque and Parent, 2005b). In addition, the rate model does not take into consideration the direct projections from the motor cortex to the STN (Nambu et al., 2000), the reciprocal connections between the STN and the GPe (Parent and Hazrati, 1995a), the feedback projections from the GPe to the striatum, the feed-forward projections from the cortex to the GABAergic interneurons of the striatum (Bolam et al., 2000), and the output to / input from the PPN (Lavoie and Parent, 1994b). Finally, the rate model does not take into account the direct effect of dopamine on STN and Gpi (Parent and Cossette, 2001).
The rate model is a static model that attempts to explain PD akinesia/bradykinesia in terms of changes in tonic firing rates. However, the model fails to account for the dynamic symptoms of PD, rigidity and resting tremor. Yet, we should keep in mind that despite the limitations discussed above, the rate model has been the predominant and pervading model over the last 20 years, as it shaped general thought on how the basal ganglia function.

1.3.2 - The centre-surround model

The centre-surround model, proposed by Mink (Mink, 1996; Mink, 2003), suggests that the basal ganglia functions through centre-surround mechanisms. Anatomical studies have shown that afferents from the STN arborize more widely and excite a large number of neurons within the GP, while the striatum exerts a more focused inhibition on a subset of these neurons (Hazrati and Parent, 1992a; Hazrati and Parent, 1992b). Based on these observations, the centre-surround model suggests that the striatal input to the GPi and SNr provides a specific, focused, context-dependent inhibition, whereas the input from STN provides a less specific, divergent excitation (see Figure 1.F3). Since the output from GPi/SNr is inhibitory, it is converted into focused facilitation and surround inhibition of motor programs in thalamocortical and brainstem circuits. This organization enables selective facilitation of desired movements while preventing potentially competing movements from interfering with the one selected.

This model is essentially different from the rate model which views the role of the inhibitory and excitatory pathways to GPi/SNr to be the scaling of parameters for the intended movement in a “push-pull” manner. In contrast to the rate model, the scheme proposed in the centre-surround model views the role of the inhibitory input to GPi/SNr to be the selection of the desired motor programs and the role of the excitatory input to GPi/SNr to be the inhibition of competing motor programs with neither being involved in the scaling of specific movement parameters (Mink, 1996).
According to the centre-surround model, abnormally increased GPi/SNr activity in Parkinson’s disease should lead to excessive inhibition of all motor programs and inability to completely turn on those involved in the desired movement. The result would be slowing of movement, decreased amplitude of movement, and in some cases an inability to move at all. In addition to changes in tonic firing rates, abnormal phasic activity of GPi neurons (Tremblay et al., 1989; Bergman et al., 1994) will result in an inability to increase the GPi output appropriately during movement to inhibit competing movements. This would explain the abnormal postures, co-contraction rigidity, and inability to suppress unwanted postural reflexes in PD. Thus, according to the present model, the abnormality in PD is two-fold. First, there is an inability to remove inhibition from desired motor programs and second, there is an inability to fully inhibit competing movements.

*The centre-surround model in relation to Parkinson’s disease*

![Diagram of the centre-surround model](image)

*Figure 1.3. Illustration of the centre-surround model for basal ganglia function. Excitatory (black) and inhibitory (gray) projections are shown. Relative magnitude of neuronal activity is represented by line thickness. GPi: globus pallidus interna; SNr: substantia nigra pars reticulata; STN, subthalamic nucleus. Modified from Mink, Arch Neurol, 2003.*
motor programs (Mink, 1996).

Shortcomings of the centre-surround model

Although the centre-surround model has some appealing features, there is no direct physiological proof of it. The model predicts a positive correlation between the discharge of pallidal neurons that participate in the same action, and a negative correlation between pallidal neurons that participate in competing actions. Physiological studies, however, have failed to reveal such relationships between simultaneously recorded basal ganglia neurons (Jaeger et al., 1994; Nini et al., 1995; Raz et al., 2000; Bar-Gad et al., 2003). Moreover, changes in discharge in pallidal neurons often lag behind movement initiation (DeLong, 1971; Anderson and Horak, 1985; Turner and Anderson, 1997).

1.3.3 - The oscillatory model

Oscillatory activity in the BG has attracted a great deal of interest in the past decade as it is thought to be important in both the normal functioning of the system as well as in the pathophysiology of PD. Findings in PD patients and animal models of PD have suggested that loss of dopamine in the striatum leads to excessive synchronized oscillatory activity in the BG which may underlie the clinical features of PD (Raz et al., 1996; Raz et al., 2000; Nini et al., 1995; Bevan et al., 2000; Boraud et al., 2002; Brown, 2003; Levy et al., 2002b) such as akinesia/bradykinesia (Chen et al., 2007; Kuhn et al., 2006b; Brown, 2006) and limb tremor (Bergman et al., 1998b; Deuschnl et al., 2000; Rivlin-Etzion et al., 2006a; Levy et al., 2000).

Oscillations of single neurons, as well as the synchronized oscillations within populations of neurons as indicated by oscillations in local field potentials (LFPs), have been studied in the GP and STN of PD patients. Recordings in patients withdrawn from their antiparkinsonian medication consistently revealed prominent oscillations between 11 Hz and 30 Hz, so-called the ‘beta band’ (Brown et al., 2001; Levy et al., 2000; Levy et al., 2002b; Kuhn et al., 2005). (This frequency range is broader than that termed beta in clinical studies of scalp electroencephalographic activity.) Pathological oscillatory
activity in the tremor frequency (3-7 Hz) has also been observed in the basal ganglia network after MPTP treatment in some monkeys (Bergman et al., 1994; Bergman et al., 1998b) and in PD patients with tremor at rest (Hutchison et al., 1997; Levy et al., 2002b; Hurtado et al., 1999; Lemstra et al., 1999).

Beta oscillations in LFPs recorded from DBS electrodes implanted in the STN of PD patients were shown to be coherent with cortical electroencephalographic (EEG) activity (Marsden et al., 2001; Williams et al., 2002) and to be intimately related to behavior. The power of beta oscillations decreases with the preparation/execution of voluntary movements (Cassidy et al., 2002; Williams et al., 2005; Kuhn et al., 2004; Amirnovin et al., 2004; Williams et al., 2003; Doyle et al., 2005a; Foffani et al., 2005c; Priori et al., 2002) and following dopamine replacement therapies such as levodopa administration (Brown et al., 2001; Williams et al., 2002; Priori et al., 2004; Doyle et al., 2005a; Levy et al., 2002a). It has been shown that the timing of the movement-related beta desynchronization in LFPs correlates with the timing of voluntary movement (Williams et al., 2005). This, together with the relative lack of beta LFP activity in the STN and GP in healthy animals, led to the hypothesis that beta activity in untreated patients is pathologically exaggerated and might be related to motor impairment. In contrast to beta oscillations, the power of LFP oscillations in the gamma range (35-90 Hz) has been shown to increase following dopaminergic medications and also before and during movement (Cassidy et al., 2002). Moreover, the increase in gamma activity before movement onset is enhanced by levodopa treatment (Androulidakis et al., 2007).

The oscillatory model for basal ganglia action proposed by Brown (Brown, 2003) suggests that basal ganglia activity may be synchronized in multiple frequency bands, each with different functional significance. In this model, beta activity plays an ‘anti-kinetic’ role that originates from the cortex while gamma activity, originating from the BG, is ‘pro-kinetic’ by virtue of its facilitation of motor cortical interaction in the gamma band (Figure 1.F4). This theory resolved the paradox of pallidotomy (an effective procedure for both hypo- and hyperkinetic movement disorders) by suggesting that pallidotomy removes abnormally high BG output in the beta and gamma bands and
therefore ameliorates motor symptoms in both types of disorders.

**Figure 1.F4.** Illustration of the oscillatory model for basal ganglia function. The arrows show the dominating direction of connectivity in each frequency band, anti-kinetic (black) and pro-kinetic (gray). Relative magnitude of connectivity is represented by line thickness. STN, subthalamic nucleus; GP, globus pallidus. Modified from Brown, Mov Disord, 2003.

The directions of connectivity in the different frequency bands, shown in Figure 1.F4, were initially speculated by Brown (2003) according to studies of coherence and phase relationships between basal ganglia and cortex. In this regard, it is important to note that phase estimates may be ambiguous in systems with bidirectional coupling, such as basal ganglia-cortical loops (Cassidy and Brown, 2003). Indeed, later studies in anaesthetized rats (Sharott et al., 2005a) and PD patients (Lalo et al., 2008) have confirmed bidirectional pattern of cortico-basal ganglia communication with a net cortical driving of STN activity at frequencies below 60 Hz. The flow of direction was found to be symmetrical for frequencies between 65 to 90 Hz (Lalo et al., 2008).

*How might prominent synchronization in the beta band impair movement?*

Generally, the information encoded by a correlated network is smaller than the sum of information encoded by its single elements because of the mutual information shared between the neurons (Brown, 2007;Hammond et al., 2007). It is therefore possible that in PD, the exaggerated beta synchronization limits the ability of neurons to code
information in time and space, as both adjacent and spatially distributed neurons are preferentially locked to the beta rhythm (Brown, 2007; Hammond et al., 2007). Only when released from this rhythm, these neurons might more effectively engage in the dynamic assembly formation and rate coding underlying the processing of movement.

Recent recordings in primates confirm an inverse relationship between oscillatory LFP activity in the beta band and focal neuronal task-related activity, so that oscillations are preferentially suppressed in the local area of the striatum showing task related increases in discharge rate (Courtemanche et al., 2003). Parallel observations have been made in the motor cortex, as exemplified by the ‘clamping’ of cortical single unit firing rates to a relatively narrow range during periods of 20-40 Hz oscillatory synchrony (Murthy and Fetz, 1996) and the tendency of firing rate modulation to occur as oscillations decrease in motor cortical LFP (Donoghue et al., 1998). In line with this, a number of studies have demonstrated the capability of synchrony to limit the coding capacity of neuronal populations in the cerebral cortex under certain circumstances (Stevens and Zador, 1998; Salinas and Sejnowski, 2000; Svirskis and Rinzel, 2000; Mazurek and Shadlen, 2002). Thus, it is possible that beta oscillatory activity in the basal ganglia might similarly limit the information coding and therefore impair movement (Brown and Williams, 2005). Indeed, it has been recently shown that STN neurons in PD demonstrate reduced response variability when compared to STN neurons in the normal monkey brain (Gale et al., 2009). Moreover, in dopamine depleted rats, there is a significant decrease in GPe network entropy (Cruz et al., 2009). These data suggest that PD is associated with a reduction in the physiological degrees of freedom of BG neurons with diminished information carrying capacity. Because in PD the baseline level of synchrony is elevated and is relatively resistant to suppression, the “reading” of cortical information can be impaired. The difficulty to initiate movements might therefore be the result of the inability of motor commands for initiation to override the enhanced oscillatory state.

As opposed to beta synchronization, the synchronization in the gamma band may share a similar role to that posited for gamma band synchronization in the visual cortex. Gamma synchronization in the visual cortex is believed to promote precise spike timing of single-
cell responses for information processing, even among spatially remote parts of an assembly (Freiwald et al., 2001). Given the reciprocal relationship between beta and gamma activities in STN LFPs (Fogelson et al., 2005a), and with respect to dopaminergic medications and movement, it is possible that extensive synchronization in the beta band interferes with the involvement of neuronal assemblies in a different pattern of synchronization (i.e. gamma) that is directly involved in information transfer (Brown and Williams, 2005). Indeed, it has been recently shown that elevations of STN gamma LFP activity do not only influence the relative timing of spikes in spike trains (Trottenberg et al., 2006), but also have a major effect on information carrying capacity (Pogosyan et al., 2006). It has been therefore suggested that subthalamic gamma oscillations can increase the information that can be transmitted and decoded by neurons downstream of STN (Foffani and Priori, 2007).

**Shortcomings of the oscillatory model**

Although the oscillatory model is based on physiological observations, it fails to provide physiological mechanisms as for why beta synchronization could be detrimental and gamma synchronization beneficial. One possible explanation can be based on the fact that the properties of oscillatory networks are the result of the physical architecture of the network and the limited speed of neuronal communication (due to axon conduction and synaptic delays) (Buzsaki and Draguhn, 2004). Because the period of oscillations is constrained by the size of the neuronal pool engaged in a given cycle, higher frequency oscillations are confined to a small neuronal space, whereas very large networks can be potentially recruited during slow oscillations. It is therefore possible that the relatively slower beta cycle can be expressed over a larger neuronal space and can thus limit the information carrying capacity of the network. Gamma oscillations, on the other hand, are fast and can only be exerted locally to increase the responsiveness to selective inputs.

Recent studies in animal models of PD present a serious challenge to the oscillatory model. In monkeys that underwent a gradual MPTP-treatment protocol that slowly induces parkinsonism, synchronous oscillatory firing in the GPe occurs after the first appearance of bradykinesia and akinesia (Leblois et al., 2007), indicating that oscillatory
firing does not contribute to early parkinsonism. Similarly, beta oscillations in STN and cerebral cortex were not exaggerated until several days after 6-OHDA injections (Mallet et al., 2008) and the appearance of akinesia (Degos et al., 2008) in rats. Moreover, it has been shown that while motor symptoms (i.e. rigidity and tremor) are improved in PD patients following administration of anticholinergic drugs, beta oscillatory activity is actually increased (Priori et al., 2004).

Considering the rate, centre-surround and oscillatory models, it appears that none of these models is truly exclusive. Another approach to modeling the BG puts forward that the normal dopaminergic system supports segregation of the functional subcircuits within the BG, and that breakdown of this independent processing (by increased synchronization between neurons) could play an important role in the pathophysiology of PD (Bergman et al., 1998a). Indeed, many studies have demonstrated that PD is associated with dramatic loss of spatial selectivity in various regions within the BG-thalamocortical circuit including the STN (Bergman et al., 1994; Abosch et al., 2002), globus pallidus (Filion et al., 1988; Boraud et al., 2000a), thalamus (Pessiglione et al., 2005) and cortex (Goldberg et al., 2002).

Taken together, it is likely that parkinsonism arises from a combination of abnormalities in neuronal activity. Increased firing rate in BG output together with increased oscillatory activity and loss of selectivity can give rise to the pathophysiology of PD.

1.3.4 - Tremor models

Although the critical role of nigrostriatal dopamine depletion in generation of PD symptoms is well accepted, the pathophysiological origin of parkinsonian rest tremor is still unclear. Two possible mechanisms have been proposed so far, the peripheral and central mechanisms (Bergman and Deuschl, 2002; Deuschl et al., 2000).

The peripheral mechanism hypothesis states that parkinsonian tremor results from an unstable long-loop transcortical reflex arc (Oguztoreli and Stein, 1976). Imposing
sinusoidal mechanical movements on the trembling limb can entrain the frequency of parkinsonian tremor with an EMG discharge that is indistinguishable from a reflex response (Rack, 1987; Rack and Ross, 1986) suggesting that peripheral reflexes are important in parkinsonian tremor. Additionally, parkinsonian tremor has been investigated in patients with the joints fixed in a cast (Burne, 1987). This has been shown to reduce tremor amplitude and change tremor frequency and, when the limbs were rigidly fixed, to stop tremor completely. It has been therefore suggested that PD resting tremor may result from oscillatory “flip-flop” inhibitions between antagonist muscles due to reduced imbalance between their spinal stretch reflexes. The supraspinal contribution to the tremor was suggested to result from non-oscillatory descending facilitation of spinal reflex pathways (Burne, 1987).

However, it becomes more and more clear that spinal reflexes play only a minor role for the generation of PD tremor (Bergman and Deuschl, 2002; Deuschl et al., 2000). Early clinical studies have demonstrated that the removal of the dorsal roots in a patient with parkinsonian tremor reduces the tremor amplitude but only slightly changes its frequency and cannot stop the tremor (Pollock and Davis, 1930). Several more recent studies show no frequency reduction after loading of the trembling limb in PD patients (Homberg et al., 1987; Timmer et al., 1993; Timmer et al., 1996). Other series of studies have dealt with the modulation of parkinsonian tremor using different stimuli such as mechanical perturbations (Lee and Stein, 1981; Britton et al., 1992), electrical stimulation of the median nerve (Britton et al., 1993b) or magnetic stimulation of the motor cortex (Britton et al., 1993a; Pascual-Leone et al., 1994). These studies failed to show a consistent resetting of the tremor when stimulating the periphery but a complete resetting when the cortex was stimulated. Thus, although PD tremor may be modulated by peripheral manipulations, it is not likely to be generated by peripheral mechanisms. Furthermore, it has been suggested that peripheral inputs can alter the tremor by modulating central oscillators (Elble et al., 1992).

It seems that the role of central generators is more important for the generation of PD tremor (Bergman and Deuschl, 2002; Deuschl et al., 2000). The central oscillator
hypothesis proposes that tremor is caused by oscillations in central structures in the absence of sensory input. The observation that deafferentation changes the frequency of parkinsonian tremor but does not suppress it (Pollock and Davis, 1930) strongly supports a central origin of PD tremor. In PD patients with tremor, PET analysis demonstrates increased metabolic activity in a network comprising the thalamus, pons, and premotor cortical regions, indicating increases in the functional activity of thalamo-motor cortical projections (Antonini et al., 1998). Consistent with this hypothesis, it has been demonstrated that magnetic brain stimulation over the motor cortex can modulate limb tremor in patients with PD (Britton et al., 1993a; Pascual-Leone et al., 1994).

Studies linking brain and muscle rhythms in PD provide supportive evidence for the role of central mechanisms in tremor. In PD patients, the normal neuromagnetic 10-Hz rhythm is suppressed during periods of tremor (Makela et al., 1993), and 3 to 6 Hz tremor-related magnetic activity can be observed over wide cortical areas (Volkmann et al., 1996). Many studies have revealed a correlation between electrical activity of neurons in the central nervous system and tremor in PD (see section 1.4). These correlation studies, however, cannot prove causal relationship and central nervous system activation could be the result of abnormal feedback from the periphery.

Different lesions within the central nervous system can suppress PD tremor. Lesioning of the motor cortex or the internal capsule have been successful in suppressing tremor but have produced other drastic side effects like paralysis (Putnam, 1940). The thalamus or the zona incerta have been successful targets during stereotactic procedures (HASSLER et al., 1960), and more recently it has been shown that chronic stimulation of these areas and also of the STN and GPi are able to efficiently suppress PD tremor (Benabid et al., 1991; Benabid et al., 1994; Hubble et al., 1997; Koller et al., 1997; Pahwa et al., 1997; Limousin et al., 1998; Plaha et al., 2008; Plaha et al., 2006; Diamond et al., 2007). Interestingly, the Vim is an effective surgical site for treating Parkinson tremor (Benabid et al., 1991; Bakay et al., 1992; Kumar et al., 1999a; Lozano, 2000), although it receives inputs from cerebellum and possibly from ascending spinal tracts, but not from the basal ganglia (Inase and Tanji, 1995). Furthermore, the suppression of parkinsonian tremor
with Vim stimulation is associated with reduced blood flow in the cerebellum (Deiber et al., 1993), suggesting that trans cerebellar pathways are entrained by the Parkinson tremor oscillator. Removal of the cerebellum, however, does not prevent PD tremor (Deuschl et al., 1999).

The motor portion of the GPi projects to ventralis oralis posterior (Vop), which is adjacent to Vim (Inase and Tanji, 1995). Stereotactic destruction of Vop does not suppress tremor but is more effective in the treatment of rigidity and bradykinesia (Bakay et al., 1992). Nevertheless, like the Vim, the Vop contains many neurons that fire in correlation with tremor (Lenz et al., 1994).

It seems that the preservation of some loops within the nervous system is critical for the occurrence of parkinsonian tremor. The question where this abnormal activity is generated is still open. The most likely location for such oscillating neuronal processes is within the basal ganglia loop (Bergman and Deuschl, 2002; Deuschl et al., 2000) (also see section 1.4.5). It is also possible that the origin of tremor oscillations might involve both peripheral and central mechanisms (Oguztoreli and Stein, 1979; Elble et al., 1992; Elble, 1996). Peripheral input might resonate with central structures and affect the strength or phase of oscillation (Lee and Stein, 1981; Rack and Ross, 1986; Lenz et al., 1993).

1.4 - Electrophysiological and behavioral findings in Parkinson’s disease patients and animal models of PD

Over the years, a broad variety of experimental models of PD were developed and applied in diverse species. The most common "classical" toxin-induced PD models are the 6-hydroxy-dopamine (6-OHDA) rat model and the MPTP monkey model. The rat model is produced by injection of the toxin 6-OHDA into the SNc and/or the medial forebrain bundle, which produces a massive lesion of nigral dopaminergic cell bodies within 2-3 days (Blandini et al., 2008). The neuronal damage induced by 6-OHDA is mainly due to the massive oxidative stress caused by the toxin. Being similar to dopamine, 6-OHDA enters the neurons via the dopamine reuptake transporters and
accumulates in the cytosol (where it promotes free radical formation) and mitochondria (where it inhibits the activity of the electron transport chain) (Schober, 2004). The injection of the toxin is commonly carried out unilaterally, with the contralateral hemisphere serving as control. The administration of dopamine agonists (i.e. apomorphine) produces a rotational behaviour contralateral to the lesioned side.

The MPTP-treated non-human primate model of PD is the most reliable model of PD discovered (Burns et al., 1983; Langston et al., 1984; Kopin and Markey, 1988; DeLong, 1990; Bloem et al., 1990). As previously mentioned in section 1.1, administration of MPTP leads to the selective loss of dopaminergic neurons in the SNc since MPTP is metabolized by monoamine oxidase B to the toxin MPP+, which is then taken up by dopaminergic neurons in SNc. The most commonly used administration modes in monkeys are multiple intraperitoneal or intramuscular injections, as well as intracarotid infusions (Petzinger and Langston, 1998). Similar to humans, a decrease of greater than 95% of striatal dopamine in monkeys with SNc neurodegeneration is required to produce parkinsonian motor abnormalities (Elsworth et al., 2000). These parkinsonian monkeys display bilateral limb dystonic postures, rigidity, and bradykinesia. Unilateral intracarotid infusion is also possible and causes mostly symptoms in the contralateral side. These animals circle towards the injured side, whereas treatment with dopaminergic medications induces circling towards the intact side (Bankiewicz et al., 1986).

The MPTP model produces symptoms that closely resemble those found in patients with PD including levodopa-induced dyskinesia and response fluctuations that are commonly observed in the later stages of PD (Clarke et al., 1987; Clarke et al., 1989). However, not all primate species develop similar parkinsonian symptoms. This is true especially with regard to the presentation of rest tremor (Bergman et al., 1998b). Vervet (African green) monkeys develop 3-6 Hz rest tremor that can be aggravated by stress (Bergman et al., 1990) thereby closely resembling that found in patients with PD. Rhesus monkeys, on the other hand, do not develop low frequency resting tremor but instead exhibit 10-16 Hz action/postural tremor (Burns et al., 1983).
Although most research has been done on experimental models of PD, the relatively recent renewal of interest in the surgical treatment of PD (Obeso et al., 1997; Quinn and Bhatia, 1998; Gross et al., 1999) has allowed an access to the human BG, since most surgical teams use microelectrode recordings to determine the exact location of the target. In addition, the advent of non-invasive techniques, such as PET, EEG, magnetic resonance imaging (MRI), magnetoencephalography (MEG), and transcranial magnetic stimulation (TMS), have also contributed to our knowledge of neurophysiology of PD in humans (Berendse and Stam, 2007; Rothwell, 2007; Nandhagopal et al., 2008; Brooks, 2008).

1.4.1 - Single unit recordings and inactivation studies

1.4.1.a - Striatum

A few studies were carried out on striatal activity in animal models of PD. One study has reported a decrease in the firing rate of striatal tonically and phasically active neurons (from ~6 to ~4 spikes/sec) following the development of parkinsonian symptoms in MPTP-treated monkeys (Yoshida et al., 1991). However, more recent reports have shown no change in the firing rate of TANs. Instead, there was an emergence of 3-19 Hz oscillatory activity in TANs that was correlated with neuronal activity in the GPi, and an increase in the number of synchronized pairs of TANs (Raz et al., 1996; Raz et al., 2001). These changes have been proposed to result from enhanced electrotonic coupling between neighbouring striatal neurons in the dopamine-depleted state (Onn and Grace, 1999). In addition, it has been shown that unilateral dopamine depletion in MPTP-treated monkeys substantially reduces the acquired sensory responsiveness of striatal neurons, and this can be reversed by administration of apomorphine (Aosaki et al., 1994).

In freely moving unilateral 6-OHDA-lesioned rats, the firing rates of medium spiny-like neurons is significantly higher on the lesioned side compared to the intact hemisphere or to normal controls (Kish et al., 1999). On the other hand, recent recordings in anesthetized 6-OHDA rats have shown decreased activity in direct pathway MSNs following dopamine depletion, while the activity of indirect pathway MSNs is increased.
(Mallet et al., 2006). It was demonstrated that striatal MSNs become more depolarized after dopamine depletion and fire more frequently in conjunction with cortical inputs (Tseng et al., 2001), suggesting that dopamine loss facilitate the passage of synchronized activity through the basal ganglia by disrupting striatal “filtering” of cortical oscillatory inputs. In addition, the normal arrangement of striatal neurons into clusters that respond to sensory inputs from single body parts appears to be fragmented in the dopamine-depleted state (Cho et al., 2002). Clusters were smaller following 6-OHDA lesion, and more neurons related to single body parts were observed in isolation, outside of clusters. In addition, more body parts were represented per unit volume, suggesting that following dopamine depletion striatal neurons may respond to multiple body parts (Cho et al., 2002). Since the physiological properties of striatal neurons are mediated by corticostriatal inputs (Liles and Updyke, 1985), it is possible that dopamine depletion resulted in a reorganization of corticostriatal connections.

The changes in dopaminergic transmission are known to affect corticostriatal function. Long-term potentiation (LTP) and long-term depression (LTD), the two main forms of synaptic plasticity, are both represented at corticostriatal synapses and strongly depend on the activation of dopamine receptors. It has been shown that in 6-OHDA-lesioned rats, corticostriatal LTP is impaired (Centonze et al., 1999), but can be restored with chronic treatment with levodopa (Picconi et al., 2003). Interestingly, with chronic levodopa treatment, a consistent number of animals develop involuntary movements, resembling human dyskinesias. In these animals, as opposed to non-dyskinetic rats, corticostriatal LTD was lost (Picconi et al., 2003). Thus, the inability of corticostriatal synapses to depotentiate might represent the cellular basis of levodopa-induced dyskinesias, with MSNs being unable to filter the redundant motor information that would normally be eliminated (Picconi et al., 2003;Pisani et al., 2005).

1.4.1.b - Subthalamic nucleus
Several lines of evidence indicate that abnormal neuronal activity of the subthalamic nucleus plays a pivotal role in the pathophysiology of parkinsonian motor symptoms. Recordings from the STN in monkeys have shown that the mean firing rate of STN
neurons increases dramatically (from 15-25 to 25-40 spikes/sec) after MPTP intoxication (Miller and DeLong, 1987; Bergman et al., 1994), and lesion or deep brain stimulation of this structure reverses parkinsonian symptoms in MPTP-treated monkeys (Bergman et al., 1990; Benazzouz et al., 1993; Guridi et al., 1994; Guridi et al., 1996). Since STN activity is increased after local injection of bicuculline (a GABA_A receptor antagonist) and decreased after local injection of muscimol (a GABA_A receptor agonist) (Bergman et al., 1994), the pathological increase in STN firing is thought to result from a decrease in the GABAergic tone arising from the GPe. However, in 6-OHDA treated rats, the increase in the firing rates of STN neurons is not solely dependent on GPe (Hassani et al., 1996) suggesting that excitatory inputs to the STN, such as the corticosubthalamic pathway, play a role in the pathology of the parkinsonian STN. In addition to increased firing rates of STN neurons, there is an increased proportion of neurons responsive to somatosensory stimulation and an increase in movement related excitability (duration and magnitude) after MPTP-treatment (Bergman et al., 1994).

In awake and unmedicated PD patients, the mean firing rates of STN neurons range between 30 and 65 Hz (Hutchison et al., 1998; Levy et al., 2000; Magarinos-Ascione et al., 2000; Magnin et al., 2000; Steigerwald et al., 2008). In has been shown that STN neurons discharge at significantly higher rates (~40 Hz) in PD patients compared to patients with essential tremor (~20 Hz), a movement disorder without any known basal ganglia pathology (Steigerwald et al., 2008). Some studies in PD patients have reported the existence of a somatotopic organization of sensorimotor fields within the STN (Rodriguez-Oroz et al., 2001; Theodosopoulos et al., 2003). Another study, however, has failed to reveal a consistent somatotopic organization, and found that STN neurons respond to more than one joint (Abosch et al., 2002). Therefore, it is possible that in PD the somatotopic organization in STN is somewhat distorted and there is a loss of spatial selectivity (Abosch et al., 2002). In PD patients, about 20% of STN neurons respond to eye movements. The majority of these neurons are located in the ventral STN, while most neurons showing somatic responses are located in the dorsal half of the nucleus (Fawcett et al., 2005).
In both animal models of PD and PD patients, the overactivity in STN is associated with a significant increase in the number of bursting cells and the occurrences of synchronized oscillatory activity among STN neurons (Bergman et al., 1994; Vila et al., 2000; Steigerwald et al., 2008). In part, this may occur because of an enhanced interaction between STN and cortical areas (Magill et al., 2000; Magill et al., 2001), but other pathological phenomena might also be involved. For example, dopamine acts locally to reduce inhibitory synaptic inputs to the STN (Shen and Johnson, 2000; Tofighy et al., 2003; Zhu et al., 2002b; Zhu et al., 2002a), and its absence may enhance the impact of synchronous GABAergic inputs on STN activity, resulting in rebound bursting (Plenz and Kital, 1999; Bevan et al., 2002b; Shen and Johnson, 2005). In parkinsonian monkeys and PD patients, abnormal oscillatory firing typically emerges in two frequency bands. The first is tremor-locked, 3-7 Hz, bursting activity (Bergman et al., 1994; Hutchison et al., 1998; Rodriguez-Oroz et al., 2001; Zhuang and Li, 2003; Amtage et al., 2008). In PD patients, the percentage of STN neurons oscillating at tremor frequencies decreases significantly following the administration of apomorphine, parallel with a reduction in limb tremor (Levy et al., 2001a). In addition to tremor-related oscillations, higher-frequency oscillations in the beta range can be observed in the STN of PD patients and parkinsonian animals (Bergman et al., 1994; Levy et al., 2002b; Moran et al., 2008; Mallet et al., 2008). It is interesting to note that, for reasons yet unknown, the frequency of beta synchronization tends to be higher in parkinsonian rodents and PD patients than in MPTP monkeys (11-30 Hz vs. 8-20 Hz respectively) (Hammond et al., 2007).

Dopamine replacement therapy in PD patients significantly reduces beta oscillatory firing in the STN (Levy et al., 2002a), suggesting a role of oscillations in the pathophysiology of PD symptoms. In support for this hypothesis, it has been shown in patients that stimulation of the STN at 10 Hz induces significant worsening of motor symptoms, especially akinesia (Timmermann et al., 2004). Similarly, stimulation of the subthalamic region at 20 Hz impairs performance in a simple tapping task (Chen et al., 2007). In these studies, however, the worsening effect is often very small compared with the beneficial effect of high frequency stimulation. Interestingly, STN stimulation at currents higher than the amount needed to produce clinical effects induces dyskinesias (Benabid et al.,...
Intentional lesioning of the STN in PD patients was usually avoided because of the possible risk of causing severe dyskinesias or ballism as was observed in normal individuals with lesions of the STN. However, local inactivation of neuronal activity by microinjections of the local anesthetic lidocaine and the GABA\textsubscript{A} agonist muscimol into the STN was shown to ameliorate parkinsonian symptoms in PD patients (Levy et al., 2001b). In addition, several reports have demonstrated good therapeutic effects of subthalamotomy in patients with PD without adverse motor effects (Gill and Heywood, 1997;Barlas et al., 2001;Alvarez et al., 2001). This anti-parkinsonian effect of STN lesions is consistent with the notion of STN hyperactivity in parkinsonism (Miller and DeLong, 1987;Bergman et al., 1994). In parkinsonian monkeys, 2-deoxyglucose metabolic mapping studies indicate that STN hyperactivity is responsible for the excessive GPi activity (Mitchell et al., 1989) (see below). Indeed, STN lesions in parkinsonian animals lead to the reduction of GPi activity (Wichmann et al., 1994b;Guridi et al., 1996;Blandini et al., 1997).

\textbf{1.4.1.c - Globus pallidus}

Most electrophysiological studies carried out in MPTP monkeys have shown that dopamine depletion induces an increase in the spontaneous activity of GPi neurons from 60-80 Hz to 80-100 Hz (Miller and DeLong, 1987;Filion and Tremblay, 1991;Boraud et al., 1996;Boraud et al., 1998;Bezard et al., 1999;Boraud et al., 2000b;Leblois et al., 2006b). A few more recent studies, however, have failed to find a significant increase in GPi firing rates (Wichmann and DeLong, 1999;Raz et al., 2000;Leblois et al., 2007). Electrophysiological recordings in the GPe have also furnished controversial results. While some studies have shown a decrease in firing rate of GPe neurons from 50-70 Hz to 30-50 Hz (Miller and DeLong, 1987;Filion and Tremblay, 1991;Boraud et al., 1998;Raz et al., 2000), others have reported no change (Bezard et al., 1999;Boraud et al., 2001). Recordings from PD patients have reported firing rates of 60-70 Hz in the GPe and 80-90 Hz in the GPi (Hutchison et al., 1994;Hutchison et al., 1997;Lozano et al., 1996). It is important to note that some studies have shown that the average spontaneous
activity of GPi neurons does not differ significantly between patients suffering from PD, dystonia and Huntington’s disease (Hutchison et al., 2003; Tang et al., 2005), suggesting that GPi firing rates do not change after dopamine depletion.

On the other hand, the firing patterns of both GPi and GPe are affected by nigral lesion. In GPe, there is an increase in the number of bursting neurons and the occurrence of pauses. Neurons that normally display low-frequency discharge bursting behavior have more frequent and longer bursts after MPTP intoxication (DeLong et al., 1985; Miller and DeLong, 1987; Filion and Tremblay, 1991; Boraud et al., 1998; Boraud et al., 2001). A similar increase in bursting activity was also observed in the GPi (Filion and Tremblay, 1991; Bergman et al., 1994; Wichmann et al., 1999; Leblois et al., 2006b) and was correlated with the degree of parkinsonism in MPTP monkeys (Filion and Tremblay, 1991). Likewise, 6-OHDA rats show a significant increase in the number of bursting cells both in the globus pallidus (Ni et al., 2000a) and entopeduncular nucleus (Ruskin et al., 2002). A high degree of bursting activity was also found in GPe and GPi neurons in PD patients (Hutchison et al., 1994).

In addition to the increased bursting firing, the proportion of oscillatory cells and of synchronized pairs has been shown to increase dramatically after nigrostriatal lesion in rats (Ruskin et al., 2002) and monkeys (Raz et al., 2000; Leblois et al., 2007). Tremor-locked oscillations of GPi neurons have been reported both in MPTP monkeys (Nini et al., 1995; Bergman et al., 1998b; Raz et al., 2000) and PD patients (Hutchison et al., 1997; Hurtado et al., 1999; Lemstra et al., 1999). Similar to STN, synchronized neuronal oscillations at beta frequencies are also observed in the GPi and GPe (Raz et al., 2000; Levy et al., 2002b; Leblois et al., 2007).

Studies that have compared GP sensorimotor fields in normal and MPTP monkeys show that parkinsonism is associated with a dramatic loss of spatial focal selectivity. In monkeys treated with MPTP, more GP neurons are activated by limb movements, and there is an increase in the duration and magnitude of the response (Filion et al., 1988; Boraud et al., 2000a; Leblois et al., 2007). In addition, the movement responses can
be linked to as many as 9 joints and 4 limbs, compared to only one joint in normal monkeys (DeLong, 1971; Filion et al., 1988; Boraud et al., 2000b; Leblois et al., 2006b). This seems also to be the case in patients with PD (Sterio et al., 1994; Taha et al., 1996). It is interesting to note that lesions of the STN in parkinsonian monkeys decrease the magnitude of GPi responses to somatosensory stimulations (Wichmann et al., 1994b). Furthermore, it has been shown that contrary to the normal situation where GPi responses to voluntary movement occur after the onset of muscle activity (Mink and Thach, 1991a), after MPTP treatment the average response starts 80 ms before the onset of muscle activity (Boraud et al., 2000b). This reversal of temporal relationship between GPi neurons and motor output may be corollary to the enhancement of inhibition exerted by the GPi neurons on their target neurons, with a consequent delay in movement initiation (Leblois et al., 2006b).

Systemic injection of levodopa or dopaminergic agonists brings down the excessive firing rate of GPi neurons by at least 50%, and increases GPe firing by up to 200% in both MPTP monkeys (Filion et al., 1991; Boraud et al., 1998; Boraud et al., 2001; Papa et al., 1999) and PD patients (Hutchinson et al., 1997; Stefani et al., 1997; Merello et al., 1999b; Merello et al., 1999a; Lozano et al., 2000; Levy et al., 2001a). Dopamine agonists also decrease the number of bursting neurons in the pallidum of MPTP monkeys (Filion et al., 1991; Boraud et al., 1998; Boraud et al., 2001) and 6-OHDA rats (Ruskin et al., 2002). Another study, in which bicuculline was injected into the GPe of intact monkeys to elicit dyskinesias, showed that dyskinesias are associated with either an increase or a decrease in the firing rate of GPi neurons, but there was a consistent modification of the firing pattern to long pauses (Matsumura et al., 1995). This suggests that firing patterns in the globus pallidus, rather than firing rates, are linked to dyskinesias.

1.4.1.d - Substantia nigra

Substantia nigra pars reticulate

Following MPTP treatment in monkeys, an increase in firing rates and bursting patterns of SNr neurons is reported (Wichmann et al., 1999). Injections of muscimol into the SNr of MPTP monkeys can reverse parkinsonian symptoms (Wichmann et al., 2001).
suggesting that the SNr is involved in the pathophysiology of PD. In PD patients, although SNr neurons fire at rates comparable to GPi firing rates (i.e. 60-90 Hz), the pattern of SNr discharges is very regular and no oscillatory activity is observed (Hutchison et al., 1998). These differences between humans and monkeys may be due to differences in parkinsonism or in the location of the sampled neurons. Rats with unilateral 6-OHDA lesion show an increase in firing rates and movement-related responses in the ipsilateral SNr (Chang et al., 2006) together with the development of bursting activity (Sanderson et al., 1986; Burbaud et al., 1995). STN lesions have been shown to decrease both firing rates and the occurrence of bursting patterns in the SNr (Burbaud et al., 1995; Tseng et al., 2000). Similar to observations in the GPi, intrastriatal injections of dopaminergic agonists decrease the firing rate and regularize the discharge pattern of SNr neurons (Akkal et al., 1996). Interestingly, it has been shown that after repeated administration of apomorphine in 6-OHDA-lesioned rats, the SNr has an increased percentage of units with bursting activity concurrently with the enhancement of behavioral responses that resemble drug-induced dyskinesias in PD patients (Lee et al., 2001). Very recently, it has been demonstrated that activity-dependent plasticity in the SNr of PD patients is remarkably enhanced with low doses of levodopa and importantly, the level of synaptic plasticity in the SNr negatively correlates with the patients’ “off” UPDRS motor scores (Prescott et al., 2009).

**Substantia nigra pars compacta**

Since the dopaminergic neurons of the SNc degenerate in PD, their activity in the course of the establishment of the disease has not yet been investigated in behaving animals. One study has examined the spontaneous and stimulation-induced overflow of endogenous dopamine from striatal slices prepared from adult rats (Snyder et al., 1990). This study has demonstrated that after lesioning with 6-OHDA, dopamine release from residual terminals is increased relative to control. This increase in dopamine release might serve a compensatory function, maintaining the control over striatal function despite the extensive loss of dopaminergic neurons (Snyder et al., 1990). It is important to note, however, that although the lesion increased dopamine release at moderate stimulation frequencies (2-8 Hz), it had reduced the effective range of frequencies over
which the dopaminergic terminals could operate.

### 1.4.1.e - Changes in basal ganglia-related structures

**Thalamus**

According to predictions of the rate model, the characteristic loss of dopaminergic innervation of the striatum is associated with an excessive tonic inhibition of thalamic motor nuclei that are part of the basal ganglia-thalamocortical circuitry, namely the ventral anterior and ventral lateral nuclei (Miller and DeLong, 1987; DeLong, 1990; Filion et al., 1991; Sterio et al., 1994; Boraud et al., 1998). This is supported by metabolic studies in MPTP-treated monkeys that show a marked increase in the metabolism of the ventral anterior and ventral lateral thalamic nuclei in parkinsonism (Mitchell et al., 1989; Rolland et al., 2007), perhaps reflecting increased basal ganglia input to this area. In PD patients, there is a correlation between increased GPi firing rates and ipsilateral ventral thalamic glucose metabolism (Eidelberg et al., 1997). Indeed, electrophysiological studies in PD patients have demonstrated a decrease of discharge rates in pallidal-receiving areas of the thalamus (Voa and Vop) (Molnar et al., 2005). Nevertheless, studies in MPTP monkeys have failed to show similar results (Pessiglione et al., 2005). There is, however, consistency among studies showing changes in thalamic firing patterns that resemble those found in basal ganglia nuclei. In MPTP-treated monkeys, the incidences of burst discharges and oscillatory firing are increased in the ventral lateral thalamus (Guehl et al., 2003; Pessiglione et al., 2005). A high level of bursting and oscillatory firing in this area was also observed in PD patients (Lenz et al., 1994; Lenz et al., 1988; Zirh et al., 1998; Raeva et al., 1999; Magnin et al., 2000; Molnar et al., 2005). Tremor-related oscillatory neuronal activity has been identified in thalamic nuclei of PD patients during parkinsonian limb tremor (Lenz et al., 1988; Zirh et al., 1998). These tremor-related neurons can also respond to sensory stimulation and voluntary movement (Lenz et al., 1994). In addition, there is an increase in the correlation of the spiking activities of neighboring neurons in the ventral anterior and ventral lateral thalamus of MPTP monkeys, and it has been demonstrated that parkinsonism is associated with reduced specificity of the sensory responses of the neurons in these areas (Pessiglione et al., 2005).
Activity changes were also observed in the CM/Pf nuclei of the thalamus, which participate in circuits by which basal ganglia output is fed back into the putamen. According to the rate model, dopaminergic loss may result in a reduction in CM/Pf activity, which may, in turn, result in reduced driving of MSNs that belong to the ‘direct’ pathway (Sidibe and Smith, 1996). In line with this prediction, there is implicit evidence that GABAergic basal ganglia output to these nuclei is increased in parkinsonism. In 6-OHDA-lesioned rats, GABA_A receptor subunit expression in Pf is decreased (Chadha et al., 2000), and the mean firing rates of Pf neurons is transiently reduced (Ni et al., 2000b). It should be noted here that the CM/Pf thalamus may also be affected by the neurodegenerative process in PD. Studies have shown more than 50% of CM/Pf neurons degenerate in parkinsonian patients and rodents (Xuereb et al., 1991; Henderson et al., 2000; Aymerich et al., 2006).

Interestingly, many of the electrophysiological changes in the thalamus do not seem to be specific to the nuclei that receive inputs from the basal ganglia. It has been shown that burst firing, oscillations and abnormal sensory processing also occur in thalamic areas that receive cerebellar input (i.e. the ventral intermediate nucleus) (Guehl et al., 2003; Pessiglione et al., 2005). Indeed, DBS in the ventral intermediate thalamus can effectively ameliorate parkinsonian tremor (Benabid et al., 1991; Blond et al., 1992; Benabid et al., 1996; Koller et al., 1997; Kumar et al., 1999a), although gait, akinesia, or the activities of daily living cannot be improved (Debievre et al., 1996; Koller et al., 1997).

Cortex
Early metabolic studies suggest that cerebral activation at rest is globally reduced in MPTP-treated monkeys (Schwartzman and Alexander, 1985). Later electrophysiological studies in MPTP-monkeys have reported a reduction in the activation of the motor cortex and the supplementary motor area (Watts and Mandir, 1992), and a loss of the reciprocal pattern of response of movement-related cells (Doudet et al., 1990). Similar to basal ganglia structures, the synchrony between cortical neurons is enhanced following MPTP treatment (Goldberg et al., 2002), and the specificity of receptive fields is markedly
decreased both in the primary motor cortex (Goldberg et al., 2002) and the supplementary motor area (Escola et al., 2002). It has been recently demonstrated that the functional connectivity between the primary motor cortex and the globus pallidus is greatly enhanced after MPTP treatment (Rivlin-Etzion et al., 2008). Microelectrode recordings in the cortex of PD patients are currently not available. Nevertheless, EEG studies in patients have demonstrated that parkinsonism may be associated with abnormal beta-band synchronization of cortical networks (see below, section 1.4.2) and a failure to modulate frontal and central beta-band activity with movement (Brown and Marsden, 1998; Brown, 2003). This is supported by studies showing significant increase in beta oscillatory activity over the sensorimotor cortex in 6-OHDA rats (Sharott et al., 2005b; Mallet et al., 2008).

Imaging studies have shown that cortical activation in PD patients, specifically in the supplementary motor area and the anterior cingulate cortex, is reduced during motor tasks, often with the activation of brain areas that are not activated in non-parkinsonian subjects (e.g. premotor cortex, cerebellum, posterior parietal cortex and occipital lobe) (Jenkins et al., 1992; Playford et al., 1992; Jahanshahi et al., 1995; Samuel et al., 1997; Brooks, 1997; Thobois et al., 2000; Haslinger et al., 2001; Turner et al., 2003). The decreased activation of the supplementary motor area and the anterior cingulate cortex in PD patients can be reversed with apomorphine (Jenkins et al., 1992) and DBS of the STN or the GPi (Limousin et al., 1997; Davis et al., 1997). These studies suggest that dopamine depletion may not only disturb frontal areas of the cerebral cortex that receive input from the basal ganglia (via the thalamus), but may also result in compensatory shifts in activation towards other areas of cortex.

Pedunculopontine nucleus

There is evidence that abnormalities in brainstem regions, specifically the PPN, may be involved in the development of parkinsonism. The PPN is tightly connected to the basal ganglia (Mena-Segovia et al., 2004). Studies in 6-OHDA-lesioned rats have suggested that PPN activity is increased in the dopamine depleted state (Breit et al., 2001), and that a lesion of the PPN can reduce some of the discharge abnormalities in STN and SNr.
On the other hand, in MPTP-monkeys, injection of the GABA receptor antagonist bicuculline into the PPN has been shown to reduce akinesia (Nandi et al., 2002a). Neuronal degeneration in the PPN itself may cause dopamine-resistant parkinsonian deficits, including gait disorders, postural instability and sleep disturbances (Zweig et al., 1989; Pahapill and Lozano, 2000). A detailed review on the PPN and its role in PD is given in the Appendix.

1.4.2 - Local field potential recordings

The physiological basis of local field potentials

Local field potentials (LFPs) are believed to be generated by the sum of numerous overlapping postsynaptic potentials distributed among many cells (ECCLES, 1951). The contribution of action potentials to the LFP is thought to be negligible for a number of reasons. First, postsynaptic potentials can propagate much further in the extracellular space compared to spikes due to the low-pass filter properties of the passive neuron. Secondly, EPSPs and IPSPs have a longer duration than the very brief action potentials, so they have a much higher chance of occurring in a temporally overlapping manner. Finally, EPSPs and IPSPs are displayed by many more neurons than spikes (because not all neurons reach the spike threshold at any given time). Thus, the extracellular field potentials are generated because the slow postsynaptic potentials allow the temporal summation of currents of relatively synchronously activated neurons (Buzsaki, 2006). It should be noted, however, than non-synaptic events such as subthreshold oscillations, calcium spikes and other intrinsic events which produce relatively long-lasting transmembrane events, can also contribute to the local field potentials. The volume of neurons that contribute to the LFP signal depends on the size and placement of the extracellular electrode. With a very fine electrode, the LFP is likely to reflect a weighted average of input signals on the dendrites and cell bodies of tens to thousands of neurons in the vicinity of the electrode (Buzsaki, 2006).

LFPs recorded from the basal ganglia

The emergence of abnormal oscillatory activity in PD occurs not only at the single-cell
level but is also reflected as changes in the LFPs. In patients, LFPs are more readily recorded from the BG than the activity of single neurons because the former recordings can be made not only intra-operatively, but also in the few days between surgical implantation of the DBS electrode and the connection of the electrode to a subcutaneous stimulator a few days later. As mentioned earlier (section 1.3.3), LFP recordings from the GPi and STN of PD patients withdrawn from their anti-parkinsonian medication have consistently revealed prominent oscillations in the 11-30 Hz range (Brown et al., 2001; Marsden et al., 2001; Cassidy et al., 2002; Levy et al., 2002a; Williams et al., 2002; Williams et al., 2002; Williams et al., 2003; Priori et al., 2002; Silberstein et al., 2003; Brown, 2003; Kuhn et al., 2004; Brown and Williams, 2005; Foffani et al., 2005c; Androulidakis et al., 2008a). These oscillatory LFPs are thought to reflect synchronized oscillatory synaptic or neuronal activity that is generated by large populations of local neural elements (Galvan and Wichmann, 2008). This notion has been strongly supported by intra-operative recordings that demonstrate locking of neuronal discharges in the STN to beta oscillatory LFPs (Kuhn et al., 2005). It is important to note that although LFP oscillations are locally generated, they might index synchronization of the whole basal ganglia-cortical loop (Brown and Williams, 2005; Hammond et al., 2007) as beta oscillatory activities in the STN, GPi and cortex are largely coherent (Brown et al., 2001; Williams et al., 2002; Marsden et al., 2001; Cassidy et al., 2002; Sharott et al., 2005b; Fogelson et al., 2005b).

Given the relation between the basal ganglia and cortex, it is not surprising that oscillatory activity in the beta band has also been observed in areas of cortex that are related to the basal ganglia in PD patients and animal models of PD (Marsden et al., 2001; Williams et al., 2002; Goldberg et al., 2002; Fogelson et al., 2005b; Sharott et al., 2005b; Silberstein et al., 2005b; Goldberg et al., 2004; Mallet et al., 2008; Costa et al., 2006). From studies of LFPs and spiking activity in the cortex and basal ganglia of parkinsonian animals (Goldberg et al., 2004; Sharott et al., 2005b; Mallet et al., 2008; Costa et al., 2006), it was concluded that in the parkinsonian conditions, cortex-basal ganglia networks are tightly locked to the beta rhythms that are echoed by the cortex and basal ganglia LFP (Hammond et al., 2007). Global engagement of the BG-
thalamocortical circuits in synchronized oscillatory activity may severely disrupt processing at all levels of the circuitry. This may reflect activities such as movement-related modulation of beta-band synchronization (see section 1.4.3), or functions such as motor planning or sequence learning (Wichmann and DeLong, 2006a). In support of this concept, 10- or 20-Hz stimulation of the subthalamic nucleus of PD patients resulted in a moderate increase in akinesia (Timmermann et al., 2004; Chen et al., 2007).

It has been speculated that DBS may act to desynchronize pathological oscillations in the basal ganglia (Brown et al., 2004). Indeed, it has been recently shown that short-train high-frequency stimulation in the STN of PD patients suppresses subthalamic LFP beta activity and decreases motor cortical-STN coherence in the beta band for up to 25 seconds after the end of stimulation (Wingeier et al., 2006; Kuhn et al., 2008). However, other studies showed that following or during STN-DBS, the power of beta oscillations remained unchanged (Foffani et al., 2006; Rossi et al., 2008), suggesting that beta oscillations in the STN do not necessarily reflect the clinical state. Investigation of the effect of TMS on beta oscillatory activity in patients revealed that stimulation of the motor cortex or SMA reduces beta activity in STN-LFPs (Gaynor et al., 2008).

1.4.3 - Oscillatory activity in relation to voluntary movements and dopaminergic medications

In PD patients and animal models of PD, the oscillatory activity in the beta-band is suppressed by treatment with dopaminergic medications in tandem with clinical improvement (Brown et al., 2001; Levy et al., 2002a; Cassidy et al., 2002; Silberstein et al., 2003; Priori et al., 2004; Sharott et al., 2005b; Kuhn et al., 2006b). Furthermore, the degree of reduction in subthalamic beta oscillatory activity following dopaminergic medications correlates with clinical improvement in bradykinesia and rigidity (Kuhn et al., 2006b). This, together with the little or no beta activity in the STN and GP of healthy animals, is the basis for presuming that beta activity might be of particular importance in the generation of motor deficits in PD. In contrast to beta suppression, gamma activity at 35-90 Hz has been reported in recordings from many PD patients following treatment with
dopaminergic medication (Brown et al., 2001; Cassidy et al., 2002; Williams et al., 2002; Androulidakis et al., 2007). There is also evidence for LFP activity at ~300 Hz in the STN of PD patients especially following treatment with levodopa (Foffani et al., 2003; Foffani et al., 2005b).

Other evidence in PD patients supports the view that beta synchronization is related to motor impairment. One of the earliest behavioral observations was that beta band oscillations picked up in STN and GPi are reduced prior to and during self- and externally-paced voluntary movements (Levy et al., 2002a; Cassidy et al., 2002; Priori et al., 2002; Williams et al., 2003; Kuhn et al., 2004; Doyle et al., 2005a; Devos et al., 2006; Kempf et al., 2007). The suppression of beta oscillation prior to movement is clear evidence that this effect is not simply the consequence of peripheral re-afference (Brown and Williams, 2005). This beta suppression does not seem related to the parkinsonian state per se, as it is also manifest in the striatum of healthy monkey (Courtemanche et al., 2003) and in healthy human putamen (Sochurkova and Rektor, 2003) and cortex (Pfurtscheller and Neuper, 1992; Toro et al., 1994; Leocani et al., 1997; Ohara et al., 2000; Alegre et al., 2002; Doyle et al., 2005b). In contrast to beta oscillatory activity, oscillations in the gamma frequencies significantly increase during voluntary movement (Cassidy et al., 2002; Kempf et al., 2007), and tend to be negatively correlated with the UPDRS motor score in PD patients (Kuhn et al., 2006b). Moreover, the increase in gamma activity before movement onset is enhanced by levodopa treatment (Androulidakis et al., 2007). Thus, synchronization at the gamma frequencies seems to have a prokinetic effect.

LFP recordings from the subthalamic nucleus of PD patients have demonstrated that during predictive trials, where a cue to move (‘go’ signal) was preceded by a warning cue, there was an obvious drop in beta power after the warning signal and an even more marked drop following the ‘go’ signal (Cassidy et al., 2002; Williams et al., 2003; Kuhn et al., 2004). This drop in beta oscillations was followed by a late post-movement increase in beta power. Interestingly, the mean timing of the drop in beta activity following a ‘go’ signal precedes and positively correlates with the mean reaction time across PD patients
(Kuhn et al., 2004) and even across single trials within individual subjects (Williams et al., 2005). In other words, earlier reductions in beta power have been observed in trials with faster motor responses. Furthermore, the power suppression prior to movement was longer in duration when greater amounts of motor processing were required after a ‘go’ cue (Williams et al., 2005). As opposed to predictive trials, in non-predictive trials, where a warning cue was followed by a cue not to move (‘nogo’ signal), the beta suppression following the ‘nogo’ signal was prematurely terminated and reversed into an early beta power increase (Kuhn et al., 2004), suggesting that increased beta activity is needed to inhibit movement. These data suggest that the degree of beta synchronization in the STN may be an important determinant of whether motor programming and movement initiation is favored or suppressed.

A drop in beta power preceding self-paced movements is also evident in STN LFP activity. Such studies demonstrate that the relative degree of pre-movement beta suppression is greater following levodopa treatment in PD patients (Doyle et al., 2005a; Devos et al., 2006). In addition, beta desynchronization and synchronization patterns during self-paced movements were very similar in the STN and primary sensorimotor cortex with post-movement beta synchronization being more prominent over contralateral structures (Devos et al., 2006). It has been shown that post-movement beta synchronization is less intense over the sensorimotor cortex in PD patients (Pfurtscheller et al., 1998), but can be increased with high frequency stimulation of the STN or treatment with dopaminergic medications (Devos et al., 2003; Devos et al., 2006).

Interestingly, desynchronization of beta oscillations in the STN has also been observed during motor imagery in PD patients (Kuhn et al., 2006a). The degree of event-related desynchronization during motor imagery was correlated with the degree of event-related desynchronization in trials of motor execution across patients and, like motor execution, was accompanied by a decrease in cortico-STN coherence. As opposed to desynchronization, event-related beta synchronization was significantly smaller in trials of motor imagery than during motor execution. It was therefore concluded that the beta desynchronization in STN during movement is related to the feedforward organization of
movement and is relatively independent of peripheral feedback. In contrast, sensorimotor feedback is an important factor in the post-movement synchronization occurring in the STN (Kuhn et al., 2006a).

It is important to keep in mind that it is still unknown whether levodopa or DBS treatment improve parkinsonism because they reduce beta activity, or whether the reduction of beta oscillations is simply correlated with, but not causal to, the beneficial motor effect (Galvan and Wichmann, 2008). Recent studies in animal models of PD shed some light on this issue. As mentioned above, monkeys that underwent a gradual MPTP-treatment, show synchronous oscillatory firing in the Gpi only after the first appearance of bradykinesia and akinesia (Leblois et al., 2007), indicating that oscillatory firing does not contribute to early parkinsonism. Similarly, beta oscillations in STN and cerebral cortex were not exaggerated until several days after 6-OHDA injections in rats (Mallet et al., 2008), suggesting that abnormally amplified beta oscillations in cortico-basal ganglia circuits do not result simply from an acute absence of dopamine receptor stimulation, but are delayed consequence of chronic dopamine depletion. This is further supported by the finding that excessive cortical beta synchronization in rats requires a prolonged interruption in dopamine transmission and is detected only several days after 6-OHDA injection and the appearance of akinesia (Degos et al., 2008).

1.4.4 - Oscillatory activity in relation to dyskinesias

Several reports have linked synchronization in the BG at low frequencies (4-10 Hz) with dystonic unwanted movements. Such activity was initially observed in the Gpi of both generalized dystonia patients and medicated PD patients (Silberstein et al., 2003). LFP power in the 11-30 Hz band was decreased and that in the 4-10 Hz band increased in medicated compared with unmedicated PD patients. Moreover, dystonia patients had less 11-30 Hz power and greater 4-10 Hz power compared with unmedicated or medicated PD patients (Silberstein et al., 2003). It therefore seems likely that synchronization in the 4-10 Hz range is associated with the symptoms of dystonia rather than with the disease itself. Indeed, it has been recently shown that 4-10 Hz oscillations in the STN of patients
with PD are increased during levodopa-induced dyskinesias (Alonso-Frech et al., 2006). In contrast, beta oscillations in the GPi of PD patients have been shown to inversely correlate with levodopa-induced dyskinesias in (Silberstein et al., 2005a). Furthermore, it has been shown that low frequency oscillations in the GPi of patients with primary dystonia are particularly associated with the mobile, rather than the postural, components of this condition (Liu et al., 2006). In agreement with this notion, mobile dystonia could be provoked by intra-operative DBS of the STN at 5 Hz (Liu et al., 2002).

It should be noted that a recent study on the relationship between neuronal firing and LFP activity recorded from the G Pi in patients with dystonia has demonstrated that neurons can be synchronized to the LFP activity over 3-12 Hz (Chen et al., 2006b).

1.4.5 - Oscillatory activity in relation to tremor

As mentioned earlier, some experimental studies suggest that parkinsonian tremor is most likely to be caused by abnormal synchronous oscillating neuronal activity within the central nervous system, and peripheral factors play only a minor role in its generation, maintenance, and modulation (Bergman and Deuschl, 2002; Deuschl et al., 2000). However, the source of oscillation in PD tremor is still uncertain. In patients and animal models, tremor-related neurons were found in the STN, G Pi and the thalamus (Lenz et al., 1988; Hutchison et al., 1997; Rodriguez et al., 1998; Bergman et al., 1994; Bergman and Deuschl, 2002; Levy et al., 2000) and either nucleus could be the origin of parkinsonian tremor. They could either be the generators themselves or may be an integral part of an oscillating network.

Studies of the correlation or coherence between tremor and BG oscillations have not been conclusive (Lemstra et al., 1999; Hurtado et al., 1999; Raz et al., 2000; Hurtado et al., 2005). One possible explanation is that PD tremor is generated by multiple segregated circuits, each involving a different limb (Alberts et al., 1965). Indeed, the frequency of tremor is often dissimilar between different sides of the body and it is generally observed that tremulous limbs oscillate independently of each other whereas muscles within a limb
are more likely to be coupled (Hurtado et al., 2000; Raethjen et al., 2000; Ben-Pazi et al., 2001). These observations support the original hypothesis by Alberts et al. (1965), that different oscillators underlie PD tremor in the different extremities. A recent study has further confirmed this hypothesis by showing that tremor-related activity in the globus pallidus can be coupled with one tremulous limb but not with the other (Hurtado et al., 2005).

Tremor-related oscillations, although common in single-unit recordings (Hutchison et al., 1997; Levy et al., 2000; Levy et al., 2002b) are not a consistent or strong feature in LFP signals, probably due to the variable phase relationships between neurons oscillating at tremor frequencies (Lenz et al., 1994; Hurtado et al., 1999; Hurtado et al., 2005). As previously mentioned, oscillatory field potential activity in PD patients ‘off” medications is particularly prominent in the beta band. Beta oscillatory activity in the basal ganglia has been previously associated with the pathology that gives rise to tremor in PD (Levy et al., 2000). However, more recent studies in PD patients failed to find a clear positive correlation between tremor severity and the degree of beta oscillations (Silberstein et al., 2003; Amirnovin et al., 2004; Wang et al., 2005; Kuhn et al., 2006b; Ray et al., 2008).

It has been recently demonstrated that the cortex-BG-periphery loops have low-pass filter properties to microstimulation patterns that contained bursts delivered at different frequencies between 1 to 15 Hz (Rivlin-Etzion et al., 2008). It has been shown that the motor cortex does not follow GP stimulation at frequencies above 5 Hz, and that stimulation at frequencies higher than 5 Hz in the motor cortex did not evoke movement. Thus, it was suggested that parkinsonian tremor is not directly driven by the BG oscillations despite their similar frequencies. Rather, these BG oscillations should be considered as disrupting the normal motor processing (Rivlin-Etzion et al., 2008). In fact, oscillatory neuronal activity can occur without overt tremor (Levy et al., 2000; Wichmann et al., 1999; Raz et al., 2001; Heimer et al., 2002; Soares et al., 2004). It is therefore possible that additional anatomic or physiological factors (poorly defined at this moment) may be necessary for tremor to occur (Wichmann and DeLong, 2006a).
1.5 - **Aim of present studies**

The overall aim of this project was to obtain a better understanding of the characteristics of the oscillatory activity recorded from the basal ganglia of PD patients and to elucidate the significance of this activity in PD. To date, local field potentials recordings from DBS electrodes implanted in the basal ganglia of PD patients are widely used as a tool to infer subthalamic and pallidal function in various different studies and these have given rise to several important claims. Thus, an important question is whether or not the LFPs recorded from DBS electrodes reflect the neuronal firing in the recorded nuclei. By recording neuronal firing and LFP activity with microelectrodes during stereotactic neurosurgery for movement disorders (Chapter 2), we examined the hypothesis that LFP oscillations recorded from the STN of PD patients are locally generated (Chapter 3). In addition, we elucidated to what extent the oscillatory LFPs reflect neuronal firing in a population of local STN neurons. The relationship between LFP activity and neuronal firing was also investigated in the GPi of PD patients (Chapter 5) and compared to that in dystonia patients in order to shed more light on the possible changes in oscillatory patterns and neuronal firing that are involved in Parkinson’s disease.

Behavioral studies in PD patients have suggested that increased beta activity is involved in movement inhibition (see section 1.4.3). This led to the hypothesis that excessive neuronal synchronization in the basal ganglia might contribute to the motor impairment in PD and especially to bradykinesia and rigidity. However, at the time, there was no clear evidence that the degree of synchronization in the beta band is related to the motor deficits in PD. We therefore considered the incidence of oscillatory neurons in the STN in relation to the patients’ motor symptoms and their benefit from dopaminergic medications (Chapter 3) in order to provide further clues into the role of beta oscillations in PD. Another common motor symptom of PD is tremor at rest. Unlike bradykinesia and rigidity, PD tremor does not correlate well with the degree of dopamine deficiency in the striatum and with the progression of the disease, and previous studies regarding the relationship between oscillatory activity in the BG and tremor have yield conflicting results (see section 1.4.5). Since tremor is considered a nonstationary phenomenon,
assessments of the interactions between basal ganglia oscillatory activities and tremor should be obtained by analyzing these signals over time. Thus, we went on to explore the temporal dynamics of oscillatory activity in the STN in relation to PD tremor (Chapter 4) in order to gain a better understanding of the role of STN and oscillatory activity in mediating parkinsonian tremor.
2 - CHAPTER 2 - GENERAL METHODS

This chapter provides a detailed description of the methods used in the studies presented in the thesis. Patients were operated by Dr. Andress Lozano and Dr. Mojgan Hodaie. Clinical assessments of the patients were performed by Dr. Elena Moro and Dr. Anthony Lang. Setup of microelectrode equipment and intraoperative microelectrode recordings were carried out by me in conjunction with Dr. William Hutchisona and Dr. Jonathan Dostrovsky. Some of the MATLAB programs used for data analysis were written by Dr. Neil Mahant. All data analyses were performed by me.

2.1 - Patients and consent

All patients underwent functional stereotactic neurosurgery for the implantation of DBS electrodes into the STN, GPi or PPN. The patients selected for STN surgery were generally PD patients treated for the cardinal signs of PD (i.e. akinesia/bradykinesia, rigidity and occasionally tremor). These patients were sensitive to levodopa and, in most cases, suffered from levodopa-induced dyskinesias. On the other hand, patients selected for DBS in the GPi were generally treated for dystonia, except for rare cases in which PD patients who greatly suffer from dyskinesias were selected for GPi surgery. Patients selected for PPN surgery were either PD patients who suffered from severe gait and postural impairment or patients with progressive supranuclear palsy (PSP) that were unable to walk.

Each patient gave his/her free and informed consent to participate in the study and all procedures were reviewed and approved by the University Health Network Research Ethics Board.

2.2 - Intraoperative neuronal recordings

2.2.1 - Operative procedures
Detailed descriptions of operative procedures are given elsewhere (Hutchison et al., 1994; Lozano et al., 1996; Hutchison et al., 1998). Briefly, on the morning of the surgery, a stereotactic frame (Leksell G, Elekta, Inc, Atlanta, Ga) was affixed to the patients’ heads and preoperative magnetic resonance (MR) images were obtained (Signa, 1.5 T, General Electric, Milwaukee, Wis). Then, a target within the nucleus was chosen using one of the two following methods. In the first method, MRI was used to locate the frame coordinates for the anterior and posterior commissures (AC, PC). These coordinates were then entered into a computer program that shrinks or expands the standard stereotactic human brain atlas map (Schaltenbrand and Wahren, 1977) to fit the patient's AC-PC length and plots the electrode trajectories. In the second method, a target can be chosen directly based on anatomical MRI visualization (Machado et al., 2006). In this method, called direct targeting, triplanar reconstruction of the MR images with the axial series parallel to the AC-PC plane is carried out on a surgical neuronavigation workstation (Mach 4.1, StealthStation, Medtronic, SNT). This method was used in most of the STN/GPi cases and in all the PPN cases.

After the initial target has been determined, the patient is brought to the operating room where one (in unilateral cases) or two (in bilateral cases) burr holes (25 mm) were made in the skull under local anesthesia. Physiological mapping of neuronal activity using microelectrode recordings was then performed through this opening. Microelectrode recording trajectories started 15 or 10 mm above the intended target and extended up to 5 mm below. A manual microdrive was used to drive the microelectrodes down through the brain. All patients were awake during the microelectrode recordings. Microstimulations were performed to elicit sensory or motor effects using an isolated stimulator (Axon system GS3000) which delivered trains of square wave pulses (pulse width 150 μs or 200 μs) at 100 to 300 Hz and up to a maximum of 100 μA. Recordings and stimulation mapping allowed the identification of physiological landmarks.

2.2.2 - Physiological targeting

2.2.2.a - Subthalamic nucleus
The use of microelectrode recordings to localize DBS electrode placement in the STN is described in detail elsewhere (Hutchison et al., 1998). Briefly, parasagittal trajectories were oriented at 12 mm from the midline. A typical microelectrode trajectory starts 10 mm above target and passes through the thalamic reticular nucleus and/or anterior thalamus, zona incerta, STN, and the SNr. Exploration of the neuronal activity was carried out along the entire dorsal/ventral extent of STN. The dorsal border of STN was noted by increase in background activity and high-frequency neuronal discharge. The main markers that were identified in order to localize the motor portion of the STN were neurons with tremor-related activity and neurons that responded to passive or active movements. As the electrodes were advanced past the ventral border of STN the background noise decreased until the electrode reached the SNr, which was characterized by higher-frequency, lower-amplitude and more regular discharges compared with STN. A representative example is shown in Figure 2.F1.

In order to determine the approximate locations of the recordings within the STN (as a function of depth within the nucleus), distances of the recordings to the dorsal border of the nucleus were calculated.
The recording procedures to localize the posteroventral GPi during stereotactic neurosurgery have been previously described (Hutchison et al., 1994; Lozano et al., 1996). Briefly, parasagittal trajectories were oriented at 20 mm from the midline. In addition to identifying GPi cells, the optic tract (located ventral to the GPi) and the internal capsule (located posterior to the GPi) were identified in order to prevent misplacement of the electrodes into these areas, which might result in reduced efficacy or side effects. Microelectrode trajectories usually start 15 mm above target so that the recordings often begin within the GPe. This would then be followed by the internal medullary lamina, which lies between the GPe and GPi. This in an area of white matter and is therefore identified by a significant decrease in overall activity. Occasionally, peripallidal ‘border’ cells, characterized by a highly regular firing pattern and slower rate, are encountered within the lamina and borders of GPi. Entrance into the GPi is marked by an overall

Figure 2.F1. Reconstruction of an electrode track through the subthalamic nucleus (STN) and examples of neuronal recordings. Sagittal 12.0 mm lateral stereotactic map (Scharfenbrand and Wahren, 1977) is shown on the right. The grey band passing through the map represents a typical microelectrode trajectory through thalamus to the STN and into the substantia nigra pars reticulata (SNr). Examples of recording traces (1-sec duration) are shown on the left. Thalamic bursting cells, the zone incerta (ZI) and the SNr are used as anatomical landmarks due to their distinct electrophysiological properties. Rt, thalamic reticular nucleus; Voa and Vop, ventro oralis anterior and posterior nuclei of the thalamus; Vim, ventro intermediate nucleus of the thalamus.

2.2.2.b - Globus pallidus internus

The recording procedures to localize the posteroventral GPi during stereotactic neurosurgery have been previously described (Hutchison et al., 1994; Lozano et al., 1996). Briefly, parasagittal trajectories were oriented at 20 mm from the midline. In addition to identifying GPi cells, the optic tract (located ventral to the GPi) and the internal capsule (located posterior to the GPi) were identified in order to prevent misplacement of the electrodes into these areas, which might result in reduced efficacy or side effects. Microelectrode trajectories usually start 15 mm above target so that the recordings often begin within the GPe. This would then be followed by the internal medullary lamina, which lies between the GPe and GPi. This in an area of white matter and is therefore identified by a significant decrease in overall activity. Occasionally, peripallidal ‘border’ cells, characterized by a highly regular firing pattern and slower rate, are encountered within the lamina and borders of GPi. Entrance into the GPi is marked by an overall
increase in background noise and high frequency discharges. The optic tract could be identified by microstimulation to elicit visual percepts and/or by using a flashing strobe light to elicit a visual evoked response in the axonal activity of the optic tract. Sometimes it can be identified by axonal electrical activity with narrow spikes or a subtle increase in high frequency noise. Another important anatomical landmark is the internal capsule that could be identified by stimulation-evoked tetanic contractions of contralateral muscles. A representative example of a microelectrode trajectory is shown in Figure 2.F2.

Figure 2.F2. Reconstruction of an electrode track through the globus pallidus internus (GPI) and examples of neuronal recordings. Sagittal 20.0 mm lateral stereotactic map (Schaltenbrand and Wahren, 1977) is shown on the right. The gray band passing through the map represents a typical microelectrode trajectory through the globus pallidus externus (GPe) to the GPI and into the optic tract (OT). Examples of recording traces (1-sec duration) are shown on the left. Border cells, the OT and the internal capsule (IC) are served as anatomical landmarks due to their distinct electrophysiological properties.

2.2.2.c - Pedunculopontine nucleus
The PPN as a surgical target for deep brain stimulation was first introduced in 2005 (Plaha and Gill, 2005; Mazzone et al., 2005). Thus, physiological landmarks for the microelectrode mapping of this region have not been yet described. Part of our studies was to characterize the neurophysiology of the human PPN region and identify neurophysiological landmarks that may aid in the localization of this target (see Appendix).
2.2.3 - Microelectrode setup

Microelectrodes were made by inserting a parylene-C coated tungsten microelectrode with an exposed tip length of 15-25 μm (Microprobe Inc.) into a 30-gauge stainless steel tube (HTX-30 tubing; Small Parts Inc., Miami Lakes, FL) that was then covered by Kapton tubing (Micro ML Tubing, #23 polyimide tubing). These parts were glued together with epoxy. Microelectrode tips were plated with gold and platinum to reduce the impedance to about 0.2-1 MΩ at 1 kHz.

A dual microelectrode set-up was employed to allow simultaneous recordings to be made from two microelectrodes (Levy et al., 2007). The two microelectrodes were independently inserted into two adjacent stainless steel guide tubes that were constructed by soldering two 23-gauge, thin walled stainless steel tubes (HTX-23; Small Parts Inc.). These tubes were positioned medial-lateral to one another relative to the patient. The dual inner guide tubes fit easily into the standard stereotactic frame outer guide tube, which, in our setup, was constructed from 17-gauge stainless steel tubing (HTX-17; Small Parts Inc.). The microelectrodes were parallel and were separated by a mediolateral distance of 600 μm. Each electrode was driven into the brain independently by a manually operated hydraulic microdrive. Therefore, it was possible to record from pairs of neurons and/or LFPs separated by about 600 μm mediolaterally and at variable distances axially (but usually at about the same height). A photograph of the dual microdrive head stage assembly is shown in Figure 2.F3.
During surgery, single unit activity and local field potentials were recorded from the microelectrodes. The monopolar recordings from both microelectrodes shared a common ground consisting of the stainless steel guide tube and frame attachments to the head. The amplifier ground was also connected to the frame. Recordings were amplified 5,000-10,000 times and filtered at 10 to 5,000 Hz (analog Butterworth filters: high-pass, one pole; low-pass, two pole; at 5 Hz amplitude decreased by roughly 50%) using two Guideline System GS3000 (Axon Instruments, Union City, CA) amplifiers. During the recordings, signals were monitored on a loudspeaker and displayed on a computer screen. Recorded signals were digitized at 10 kHz and directly stored onto a computer hard drive with a CED 1401 data acquisition system running Spike2 (Cambridge Electronic Design, Neurosurgery 60(4 Suppl 2):277-284.

2.2.4 - Single unit and local field potential recordings

During surgery, single unit activity and local field potentials were recorded from the microelectrodes. The monopolar recordings from both microelectrodes shared a common ground consisting of the stainless steel guide tube and frame attachments to the head. The amplifier ground was also connected to the frame. Recordings were amplified 5,000-10,000 times and filtered at 10 to 5,000 Hz (analog Butterworth filters: high-pass, one pole; low-pass, two pole; at 5 Hz amplitude decreased by roughly 50%) using two Guideline System GS3000 (Axon Instruments, Union City, CA) amplifiers. During the recordings, signals were monitored on a loudspeaker and displayed on a computer screen. Recorded signals were digitized at 10 kHz and directly stored onto a computer hard drive with a CED 1401 data acquisition system running Spike2 (Cambridge Electronic Design, Neurosurgery 60(4 Suppl 2):277-284.)
Cambridge, UK). All neuronal data were recorded simultaneously with wrist flexor/extensor EMG and accelerometers (sampled at 1 kHz) to monitor muscle activity and limb movement, respectively.

2.3 - **Data analysis**

2.3.1 - **Spectral analysis of neuronal discharges and local field potentials**

Only periods without voluntary movements or artifacts were analyzed. Single and multiunit activity was discriminated using the wavemark template-matching tool in Spike2 (Cambridge Electronic Design, Cambridge, UK). Only sites where good unit recordings were obtained were analyzed. Spike times and unfiltered LFP data were imported into MATLAB (version 6.5 or 7.1, The Math Works, Natick, MA) for further analysis.

2.3.1.a - **Power spectrum**

*Local field potentials*

The main statistical tool for analyzing LFP recordings was the discrete Fourier transform and its derivations calculated according to Halliday et al., (1995). After signals were down-sampled to 1 kHz, spectra of LFP power were estimated by dividing the waveform signal into a number of sections of equal duration of 1.024 seconds (1024 data points, 512 point overlap), each section was windowed (Hanning window) and the magnitudes of the 1024 discrete Fourier transform of each section were squared and averaged to form the power spectrum, yielding a frequency resolution of 0.97 Hz. The power was transformed to a logarithmic scale and shown in decibels (dB). Because the estimated power spectrum has a distribution analogous to a $\chi^2$ distribution, the 95% confidence intervals were given on the basis of the $\chi^2$ distribution (Jarvis and Mitra, 2001), whereas degrees of freedom values are based on the number of windowed sections.

In the case of time series, the Fourier transform can be thought of as performing a Fourier decomposition of the sampled waveform into constituent frequency components, which
should highlight any distinct periodic components in the data (Halliday et al., 1995). Since the signal was sampled at 1000 Hz, the Nyquist frequency was 500 Hz.

**Neuronal discharges**

Similarly to the LFP, spectral analysis of spike trains was performed using the Fourier transform according to Halliday et al. (1995). Before the power spectral density was estimated, spike times were converted to a logical function (zeros and ones) where each neuronal spike is stored as logical pulse together with a vector of their relative times. The spectral analysis, in case of point process, can be thought of as performing a correlation between the sinusoids and co-sinusoids of the complex Fourier exponential with the times of occurrence of the events. The presence/absence of particular periodicities in the spike timings will lead to increase/decrease of correlation at the frequency of periodicity from that expected by chance alone, which would highlight periodic components in the discharge (Halliday et al., 1995).

Significant oscillations in the power spectra were detected using shuffling of the interspike intervals (ISIs) of the spike trains (Rivlin-Etzion et al., 2006b). Interspike interval shuffling generates a new spike train by using the time differences between adjacent spikes (first-order ISIs). Thus the spectrum of the new spike train is determined solely by the first-order ISIs of the original spike train, whereas higher-order effects (i.e., the time difference between spikes that are separated by one spike or more) are abolished by the shuffling process. Comparing the original spectrum to the new one enables one to detect patterns such as oscillations that are generated by higher-order ISIs. To obtain an accurate and less-noisy estimate, we repeated the shuffling process 100 times and averaged the results. Subtraction of the new estimated spectrum from the original spectrum resulted in a corrected spectrum, in which peaks were considered significant when they exceeded the upper 95% confidence limit. The confidence limit was estimated from the mean spectrum of the shuffled ISIs based on the $\chi^2$ distribution as described above. Because the the variance for the shuffled ISIs spectrum is the same for all frequencies, the confidence interval depends solely on the degrees of freedom and the term is a constant that does not depend on the frequency.
2.3.1.b - Coherence and cross-correlation

Coherence analyses (Rosenberg et al., 1989; Halliday et al., 1995) were used to evaluate the relationship between simultaneously recorded data from separate electrodes and between LFP and spike data recorded from the same microelectrode. The coherence function provides a frequency domain bounded measure of association, taking on values between 0 and 1, with 0 in the case of independence and 1 in the case of a perfect linear relationship. Coherence can be estimated by direct substitution of the appropriate spectra as: $|f_{xy}|^2/f_{xx}f_{yy}$ with 95% confidence level of $1-(0.05)^{1/(L-1)}$; where $f_{xx}$ and $f_{yy}$ are the autospectra of the component processes, $f_{xy}$ is the cross-spectral density, and $L$ is the number of windowed sections.

It should be noted that since the LFP signals (sampled at 1000 Hz) did not undergo additional low-pass filtering prior to the analyses, they were still likely to contain a bit of information about the neuronal firing recorded from the same microelectrode. This was often noticeable as significant coherence at frequencies above 40 Hz between LFP and spike data that were recorded from the same microelectrode. Thus, significant coherence at higher frequencies between neuronal firing and LFP from the same electrode might be the result of spike contamination. However, in these studies the coherence between neuronal firing and LFP data were only assessed for frequencies up to 30 Hz.

Cross-correlation provides another means of calculating the relationship between simultaneously recorded data as it provides time-domain measure of dependency between random processes. Cross-correlation is defined by the inverse Fourier transform of the cross-spectrum $f_{xy}$, but can also be estimated directly in the time domain. The estimation throughout the frequency domain, however, facilitates the construction of confidence limits (Halliday et al., 1995). To estimate the 95% confidence limits, the variance of the cross-correlation ($\text{var}_{\text{cross-corr}}$) was approximated based on the autospectra of the component processes ($f_{xx}$ and $f_{yy}$). Under the hypothesis that in the case of two independent processes the value of the cross-correlation function is zero, the upper and lower 95% confidence limits for the estimated cross-correlation are given by $0 \pm$
1.96(var_{cross-corr})^{1/2}. In these studies correlation histograms were plotted for delays of 500 ms (1-ms bin width) and their main purpose was to provide as an additional way to evaluate the correlation between signals at specific frequency bands, as LFP data were band-pass filtered prior to the calculation. However, only the coherence estimates were used to determine whether a relationship was significant or not.

It should be noted that phase relationships were not considered in this thesis. Although information about the phase shifts between simultaneously recorded data could have added to our results, we feel that such analyses will not contribute much to our overall conclusions since we only used two microelectrodes that were very close to each other (~1 mm) and the phase shifts are expected to be centered around zero (see Levy et al. 2000).

2.3.1.c - Time frequency analysis

We used the continuous wavelet transform to analyze changes in LFP power in the time-frequency domain (Wang et al., 2005). The wavelet function is convolved with the observed data at each time point and across a range of scales, allowing the identification of specific frequency components (ω) over time (t). In the present study we used the Morlet wavelet function, which is a sine wave (e^{iωt} = cos(ωt) + i sin(ωt)) that is windowed by a Gaussian bell curve (e^{-t^2}) to give a brief oscillation localized in both time and frequency. At higher frequencies, time resolution increases where frequency resolution decreases. A constant, the Morlet parameter, determines the number of sine waves within the wave packet, and higher values give greater frequency resolution at the expense of time resolution. The absolute value of the transform is then squared to give a continuous time-frequency representation of the power content of the signal (Torrence and Compo, 1998).

Wavelet-based coherence was calculated to characterize the temporal relationship between neuronal firing and the simultaneously recorded LFP or tremor. The classical coherence definition was modified utilizing the smoothed Morlet wavelets to yield time-frequency coherence, defined as the ratio of the wavelet cross spectrum to the product of
the wavelet autospectra of the two signals (Gurley et al., 2003). Areas of significant coherence were detected with 95% confidence level of \(1-(0.05)^{1/(df-1)}\), where df is the number of degrees of freedom required in order to detect coherence values above 0.1. The percent time during the overall record when the two signals were significantly coherent with each other for a given frequency band was calculated. A Morlet parameter of 16 was used to investigate the coherence between a tremor-frequency neuron and the simultaneously recorded LFP or between two LFPs, whereas a parameter of 8 was used to investigate coherence between a tremor-frequency firing neuron and limb tremor (which required a better time resolution). These parameters were chosen because they were determined to give the most optimal time and frequency resolution over the frequency range inspected.

### 2.3.2 - Statistical comparisons

All standard statistical tests were performed using SigmaStat software (version 3.1, Systat Software, Richmond, CA). Comparisons of firing rates, power of oscillations, or recording locations (along the microelectrode track) between two groups were subjected to the student t-test if distributions of data were normal; otherwise, Mann-Whitney rank sum tests were performed. Paired t-tests / Wilcoxon signed-rank tests were also used to compare neuronal and LFP measurements in differing circumstances (e.g. without/during tremor). Chi-square comparisons were performed to compare proportions of observations of different categories, but if the sample size was small Fisher’s exact test was used instead. In all statistical tests, a P value less than 0.05 was considered to be a significant difference. Values are expressed in either mean ± standard error (SE) or mean ± standard deviations (SD) as appropriate.
3 - CHAPTER 3 - BETA OSCILLATORY ACTIVITY IN THE SUBTHALAMIC NUCLEUS AND ITS RELATION TO DOPAMINERGIC RESPONSE

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Abstract
Recent studies suggest that beta (15-30 Hz) oscillatory activity in the subthalamic nucleus (STN) is dramatically increased in Parkinson's disease (PD) and may interfere with movement execution. Dopaminergic medications decrease beta activity and deep brain stimulation (DBS) in the STN may alleviate PD symptoms by disrupting this oscillatory activity. Depth recordings from PD patients have demonstrated beta oscillatory neuronal and local field potential (LFP) activity in STN, although its prevalence and relationship to neuronal activity are unclear. In this study, we recorded both LFP and neuronal spike activity from the STN in 14 PD patients during functional neurosurgery. Of 200 single- and multiunit recordings 56 showed significant oscillatory activity at about 26 Hz and 89% of these were coherent with the simultaneously recorded LFP. The incidence of neuronal beta oscillatory activity was significantly higher in the dorsal STN (P = 0.01) and corresponds to the significantly increased LFP beta power recorded in the same region. Of particular interest was a significant positive correlation between the incidence of oscillatory neurons and the patient's benefit from dopaminergic medications, but not with baseline motor deficits off medication. These findings suggest that the degree of neuronal beta oscillatory activity is related to the magnitude of the response of the basal ganglia to dopaminergic agents rather than directly to the motor symptoms of PD. The study also suggests that LFP beta oscillatory activity is generated largely within the dorsal portion of the STN and can produce synchronous oscillatory activity of the local neuronal population.
3.1 - Introduction

Damage to the basal ganglia (BG) is well known to result in a variety of movement disorders highlighting the important although still poorly understood function of these nuclei in the motor system. The loss of nigral dopaminergic input to the striatum in Parkinson's disease (PD) leads to a poverty and slowness of movements, rigidity, and frequently also postural instability and tremor, but the mechanisms underlying these abnormalities remain unclear. Studies in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–treated monkeys and in PD patients suggest that one of the consequences of loss of dopaminergic inputs to the BG is increased synchronized oscillatory activity in the subthalamic nucleus (STN) and globus pallidus interna (GPi) (Bergman et al., 1994; Levy et al., 2000; Levy et al., 2002a; Marceglia et al., 2006; Nini et al., 1995).

Depth recordings of local field potentials (LFPs) in STN of PD patients have demonstrated prominent oscillatory activity in the beta frequency band (15–30 Hz) (Brown et al., 2001; Cassidy et al., 2002; Kuhn et al., 2004; Levy et al., 2002a), which is consonant with cortical EEG activity (Marsden et al., 2001; Williams et al., 2002). The STN beta LFPs are decreased by dopaminergic medication and active movements (Alegre et al., 2005; Alonso-Frech et al., 2006; Cassidy et al., 2002; Doyle et al., 2005a; Foffani et al., 2005c; Kuhn et al., 2004; Levy et al., 2002a; Priori et al., 2004; Priori et al., 2002; Williams et al., 2003). It was previously suggested that the therapeutic effects of deep brain stimulation (DBS) arise from disrupting this pathologically increased synchronized beta activity (Brown et al., 2004; Filali et al., 2004; Jahanshahi et al., 2000; Lozano et al., 2002; Wingeier et al., 2006).

The origin of the beta LFP activity is still poorly understood, although studies by Levy et al. (2000) demonstrated the existence of neurons in STN that fired rhythmically at frequencies within the beta band and many pairs of neurons were shown to fire synchronously in the beta range. Moreover, the STN oscillatory neurons fire in synchrony with the simultaneously recorded beta LFP (Kuhn et al., 2005; Levy et al., 2002a) and the beta LFP activity is maximal in the dorsal part of STN (Kuhn et al., 2005).
To further elucidate the relationship of the STN oscillatory neuronal activity and LFPs and their possible role in mediating the motor symptoms of PD, we simultaneously recorded neuronal and LFP activity from pairs of microelectrodes in the STN of PD patients at rest and off medication. Moreover, investigation of the changes in the neuronal and LFP beta activity with depth along the electrode trajectories, above and within the STN, allowed analysis of the distribution of these activities within the nucleus. Also of interest was whether there is a relationship between the incidence of beta oscillatory cells and the patient's motor impairment and/or the effectiveness of dopaminergic medication in alleviating the patient's parkinsonian symptoms. Thus we examined the relationship between the percentage of STN oscillatory cells and the degree of motor disability on and off dopaminergic medication. Clarification of the relationship between the recorded LFP, local neuronal discharge, and clinical symptoms will provide further insight into the possible roles of these beta rhythms and their relationship to the therapeutic effectiveness of dopaminergic medication.

3.2 - Methods

Patients
We studied 14 patients with advanced PD who were undergoing stereotactic surgery for implantation of bilateral STN DBS electrodes. The group consisted of five women and nine men who, at the time of operation, had a mean age of 57 yr (range 46–68) and a mean duration of PD of 14.1 ± 5.2 (mean ± SD) years. All patients were assessed preoperatively using the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn et al., 1987) before and after an acute levodopa challenge (Moro et al., 2002). During surgery the patients were awake and off dopaminergic medications for at least 12 hours from the last oral dose of antiparkinsonian medications. Demographic details of the patients are given in Table 3.T1.
The electrophysiological mapping procedure used to obtain physiological data for localizing the target for the DBS electrode placement in the STN is described in detail in the General methods (section 2.2.2.a). Extracellular recordings of neuronal firing and LFPs were obtained simultaneously from two independently driven microelectrodes (about 25 µm tip length, axes 600 µm apart, about 0.2-MΩ impedance at 1 kHz) as described in detail in the General Methods (sections 2.2.3/4). All recordings were performed in the resting state and under local anesthesia. Patients were not asked to perform any task and epochs with movements were excluded.

Pairs of simultaneous recordings of neurons and/or LFPs were generally at roughly the same depth. The mean linear distance between the pairs of recording sites was 0.83 ± 0.3 mm (mean ± SD, n = 195). An example of simultaneously recorded LFP and neuronal discharge is shown in Figure 3.F1A.

Data analysis

Single- and multiunit activity was discriminated using the wavemark template matching
tool in Spike2 (Cambridge Electronic Design, Cambridge, UK). Only sites where good unit recordings were obtained were analyzed. At 24% of the sites the action potentials were well discriminated and deemed to be from a single unit. At the remainder of the sites we could not rule out the contribution from an additional one or more units and these were then termed multiunit recordings. However, the difference between the mean (±SD) firing rate of the single units and the multiunit sites was relatively small (39.1 ± 18.6 vs. 49.1 ± 24.5 Hz), suggesting that even in the multiunit cases, most of the discriminated spikes were probably from a single cell. Throughout this chapter the use of the term "cells" refers to both single units and multiunit recording sites. Spike times and unfiltered LFP data were imported into MATLAB (version 6.5, The MathWorks, Natick, MA) for further analysis.

Recordings from 21 sides in 14 patients were analyzed. In seven patients both right and left sides were analyzed; only left STN recordings were analyzed in four patients and right STN in three patients. Only data of ≥17-s duration during periods without movements (based on EMG recordings and observations) or artifacts were analyzed (range: 17–214 s, mean ± SD: 36.5 ± 22.9 s). Recording depths were realigned to the top of STN in each track, where 0 is the dorsal border of STN and negative values are ventral to this border.

Spectral analysis of spike trains and LFPs were performed using the discrete Fourier transform and its derivations calculated according to Halliday et al. (1995) and Rivlin-Etzion et al. (2006). These calculations included power spectra of recorded activity (spike trains and LFPs) as well as coherence and cross-correlations between simultaneously recorded data, as previously described in the General Methods (section 2.3.1.a/b). To estimate the relative LFP beta power according to the distance above or below the top of STN, we identified the frequency of the beta peak of each STN trajectory, and the mean power across a 10-Hz window centered on the peak frequency was calculated at each recording site. LFP power at each recording site was then expressed as the percentage of the maximum power observed in the trajectory. Percentages of maximum power were averaged across subjects to give mean percentage of LFP beta power (Kuhn et al., 2005).
Relative beta power according to depth for neuronal firing was estimated from the original power spectra in the same manner.

Changes in LFP power were evaluated by change-point analysis and control limits (Change-Point Analyzer 2.0 shareware program; Taylor Enterprises, Libertyville, IL). Change-point analysis iteratively uses a combination of cumulative sum (cusum) and bootstrapping to detect changes and is more sensitive to change than control limits, based on plots of serial deviations from the mean (Taylor, 2000). Cusums were determined by plotting the sequentially summed deviation from the averaged power. Significant changes were determined by control limits that give the maximum range over which values are expected to vary (with 95% probability). Application of this technique to measure power changes in the basal ganglia was used in previous studies (Cassidy et al., 2002; Kuhn et al., 2004; Williams et al., 2003).

To examine the relationship between incidence of oscillatory cells and clinical motor symptoms and effectiveness of medications we used part III (motor) of the UPDRS "on" and "off" total scores as well as subscores as evaluated 8.1 ± 7.8 days (mean ± SD) before surgery. The effectiveness of the antiparkinsonian medications was calculated as the difference between the "on" and "off" scores, divided by the "off" score to give the percentage of benefit. Nonlinear regression statistics in addition to linear regression were used to better describe the correlations using SigmaStat (version 3.1, Systat Software, Richmond, CA).

3.3 - Results

3.3.1 - Coherence between neuronal and beta LFP oscillatory activity in the STN

Of 200 single- and multiunit recordings in 14 patients, 56 (28%) displayed significant beta (15–30 Hz) oscillatory activity at 25.8 ± 3.7 Hz (mean ± SD). There was no significant difference in the fraction of oscillatory cells between the single- and the multiunit recordings (31.3% vs. 27%, respectively, \( \chi^2 \) test). However, oscillatory cells had
higher firing rates than the non-oscillatory cells (medians: 52.8 vs. 40.9 Hz respectively, \( P = 0.005 \)). In 50 (89.3\%) of the oscillatory cells, the oscillatory activity was coherent with the LFP recorded from one or both electrodes. Neuronal oscillatory activity at other frequencies was only rarely encountered. We observed significant coherence in the beta range between the LFPs at all the STN sites where LFP activity was recorded from both electrodes, even in cases where no peak in the beta band was observed in the LFP power spectrum. In contrast, the firing of only 17 of the 67 (25.4\%) pairs of cells recorded from the two electrodes was significantly coherent in the beta range. Coherence was present in nine of ten cases where beta oscillations were detected in both cells and in six of 15 cases where beta oscillations were detected in just one cell; the firing activity in two pairs of cells was coherent even though no beta oscillations were detected in either cell. Figure 3.F1B shows an example of coherence plots and correlograms from simultaneous recordings of neuronal and LFP activity where there was significant neuronal and LFP beta activity.

### 3.3.2 - Incidence of neuronal beta oscillatory activity is higher in the dorsal STN

The majority of the beta oscillatory cells was localized in the dorsal STN. The mean depths from the top of STN of the oscillatory (\( n = 56 \)) and non-oscillatory (\( n = 144 \)) cells (\( \pm SD \)) were \(-1.5 \pm 1.1 \) and \(-2.1 \pm 1.3 \) mm, respectively (\( P = 0.001 \), t-test). Figure 3.F2A shows the distributions of the locations of the oscillatory and non-oscillatory cells at successive 0.3-mm intervals. The two distributions are illustrated by box plots in Figure 3.F2B (median locations: \(-1.3 \) and \(-2.0 \) mm for oscillatory and non-oscillatory cells, respectively). Seventy-five percent of the oscillatory cells were found in the dorsal STN, whereas 25\% were in the ventral STN (\( P = 0.01, \chi^2 \) test). On the other hand, there was no significant difference in the incidence of non-oscillatory cells in the dorsal and ventral STN (54\% vs. 46\%, respectively). There was a significant relationship between the dorsal/ventral location and the presence/absence of neuronal beta activity (\( P = 0.003, \chi^2 \) test).

### 3.3.3 - LFP beta oscillations are greatest in the dorsal STN
The power of the beta activity recorded from the pair of microelectrodes in the 14 patients was calculated for 351 sites located from 5 mm dorsal to 5 mm ventral to the dorsal border of STN. Figure 3.F3A plots the changes in the percentage maximum LFP power from each individual patient, and Figure 3.F3B shows the average percentage maximum LFP power for all 14 subjects as a function of depth in 0.5-mm intervals. Note that LFP power exceeds the upper 99% confidence limit (mean + 2.58 SD) for the intervals between –0.5 and –2.5 mm in STN. A similar distribution of the beta LFP power was also observed when recording depths were aligned to the bottom instead of the top of STN. Averaging the percentage of beta LFP power every 2.5 mm reveals significantly greater power in the dorsal part (P < 0.001, Mann–Whitney rank-sum test) (Figure 3.F3C). The power in the ventral STN was significantly higher than the power above STN (P = 0.002). No significant change was observed between the 2 segments above STN. These results were confirmed by control charts and change-point analysis. Analyses were performed on the median LFP beta power in each site (Figure 3.F3D). Two significant changes were detected: the first change was an increase in power in the 0.5-mm interval between –0.5 and –1.0 mm and the second change was a decrease in power at the –2.5- to –3.0-mm interval. A similar analysis of the neuronal oscillatory power according to depth revealed a gradual reduction in the mean beta power from the dorsal to the ventral border of STN (data not shown) resembling that found for LFP power.

The distributions of oscillatory cells and mean LFP beta power by depth within the STN reveal the same pattern of greater beta activity dorsally. Figure 3.F4A shows the variations in mean percentage LFP beta power and percentage of oscillatory cells with depth within the STN in 0.5-mm intervals and the corresponding regression lines (linear regression, $R^2 = 0.63$ and $R^2 = 0.71$, respectively). The relationship between depth within the STN and amount of beta activity is not significantly different for cells and LFPs (t-test for difference in slope, P = 0.23). Figure 3.F4B shows the strong linear correlation between the percentage of oscillatory cells and the mean LFP beta power at different depths within the STN (linear regression, $R^2 = 0.63$).
3.3.4 - Neuronal oscillations correlate with levodopa response

The percentage of beta oscillatory cells in STN (including both sides) was found to vary substantially between patients (see Figure 3.F5), but interestingly was negatively correlated with the "on" drug UPDRS score (Figure 3.F5B) and positively correlated with the magnitude of the preoperative levodopa response (Figure 3.F5C) (nonlinear regression, $R^2 = 0.49$, $P < 0.05$ and $R^2 = 0.62$, $P < 0.005$, respectively; linear regression, $R^2 = 0.37$, $P = 0.02$ and $R^2 = 0.46$, $P < 0.01$, respectively). Similar significant correlations occurred between the percentage of oscillatory cells and medication response on tremor and on the other non-tremor subscores of the UPDRS (data not shown). However, no significant correlation was observed between the percentage of oscillatory cells and the "off" drug UPDRS score (Figure 3.F5a). Moreover, no significant correlations were observed with the different "off" drug UPDRS subscores such as tremor, rigidity, and postural instability. Figure 3.F6 demonstrates the lack of association between the percentage of oscillatory cells and "off" total tremor scores.

We did not use the LFP beta power for these analyses because we did not have a valid way to normalize the LFP power in a way that enables to compare between patients. In each patient, the LFP was recorded in different sites within the STN and its power over the beta frequencies differ according to the location of the microelectrode within the nucleus (see previous section) and also according to the microelectrode impedance.
Figure 3.1. Example of synchronized neuronal and local field potential (LFP) beta oscillatory activity. [A] raw data showing LFP and multiunit neuronal discharge recorded simultaneously from the 2 microelectrodes. Both electrodes were –2.5 mm within the STN. Spikes and LFP activity were derived by high- and low-pass filtering the raw signals at 125 and 100 Hz, respectively. [B] LFP and multiunit neuronal discharge power spectra, obtained from the pair of recording sites, and their corresponding coherence and cross-correlation functions. [a, b] LFP power spectra. Dotted line indicates 95% confidence interval of the estimated spectrum. [c, d] neuronal power spectra. Shaded area indicates 95% confidence interval for the absence of oscillatory activity. [e–j] coherence functions for each combination. Dotted line indicates 95% confidence limit for the absence of coherence. [k–p] cross-correlograms for each combination (LFPs were band-passed filtered between 11- to 35-Hz). Dotted lines indicates the 95% confidence interval. Red vertical line indicates zero time delay.
Figure 3.F2. Distribution of the total number of oscillatory and non-oscillatory cells located within the STN. [A] reconstruction of an electrode track on the sagittal 12.0-mm lateral stereotactic STN map (Schaltenbrand and Wahren, 1977) showing the total number of oscillatory (n = 56) and non-oscillatory (n = 144) cells located within the STN from top to bottom (0 to -5 mm, respectively) in 0.3-mm intervals. [B] box plots of oscillatory and non-oscillatory cells' distribution within the STN. Solid and dashed lines indicate the median and the mean depths, respectively (means ± SD: -1.5 ± 1.1 and -2.1 ± 1.3 mm for oscillatory and non-oscillatory cells, respectively; P = 0.001, t-test). Note the smaller number of observations in the last millimeter of STN attributed to the fact that in many cases the extent of the STN is less than 5 mm.
Figure 3.F3. Distribution of LFP beta power from 5 mm above STN to the bottom of STN (~5 mm). [A] examples of the pattern of variations in the LFP beta power with depth as seen in each patient. Each line represents one microelectrode recording track from one STN side in one patient. Power was expressed as the percentage of the greatest beta LFP power recorded during each track. [B] LFP power was averaged across subjects (n = 14, 21 sides x two electrodes) to give mean percentage maximum beta frequency (±SE) within 0.5-mm intervals. Dashed line indicates the upper 99% confidence limit. [C] bar graph represents the mean percentage LFP power every 2.5 mm. [D] graphical representation of the results of change-point and control chart analysis. Shifts in the shaded background represent the 2 changes in the median percentage LFP beta power according to change-point analysis. Dotted lines indicate 95% control limits. Vertical dashed line represents the top of STN.
Figure 3.F4. Relationship between mean percentage LFP beta power and percentage of beta oscillatory cells distribution within the STN. [A] scatterplot showing the distributions of the mean percentage LFP beta power and the percentage of oscillatory cells every 0.5 mm with their corresponding regression lines. Solid and dashed lines represent linear regression of mean LFP power ($R^2 = 0.63$) and percentage of oscillatory cells ($R^2 = 0.71$) distributions, respectively. There is no significant difference between the 2 distributions (t-test for difference in slope, $P = 0.23$). [B] scatterplot shows strong correlation between the percentage of oscillatory cells and the percentage LFP beta power. Solid line indicates linear regression ($R^2 = 0.63$).
Figure 3.5. Correlation of the percentage of beta oscillatory cells, observed in each patient, with total motor Unified Parkinson's Disease Rating Scale (UPDRS) scores and clinical efficacy of levodopa medication, assessed preoperatively (n = 14). [A] Scatterplot showing no relationship between the amount of oscillatory cells and "off" motor UPDRS scores. [B] A negative relationship was observed with "on" motor UPDRS scores. Solid line indicates exponential decay regression curve (R² = 0.49, P < 0.05). [C] A positive relationship was observed with levodopa response, which was expressed as the percentage of improvement in total UPDRS score after levodopa intake. Solid line indicates logarithmic regression curve (R² = 0.62, P < 0.005).
3.4 - Discussion

This study provides new data documenting the relationship of neuronal beta oscillatory activity to local field potential activity at the recording site and at a distance of about 1 mm. In addition, we describe the distribution of neurons with oscillatory activity within STN. Most interestingly, our data show a significant positive correlation of neuronal oscillatory activity with the patient's benefit from dopaminergic medication. The study also confirmed previous reports that oscillatory LFP activity in the beta range is consistently observed in all PD patients off dopaminergic medications (Alonso-Frech et al., 2006; Brown et al., 2001; Cassidy et al., 2002; Kuhn et al., 2004; Levy et al., 2002a; Marceglia et al., 2006; Priori et al., 2004). Unexpectedly, however, the incidence of cells with oscillatory firing in the beta range varied considerably between patients.

Our results demonstrating coherence between neuronal discharge and LFPs in the beta range confirm and extend the findings of two previous reports (Kuhn et al., 2005; Levy et al., 2002a). The study by Levy et al. reported data from only a single case showing statistically significant coherence between the LFP recorded from a macroelectrode and the discharge of a cell recorded about 1 mm away. The study by Kuhn et al. used spike-triggered averaging (equivalent to cross-correlation) of the LFP recorded from a different
contact at the microelectrode tip <30 µm away in six patients, to show that the activity of some of the neurons was time locked to the beta oscillations in the LFP. Our study is the first to examine the coherence between unit activity and LFPs recorded from both the recording microelectrode and another microelectrode located about 1 mm away, showing that neuronal activity was frequently coherent with beta oscillatory activity recorded ~1 mm away. Furthermore, the firing of 90% of the cells with beta oscillatory activity was coherent with the LFP in the beta range. Interestingly, the LFPs at all pairs of recording sites within STN showed significant coherence in the beta range even in cases where no peak in the beta band was observed in the LFP power spectrum. These observations suggest that the generators of the beta LFP oscillations are distributed and synchronized over a large region (at least several millimeters) of the STN.

The present study confirmed the observation in the Levy et al. (2000) study that pairs of cells in STN can fire rhythmically and synchronously at beta frequencies. In total we found that 25% of the pairs fired coherently at beta frequencies, which is comparable to the 30% in the Levy et al. study. However, the findings of the Levy et al. study suggested that beta oscillatory activity was present primarily in patients with tremor because all but one of the coherent pairs were from the patients with tremor where 47% of the pairs were found to fire coherently. In contrast, the current study, which included a larger and different population of patients, failed to find such a relationship. The tremor patients in the Levy study were identified on the basis of their having tremor during the operation, whereas in our study patients were defined as having tremor on the basis of the preoperatively evaluated UPDRS tremor subscores. However, this is unlikely to have had a major impact on the conclusions of the two studies and we ascribe the difference to the lower number of patients in the Levy study where only three patients were in the non-tremor group.

Unexpectedly, the percentage of cells with beta oscillatory activity varied greatly and ranged from a low of no cells to a high of 90% of STN cells per patient, even though in all cases the local field potentials revealed the presence of beta oscillatory activity. It is possible that the variability in the number of oscillatory cells is a confound resulting from
limited sampling. However, this would imply that only a relatively small part of the STN contains cells firing rhythmically at beta frequencies, which would seem unlikely and inconsistent with the data and conclusions discussed above. A more likely possibility is that STN neuron membrane potential oscillations that generate the beta LFPs are largely confined to the dendrites and do not necessarily strongly influence the probability of the neuron firing. Thus neurons with non-significant beta oscillatory firing may nonetheless contribute to the generation of beta oscillatory LFPs. With increased oscillatory synaptic inputs and/or somatodendritic coupling there would be increased probability and power of oscillatory spike activity. Even in the cases where there was only a small number of oscillatory neurons, their activity could still produce a significant effect on STN target nuclei because a weak correlation among very many neurons could add up to become prominent at the population level (Schneidman et al., 2006).

Because only some of the cells in the dorsal STN fired in synchrony with LFP beta oscillations and in some patients with clear beta LFPs there were only very few oscillatory firing cells, it would appear that the oscillatory firing in STN is not the source of beta LFPs. However, we cannot rule out the possibility that some of the non-synchronous cells did have a weak synchronicity but that the coherence failed to reach significance. Nevertheless, we feel that a more likely source is oscillatory afferent inputs, which are generated by oscillatory firing cells outside of the STN, possibly in cortex. For example, in vivo recordings from the rat STN demonstrate a close correspondence between synchronized neuronal and LFP activity after cortical stimulation (Magill et al., 2004). Phase estimates between the subthalamic area and cortical EEG suggest that cortical inputs drive STN LFP beta oscillatory activity (Fogelson et al., 2006; Marsden et al., 2001; Williams et al., 2002) by two possible routes, either indirectly by the striatum/globus pallidus externus (GPe) or by a direct projection to the subthalamic nucleus (Parent and Hazrati, 1995a). Moreover, previous studies in a rodent model of parkinsonism established that the cerebral cortex can induce pathological patterns of neuronal activity in the STN, perhaps as the result of greater sensitivity of the STN to rhythmic cortical inputs (Magill et al., 2001). It was recently suggested by that feedback GABAergic inhibition from the reciprocally connected GPe can prime STN neurons to
respond more efficiently to excitatory input by increasing the availability of the postsynaptic voltage-dependent Na\(^+\) channels and is critical for the emergence of coherent beta oscillations between the cortex and STN in PD (Baufreton et al., 2005a).

Our analysis of the distribution of local field potential beta oscillations revealed that they are maximal within the dorsal portion of the subthalamic nucleus and confirm similar recent findings by Kuhn et al. (2005). That study, however, did not find a significant difference in power between the dorsolateral and the ventromedial STN, probably as a result of the limited number of recordings in the ventral part (Kuhn et al., 2005). This finding is also supported by our data showing that most of the beta oscillatory cells were observed within the dorsal part of the nucleus. Moreover, we were able to demonstrate a significant positive relationship between the percentage of oscillatory cells and the LFP beta power within the STN. This suggests that there is a close association between the power of the LFP beta oscillations and the neuronal beta oscillations. The LFP is likely to be generated by the postsynaptic potentials produced by rhythmic activity in STN afferents that terminate preferentially on neurons in the dorsal STN. The dorsal region is known to receive inputs from the primary motor cortex in the monkey (Monakow et al., 1978; Nambu et al., 1996) and to contain neurons responsive to passive and active movements (Abosch et al., 2002; DeLong et al., 1985; Rodriguez-Oroz et al., 2001). On the other hand, neurons in the more ventromedial STN are associated with the limbic and associative cortical regions and have no sensorimotor response (Maurice et al., 1998; Parent and Hazrati, 1995a). Thus these results, showing the largest amount of oscillatory cells and the greatest LFP oscillations in the dorsal part, provide further evidence for the association of this region with motor functions and the target for DBS stimulation to alleviate PD motor symptoms.

The oscillatory activity is thought to interfere with initiation and regulation of movements and, if this were the case, one might expect to find a positive correlation between the incidence of oscillatory neurons and the baseline UPDRS score off medication. Interestingly, we did not observe a significant relationship between the "off" UPDRS scores and degree of oscillatory activity. This observation is somehow inconsistent with
the oscillatory model, which holds that exaggerated beta activity plays an anti-kinetic role. The clinical features remaining after the levodopa response are considered "non-dopaminergic" (particularly those involving axial structures) and in part may be generated from pathology in other brain (particularly brain stem) regions that are affected in parallel to the nigrostriatal pathology (Schapira, 2005). This may account for the correlation between the beta oscillatory activity and levodopa response (i.e., dopaminergic features) and not with the “off” motor scores, which combine both dopaminergic and non-dopaminergic features. In this study, however, no correlation with symptom severity was found even when considering each of the motor sub-scores alone (see section 3.3.4).

Dopaminergic medication and active movements are known to decrease STN beta synchronization (Cassidy et al., 2002; Alonso-Frech et al., 2006; Alegre et al., 2005; Brown et al., 2001; Foffani et al., 2005c; Kuhn et al., 2004; Levy et al., 2002a; Marsden et al., 2001; Priori et al., 2002; Priori et al., 2004; Williams et al., 2003) and deep brain stimulation in the STN may alleviate PD symptoms by disrupting this oscillatory activity (Brown et al., 2004; Filali et al., 2004; Jahanshahi et al., 2000; Lozano et al., 2002; Wingeier et al., 2006). It was previously hypothesized that dopamine action on the striatum acts as a filter for cortical input to the STN (Doyle et al., 2005a; Magill et al., 2001; Magill et al., 2004; Sharott et al., 2005b). Thus the increase in number of STN cells with oscillatory beta activity might reflect the degree of nigrostriatal dopamine deficiency. The positive correlation between the magnitude of the levodopa response and the oscillatory activity we observed may be related to the increase in the magnitude of the levodopa response with progression of PD (Zappia et al., 1997). This is consistent with the idea that abolishing the excessive beta activity by levodopa or DBS produces an improvement in parkinsonian symptoms. Our findings are also consistent with the recently reported findings that levodopa-induced reduction in subthalamic LFP power in the 8- to 35-Hz band as recorded postoperatively from DBS electrodes correlates with the simultaneously observed clinical improvement in PD patients (Kuhn et al., 2006b).

However, the fact that severity of the patients' motor symptoms did not relate to the percentage of oscillatory cells suggests that beta oscillatory neuronal activity, alone, may
not reflect the clinical state of the patient and other mechanisms must also be involved in the pathophysiology and the contribution of each to the patient's symptoms can vary. A dissociation between beta oscillatory LFPs and the clinical symptoms was also observed by Priori et al. (2004), who showed that the anticholinergic drug orphenadrine, in contrast to levodopa, increases rather than decreases STN beta oscillations while decreasing tremor and rigidity.

It has been suggested by Bevan et al. (2002b) that both the pattern of inhibitory input from the GPe and the polarization level of STN neurons are crucial in determining whether STN neurons fire in a single-spiking or oscillatory pattern. This implies that neuromodulators, such as dopamine, that influence the membrane potential of STN neurons will have a profound effect on the activity in the GPe–STN network (Bevan et al., 2002b). Based on this, we hypothesize that in the subgroup of patients who have a greater amount of neuronal oscillatory activity, the dopamine can act both by suppressing the overactivity of the indirect pathway at the level of the striatum and by changing the polarization level of STN neurons and blocking the oscillatory firing. In these patients, the STN might be more susceptible to rhythmic cortical inputs and therefore show a better response to levodopa in relation to those patients with smaller numbers of oscillating cells.

It should be noted that some later studies are consistent with our findings. In a recent study, the LFP power over the 8-35 Hz band measured “off” medication positively correlated with the improvements in motor symptoms after dopamine intake. In addition, similarly to our findings, no correlation with baseline motor symptoms was found (Ray et al., 2008). This was true even when considering only dopamine-responsive motor features (i.e. bradykinesia+rigidity). Interestingly, both the baseline power and reduction of 8-35 Hz LFP after dopamine therapy correlated with the degree of improvement in bradykinesia/rigidity but not tremor (Kuhn et al., 2006b; Ray et al., 2008; Kuhn et al., 2009).
4 - CHAPTER 4 - THE RELATIONSHIP BETWEEN SUBTHALAMIC OSCILLATORY ACTIVITY AND TREMOR

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Abstract

Rest tremor is one of the main symptoms in Parkinson's disease (PD), although in contrast to rigidity and akinesia, the severity of the tremor does not correlate well with the degree of dopamine deficiency or the progression of the disease. Studies suggest that akinesia in PD patients is related to abnormal increased beta (15-30 Hz) and decreased gamma (35-90 Hz) synchronous oscillatory activity in the basal ganglia. Here we investigated the dynamics of oscillatory activity in the subthalamic nucleus (STN) during tremor. We used two adjacent microelectrodes to simultaneously record neuronal firing and local field potential (LFP) activity in 9 PD patients who exhibited resting tremor during functional neurosurgery. We found that neurons exhibiting oscillatory activity at tremor-frequency are located in the dorsal region of STN, where neurons with beta oscillatory activity are observed, and that their activity is coherent with LFP oscillations in the beta frequency range. Interestingly, in 85% of the 58 sites examined, the LFP exhibited increased oscillatory activity in the low gamma frequency range (35-55 Hz) during periods with stronger tremor. Furthermore, in 17 of 26 cases where two LFPs were recorded simultaneously, their coherence in the gamma range increased with increased tremor. When averaged across subjects, the ratio of the beta to gamma coherence was significantly lower in periods with stronger tremor compared to periods of no or weak tremor (P = 0.003). These results suggest that resting tremor in PD is associated with an altered balance between beta and gamma oscillations in the motor circuits of STN.
4.1 - **Introduction**

A severe loss of midbrain dopaminergic neurons is the pathological hallmark of Parkinson’s disease (PD). Despite significant advances in our understanding of the anatomy and cellular physiology of the basal ganglia, the casual link between the pathology and the symptoms is still unclear. Tremor at rest is a well recognized cardinal symptom of Parkinson’s disease affecting roughly 70% of PD patients. It is classically defined as a 4-6 Hz tremor, with or without postural/kinetic tremor. Unlike rigidity and akinesia, tremor does not necessarily get worse with disease progression and the severity of tremor does not correlate with dopamine deficiency in the striatum (Deuschl et al., 2000; Deuschl et al., 2001). Post-mortem and imaging studies suggest that the pathophysiology of rest tremor may be distinct from that of rigidity and akinesia (Jellinger, 1999; Pavese et al., 2006). However, the mechanisms underlying parkinsonian tremor remain to be elucidated.

Most recently, hypotheses regarding the pathophysiology of PD have emphasized dynamic changes in the basal ganglia network. Changes in firing patterns and in particular oscillatory activity, in addition to mean firing rates, are considered critical in PD pathophysiology (Bevan et al., 2002b; Brown, 2003; Hammond et al., 2007; Brown, 2006). Studies in monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism suggest that one of the consequences of loss of dopaminergic inputs to the basal ganglia is increased oscillatory firing and synchronization in basal ganglia nuclei, i.e. the subthalamic nucleus (STN) and globus pallidus (Bergman et al., 1994; Heimer et al., 2006; Nini et al., 1995; Raz et al., 2000). Oscillatory neuronal firing at the frequency of tremor and the beta frequency band (15-30 Hz) in these structures has also been observed in PD patients undergoing functional neurosurgery and presumed to be related to the pathophysiological changes (Hurtado et al., 1999; Hutchison et al., 1997; Hutchison et al., 1998; Levy et al., 2000; Levy et al., 2002b; Magnin et al., 2000; Weinberger et al., 2006).

Beta oscillatory activity in the basal ganglia has been previously associated with the
pathology that gives rise to tremor in PD (Levy et al., 2000). However, more recent studies in PD patients failed to find a clear positive correlation between tremor severity and the degree of beta oscillations (Amirnovin et al., 2004;Kuhn et al., 2006b;Ray et al., 2008;Silberstein et al., 2003;Wang et al., 2005;Weinberger et al., 2006). Instead, there is growing evidence suggesting that beta oscillations play an anti-kinetic role and contribute to bradykinesia and rigidity in PD (Brown, 2003;Chen et al., 2007;Kuhn et al., 2006b;Kuhn et al., 2008;Ray et al., 2008). On the other hand, oscillatory firing in the tremor frequency range is frequently coherent with the tremor of a particular limb, although uncorrelated oscillations are commonplace as well (Hurtado et al., 1999;Hurtado et al., 2005;Lemstra et al., 1999;Raz et al., 2000). It was originally suggested by Alberts et al. (1969) that PD tremor is generated by segregated parallel networks, each involving a different limb. This idea was supported by studies showing that different limbs oscillate independently of each other (Ben-Pazi et al., 2001;Hurtado et al., 2000;Raethjen et al., 2000). A recent study has further confirmed this hypothesis by showing that tremor-related activity in the globus pallidus can be coherent with one tremulous limb but not with the other (Hurtado et al., 2005).

In spectral analysis terms, tremor is considered a nonstationary phenomenon since it comes and goes over time (Hurtado et al., 2005). Therefore, assessments of the interactions between basal ganglia oscillatory activities and tremor should be obtained by analyzing these signals over time. One means of studying changes in the pattern of local neuronal synchrony is through a frequency-based analysis of local field potential (LFP) signals. The LFP is believed to reflect synchronized dendritic currents in a group of neurons. Tremor-related oscillations, although common in single-unit recordings (Hutchison et al., 1997;Levy et al., 2000;Levy et al., 2002a;Levy et al., 2002b) are not a consistent or strong feature in LFP signals, probably due to the variable phase relationships between neurons oscillating at tremor frequencies (Hurtado et al., 1999;Hurtado et al., 2005;Levy et al., 2000). Instead, oscillatory field potential activity in PD patients off medications is particularly prominent in the beta band (Brown et al., 2001;Kuhn et al., 2005;Levy et al., 2002a;Weinberger et al., 2006) and, to a lesser extent, in the gamma band (35-90 Hz) (Fogelson et al., 2005a;Pogosyan et al., 2006;Trottenberg
et al., 2006). In contrast to the beta activity, gamma band oscillations in the basal ganglia have been hypothesized to play a pro-kinetic role and to contribute to movement generation (Brown, 2003; Brown and Williams, 2005), as these oscillations are increased during movement and by treatment with dopaminergic medications, in tandem with clinical improvement (Alonso-Frech et al., 2006; Androulidakis et al., 2007; Cassidy et al., 2002; Williams et al., 2002). However, their possible involvement in PD tremor has not been reported.

In order to gain a better understanding of the role of STN and oscillatory activity in mediating parkinsonian tremor, we studied the relationship between tremor and oscillatory activities in the STN, at both gamma and beta frequencies, and how these change with time and in relation to variations in tremor intensity. In addition, we examined the coherence of STN neurons displaying oscillatory firing at the tremor frequencies with the simultaneously recorded limb tremor and how the coherence varied over time. Also, we examined and compared the spatial distribution of these neurons and beta oscillatory neurons along the dorso-ventral STN axis and their relation to the simultaneously recorded LFP.

4.2 - Methods

Patients
We studied 9 patients with advanced PD who exhibited intermittent periods of resting tremor during stereotactic surgery for the implantation of deep brain stimulating electrodes in the STN. The group consisted of 2 women and 7 men who, at the time of operation, had a mean age of 59.5 years (range 55–67) and a mean duration of PD of 10.7 ± 3.7 (mean ± SD) years. All patients were assessed preoperatively using the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn et al., 1987) before and after an acute levodopa challenge (Moro et al., 2002). During surgery the patients were awake and off dopaminergic medications for at least 12 hours from the last oral dose of antiparkinsonian medications. Demographic details of the patients are given in Table 4.T1.
Recordings

Neuronal activity and LFPs were recorded simultaneously from each of two independently driven microelectrodes during the electrophysiological mapping procedure used to obtain physiological data for localizing the STN for the deep brain stimulation electrode placement. The localization procedure of the STN using microelectrode recording is described in detail in the General methods (section 2.2.2.a), as well as the methods for microelectrode recordings of neuronal firing and LFP (sections 2.2.3/4). Limb tremor was measured with EMG and/or triaxial accelerometers (with summated x-y-z signals) that were recorded simultaneously with neuronal activity and were sampled at 1000 Hz. In all cases the tremor was contralateral to the side of the recordings.

Data analysis

Neuronal discharges were discriminated using spike sorting algorithms in Spike2 (Cambridge Electronic Design, Cambridge, UK). Spike times, unfiltered LFP and accelerometer/EMG data were imported into MATLAB (version 7.1, The MathWorks, Natick, MA) for further analysis. Recordings from 13 sides in 9 patients were analyzed. In four patients, both right and left STN were analyzed; only left STN was analyzed in 3 patients and right STN in two patients. Only periods without voluntary movements or artifacts were analyzed. Recording depths were realigned to the top of STN in each track, where 0 is the dorsal border of STN and negative values are ventral to this border.

Table 4.T1: Demographic and clinical characteristics of the patients

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age (years) and gender</th>
<th>Disease duration</th>
<th>Motor UPDRS on/off drugs pre-op</th>
<th>Number of cells sampled</th>
<th>Number of neurons with tremor/beta activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55 M</td>
<td>11</td>
<td>11 / 28</td>
<td>7</td>
<td>1 / -</td>
</tr>
<tr>
<td>2</td>
<td>59 F</td>
<td>14</td>
<td>10 / 52.5</td>
<td>16</td>
<td>5 / 1</td>
</tr>
<tr>
<td>3</td>
<td>65 M</td>
<td>7</td>
<td>19 / 48</td>
<td>20</td>
<td>- / -</td>
</tr>
<tr>
<td>4</td>
<td>59 M</td>
<td>17</td>
<td>19 / 60.5</td>
<td>13</td>
<td>1 / 3 *</td>
</tr>
<tr>
<td>5</td>
<td>58 M</td>
<td>9</td>
<td>6 / 33</td>
<td>14</td>
<td>1 / 5</td>
</tr>
<tr>
<td>6</td>
<td>55 F</td>
<td>10</td>
<td>18 / 41</td>
<td>4</td>
<td>2 / -</td>
</tr>
<tr>
<td>7</td>
<td>67 M</td>
<td>6</td>
<td>14 / 33</td>
<td>11</td>
<td>- / -</td>
</tr>
<tr>
<td>8</td>
<td>55 M</td>
<td>14</td>
<td>4.5 / 39</td>
<td>8</td>
<td>1 / 3 *</td>
</tr>
<tr>
<td>9</td>
<td>63 M</td>
<td>8</td>
<td>17 / 42</td>
<td>9</td>
<td>4 / -</td>
</tr>
</tbody>
</table>

* Including a neuron with both tremor and beta activity
Tremor amplitude was quantified off-line by calculating the root mean square (RMS) value of the sampled accelerometer signal. For frequency domain analysis of spike trains and LFP signals we calculated power spectra and coherence according to Halliday et al. (1995) and Rivlin-Etzion et al. (2006). These techniques are described in the General Methods (sections 2.3.1.a/b). To analyze changes in LFP power in the time-frequency domain, we used the continuous wavelet transform (Morlet wavelet). The wavelet technique has been previously used to analyze LFP signals recorded from the STN (Wang et al., 2005) and is described in the General Methods (section 2.3.1.c). In addition, wavelet-based coherence was calculated to characterize the temporal relationship between neuronal firing and the simultaneously recorded LFP and tremor (see General Methods, section 2.3.1.c). A Morlet parameter of 16 was used to investigate the coherence between a tremor-frequency neuron and the simultaneously recorded LFP or between two LFPs, whereas a parameter of 8 was used to investigate coherence between tremor-frequency neuron and limb tremor. These parameters were determined to give good frequency resolution over the 0.5-35 Hz without sacrificing time resolution. The percent time during the overall record when the two signals were significantly coherent with each other for a given frequency band was calculated.

4.3 - Results

4.3.1 - Incidence of neurons with tremor and/or beta oscillatory firing is higher in the dorsal STN

A total of 102 neurons was recorded from the STN during periods of relatively constant tremor amplitude. The mean (± SD) duration of recordings was 34.6 ± 20.5 sec (range: 14-129 sec). Of these 102 neurons, 15 neurons exhibited significant oscillatory firing at a frequency in the range of parkinsonian tremor (tremor-frequency activity) (mean oscillation frequency ± SD: 4.1 ± 0.6 Hz). In addition, 12 neurons exhibited significant oscillatory firing in the beta frequency range (mean frequency ± SD: 22.1 ± 8 Hz). Two of these neurons had both beta and tremor-frequency activity. Examples of power spectra
of neurons firing at tremor and/or beta frequencies are shown in Figures 4.F1A and 4.F2. There was no significant difference between the mean firing rate of neurons with tremor-frequency activity and neurons with beta activity (mean ± SD: 55.7 ± 30 and 67.8 ± 27 Hz respectively, t-test), however the oscillatory neurons (n = 25) had a significantly higher mean firing rate than non-oscillatory neurons (n = 77) (medians: 60.5 and 36 Hz respectively, P = 0.007, Mann-Whitney rank-sum test). The distribution of the observed neurons among patients is indicated in Table 4.T1.

The majority (13/15) of the neurons with tremor-frequency activity was localized in the dorsal 2.5 mm of STN. This distribution was similar to that of the beta oscillatory neurons. The mean location within the STN (where 0 indicates the dorsal border) of neurons with tremor-frequency activity was not significantly different than that of neurons with beta activity (mean ± SD: –1.3 ± 1.0 and –1.7 ± 1.0 mm respectively, t-test). Figure 4.F1B shows the distributions of the oscillatory and non-oscillatory neurons within the STN at successive 0.5-mm intervals.

4.3.2 - Neurons with tremor-frequency oscillations are coherent with the LFP at the beta frequencies

Seven of the 15 neurons (46.6%) with tremor-frequency oscillatory firing showed significant coherence with the simultaneously recorded LFP in the tremor frequency range (Figure 4.F2A and B). Interestingly however, 13 tremor-frequency neurons (86.6%) showed significant coherence with the LFP in the beta frequency range. Eight of these neurons were coherent with the LFPs recorded from both microelectrodes (including the two neurons with both tremor and beta activity) (Figure 4.F2). The electrophysiological characteristics of the neurons with tremor-frequency activity are summarized in Table 4.T2.
Of the 12 neurons with beta oscillations, 11 (92%) showed significant coherence with the LFP, and 10 of these neurons were coherent with the LFPs from both microelectrodes. It is important to note that the values of coherence, at the beta frequencies, between the tremor-frequency neurons and the LFP were significantly lower than the values of coherence between the beta oscillatory neurons and the LFP (median: 0.17 and 0.35 respectively, \( P \leq 0.001 \), Mann-Whitney rank-sum test, excluding neurons with both tremor and beta activity). In contrast to oscillatory neurons, only 19 out of the 77 non-oscillatory neurons (25%) showed coherent firing with the LFP at the beta frequencies. These neurons might exhibit weak beta oscillatory firing that failed to reach significance but nonetheless had significant coherence when compared to the LFP.

### 4.3.3 - Coherence between neuronal firing and LFP varies over time

To further characterize the coherence between neurons and the simultaneously recorded LFP, we used wavelet-based coherence, which allowed us to estimate the percentage of time during which the two signals were significantly related. Examples of temporal coherence between tremor / beta oscillatory neurons and the simultaneously recorded LFP are shown in Figures 4.F3A and 4.F3B respectively. During these periods, tremor
amplitude was relatively constant. Only 8 out of the 15 tremor-frequency neurons displayed periods of significant coherence with the LFP recorded from one or both microelectrodes at the tremor frequencies. On average, this coherence lasted for 68 ± 23% (mean ± SD) of the time. In addition, 14 tremor-frequency neurons were coherent with the LFP at the beta frequencies for an average of 50 ± 26% of the time. It is important to consider that although more neurons displayed coherence with the LFP at the beta frequencies, there was no significant difference between the durations of coherence in the two frequency bands (P = 0.13, t-test). Beta oscillatory neurons were coherent with the LFP for 77 ± 33% of the time and only one neuron was not coherent with the LFP. At the beta frequencies, beta oscillatory neurons were coherent with the LFP for a significantly longer duration relative to tremor-frequency neurons (P = 0.03, t-test). The data are summarized in Figure 4.F3C. Note that neurons displaying only beta oscillatory activity were not coherent with the LFP at the tremor frequencies.

4.3.4 - Neurons with tremor-frequency oscillations are not constantly correlated with tremor

Of the 15 neurons with tremor-frequency oscillations, only 7 were coherent with the simultaneously recorded tremor (EMG and/or accelerometer) (Table 4.T2, Figure 4.F2). However, the frequency of oscillations of 12 out of the 15 neurons was similar to the frequency of tremor, ranging between 4 to 4.9 Hz. In the remaining three neurons, the frequency of oscillations was 3 Hz and was lower than the frequency of tremor (about 4.8 Hz). Nevertheless, the activity of one of these neurons was coherent with the EMG at 4.8 Hz (see Figure 4.F2C) suggesting that some of its activity was nevertheless related to the tremor.

Since the relationship between tremor-frequency neuronal activity in the STN and tremor is most likely a nonstationary process, we examined this relationship over periods of relatively constant tremor amplitude. Consistent with our conventional coherence analyses, only 7 out of the 15 tremor-frequency neurons were significantly related to the tremor over time. These neurons were coherent with the tremor for 86 ± 23 % (mean ±
SD) of the time. Figure 4.F4A shows an example of temporal coherence between a tremor-frequency neuron and the simultaneously recorded tremor.

Six tremor-frequency neurons were recorded during periods of altering tremor amplitude. Three of these neurons were oscillating significantly only during periods of stronger tremor, whereas 2 neurons were oscillating significantly only when tremor ceased. One neuron was oscillating during both periods of tremor and non-tremor. Figure 4.F4B shows an example of the activity of a tremor-frequency neuron over time. This neuron exhibited significant tremor-frequency oscillations only during episodes of simultaneous limb tremor (although, in this case, there was no significant coherence with the recorded tremor). The tremor-frequency activity could be observed together with significant beta oscillations.

4.3.5 - LFP oscillatory activity in the 35-55 Hz gamma frequency band is increased during tremor

LFP signals during periods with altering tremor amplitude were recorded from 58 sites within the STN (mean recording duration ± SD: 91.1 ± 69.7 sec, range: 34-436 sec). Interestingly, in 49 out of the 58 sites (85%), we observed an increase in the LFP power in the low gamma-frequency range (35-55 Hz) during periods of stronger tremor compared to periods of weak or no tremor. Examples of increased LFP gamma power with increased tremor amplitude are shown in Figures 4.F5 and 4.F6. The majority of these sites (40/49) were located in the dorsal 3.0 millimeters of the STN. In contrast, 7 out of the 9 sites where the LFP gamma power did not increase during periods of stronger tremor were in the ventral 3.0 millimeters. The distributions of sites where gamma activity increased and sites with no increase were significantly different (mean depths ± SD: -2.0 ± 1.4 vs. -3.3 ± 1.5 mm respectively, P = 0.013, t-test). The increase in the LFP power in the low gamma-frequency range was observed in each of the 9 patients (Figure 4.F7A).

In 37 out of the 58 sites, neuronal spiking activity was recorded simultaneously with the
LFP. The mean firing rate of these neurons was significantly higher during periods of stronger tremor amplitude relative to periods of weak/no tremor (mean ± SD: 48.7 ± 27 and 38.3 ± 23.9 Hz respectively, P ≤ 0.001, paired t-test).

The LFP power over the gamma frequencies was then averaged across patients for the periods of weak tremor (mean tremor RMS = 0.14) and periods of stronger tremor (mean tremor RMS = 1.12). On average, the gamma power was significantly higher during the periods of stronger tremor (median power: 0.014 and 0.026 for weak and stronger tremor respectively; n = 58, P ≤ 0.001, Wilcoxon singed rank test) (Figure 4.F7B). Figure 4.F7C shows an example of time frequency analysis revealing increased low gamma activity during tremor.

An increase in the LFP power in the tremor frequencies was also observed, however, we cannot rule out the possibility that this increase is due to tremor-movement related artifacts.

4.3.6 - Altered balance between beta and gamma rhythms during periods of stronger tremor

In addition to the increased power of individual LFPs, the coherence between two LFPs in the low gamma frequency range was also increased during periods of stronger tremor (see Figures 4.F5 and 4.F6: B3, C3). This was observed in 17 (65%) of the 26 sites where the two LFPs were recorded simultaneously. In comparison, the coherence between LFPs in the beta frequency range was increased in 46% of the cases (12/26 sites). When averaging across sites for the periods of weak tremor (mean RMS = 0.13) and stronger tremor (mean RMS = 1.12), we observed that the gamma coherence was significantly increased with increasing tremor (medians: 0.01 and 0.23 for weaker and stronger tremor respectively; n = 26, P ≤ 0.001, Wilcoxon singed rank test). On the other hand, beta coherence did not change significantly (means: 0.38 and 0.33; P = 0.28, paired t-test). Figure 8A shows the relative changes in beta and gamma coherence that were calculated by normalizing the coherence values during periods of strong tremor to the values that
were measured during weak tremor. On average, the coherence in the beta frequencies decreased only by 0.04 ± 0.2, whereas the coherence in the gamma frequencies increased by 0.14 ± 0.2 (mean ± SD). In addition, the averaged ratio of the coherence value in the beta frequencies and the coherence value in the gamma frequencies was significantly lower during periods of strong tremor versus weak tremor (medians: 3.95 and 1.28; P = 0.003, Wilcoxon singed rank test) (Figure 4.F8B).

To provide further support for the relative increase in gamma coherence compared to beta coherence, we used temporal coherence to calculate the percentage of time during which the simultaneously recorded LFPs were coherent with each other. We found that during the periods of stronger tremor, gamma coherence significantly increased in duration and lasted for 58.7 ± 33.5% of the time (mean ± SD) compared to 34.5 ± 32.8% during periods of weaker tremor (P ≤ 0.001, paired t-test). The duration of beta coherence, on the other hand, did not alter significantly (mean ± SD: 83.5 ± 34.2 and 93.1 ± 13.1% for weak and stronger tremor respectively) (Figure 4.F8C). An example of an increase in gamma coherence with increasing tremor amplitude is shown in Figure 4.F8D.
Figure 4F1. [A] Examples of the power spectrum of a neuron with tremor-frequency activity (top), beta activity (middle) and of a neuron with both tremor-frequency and beta activity (bottom, n = 2). Shaded areas indicate 95% confidence interval. [B] Distribution of neurons with tremor-frequency activity (n = 13), beta activity (n = 12) and non-oscillatory activity (n = 79) within the STN from 0 (top of STN) to -6 mm (bottom of STN) within 0.5 mm intervals.
Figure 4.F2. [A-C] Examples of neurons with tremor-frequency activity (top) (shaded areas indicate 95% confidence interval) and their coherence with the LFPs recorded from the two microelectrodes (middle) and with the EMG recorded form the tremulous joint (bottom) (dotted lines indicate 95% confidence limit). These neurons are represented in Table 4.T2 as #3, #14 and #1 respectively.
Figure 4.F3. [A] Wavelet-based coherence (Morlet parameter = 16) showing 58 seconds of temporal relationship between a tremor-frequency neuron (#3 in Table 4.T2) and the simultaneously recorded LFP. Gray boundaries indicate areas of 95% significance level. [B] Wavelet-based coherence showing 65 seconds of temporal relationship between beta oscillatory neuron and the simultaneously recorded LFP. Accelerometer traces in A and B indicate a relatively constant tremor state in patients #2 and #4 respectively. [C] Bar graph showing the mean duration (+SE) of significant coherence with the LFP for tremor and beta oscillatory neurons. Note that each group contains only the neurons that showed significant coherence with the LFP. * P = 0.03 (t-test).
Figure 4.4. [A] Accelerometer trace showing a total of 40 seconds of resting tremor of the contralateral arm in patient #1 (top). Wavelet-based coherence (Morlet parameter = 8) showing the temporal relationship between the accelerometer signal and a simultaneously recorded tremor-frequency neuron (#10 in Table 4T.2) (bottom). Significant coherence is indicated by the gray boundaries. [B] Accelerometer traces showing a total of 53 seconds of intermittent resting tremor of the contralateral arm in patient #8 (top) together with the simultaneously recorded spike trains (middle) and their corresponding power spectra (bottom). The recorded neuron (#11 in Table 4T.2) shows transient periods of tremor and/or beta oscillations over time. Shaded areas indicate 95% confidence interval.
Figure 4.F5. [A] Accelerometer trace showing 70 seconds of intermittent resting tremor of the contralateral arm in patient #3. [B1-3] LFP power spectra of the first 35 sec (RMS = 0.17) obtained from the pair of recording sites, with their corresponding coherence function. [C1-3] LFP power spectra of the last 35 sec (RMS = 0.72), with their corresponding coherence function. Dotted lines indicate 95% confidence limit.

Figure 4.F6. [A] Accelerometer trace showing 68 seconds of intermittent resting tremor of the contralateral arm in patient #4. [B1-3] LFP power spectra of the first 34 sec (RMS = 0.04) obtained from the pair of recording sites, with their corresponding coherence function. [C1-3] LFP power spectra of the last 34 sec (RMS = 2.25), with their corresponding coherence function. Dotted lines indicate 95% confidence limit.
Figure 4.7. [A] Examples of the increase in the LFP gamma power in each patient. Each line represents one microelectrode recording during a period of alternating tremor amplitude from one patient. [B] Box plots of the mean LFP power in the gamma range during the episodes of weak and stronger tremor (medians 0.014 and 0.026 for weaker and stronger tremor respectively; $P \leq 0.001$, Wilcoxon signed rank test, $n = 58$). [C] Accelerometer trace showing 20 seconds of intermittent resting tremor of the right arm (top) with a wavelet spectrogram of the LFP (bottom).
Figure 4.F8. [A] The relative changes (+SE) in beta and gamma coherences between episodes of weak and stronger tremor. The coherence values during episodes of strong tremor (mean RMS = 1.12) were normalized to the values that were measured during weak tremor (mean RMS = 0.13). On average, beta coherence decreased only slightly (by 0.04 ± 0.2, mean ± SD), whereas gamma coherence increased significantly (by 0.14 ± 0.2, mean ± SD). * P ≤ 0.001 (Wilcoxon singed rank test). [B] Box plots of the ratios of the beta to gamma coherence during episodes of weak and stronger tremor (medians: 3.96 and 1.28 respectively; P = 0.003, Wilcoxon singed rank test). [C] Bar graph showing the mean duration (+SE) of significant coherence between the two LFPs in the beta and gamma frequencies during periods of weak and stronger tremor. * P ≤ 0.001 (paired t-test). [D] Accelerometer trace showing 52 seconds of intermittent resting tremor of the left leg in patient #3 (top) with a wavelet-based coherence (Morlet parameter = 16) between the two simultaneously recorded LFPs (bottom). Significant coherence is indicated by the gray boundaries.
4.4 - **Discussion**

This study provides data documenting the relationship of STN neuronal and LFP oscillatory activity to rest tremor in PD. This is the first study to show that STN neurons oscillating at PD tremor frequencies are not only located in the same region as beta oscillatory neurons (in dorsal STN) but are also coherent with the simultaneously recorded local field potentials in the beta frequency band. In addition, we have shown that the oscillatory LFP activity in the low-gamma frequency band is enhanced during periods when patients exhibit tremor at rest.

4.4.1 - **Tremor-frequency neuronal activity: relation to tremor**

In the present study we found that in tremulous PD patients, nearly 15% of STN neurons exhibit significant oscillations at the tremor frequency and that in 80% of the cases, the frequency of oscillations was similar to the frequency of limb tremor suggesting that this oscillatory activity might be tremor-related. However, significant coherence between tremor-frequency oscillation and limb tremor was only observed in 47% of the cases (Table 4.T2). Tremor characteristically varies in location and timing, and practical limitations preclude simultaneous monitoring of all somatic muscles that may be tremulous. Also, in our study in many cases accelerometer signals were used to determine coherence with firing and may not detect low intensity tremors. This may account at least in some of the cases for the lack of correlation between the STN tremor-frequency neuronal activity and the tremor recorded in the sampled muscles. Our observation is consistent with previous reports showing that neuronal oscillations in the globus pallidus are not always coherent with the tremulous limb (Hurtado et al., 1999; Lemstra et al., 1999; Raz et al., 2000), although their oscillation frequency is similar to the frequency of the limb tremor (Hutchison et al., 1997). Several studies of multilimb EMG recordings in PD patients also indicated that tremor in different limbs is largely uncorrelated (Ben-Pazi et al., 2001; Hurtado et al., 2000; Raethjen et al., 2000). These studies, together with the fact that only half of the tremor-frequency neurons fire coherently with the local field potential, support the earlier hypothesis that independent
Oscillatory circuits may underlie parkinsonian tremor in different extremities (Alberts et al., 1969). This idea was further strengthened by Hurtado et al. (2005) who showed that tremor-related activity in the globus pallidus can be coherent with one tremulous limb but not with the other. A multichannel recording method might be able to reveal neuronal sharing and network movement of oscillations amongst different cells.

Interestingly, we found that oscillatory activity in single cells, as well as their coherence with limb tremor, occurs intermittently, and in some cases independently from the fluctuations in tremor amplitude. This finding suggests that tremor oscillatory circuits are not only independent but that their oscillatory activity and synchrony with tremor can fluctuate over time. These findings are similar to those of Hurtado et al. (1999, 2005) who reported transient synchronization between limb tremor and neuronal oscillations in globus pallidus of PD patients. This data suggest that tremor might be a property of the population activity in an oscillatory network, as oppose to a property of the individual cells (Hurtado et al., 2005).

4.4.2 - Tremor-frequency neuronal activity: relation to beta oscillations

We have demonstrated that the vast majority (~87%) of the tremor-frequency neurons are located in the dorsal part of STN where neurons firing with beta oscillatory rhythms are observed. This observation is consistent with an earlier report that 84% of the tremor-related neurons are located in the dorsolateral STN (Rodriguez-Oroz et al., 2001) and with our previous study showing that most of the beta oscillatory neurons are located in the dorsal portion of STN (Weinberger et al., 2006) where also the beta LFP oscillatory activity is maximal (Kuhn et al., 2005; Weinberger et al., 2006). Anatomical studies indicate that the dorsolateral region receives inputs from the primary motor cortex (Monakow et al., 1978; Nambu et al., 1996), whereas inputs from the premotor and supplementary motor areas project mainly to the medial region of the STN (Nambu et al., 1997). The dorsolateral STN contains neurons responsive to passive and active movements in both monkey and human (Abosch et al., 2002; DeLong et al., 1985; Rodriguez-Oroz et al., 2001). In contrast, neurons located in the ventral and medial
region of the STN have relatively weak or no sensorimotor responses and are reciprocally connected with the associative and limbic cortical regions (Maurice et al., 1998; Parent and Hazrati, 1995a). It is therefore not surprising that neurons with tremor-related activity are clustered in the dorsal STN, which is most likely to be the STN region involved in mediating the cardinal motor features of Parkinson's disease. It has been shown that microstimulation in the dorsolateral STN can induce tremor arrest with a short latency of <200 ms (Rodriguez-Oroz et al., 2001), and that local block of this region by lidocaine or muscimol microinjections reduces tremor (Levy et al., 2001b). Our results provide further support for the association of this region with tremor and as a clinically effective target for deep brain stimulation to alleviate PD tremor (Herzog et al., 2004).

Interestingly, apart from the similar distribution of tremor-frequency and beta oscillatory neurons within the STN, our results demonstrate that while only half of the tremor-frequency neurons oscillate coherently with the local field potential at the tremor frequencies, the majority fire in coherence with the LFP at the beta frequencies. This is surprising since only 2/25 showed both tremor and beta oscillatory activity and also in light of the growing evidence for the lack of relationship between beta oscillations and tremor (Amirnovin et al., 2004; Kuhn et al., 2006b; Ray et al., 2008; Silberstein et al., 2003; Wang et al., 2005; Weinberger et al., 2006). Instead, beta oscillations have been suggested to contribute to bradykinesia and rigidity (Brown, 2003; Chen et al., 2007; Kuhn et al., 2006b; Kuhn et al., 2008; Ray et al., 2008). The low incidence of neurons with both tremor and beta oscillation could indicate the existence of two separate but interdigitated populations of neurons with different functions and connectivity within the same region. However, the fact that tremor-activity neurons are coherent with LFP beta oscillatory activity suggests the opposite – i.e. that most if not all of the neurons in the dorsal region receive oscillatory inputs in both frequencies. The tremor-frequency neurons might therefore exhibit weak beta oscillations that failed to reach significance. The coherence analysis used in this study is more sensitive and reveals activity that may not reach significance in the power spectrum.

In light of these results, we hypothesize that those neurons in STN that are likely to
oscillate at tremor frequencies, are also those prone to oscillate at beta frequencies. In other words, the same STN neuron that oscillates at tremor frequencies during tremulous behavior can oscillate at beta frequencies during akinetic/rigid behavior. When examining the distribution of the neurons among our tremulous group of patients, it can be observed that tremor oscillatory activity was detected in most patients (7 out of 9), whereas beta oscillatory neurons were only observed in 4 patients (see Table 4.T1). It is possible that in tremulous patients, neurons in the dorsal STN are more likely to oscillate at the tremor frequencies since cortical oscillations at these same frequencies are also increased and this region receives direct projections from the corresponding cortical regions (Hellwig et al., 2000). In our previous study (Weinberger et al., 2006) we found that 28% of the neurons in STN display significant beta oscillatory activity (also see Chapter 3). On the other hand, in this study, which included a different population of patients, only 12% of the neurons were oscillating at beta frequencies. However, overall 24.5% of the neurons were oscillatory at either tremor or beta frequencies. These neurons are perhaps those which, in the dopamine depleted state, become more sensitive to rhythmic cortical inputs (Bevan et al., 2007; Magill et al., 2001; Paz et al., 2005; Sharott et al., 2005b). Supporting this hypothesis and consistent with previous finding by Levy et al. (2000), there was no significant difference between the mean firing rate of neurons with tremor activity and neurons with beta activity. These neurons fire at a faster rate relative to the non-oscillatory neurons. The higher mean firing rates of the oscillatory neurons is perhaps due to a greater efficacy of the excitatory cortico-subthalamic inputs on those neurons following dopamine depletion (Bevan et al., 2007; Magill et al., 2001). The chronic loss of dopamine in the STN (in addition to its loss in the striatum) might further promote the capability of the cortex to drive rhythmic STN activity through actions both on the excitability of STN neurons and on the activity-dependent plasticity at synapses in the STN (Bevan et al., 2006).

4.4.3 - Low-gamma LFP oscillatory activity: relation to tremor

Increased LFP power in the low gamma frequency range (35-55 Hz) was observed during periods of stronger tremor. We have shown that the increase in gamma activity during
tremor is most likely to occur at sites in the dorsal STN suggesting it is related to sensorimotor processing. It is yet to be elucidated, however, whether this increase is associated with the mechanism of generation of resting tremor in PD patients, or is simply a result of increased motor drive to, or output from, STN during tremor. In addition to the increased low gamma power at individual sites within STN, we also observed increased coherence between nearby sites, suggesting that the low gamma LFP oscillations are distributed and synchronized over at least a few millimeters within the STN. It has been previously demonstrated that subthalamic gamma LFP oscillations recorded from PD patients during rest are greater within the upper STN and bordering zona incerta, where they are consistently synchronized to neuronal discharge and might therefore reflect synchronized population activity of local neurons (Trottenberg et al., 2006).

In our study, the mean firing rate of local neurons was significantly higher during stronger tremor, suggesting that the elevated gamma LFP activity is associated with changes in STN neuronal firing rates during tremor. Indeed, it has been recently shown that elevations of STN gamma LFP activity do not only influence the relative timing of spikes in spike trains (Trottenberg et al., 2006), but also have a major effect on information carrying capacity (Pogosyan et al., 2006). It has been argued that STN gamma oscillations can increase the information that can be transmitted and decoded by neurons downstream of STN (Foffani and Priori, 2007). Thus, exaggerated increase in overall information flow in downstream neurons may indirectly contribute to the generation of rest tremor in PD. In line with our findings, it has been recently shown that deep brain stimulation in the zona incerta, where gamma oscillations are maximal (Trottenberg et al., 2006), improves PD resting tremor by 95% (Plaha et al., 2008). Moreover, an earlier study showed that electrical stimulation of primary motor cortex during neurosurgery at a frequency of 60 Hz evoked 5 Hz tremor, whereas 1-8 Hz stimulation resulted in movements of the same frequency as the stimulation (Alberts, 1972), suggesting that abnormal high frequency synchronization may indirectly cause tremulous movements.
We found that the ratio of the beta to gamma coherence was significantly lower during stronger tremor, suggesting that alteration of the balance between beta and gamma synchronization in the STN is related to tremor and/or its magnitude. STN beta oscillations are prominent in PD patients withdrawn from dopaminergic therapy (Brown et al., 2001; Levy et al., 2002a; Priori et al., 2004; Williams et al., 2002) and are thought to play an anti-kinetic role (Brown, 2003; Kuhn et al., 2004; Williams et al., 2005). As mentioned above, increasing evidence suggests that beta oscillations contribute to bradykinesia and rigidity rather than to tremor in PD (Chen et al., 2007; Kuhn et al., 2006b; Kuhn et al., 2008; Ray et al., 2008; Kuhn et al., 2009). In contrast, STN oscillations in the gamma range are increased following dopaminergic therapy and during movement, and have been related to specific coding of movement related parameters (Androulidakis et al., 2007; Brown, 2003; Cassidy et al., 2002; Williams et al., 2002). Negative correlation between beta and gamma oscillations in STN has been shown to occur after treatment with dopaminergic medications (Alonso-Frech et al., 2006; Fogelson et al., 2005a) providing further evidence for the reciprocal interactions between these two functionally different oscillatory patterns. It has been argued that the balance between beta and gamma oscillations determines the effects of basal ganglia-thalamocortical projections on the motor areas of the cortex (Brown, 2003). Our results may therefore suggest that altered balance between these two modes of activity may also be related to resting tremor in PD. It is important to mention that the baseline levels of gamma LFP in our study may be relatively low as the recordings were done during off medication state. The elevated gamma levels together with the relatively high beta activity (that did not decrease during tremor episodes) may characterize the STN state during resting tremor in PD.

It is important to note, however, that the gamma oscillatory activity we observed was in the low gamma range (35-55 Hz) and we did not see activity at the higher range of gamma frequencies (up to 90 Hz) usually observed in STN following levodopa treatment and with movement. In fact, parkinsonian rest tremor is known to be suppressed with onset of voluntary movement of the same limb. Thus, the low-gamma activity observed during tremor might not necessarily be movement-related. Parkinsonian rest tremor is diagnosed only if its amplitude increases during mental stress (Deuschl et al., 1998).
Therefore, an alternative hypothesis is that subthalamic low-gamma oscillations are related to mental processes that lead to fluctuations in STN state during tremor. Gamma-band synchrony in the 40-60 Hz range has been implicated in intense mental activity and in various cognitive functions such as memory and attention (Fitzgibbon et al., 2004; Kaiser and Lutzenberger, 2005; Kissler et al., 2000; Tallon-Baudry et al., 1998; Tiitinen et al., 1993). Attention-related gamma power modulations were reported in humans especially over the frontal and central cortices (Tiitinen et al., 1993; Ward, 2003). The cerebral cortex is known to influence the activity of the STN directly via monosynaptic projections (Kitai and Deniau, 1981). As mentioned above, in the dopamine depleted state, there is an increased coupling between cortex and STN activities (Magill et al., 2001) perhaps due to a greater efficacy of the excitatory synaptic inputs (Bevan et al., 2007). It has been previously suggested that basal ganglia beta- and gamma-band oscillatory activity may arise in the cortex (Hammond et al., 2007). Thus, the low-gamma synchrony observed in STN during tremor might reflect an enhanced response to rhythmic cortical input during periods when there is increased low-gamma cortical activity.

The significance of increased low-gamma activity during tremor requires future studies, but we believe that this might represent an important step to better understand tremor in PD. One way to test the hypothesis that low-gamma activity is related to attentional processes is to analyze the latency of the appearance of low-gamma oscillations in STN, and possibly in the cortex, while patients are asked to perform mental arithmetics.
Abstract
Deep brain stimulation in the globus pallidus internus (GPI) is used to treat the motor symptoms of both Parkinson's disease (PD) and dystonia. It has been suggested that PD and dystonia are characterized by different temporal patterns of synchronized oscillatory activity in the GPI. We hypothesize that distinct patterns of oscillations can be observed in the neuronal firing and local field potentials (LFPs) in GPI of PD compared to dystonia patients. To test this hypothesis, extracellular recordings of GPI neuronal firing and LFPs were simultaneously obtained using two microelectrodes separated by about 1 mm during stereotactic surgery for the implantation of DBS electrodes in two PD patients and six dystonia patients. In the PD patients, beta (11-30 Hz) oscillations were observed in the LFPs and these were coherent between the 2 electrodes. Furthermore, of the 73 neurons recorded in these patients, the firing activity of 21 (~29%) was significantly coherent with the LFP recorded from one or both microelectrodes. In addition, the average beta LFP power in the dorsal and ventral GPI of each patient was closely related to the percentage of correlated neurons within each region. In contrast, in the dystonia patients, the peak frequency of LFP oscillations was lower (8-20 Hz) and the activity of only 27 (10.5%) of the 257 neurons recorded in these patients, was coherent with the LFP oscillations. This proportion was significantly lower than in PD (P ≤ 0.001). These findings suggest that the oscillatory LFPs recorded from the GPI in PD patients are closely associated with beta oscillatory synchronous activity in populations of GPI neurons. In dystonia, however, the frequencies of LFP oscillations are lower than in PD and are not as closely related to the neuronal firing. It is possible that in PD synchronized oscillatory inputs to the GPI are more likely to influence the neuronal firing than in dystonia perhaps due to a stronger influence of dendritic potentials on the soma in the dopamine depleted state and/or a differential source of oscillatory input.
5.1 - Introduction

Parkinson’s disease (PD) and dystonia are two movement disorders that are known to be of basal ganglia (BG) origin. PD is a neurodegenerative disorder, which is characterized by a severe loss of dopaminergic neurons in the substantia nigra pars compacta and motor symptoms such as rigidity and akinesia. Classical models of basal ganglia function are based on discharge rates in the BG structures and predict that in PD, the dramatic decrease in dopamine concentration in the striatum results in an increased activity in the output nuclei, the globus pallidus internus (GPI) and substantia nigra pars reticulata, and over-inhibition of the thalamocortical drive (Albin et al., 1989; DeLong, 1990). Conversely, dystonia, which is characterized by sustained co-contractions of agonist and antagonist muscles that lead to abnormal posture and involuntary movements, is believed to result from decreased basal ganglia output that, in turn, leads to decreased inhibition of thalamic activity and consequently to increased excitability of the motor cortex (Vitek, 2002). These predictions of the so-called ‘rate model’ have been generally confirmed in animal models of PD (Miller and DeLong, 1987; Filion and Tremblay, 1991; Boraud et al., 1998) and are also supported by recording studies in humans undergoing functional neurosurgery as a treatment for PD or dystonia (Lenz et al., 1998; Vitek et al., 1998; Vitek et al., 1999; Merello et al., 2004; Zhuang et al., 2004; Starr et al., 2005; Tang et al., 2007).

It is noteworthy, however, that the predicted rate changes have not been found in all studies (Bergman et al., 1994; Hutchison et al., 2003; Boraud et al., 2002; Lenz et al., 1999; Pessiglione et al., 2005; Raz et al., 2000; Wichmann et al., 1999). Instead, there is increasing evidence for changes in the activity patterns of basal ganglia neurons. In parkinsonism, the incidence of bursts in the globus pallidus and subthalamic nucleus (STN) is increased (Filion and Tremblay, 1991; Boraud et al., 1998; Hutchison et al., 1994), most often in the context of synchronous oscillatory activity, which is pathologically high in both the STN and GPI (Bergman et al., 1994; Nini et al., 1995; Bergman et al., 1998b; Raz et al., 2000; Levy et al., 2002b; Magnin et al., 2000) (Brown et al., 2001; Silberstein et al., 2003; Levy et al., 2002a). This might be, in part, due to an enhanced interaction between cortical areas and basal ganglia structures (Magill et
al., 2000; Magill et al., 2001; Sharott et al., 2005b; Baufreton et al., 2005a; Walters et al., 2007), although other pathological phenomena such as the STN-GPe oscillatory network (Plenz and Kital, 1999) may also play a role. The oscillatory discharges are rapidly reversed by treatment with dopaminergic medications (Levy et al., 2002a), and are positively correlated with the patients’ response to the medications (Weinberger et al., 2006), suggesting that these oscillations might be a direct effect of dopamine deficiency.

Importantly, abnormal oscillatory firing in the GPi has also been observed in patients with dystonia (Starr et al., 2005). It is known that a lesion or high-frequency stimulation in the GPi ameliorates the motor disabilities of both PD and dystonia (Marsden and Obeso, 1994b; Lang et al., 1997; Lozano et al., 1997; Vidailhet et al., 2005; Lozano, 2001). This paradoxical improvement, which is inconsistent with the rate model, might be explained if the different symptoms are dependent on abnormal, but different, patterns of synchronized oscillations in the basal ganglia circuits, so that eliminating this activity by lesion or high frequency stimulation results in decreased disability.

One means of studying changes in the pattern of local neuronal synchrony is through a frequency-based analysis of local field potentials (LFPs). These signals are believed to reflect synchronized dendritic currents averaged over a larger neuronal population. In a relatively recent study carried out by Silberstein et al. (2003), it has been shown that PD and dystonia are indeed characterized by different oscillatory patterns. The LFPs recorded from the deep brain stimulation (DBS) electrodes implanted in the GPi reveal that in PD the dominant frequency of oscillation is between 11 to 30 Hz, known as the beta range. In dystonia patients, the power of LFP beta oscillations are weaker and there is increased oscillatory activity in the lower frequencies (4-10 Hz) (Silberstein et al., 2003). In contrast to beta oscillatory activity which is related to rigidity and bradykinesia (Kuhn et al., 2006b; Kuhn et al., 2008; Chen et al., 2007; Ray et al., 2008; Kuhn et al., 2009), the 4-10 Hz oscillations have been implicated in dyskinesias (Silberstein et al., 2003; Silberstein et al., 2005a).

In PD patients, beta activity in the GPi is mainly evident in the local field potentials
recorded from the macroelectrodes used for therapeutic high-frequency stimulation of this region (Brown et al., 2001; Priori et al., 2002; Silberstein et al., 2003; Silberstein et al., 2005a). Although synchronized beta oscillatory neuronal firing in the GPi has been previously reported (Levy et al., 2002b; Tang et al., 2007), the relationship between neuronal firing and the LFP in GPi remains to be elucidated. Previous studies in STN of PD patients have demonstrated a close association between oscillatory neuronal firing and the LFP in the beta frequencies (Kuhn et al., 2005; Weinberger et al., 2006). This phenomenon was hypothesized to result from a greater sensitivity of basal ganglia neurons to oscillatory inputs in the dopamine depleted state (Magill et al., 2001; Sharott et al., 2005b; Baufreton et al., 2005a; Walters et al., 2007). This hypothesis can be examined in the GPi by comparing the relationship between neuronal firing and LFP in PD and dystonia. This comparison is possible since the GPi is widely targeted to treat dystonia (Vidailhet et al., 2005) and in some cases, levodopa-induced dyskinesias in PD patients (Krack et al., 1998).

In the present study, we investigated the relationship between neuronal discharges and the oscillatory local field potentials in the GPi in both PD and dystonia patients. The degree of association between neuronal firing and LFP, as well as the predominant frequency of oscillations, was compared between PD and dystonia. In addition, we investigated the changes in GPi oscillatory activity during levodopa-induced dyskinesias in PD.

5.2 - Methods

Patients

We studied two PD patients undergoing stereotactic neurosurgery for the implantation of DBS electrodes in the left GPi, mainly to treat their severe levodopa-induced dyskinesias and motor fluctuations. The second patient (Table 5.1) also suffered from off-period dystonic cramping in the lower limbs, and had previously undergone bilateral implantation of DBS electrodes in the STN. These two patients were the only PD cases selected for GPi surgery recently in our center. Both patients were women who, at the
time of operation, reached an age of 70 and 40 years and PD duration of 27 and 8 years respectively. For comparison purposes, we also studied one male and five female dystonia patients with a mean age of 55.8 ± 9 (±SD) years and disease duration of 16.2 ± 14 years. No sedatives or anesthetics (e.g. propofol) were administered during or before surgery. Pre-surgical clinical assessments of all patients were performed by movement disorder specialists at the Toronto Western Hospital. PD patients were assessed according to the Unified Parkinson’s Disease Rating Scale (UPDRS) (Fahn et al., 1987) before and after acute levodopa challenge (Moro et al., 2002), whereas dystonia patients were evaluated according to the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) (Comella et al., 1997) and the Burke-Fahn-Marsden (BFM) rating scale (Burke et al., 1985). Clinical and demographic details of the patients are given in Table 5.T1.

Table 5.T1: Demographic and clinical characteristics of the patients

<table>
<thead>
<tr>
<th>Patient # and disease</th>
<th>Age (years) and sex</th>
<th>Disease duration (years)</th>
<th>Pre-op motor scores: UPDRS on/off (PD) TWSTRS/BFM (dystonia)</th>
<th>Microelectrode trajectories (and clinical state)</th>
<th># of GPi neurons analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - PD</td>
<td>70 F</td>
<td>27</td>
<td>34.5/80</td>
<td>L - on</td>
<td>17</td>
</tr>
<tr>
<td>2 - PD*</td>
<td>40 F</td>
<td>8</td>
<td>21.5/56</td>
<td>L - semi-off</td>
<td>31</td>
</tr>
<tr>
<td>3 - Generalized dystonia</td>
<td>54 M</td>
<td>40</td>
<td>22/34</td>
<td>R×2 + L</td>
<td>36</td>
</tr>
<tr>
<td>4 - Segmental dystonia</td>
<td>57 F</td>
<td>11</td>
<td>18/23</td>
<td>R + L</td>
<td>23</td>
</tr>
<tr>
<td>5 - Cervical dystonia</td>
<td>48 F</td>
<td>5</td>
<td>23/NA</td>
<td>R</td>
<td>15</td>
</tr>
<tr>
<td>6 - Cervical dystonia</td>
<td>73 F</td>
<td>NA</td>
<td>NA</td>
<td>R + L</td>
<td>36</td>
</tr>
<tr>
<td>7 - Cervical dystonia</td>
<td>53 F</td>
<td>17</td>
<td>27/7.5</td>
<td>R + L</td>
<td>62</td>
</tr>
<tr>
<td>8 - Cervical dystonia</td>
<td>50 F</td>
<td>8</td>
<td>24/NA</td>
<td>R + L</td>
<td>50</td>
</tr>
</tbody>
</table>

* Patient had bilateral STN-DBS; NA, not available

**Recordings**

The methods of microelectrode-guided stereotactic neurosurgery for the implantation of DBS electrodes into the Gpi are described in detail in the General Methods (Section 2.2.2.b). Extracellular recordings of neuronal firing and LFPs were obtained simultaneously using two independently driven microelectrodes (about 25 µm tip length, axes 600 µm apart, ~0.2-MΩ impedance at 1 kHz) as described in the General methods (sections 2.2.3 and 2.2.4). The distinction between the external and internal segments of the pallidum was determined based on characteristics of neuronal activity such as firing patterns and background noise, and the existence of border cells and white matter. The
bottom of the GPi was determined based on white matter and identification of the optic tract, which lies close to the ventral border of the GPi. An example of simultaneous GPi recordings of LFP and neuronal discharge is shown in Figure 5.F1A. All recordings were performed in the resting state while the patients were awake and with local anesthesia. Patients were not asked to perform any task and epochs with voluntary movements were excluded. Limb movements were measured using electromyography (EMG) and/or triaxial accelerometers (with summated x-y-z signals) that were recorded simultaneously with the neuronal activity.

The first PD patient (#1) was given 1 tablet (100 mg) of levodopa (Sinemet®, levodopa/carbidopa 100/25) in the morning prior to the surgery as it was deemed medically necessary for the patient. Microelectrode recordings started approximately two hours later so that during the first trajectory, which lasted for about 1.5 hours, the patient was generally “on” and displayed levodopa-induced dyskinesias almost continuously during the recording session. During the second microelectrode trajectory, which was performed at least 3.5 hours after the last dose of levodopa, there was an eradication of dyskinesias together with recurrence of rest tremor in the left foot. This track was therefore considered to be in the “semi-off” state. The second PD patient (#2) was given 1 tablet of Sinemet® (100/25) approximately 4 hours before surgery. In this patient, only one microelectrode trajectory was performed (see Table 5.T1), during which the patient was very stiff and was considered to be “semi-off”. The term “semi-off” was chosen in order to distinguish the patients’ state in the current study from the classical “off”, in which patients are withdrawn from their anti-parkinsonian medication for at least 12 hours before surgery (also see Discussion). Dyskinesias were not observed during the “semi-off” state in either of the PD patients, and importantly, dystonic activity was not observed during the recordings in any of the dystonia patients.

Data analysis

Action potentials arising from single- and multiunits were discriminated using template matching tools in Spike2 (Cambridge Electronic Design, Cambridge, UK). Only recordings ≥17 seconds and during periods without voluntary movements (based on EMG
recordings and observations) or artifacts were analyzed. Border cells were identified and excluded from the analyses (see (Lozano et al., 1998)). Spike times and unfiltered LFP data were imported into MATLAB (version 7.1, The MathWorks, Natick, MA) and spectral analyses were performed using the discrete Fourier transform and its derivations calculated according to Halliday et al. (1995) and Rivlin-Etzion et al. (2006). These calculations included power spectra of recorded activity (spike trains and LFPs) as well as coherence and cross-correlations between simultaneous recordings, as previously described in the General methods (section 2.3.1.a/b).

Locations of recording sites were determined retrospectively according to the physiological landmarks observed intraoperatively and a reconstruction of the electrode trajectory using the stereotactic atlas of Schaltenbrand and Wahren (1977). From these reconstructions, recording sites were approximated to be either in the dorsal or ventral part of the GPi. In order to compare the relative LFP beta power in the dorsal and ventral GPi in each PD patient, the mean power across the 10-Hz band centered on the frequency of the peak power was calculated at each recording site. The LFP power at each recording site was then expressed as the percentage of the maximum power observed in the trajectory. Finally, the percentage values were averaged in each half of the GPi to give mean percentage of LFP beta power in the dorsal and ventral GPi. This method of measuring LFP power changes in basal ganglia nuclei was used previously (Kuhn et al., 2005; Weinberger et al., 2006; Chen et al., 2006b).

5.3 - Results

We analyzed recordings from 73 GPi neurons along 2 tracks in two PD patients “semi-off” medications, and from 257 neurons along 12 tracks in six dystonia patients. The mean recording durations (±SD) were 43.7 ± 18.1 and 41.9 ± 26.8 respectively (no statistical difference). In addition, 17 neurons were recorded in track 1 of PD patient #1 when the patient was “on” and the findings are reported separately in section 5.3.5. The number of microelectrode trajectories that were analyzed in each patient and the number of neurons obtained are given in Table 5.T1.
5.3.1 - Firing rates of GPi neurons

The mean firing rate (±SD) of GPi neurons recorded from PD patients “semi-off” medication was 79.3 ± 39.7 Hz. In the dystonia group, the mean firing rate was 60.4 ± 30.8 Hz and was significantly lower than that from PD patients (P ≤ 0.001, Mann-Whitney rank-sum test on the medians: 55.1 and 71.8 Hz respectively).

5.3.2 - Coherence between neuronal firing and oscillatory LFPs in PD patients

In the PD patients, beta (11-30 Hz) oscillatory activity was observed in the LFPs recorded from the GPi, and there was significant coherence between pairs of LFPs in 35 out of 42 sites (~83%) where LFPs were recorded simultaneously from the two microelectrodes. Of the 73 neurons recorded in the PD patients, the firing of 21 (~29%) neurons was significantly coherent with the simultaneously recorded LFP at the beta frequencies. The frequency of coherence ranged between 15 to 30 Hz with a mean of 24.3 ± 4.8 Hz. Seven of these neurons were coherent with the LFPs recorded from both microelectrodes, whereas 11 neurons were coherent only with the LFP recorded from the same electrode. The remaining 3 neurons were coherent only with the LFP recorded from the other electrode. Figure 5.F1 shows an example of spectral analysis of simultaneous recordings of neuronal firing and LFP activity, in which significant coherence in the beta range was observed.

It should be noted, however, that although ~29% of the neurons displayed significant coherence with the LFP, significant oscillatory firing was only observed in 11 (15%) neurons, of which 9 were coherent with the LFP (see Figure 5.F1B for an example). The mean frequency of neuronal oscillations was 24.4 ± 5.9 Hz, and this was similar to the mean frequency of coherence with the LFP (P = 0.8, Mann-Whitney rank-sum test). Coherence between simultaneously recorded neurons was observed in only 3 out of 38 pairs.
5.3.3 - The averaged beta LFP power in the GPi is closely related to the percentage of correlated neurons

Interestingly, we found that in each of the PD patients, the average beta LFP power in the dorsal and ventral GPi is closely related to the percentage of oscillatory and correlated neurons within that region. In patient #1, the firing of 13 out of 31 neurons was oscillatory and/or correlated with the LFP. Seven of these neurons were located in the dorsal GPi, where they comprised 37% of the total number of neurons, and 6 were in the ventral GPi where they comprised 50% of the neurons. These proportions were not significantly different (P > 0.05, Fisher exact test). Likewise, the average beta LFP power was not significantly different between the dorsal and ventral GPi (medians: 38.2 and 45.1% respectively; P = 0.4, rank-sum test) (see Figure 5.F2). In patient #2, 10 out of 42 neurons were oscillatory and/or correlated with the LFP. But in this case, these neurons were unevenly distributed within the GPi: 9 neurons were located in the ventral GPi (37.5% of the neurons there) whereas only one neuron was found in the dorsal part (5.5%) (P < 0.03, Fisher exact test). In this patient, the average LFP beta power was significantly greater in the ventral part (medians: 4.7 and 11.4 respectively; P ≤ 0.001, rank-sum test) (Figure 5.F2).

5.3.4 - Neurons are less correlated to oscillatory LFPs in dystonia patients

In the dystonia group, oscillatory activity was observed in the LFPs mostly in the frequencies between 8 to 20 Hz. Of 200 sites where LFPs were recorded simultaneously from the two microelectrodes, 177 (88.5%) showed significant coherence across this frequency band. Examples of the coherence between the two simultaneously recorded LFPs in each of the six dystonia patients are shown in Figure 5.F3. Of 257 neurons recorded in these patients, the firing activity of 27 (10.5%) was significantly coherent with the simultaneously recorded LFP. This proportion, however, was significantly lower than that observed in PD (P ≤ 0.001, χ² test). Of these neurons, 6 were coherent with the LFPs recorded from both microelectrodes, whereas the vast majority (n = 21) were
coherent only with the LFP recorded from the same electrode. In this group of patients, the mean frequency of coherence was $18 \pm 4.2$ Hz, and was determined to be significantly lower than the frequencies observed in PD ($P \leq 0.001$, t-test).

The number of correlated neurons in each of the dystonia patients varied from 0 to 11 with an average of $4.5 \pm 4$ neurons per patient. In general, these neurons were evenly distributed within the dorsal and ventral halves of the GPi. In the dorsal GPi, the activity of 12 out of the 137 neurons (8.5%) was correlated with the LFP, whereas in the ventral part, the activity of 15 of the 120 neurons (12.5%) was correlated with the LFP. Significant oscillatory activity of neuronal discharges at the 8-20 Hz band was not observed in any of the dystonia patients. Because of the relatively small number of neurons that fired coherently with the LFP in each dystonia patient, we did not compare their distribution to that of the LFP power within the GPi.

5.3.5 - Changes in GPi oscillatory activity during levodopa-induced dyskinesias

In one of the PD patients (patient #1), we had the opportunity to perform one microelectrode trajectory through the GPi during levodopa-induced dyskinesias. In this trajectory, the LFPs revealed oscillatory activity at frequencies around 9 and 70 Hz. In addition, all sites ($n = 16$) in which LFPs were recorded simultaneously from the two electrodes showed significant coherence at these frequencies, whereas beta coherence was significantly reduced (from a median value of 0.5 to 0.2; $P \leq 0.001$, rank-sum test). Figure 5.F4 shows examples of power spectra of simultaneously recorded LFPs and their corresponding coherence and cross-correlation functions, as were observed in each of the two microelectrode trajectories performed in this patient. Importantly, we did not find correlation between the recorded LFP and limb dyskinesias.

Two out of the 17 (~12%) neurons that were recorded in this track, showed significant coherence with the LFPs recorded from both microelectrodes at a frequency around 9 Hz. One of these two neurons also exhibited significant oscillatory firing at this frequency (see Figure 5.F5). Examination of the firing rates of neurons recorded during levodopa-
induced dyskinesias (n = 17) revealed no significant difference from the neurons recorded during the “semi-off” state (n = 73) in the two PD patients (mean firing rate of 74.9 ± 29.8 Hz compared to 79.3 ± 36.7 Hz respectively; P = 0.66 t-test).
Figure 5.1. Example of synchronized neuronal and local field potential (LFP) beta oscillatory activity recorded from the GPi of a Parkinson’s disease patient (#1) during the “semi-off” state. [A] raw data showing LFP and neuronal discharge recorded simultaneously from the 2 microelectrodes. Spikes and LFP activity were derived by high- and low-pass filtering the raw signals at 125 and 100 Hz, respectively. [B] LFP and neuronal discharge power spectra, obtained from the pair of recording sites, and their corresponding coherence and cross-correlation functions. [a, b] LFP power spectra obtained from the recorded LFPs. Dotted line indicates 95% confidence interval of the estimated spectrum. [c] neuronal power spectrum. Shaded area indicates 95% confidence interval for the absence of oscillatory activity. [d-f] coherence functions for each combination. Dotted line indicates 95% confidence limit for the absence of coherence. [g-i] cross-correlograms for each combination (LFPs were band-pass filtered between 11- to 35-Hz). Dotted lines indicate the 95% confidence interval. Vertical red line indicates zero time delay.
Figure 5.F2. Left: reconstruction of the microelectrode tracks through the globus pallidus in both PD patients. The globus pallidus internus (GPi) is colored in yellow. The neurons recorded during the “semi-off” state were plotted along the tracks where dark dots represent neurons that were not oscillatory or correlated with the LFP and red dots represent neurons that were oscillatory/correlated with the LFP. Open circles represent neurons that were recorded outside the GPI. Dotted line illustrates the trajectory performed during the “on” state in patient #1. Dashed line illustrates the division of the GPI into dorsal and ventral halves. Right: box plots of the relative LFP beta power in the dorsal and ventral GPI. LFP beta power was expressed as a percentage of the maximum power observed in each trajectory.
Figure 5.F3. Examples of the coherence between simultaneously recorded LFPs that were obtained from the GPi in each of the six dystonia patients. Dotted line indicates 95% confidence limit.

Figure 5.F4. Examples of synchronized LFP activity recorded from the GPi of a Parkinson’s disease patient (#1) during the “semi-off” and “on” levodopa states. [A] LFP power spectra, obtained from a pair of recording sites during the “semi-off” state (no-dyskinesias - second trajectory), and their corresponding coherence and cross-correlation functions. [B] power spectra obtained from LFPs that were recorded simultaneously during the “on” state (levodopa-induced dyskinesias - first trajectory).
Figure 5.5. Example of synchronized neuronal and LFP oscillatory 10 Hz activity recorded from the GPi of a Parkinson’s disease patient (#1) during levodopa-induced dyskinesias. [a, b] LFP power spectra. Dotted line indicates 95% confidence interval of the estimated spectrum. [c] Neuronal power spectrum. Shaded area indicates 95% confidence interval for the absence of oscillatory activity. [d–f] Coherence functions for each combination. Dotted line indicates 95% confidence limit for the absence of coherence. [g–i] Spike-triggered averages with, and cross-correlograms of, the 8- to 20-Hz band-pass filtered LFPs. Dotted line indicates the 95% confidence interval. Vertical red line indicates zero time delay.
5.4 - Discussion

5.4.1 - Methodological constraints

The number of Parkinson’s disease patients (n = 2) that participated in this study is very limited because of the low prevalence of patients with PD that are selected for GPi surgery in our center. PD patients normally receive bilateral STN implantation as a standard therapy. These exceptional two cases were selected for GPi surgery due to their severe drug-induced dyskinesias. In addition, patient #2 also suffered from off-period dystonic cramping in her lower limbs. Thus, the pathology in this patient might have some similarities to the pathology in dystonia, which might influence our results. Moreover, this patient has been previously treated with bilateral deep brain stimulation of the STN. Therefore, the possibility that chronic stimulation of the STN have resulted in some long-term physiological changes in the circuit should be considered. Another limitation was that these data were not obtained after overnight withdrawal from dopaminergic medication since full medication withdrawal was contraindicated in these severely affected patients. The patients were considered to be either “on” (during levodopa-induced dyskinesias) or “semi-off” in the absence of dyskinesias and reappearance of symptoms such as rigidity and tremor, but the exact clinical state was not evaluated. Despite these limitations, we feel that our results should be reported in view of their uniqueness and since they provide interesting insights regarding the pathophysiology of PD and dystonia.

5.4.2 - GPi firing rates

In the current study, the firing rates of GPi neurons were significantly higher in the PD group compared to the dystonia group. This is consistent with the predictions of the rate model of the basal ganglia for PD (Albin et al., 1989; DeLong, 1990) and dystonia (Vitek, 2002), although the absence of control data limits our ability to determine whether the firing rates observed in PD/dystonia were respectively higher/lower than normal. In the normal monkey, the mean GPi firing rate was found to be around 80 Hz (Filion and
Tremblay, 1991; Starr et al., 2005), which is similar to the mean rate of 79 Hz found in our PD patients. This might imply that the firing rates observed in our PD patients are close to normal. Indeed, previous studies in PD patients that were completely off dopaminergic medication have reported higher GPi firing of about 90-95 Hz (Starr et al., 2005; Tang et al., 2005; Tang et al., 2007), which is comparable to the firing observed in the GPi of MPTP monkeys (~95 Hz) (Filion and Tremblay, 1991). This suggests that the “semi-off” state of our PD patients (about 4 hours after the last dose of levodopa) might not be comparable to the practically-defined off state, in which patients are withdrawn from their anti-parkinsonian medication for at least 12 hours prior to the recordings. In fact, the mean firing rate recorded during the “on” levodopa state (~75 Hz) was not significantly different than that recorded during the “semi-off” state.

Because in our study the patients’ clinical motor scores were not evaluated intraoperatively, it was not possible to directly compare their “semi-off” state to the complete off state. According to pharmacokinetic and pharmacodynamics studies, levodopa plasma levels peak 0.5 to 2 hours after its administration, when the patients’ best motor status is reached, and may take 5-6 hours to wear off completely even though motor scores decline to baseline after approximately 3 hours (Fabbrini et al., 1987; Deleu et al., 2002). Therefore, it is likely that the “semi-off” state in this study was associated with residual levels of dopamine in the system, which might explain the relatively low firing rates observed in our PD patients. Indeed, it has been recently shown in PD patients that basal ganglia output neurons are sensitive to even low doses of levodopa, as observed in the form of synaptic plasticity (Prescott et al., 2009). Alternatively, the low firing rates might be due to the fact that the two PD patients in this study suffered from severe dyskinesias. Some studies have reported decreased firing rate in GPi neurons in PD patients with off-period dystonia and levodopa-induced dyskinesias (Hallett, 2000; Hashimoto et al., 2001).

In the dystonia group, on the other hand, the mean GPi firing rate found (~60 Hz) is similar to that reported previously (Vitek et al., 1999; Merello et al., 2004; Starr et al., 2005) and is lower than the mean firing observed in normal monkeys (Filion and...
Tremblay, 1991; Starr et al., 2005), suggesting that it might be abnormally reduced (Vitek, 2002). It is important to note, however, that in some studies the firing rates in the GPi of dystonia patients were closer to normal with a mean of about 75 Hz (Hutchison et al., 2003; Tang et al., 2007).

5.4.3 - GPi LFP oscillatory activity and its relationship to neuronal discharges in PD

We found that in PD patients, oscillatory activity in the local field potentials recorded from the GPi during the “semi-off” levodopa state is mostly prominent in the beta-frequency band. This finding is consistent with previous studies that have reported beta oscillatory activity in GPi of PD patients off medications, in whom local field potentials were recorded from the large contact DBS macroelectrodes (Brown et al., 2001; Silberstein et al., 2003; Silberstein et al., 2005a; Foffani et al., 2005a). The present study is the first to demonstrate beta oscillations in GPi LFPs recorded from the more focal, higher-impedance, microelectrodes. In addition, we were able to reveal significant coherence between simultaneously recorded LFPs, suggesting that the beta LFP oscillations are distributed and synchronized over at least a few millimeters within the GPi. This finding is similar to our previous observation in the STN of PD patients off dopaminergic medication (Weinberger et al., 2006), implying that STN and GPi share similar oscillatory patterns in PD.

Our results demonstrate for the first time that in PD, about 30% of the neurons in the GPi are significantly coherent with the simultaneously recorded LFP and there appears to be a spatial association within the GPi between the percentage of correlated neurons and the relative LFP power in the beta range. These data further confirm that the beta LFP recorded from the basal ganglia reflects, at least in part, synchronized activity in a population of local neurons (Kuhn et al., 2005; Chen et al., 2006b; Weinberger et al., 2006). However, although 29% of the neurons fired coherently with the beta LFP oscillations, only 15% displayed significant beta oscillatory firing. This can be attributed to the fact that coherence analysis is generally more sensitive and reveals activity that may not reach significance in the power spectrum. Thus, we cannot rule out the
possibility that the correlated, non-oscillatory, neurons might exhibit weak beta oscillations that failed to reach significance. Nevertheless, in our previous study on the relationship between neuronal firing and beta oscillatory LFPs in the STN (Weinberger et al., 2006), we found that as many as 28% of the neurons displayed significant oscillatory firing, and 25% of the simultaneously recorded pairs fired in synchrony (compared to 8% in the present study). It is possible that the relatively small number of oscillatory neurons found in the present study is due to the “semi-off” state of the patients, as opposed to the overnight withdrawal from dopaminergic medication in our previous study.

Alternatively, the difference between these results and those observed in STN may reflect anatomical and physiological differences between STN and GPi. This, however, seems unlikely since a tendency toward neuronal synchronization has been demonstrated in the monkey GPi following MPTP treatment (Nini et al., 1995; Raz et al., 2000) and in PD patients (Levy et al., 2002b). Such synchronization has been suggested to result, at least in part, from a greater influence of striatal tonically active neurons that, in the dopamine depleted state, display oscillatory activity that is correlated with neuronal activity in the GPi (Raz et al., 1996; Raz et al., 2001). These changes have been proposed to result from enhanced electrotonic coupling between neighbouring striatal neurons in the absence of dopamine (Omn and Grace, 1999), which has been shown to directly modulate the activity of striatal tonically active neurons (Watanabe and Kimura, 1998). A significant increase in the occurrences of synchronized beta oscillatory firing was also observed among STN neurons (Bergman et al., 1994; Vila et al., 2000; Steigerwald et al., 2008), and coherence studies have documented that the activity in STN and GPi of PD patients are well synchronized at the beta frequencies (Brown et al., 2001; Cassidy et al., 2002; Foffani et al., 2005a). It has been suggested that dopamine depletion at the level of the STN and globus pallidus may enhance the sensitivity of the network to rhythmic activity originating from the cortex (Magill et al., 2001; Sharott et al., 2005b; Baufreton et al., 2005a). Since the nigro-striatal dopaminergic projection has axon collaterals to the STN and pallidum (Lavoie et al., 1989; Hassani et al., 1997; Cossette et al., 1999; Hedreen, 1999; Francois et al., 2000), extrastriatal dopaminergic modulation may facilitate the normal interaction of subthalamic and pallidal neurons and its loss in PD may contribute
to the pathological synchronization within and between these structures (Bevan et al., 2006).

Taken together, it is possible that in our study, the residual amount of dopamine that might have remained in the system during the “semi-off” state (see above) resulted in reduced oscillatory firing and synchrony among GPi neurons by means of striatal and/or extrastriatal mechanisms. In addition, we speculate that in some of the neurons that showed significant coherence with the LFP, the oscillatory membrane potentials were largely confined to the dendrites and had only a relatively small influence on the probability of the neuron firing. Therefore, even neurons with non-significant beta oscillatory firing may nonetheless contribute to the generation of beta oscillatory LFPs. This hypothesis is further supported by the association observed between the distribution of correlated neurons within the GPi and the relative LFP power at the beta frequencies.

5.4.4 - Modulation of GPi oscillatory activity during levodopa-induced dyskinesias

Interestingly, we found that although the firing rates of GPi neurons were not significantly different between the “semi-off” and the “on” states, the oscillatory patterns were different. During periods of levodopa-induced dyskinesias, beta activity in the local field potential was largely reduced and there was an emergence of new peaks around 9 and 70 Hz. High gamma activity around 70 Hz in the GPi (and also in STN) has been shown to increase in PD patients following dopaminergic medication (Brown et al., 2001) and during voluntary movement (Cassidy et al., 2002) in concurrence with a reduction in beta activity. This led to the hypothesis that beta synchronization may be essentially anti-kinetic perhaps by limiting the ability of neurons to code information in time and space, as both adjacent and spatially distributed neurons are preferentially locked to the beta rhythm (Brown, 2003; Williams et al., 2005; Brown, 2007; Hammond et al., 2007). Gamma activity, on the other hand, has been considered to be pro-kinetic and related to movement demands, as it may share similar role to that posited for gamma band synchronization in the visual cortex (Freiwald et al., 2001). Our finding is therefore consistent with previous studies demonstrating decreased beta activity and increased
gamma during the “on” state (Brown et al., 2001; Cassidy et al., 2002), and suggest that gamma activity could also be observed during epochs of levodopa-induced dyskinesias.

Furthermore, it has been shown by Silberstein et al. (2005) in two PD patients that oscillatory pallidal LFP activity over 8-30 Hz is inversely correlated to the degree of levodopa-induced dyskinesias, suggesting that the levodopa-induced suppression of neuronal synchronization in the basal ganglia may play a role in the origin of dyskinesias (Silberstein et al., 2005a). However, the reduction in 8-30 Hz synchronization alone cannot account for dyskinesias, particularly since beta suppression occurs following levodopa in PD patients without dyskinesias. Our results demonstrate that together with beta suppression, there is increased synchronization at lower frequencies around 9 Hz during periods of levodopa-induced dyskinesias. These data are consistent with a recent LFP study in a PD patient exhibiting unilateral off-period dyskinesias (Foffani et al., 2005a). That study demonstrated that contralateral to the dyskinetic side of the body, there was a reduced coherence between GPi and STN in the high-beta frequencies (20-30 Hz) and an enhanced coherence at low frequencies (<10 Hz) compared to the GPi and STN ipsilateral to dyskinesias. In addition, 4-10 Hz oscillations in the LFPs were also recorded from the STN during levodopa-induced dyskinesias, and this was observed only contralateral to the side of dyskinesias (Alonso-Frech et al., 2006). Here, we also revealed that during levodopa-induced dyskinesias, neuronal firing can oscillate and be coherent with the LFP at low frequencies. This observation, together with the lack of correlation between the low-frequency LFP oscillatory activity and limb dyskinesias, rules out the possibility that these LFPs reflect movement-related artifacts. In fact, it has been previously shown that the spectral content of dyskinetic movements in PD patients is generally between 1 to 3 Hz (Manson et al., 2000).

Taken together our findings suggest that parkinsonian dyskinesias are related to an altered balance between oscillatory rhythms within the basal ganglia network rather than to a further decrease in firing rates. These findings also support the hypothesis that the balance between oscillatory rhythms in the basal ganglia determines the effects of basal ganglia-thalamocortical projections on the motor areas of the cortex (Brown, 2003). We
have previously demonstrated that altered balance between rhythms of oscillations can also characterize the state of the subthalamic nucleus during rest tremor in PD patients (Weinberger et al., 2009).

5.4.5 - Gpi oscillatory activity in dystonia and its comparison to PD

Our spectral analysis of the local field potentials in the Gpi of dystonia patients revealed oscillatory activity at frequencies between 8 to 20 Hz, which are higher than those (3-15 Hz) previously reported in dystonia (Liu et al., 2002; Silberstein et al., 2003; Chen et al., 2006b; Liu et al., 2006; Foncke et al., 2007). By using microelectrode recordings, Chen et al. (2006b) showed that in dystonia patients, 3-12 Hz oscillatory LFPs are maximal in the Gpi and can be synchronized with the activity of the neurons. This activity was also shown to be coherent with dystonic EMG activity in the contralateral muscles (Liu et al., 2002; Liu et al., 2006; Chen et al., 2006a; Foncke et al., 2007). Furthermore, it has been demonstrated by Silberstein et al. (2003) that the LFPs recorded from the DBS electrodes implanted in the Gpi in dystonia patients have higher power over the 4-10 Hz band and less power over the 11-30 Hz band compared to PD patients, suggesting that the two diseases are characterized by different patterns of pallidal activity. It should be noted, however, that although the percentage of LFP power in the 4-10 Hz band was higher in patients with dystonia, a discrete peak was found consistently only in the 11-30 Hz band and not in the 4-10 Hz band (Silberstein et al., 2003). Studies that have investigated movement-related changes in LFPs recorded from the Gpi of dystonia patients revealed that movements cause modulation of oscillatory activity in the range of 8-20 Hz, which is equivalent to the frequency range observed in our dystonia patients. In comparison with rest conditions, a significant decrease in the oscillatory power of pallidal LFPs at 8-20 Hz was induced by active and passive movements as well as by vibrations (Liu et al., 2006; Liu et al., 2008; Brucke et al., 2008).

Here, not only did we observe 8-20 Hz LFP oscillations but we also found that the firing of 10.5% of the neurons was correlated with the LFP at those frequencies. In Chen et al. (2006b), spike triggered averages with significant activity were observed in 28% of the
sites recorded in the GPi and the synchronization was mainly evident in the 3-12 Hz band. These differences might be related to the fact that some of the patients in their study experienced spontaneous dystonic movements during the recordings (Chen et al., 2006b). In our study, patients exhibited neither voluntary nor dystonic movements, so that the level of 8-20 Hz synchronization within the GPi might have remained relatively high compared to the lower frequencies.

Importantly, we found that the percentage of neurons that were correlated with the LFP in the dystonia group was significantly lower than that observed in PD. A possible explanation is that synchronized activity in dystonia tends to not be oscillatory and thus, could not be detected by our coherence analysis. Whether this is the case or not, our findings provide further evidence for the hypothesis that dopaminergic loss at the level of the STN and globus pallidus in Parkinson’s disease may increase the sensitivity of the network to rhythmic, perhaps cortical, oscillatory inputs (Magill et al., 2001; Sharott et al., 2005b; Baufreton et al., 2005a; Walters et al., 2007), and might contribute to the pathological synchronized oscillations within and between these structures (Bevan et al., 2006).

In summary, our findings suggest that the oscillatory LFPs recorded from the GPi in PD patients are closely associated with both significant and subthreshold beta oscillatory activity in populations of GPi neurons. The relatively low firing rate and the small number of oscillatory neurons observed in these patients may be attributed to their “semi-off” state during the recordings. In dystonia, however, the firing rates of GPi neurons and the frequencies of LFP oscillations are significantly lower than in PD, and the oscillatory LFPs are not as closely related to the neuronal firing. It is possible that in PD synchronized oscillatory inputs to the GPi are more likely to influence the neuronal firing than in dystonia, perhaps due to a stronger influence of dendritic potentials on the soma in the dopamine depleted state.
6 - CHAPTER 6 - GENERAL SUMMARY AND SIGNIFICANCE

6.1 - Oscillatory firing in the basal ganglia and its relationship to the local field potential and dopamine

The results of these studies provide important data documenting the nature of synchronized oscillations in PD. These data add new insights on the relationship of neuronal beta oscillatory discharges in the basal ganglia to local field potential activity in Parkinson’s disease patients. The coherence between neuronal discharges and LFPs in the beta range suggests that LFP beta oscillations recorded from the basal ganglia reflect, to some extent, rhythmic activity in a population of local neurons. This conception was strengthened even further by our results showing topographical association between the power of LFP beta oscillations and the locations of oscillatory and/or coherent neurons within the STN or GPi. These findings are particularly important because they suggest that LFP recordings from the basal ganglia, which are often used in various recent studies, essentially reflect the activity within its nuclei. Importantly, however, it seems from our studies that there are certain conditions during which beta oscillatory LFPs are likely to reflect only the oscillatory synaptic inputs but not neuronal firing.

When patients were studied off medications (and only 3 out of 14 exhibited tremor during the recordings) we found that approximately 30% of the neurons in STN display significant beta oscillatory firing and there is a significant correlation between the locations of oscillatory neurons within the STN and the LFP power (Chapter 3). In comparison, we found that the proportion of beta oscillatory neurons in the STN is somewhat lower in tremulous PD patients, and perhaps there are more neurons that oscillate at the frequency of tremor (Chapter 4). Our data show for the first time that even the tremor-frequency neurons can be coherent with the LFP oscillations over the beta frequency band, suggesting that they may still contribute to the generation of beta oscillatory LFPs. We speculate that during periods of rest tremor, beta oscillatory LFPs are more confined to the dendrites relative to periods without tremor. Similarly, our GPi analyses revealed that although ~30% of the neurons were coherent with the LFP, only
half of these displayed significant beta oscillatory firing (Chapter 5), leading to the conclusion that even non-oscillatory neurons might contribute to the generation of beta LFPs. In this case, the relatively small number of oscillatory neurons in the GPi was attributed to the dopaminergic state of the patients that were considered to be “semi-off”. Taken together, our data suggest that beta oscillatory LFPs are likely to reflect oscillatory synaptic inputs to the neurons in the recorded area and these may or may not influence the probability of firing depending on the clinical state.

If the “semi-off” state (Chapter 5) was indeed associated with residual amounts of dopamine in the basal ganglia (see section 5.4.2), we may conclude that the oscillatory firing in the basal ganglia is a consequence of dopaminergic loss. This conclusion is also supported by the finding that the proportion of oscillatory neurons in the STN of PD patients can predict the patients’ response to dopaminergic medication (Chapter 3). Thus, oscillatory firing in the basal ganglia might, at least in part, reflect the degree of dopamine deficiency in the system. Indeed, when the oscillatory activity in the GPi of PD patients was compared to that of dystonia patients (Chapter 5), we found that in dystonia a significantly smaller number of neurons are coherent with the LFP. Taken together, our data add to the growing evidence (emerging from animal models and in vitro studies) that dopaminergic loss in Parkinson’s disease may increase the sensitivity of the network to rhythmic, perhaps cortical, oscillatory inputs (Tseng et al., 2001; Magill et al., 2001; Sharott et al., 2005b; Baufreton et al., 2005a; Walters et al., 2007), and might contribute to the pathological synchronization within and between these structures (Bevan et al., 2006). Figure 6.F1 summarizes some possible mechanisms involved in the generation of oscillatory activity in the BG network following dopamine depletion.

6.2 - Spatial distribution of beta oscillations

We found that beta oscillations in both neuronal firing and LFPs are maximal in the motor portion of STN (i.e. the dorsal part) (see Chapters 3 and 4). In addition, we also obtained data suggesting that beta oscillatory activity tends to be greater in the motor portion of GPi (i.e. the ventral part) (Chapter 5). In the GPi, however, although a trend
was observed in the two PD patients studied, it was only significant in one of the patients. Future studies that include larger number of patients will have to confirm that in the GPi, beta oscillatory activity is maximal in the ventral part. It has been recently reported that the ventral GPi is the region that shows differential changes (i.e. in firing rates and patterns) in various movement disorders including PD (Tang et al., 2007). Overall, our results provide important evidence that beta oscillatory activity in the basal ganglia might be related to motor functions, therefore supporting its potential role in mediating some of the motor symptoms in Parkinson’s disease (Brown and Williams, 2005).

6.3 - The oscillatory neuronal population

In general, oscillatory firing in the basal ganglia of PD patients has been shown to occur in two major frequency bands. Neurons can fire at either the beta or the tremor frequencies (Levy et al., 2000; Levy et al., 2002b), and some neurons can display significant oscillatory firing at both frequencies (see for example Figures 3.F1, 4.F1 and 4.F2). Our findings show that STN neurons that exhibit oscillatory firing in either the tremor or the beta band are located in the same region within the STN and more interestingly, neurons with tremor-frequency activity are coherent with the LFP over the beta band. These observations suggest that tremor-frequency neurons might also exhibit weak beta oscillations and lead us to hypothesize that the neurons that are likely to oscillate at tremor frequencies are also those prone to oscillate at the beta frequencies and vice versa. This hypothesis is supported by the fact that although in tremulous patients only 12% of the neurons in STN have beta oscillatory activity, a total of 24.5% of the neurons were oscillatory at either tremor or beta frequencies, and this percentage is comparable to the 28% oscillatory neurons observed in Chapter 3 in which the majority of the patients did not exhibit tremor during the recordings.

Our data therefore suggest that irrespective of the frequency of oscillations, between a quarter to two thirds of the neurons in the STN are capable of oscillatory firing and these neurons are more likely to be found in the motor portion of STN. We believe that these neurons reflect the neuronal population that, in the dopamine depleted state, become
more sensitive to rhythmic cortical inputs (Bevan et al., 2007; Magill et al., 2001; Paz et al., 2005; Sharott et al., 2005b), which then determine their frequency of oscillations. In agreement with this hypothesis, the firing rates of STN neurons with tremor-frequency activity are similar to those with beta activity, and are significantly higher than those of the non-oscillatory neurons (see Chapters 3 and 4). The higher mean firing rates of the oscillatory neurons is perhaps due to a greater efficacy of the excitatory cortico-subthalamic inputs on those neurons following dopamine depletion (Bevan et al., 2007; Magill et al., 2001). Thus, our data suggest that in addition to the increased inhibition of the GPe, the increased sensitivity of STN neurons to cortical inputs (see Figure 6.F1) might also contribute to the established STN hyperactivity in PD (Miller and DeLong, 1987; Bergman et al., 1994).

6.4 - The relation of oscillatory activity to clinical aspects in PD patients

In these studies, the oscillatory activity in the basal ganglia was considered in relation to various clinical aspects. In Chapter 3, the incidence of beta oscillatory neurons in the STN was studied in relation to the patients’ motor symptoms and their benefit from dopaminergic medications. Interestingly, the percentage of neurons exhibiting oscillatory firing in the beta range correlated well with the degree by which PD motor symptoms improved after dopamine replacement therapy, whereas the incidence of oscillatory neurons did not correlate with the severity of symptoms before treatment. This suggests that beta oscillatory activity does not characterize motor impairment per se. Instead, it might be related to the magnitude of the response of the basal ganglia, or at least STN, to dopaminergic agents. These novel findings were later supported by newer studies showing that the LFP power over the 8-35 Hz band (measured “off” medication) is positively correlated with the improvements in motor symptoms after levodopa intake (Ray et al., 2008) and that levodopa-induced reduction in subthalamic LFP beta power correlates with the simultaneously observed clinical improvement in bradykinesia and rigidity, but not tremor (Kuhn et al., 2006b; Ray et al., 2008; Kuhn et al., 2009). Similar to our findings, no correlation between beta power and baseline motor symptoms was found.
The lack of correlation between beta oscillations and motor impairment is also in line with recent studies in animal models of PD. In monkeys that underwent a gradual MPTP-treatment protocol that slowly induces parkinsonism, except for a slight increase in the very beginning, robust oscillatory firing in the GPi appears only after first motor symptoms have already developed (Leblois et al., 2007), suggesting that oscillatory firing is not directly related to the early manifestations of parkinsonian motor symptoms.

Similarly, beta oscillations in STN and cerebral cortex were not exaggerated until several days after 6-OHDA injections in rats (Mallet et al., 2008) and are detected only several days after the appearance of akinesia (Degos et al., 2008). These results suggest that abnormally amplified beta oscillations in cortico-basal ganglia circuits do not result simply from an acute absence of dopamine receptor stimulation, but are a delayed consequence of chronic dopamine depletion.

In Chapter 4, the temporal dynamics of oscillatory activity in the subthalamic nucleus was investigated in relation to parkinsonian resting tremor. After establishing that beta oscillatory firing in the STN does not correlate with tremor severity (Chapter 3) we observed that in fact, during periods of tremor patients tend to have less beta oscillatory firing and in addition, there are neurons firing at the frequency of limb tremor. Interestingly, however, their oscillations, as well as their coherence with limb tremor, occur intermittently and in some cases independently from the fluctuations in tremor amplitude. This observation is in line with previous studies showing transient synchronization between limb tremor and neuronal oscillations in globus pallidus of PD patients (Hurtado et al., 1999;Hurtado et al., 2005) and supports the hypothesis that tremor might be a property of the population activity in an oscillatory network, as opposed to a property of the individual cells (Hurtado et al., 2005). In addition, it has been suggested that independent oscillatory circuits may underlie parkinsonian tremor in different extremities (Alberts et al., 1969). Indeed, tremor-frequency neurons tend to oscillate in different phases (Levy et al., 2000;Moran et al., 2008), which is perhaps why tremor-frequency oscillations are not easily detected by LFP recordings in the STN.

Temporal examination of the LFPs recorded in our tremulous patients revealed, for the
first time, that during periods of stronger tremor the oscillatory power in the LFP is
preferentially increased in the low gamma range (35-55 Hz). This is an intriguing finding
in light of the fact that parkinsonian rest tremor is known to increase in amplitude during
mental stress (Deuschl et al., 1998), which has been previously associated with increased
low-gamma synchrony across cortical areas (Tiitinen et al., 1993; Ward, 2003). Thus, it is
possible that the low-gamma synchrony observed in STN during tremor reflects rhythmic,
perhaps attentional-related, cortical input to the STN.

In addition to the increased LFP power in the low-gamma range, the ratio of the beta to
gamma coherence was significantly lower during stronger tremor, suggesting that
alteration of the balance between beta and gamma oscillations in the STN might be
related to tremor and/or its magnitude. These data are consistent with the notion that the
balance between oscillatory rhythms within the basal ganglia determines its effects on the
motor areas of the cortex (Brown, 2003). Similarly, overt changes in oscillatory activities
within the basal ganglia were observed during levodopa-induced dyskinesias (Chapter 5).
Beta activity in the local field potential was largely reduced and there was an emergence
of new peaks around 9 and 70 Hz. Importantly, however, there was no significant change
in firing rates, suggesting that parkinsonian dyskinesias are related to an altered balance
between oscillatory rhythms within the basal ganglia network rather than to a further
decrease in firing rates.

6.5 - Implications to basal ganglia models

Although these studies were not aimed to directly test the predictions of the rate model of
basal ganglia function (Albin et al., 1989; DeLong, 1990), results from Chapter 5 seem to
confirm that hyperkinetic movement disorders, such as dystonia, are associated with low
firing rates in GPi output neurons. Similar to hyperkinetic movement disorders,
levodopa-induced dyskinesias in PD were predicted to result from a substantial reduction
in GPi firing rates. This, however, was not supported by our data showing that GPi firing
rates remain unchanged during periods of levodopa-induced dyskinesias relative to
periods without dyskinesias. Thus, in contrast to the predictions of the model,
hyperkinetic motor symptoms in PD might not necessarily result from reduced neuronal firing rates in the Gpi and other mechanisms must be involved.

Our results from PD patients that were off dopaminergic medications for at least 12 hours (Chapters 3 and 4) have established that neurons in the dorsal STN tend to exhibit significantly elevated firing rates together with pathological oscillatory activity. This observation is not only consistent with the rate model, which predicts hyperactivity of the subthalamic nucleus in PD, but also suggests that oscillatory neurons tend to be hyperactive perhaps due to their greater sensitivity to excitatory cortical inputs (see above).

The overt beta band synchronization observed in the STN and Gpi of PD patients is in concurrence with the oscillatory model of basal ganglia (Brown, 2003), which hypothesizes that in PD the beta oscillations are enhanced and that dopaminergic medications decrease the pathological beta band oscillations and enhance the gamma band oscillations. Our results confirm this prediction as a reduction in beta activity was observed in the Gpi right after levodopa administration together with an increase in high gamma activity. The oscillatory model also proposes that STN and Gpi activity in the beta band is driven from the motor areas of the cortex. This is also supported by our findings showing that beta oscillatory activity was observed in the LFPs (likely to reflect oscillatory inputs) even in the absence of oscillatory firing. Our data expand on the oscillatory model by suggesting that beta oscillatory inputs to STN do not always influence the neuronal firing depending on the clinical state (see above) and in particular, might reflect the amount of dopamine deficiency in the system. As opposed to beta oscillations, the oscillatory model suggests that oscillations at tremor frequencies arise within the basal ganglia and spread to the cortex. Our data do not directly support this prediction as they suggest instead that neurons oscillating at either tremor or beta frequencies might be influenced by cortical input. Indeed, recent findings in anaesthetized rats (Sharott et al., 2005a) and PD patients (Lalo et al., 2008) have confirmed a net cortical driving of STN activity at frequencies below 60 Hz.
According to the oscillatory model, in PD beta oscillations are enhanced to such an extent that the desynchronization required to initiate movement cannot "break through" the elevated threshold, leading to the poverty of movement (Brown, 2003). This idea is challenged by our data showing lack of correlation between symptom severity and prevalence of oscillatory neurons (presented in Chapter 3). Indeed, some evidence suggests that pathological oscillations may occur after the appearance of first motor symptoms (Leblois et al., 2007; Degos et al., 2008). In this regard, it should be mentioned that the occurrence of motor impairment before the appearance of oscillations has been previously predicted by a theoretical model that suggests that pathological synchronization may be driven at the level of the BG-cortical loops (Leblois et al., 2006a). According to this model, oscillatory activity emerges in the pathological condition as a dynamic imbalance between two feedback BG–cortical loops (i.e. the direct and hyperdirect loops), but it is not related to parkinsonian motor symptoms that may be expressed in the absence of oscillations.

In 1998, Harris and Wolpert have suggested that signal-dependent noise plays a fundamental role in motor planning. According to their theory, biological noise, which is present in the neural control signals and whose variance increases with the size of the control signal, shapes the trajectory planning of a movement to minimize the variance of the final eye or arm position (Harris and Wolpert, 1998). Based on this theory, it is tempting to hypothesize that elevated levels of synchronization within the basal ganglia may interfere with motor planning by reducing noise levels (but this is not to suggest that the basal ganglia is simply devoted to a noise-making function). The idea that under normal conditions the basal ganglia uses noise for motor planning is similar to the role proposed for the avian homologue of the basal ganglia, in which residual “noisy” variability in well learned skills reflects meaningful motor exploration that can support optimization of performance (Tumer and Brainard, 2007). Indeed, PD patients become even slower with increased task complexity which requires more motor planning.

Alternatively, it has been proposed that segregation of the functional subcircuits of the basal ganglia (i.e. de-correlations) is an important aspect of normal BG function, and that
a breakdown of this independent processing could play an important role in the pathophysiology of PD (Bergman et al., 1998a). As mentioned in the General Introduction, the specificity of receptive fields in the BG-thalamocortical network is markedly reduced in PD. The loss of functional segregation within the motor circuit can be partly due to the increased sensitivity of the BG to oscillatory inputs in the beta and tremor frequencies. The excess synchronized oscillations might result in co-selection of antagonist motor programs resulting in rigidity or tremor, depending on the oscillation frequency. These frequencies (i.e. beta and tremor) are relatively low compared to the gamma band, and are therefore capable of synchronizing information channels over larger distances because their synchronization is less susceptible to long conduction delays (Buzsaki and Draguhn, 2004).

To summarize, these studies indicate that beta oscillatory activity in the STN and GPi might be involved in the pathological functioning of the basal ganglia in Parkinson’s disease and may reflect the degree of striatal and/or extra-striatal dopamine deficiency. The beta oscillatory LFPs recorded from the STN and GPi may or may not reflect the degree of synchronized firing among population of local neurons depending on factors such as the existence of tremor and the amounts of dopamine in the system. In addition, the balance between oscillatory rhythms may play an important role in the pathological functioning of the basal ganglia in PD. It seems likely that alterations in both serial and parallel information processing are involved in the pathological functioning of the BG in PD.
Figure 6.F1. Possible mechanisms involved in the generation of oscillatory rhythms in the basal ganglia following dopaminergic loss. In normal conditions, cortical oscillations do not have strong impact on the firing patterns of neurons in the basal ganglia. Because dopamine enhances the up and down states of MSNs neurons, the firing probability of MSNs is too low to encode cortical rhythms. Nigrostriatal dopamine deficiency liberates MSNs from the continuous restraining action of dopamine, allowing the transfer of cortical rhythms through MSNs to other BG nuclei (Tseng et al., 2001). In extra-striatal sites, dopamine acts on both pre- and postsynaptic receptors. In STN, dopamine converts burst firing to tonic by causing depolarization and suppressing GABA and glutamate release from presynaptic terminals (Baufreton et al., 2005b). Thus, loss of dopamine in the STN might amplify feedback inhibition from the GPe by hyperpolarizing STN neurons and enhancing the release probabilities at synapse in STN. These effects can also unmask the intrinsic oscillatory potential of the GPe-STN network (Plenz and Kitai, 1999). Moreover, the feedback inhibition from GPe, together with the increased release probability of glutamate in STN, enables cortical inputs to generate action potentials at reduced threshold, latency and variability (Baufreton et al., 2005a). This, together with the increased striatal-GPe and STN-GP transmission might lead to pathological expression of oscillatory activity in the STN and other BG nuclei.
APPENDIX - RECORDINGS FROM THE PEDUNCULOPONTINE NUCLEUS

The work described in this appendix was published by Weinberger et al. (2008), and is used with kind permission from Springer Science+Business Media: Experimental Brain Research, Pedunculopontine Nucleus Microelectrode Recordings in Movement Disorders Patients, 188(2):165-74, 2008. Weinberger M, Hamani C, Hutchison WD, Moro E, Lozano AM, Dostrovsky JO, Figures 1-5, Tables 1-2.

Abstract
The pedunculopontine nucleus (PPN) lies within the brainstem reticular formation and is involved in the motor control of gait and posture. Interest has focused recently on the PPN as a target for implantation of chronic deep brain stimulation (DBS) electrodes for Parkinson's disease (PD) and progressive supranuclear palsy (PSP) therapy. The aim of this study was to examine the neurophysiology of the human PPN region and to identify neurophysiological landmarks that may aid the proper placement of DBS electrodes in the nucleus for the treatment of PD and PSP. Neuronal firing and local field potentials were recorded simultaneously from two independently driven microelectrodes during stereotactic neurosurgery for implantation of a unilateral DBS electrode in the PPN in five PD patients and two PSP patients. Within the PPN region, the majority (57%) of the neurons fired randomly while about 21% of the neurons exhibited 'bursty' firing. In addition, 21% of the neurons had a long action potential duration and significantly lower firing rate suggesting they were cholinergic neurons. A change in firing rate produced by passive and/or active contralateral limb movement was observed in 38% of the neurons that were tested in the PPN region. Interestingly, oscillatory local field potential activity in the beta frequency range (at approximately 25 Hz) was also observed in the PPN region. These electrophysiological characteristics of the PPN region provide further support for the proposed role of this region in motor control. It remains to be seen to what extent the physiological characteristics of the neurons and the stimulation-evoked effects will permit reliable identification of PPN and determination of the optimal target for DBS therapy.
A.1 - Literature overview

A.1.1 - Functional anatomy of the PPN

The PPN is located in the mesopontine tegmentum and can be divided into two territories based on cell density and neurochemical characteristics: the pars compacta (PPNc) and the pars dissipatus (PPNd) (Matsumura, 2005; Pahapill and Lozano, 2000). The PPNc is located within the caudal half of the nucleus in its dorsolateral aspect and mainly contains cholinergic neurons (Mesulam et al., 1989). However, some non-cholinergic cells are also present. The PPNd contains a nearly equal number of cholinergic and non-cholinergic neurons that are distributed sparsely within the rostral half of the nucleus (Kus et al., 2003). Non-cholinergic PPN neurons are mostly glutamatergic (Lavoie and Parent, 1994c), but also noradrenergic, dopaminergic, GABAergic and peptidergic (Pahapill and Lozano, 2000).

Inputs to the PPN
Inhibitory GABAergic projections from the GPi and the SNr to the PPN are well established (Noda and Oka, 1986; Granata and Kitai, 1991) and are the most widely studied projection to the primate PPN. In monkeys, pallidal afferents to the PPN terminate preferentially on the non-cholinergic neurons of the PPNd (Shink et al., 1997) and their majority also project to the ventrolateral thalamus (Harnois and Filion, 1982; Parent et al., 2001). Projections from the SNr also appear to terminate mainly but not exclusively on the non-cholinergic neurons of the PPNd in rats (Kang and Kitai, 1990; Spann and Grofova, 1991). It is not known, however, to what extent GPi and SNr afferents are segregated within the PPN or converge onto the same neurons (Pahapill and Lozano, 2000).

Cortical inputs from the primary motor cortex innervate the dorsal aspect of the PPN in non-human primates, with representations of orofacial, forelimb and hindlimb structures arranged somatotopically from medial to lateral respectively (Matsumura et al., 2000). Terminals from the supplementary motor areas, as well as dorsal and ventral premotor...
cortices overlap this mediolateral arrangement in the middle portion of the nucleus. Frontal eye field projections are scattered through the PPN (Matsumura et al., 2000). The cortical afferents to the PPN are likely glutamatergic.

Glutamatergic inputs from the STN to the PPN have been described in rats (Kita and Kitai, 1987; Hammond et al., 1983; Steininger et al., 1992; Granata and Kitai, 1989) and, to a small degree, in non-human primates (Smith et al., 1990). However, the different subpopulations of PPN neurons that receive input from STN have not been established. Brainstem afferents to the PPN have been described in cats and rodents but not in non-human primates (Inglis and Winn, 1995).

Outputs from the PPN
The efferent projections of the PPN can be divided into descending and ascending, with cholinergic and non-cholinergic neurons contributing to both (Lavoie and Parent, 1994b). Although some PPN neurons have specific ascending projections and others specific descending projections, some neurons collateralize and project in both directions (Pahapill and Lozano, 2000).

Ascending cholinergic PPN neurons project to all thalamic nuclei in the rat (Hallanger et al., 1987; Rye et al., 1987; Sofroniew et al., 1985), cat (Steriade et al., 1988) and monkey (Lavoie and Parent, 1994b), and in particular to the associative and non-specific midline and intralaminar nuclei. In addition, ascending PPN projection provide dense innervation to basal ganglia structures via the ventral tegmental bundle (Lavoie and Parent, 1994a; Lavoie and Parent, 1994b). The most densely innervated structures are the SNC, the STN and the globus pallidus. PPN projections to the SNC in non-human primates and rats have been shown to consist of both cholinergic and glutamatergic neurons. Only a minor contingent of PPN cholinergic neurons project to SNr (Lavoie and Parent, 1994a; Charara et al., 1996; Takakusaki et al., 1996). The STN receives bilateral projections from the PPN in the rat (Woolf and Butcher, 1986), cat (Edley and Graybiel, 1983) and monkey (Lavoie and Parent, 1994b). In the rat, these connections have been shown to be both cholinergic (Woolf and Butcher, 1986) and non-cholinergic (Lee et al.,
1988). Other studies in the rat suggest that PPN efferents to the STN and adjacent zona incerta are collaterals of PPN projections to the globus pallidus (Hammond et al., 1983; Hallanger and Wainer, 1988). In the monkey, the PPN projections to the pallidal complex are less dense than those of the STN and SNc, and appear to arborize more profusely in the GPi than the GPe (Lavoie and Parent, 1994b). The neurotransmitter involved in the PPN-pallidal connections has not yet been identified with certainty (Pahapill and Lozano, 2000). Other ascending targets thought to exist in non-human primates include the striatum, superior colliculus and a number of limbic structures such as the basal forebrain, hypothalamus and amygdala (Inglis and Winn, 1995; Pahapill and Lozano, 2000; Reese et al., 1995b).

Although not as numerous as ascending projections, descending PPN efferents travel to several midbrain, pontine, medullary and spinal cord areas, including several nuclei of the reticular formation and deep cerebellar nuclei (Inglis and Winn, 1995; Reese et al., 1995b). These descending projections are thought to collateralize extensively to caudal structures (Rye et al., 1988) as well as to the thalamus (Semba et al., 1990). In rodents, it has been shown that most of the descending cholinergic PPN neurons travel a shorter distance to the medullary reticular formation, which in turn provides bilateral outputs to the spinal cord. The direct PPN projections to the spinal cord are thought to be mainly non-cholinergic, although a small number of cholinergic neurons may project as far as the spinal cord (Rye et al., 1988; Skinner et al., 1990a; Skinner et al., 1990b; Goldsmith and van der Kooy, 1988).

A.1.2 - Regulation of locomotion and gait in the PPN

The role of the PPN in locomotion is bound up with the fact that the PPN is part of the so-called mesencephalic locomotor region, a functionally defined area of the brainstem within which it is possible to elicit controlled locomotion in decerebrate animals by electrical stimulation (Shik et al., 1969). The effects of stimulation on locomotion and muscle tone seem to be dependent on the parameters employed. For instance, continuous stimulation of the mesencephalic locomotor region at frequencies between 20-60 Hz for
several seconds is effective in eliciting locomotor activity, whereas stimulation at
frequencies higher than 100 Hz suppress muscle tone (Garcia-Rill and Skinner,
1987a; Garcia-Rill and Skinner, 1987b; Lai and Siegel, 1990). In the area of the PPN,
there are three separate populations of neurons displaying rhythmic activity in relation
to locomotion in the decerebrate cat (Garcia-Rill and Skinner, 1988). The first group of
neurons displays tonic firing during locomotion, which decreases in frequency or stops
completely with the termination of the locomotor episode. These neurons have been
termed ‘on’ cells. The second group of neurons also displays tonic firing, but the firing
rate decreases as the locomotion frequency increases. These neurons are called ‘off’ cells.
The third group of neurons displays a bursting pattern of firing during locomotion. It has
been suggested that the ‘on’/’off’ cells (though to be cholinergic) might modulate the
duration of the stepping episode, while the bursting cells (though to be non-cholinergic)
may be involved in modulating the frequency (and possible initiation) of stepping
(Garcia-Rill and Skinner, 1991).

Although the optimal sites for the stimulus induction of locomotion appear to be within
the cholinergic neuron mass of the PPNc (Garcia-Rill et al., 1987), there are several
brainstem regions, including prominent sensory nuclei, which can be stimulated to
initiate locomotion. Each of these areas project to the spinal cord and none of these can
be considered specific to locomotion. Indeed, bilateral excitotoxic lesions of the whole
PPN have repeatedly failed to affect locomotor activity in rodents (Inglis et al.,
1994a; Inglis et al., 1994b; Olmstead and Franklin, 1994; Steiniger and Kretschmer,
2004; Swerdlow and Koob, 1987). However, unilateral microinjections of the cholinergic
antagonist cabachol into the PPN decreased spontaneous locomotor activity of rats
(Brudzynski et al., 1988). In non human primates, excitotoxic lesions of the PPN (with
kainic acid injections) have been shown to produce contralateral hemiparkinsonism
characterized by flexed posture and hypokinesia (Kojima et al., 1997; Munro-Davies et al.,
1999). In addition, radiofrequency lesions in the PPN significantly reduced motor activity
in monkeys, as reflected by generalized bradykinesia that resembled Parkinson’s disease
(Aziz et al., 1998). Although animals recovered to a near normal condition after
approximately one week following unilateral lesions, bilateral lesions induced
significantly long-lasting effects (Munro-Davies et al., 1999).

In the normal monkey, nearly half of the recorded PPN neurons changed their firing rates in response to voluntary movement of the ipsilateral and/or contralateral arm (Matsumura et al., 1997), as well as in response to voluntary guided saccades (Kobayashi et al., 2002). In the cat it has been shown that PPN neurons change their firing activity during a conditioned movement of the forelimb (Dormont et al., 1998) and that pharmacological inactivation of the PPN by injection of muscimol induces prolonged arrest in the performance of a conditioned motor task (Conde et al., 1998). These findings suggest that the PPN could influence more widespread somatic movements (Matsumura, 2005). Interestingly, unilateral electrical stimulation of the PPN in a normal monkey results in frequency dependent motor effects: low frequencies (<30 Hz) elicit contralateral proximal limb tremor while high frequencies (>45 Hz) cause loss of postural control and severe akinesia (Nandi et al., 2002b).

Neurons in the PPN were also shown to respond to somatosensory stimuli (especially in contralateral trigeminal areas) (Grunwerg et al., 1992) and auditory inputs (Reese et al., 1995a). The responsiveness of PPN neurons to somatosensory stimuli, together with the proposed inputs to the PPN from lamina I of the cat spinal cord (Hylden et al., 1985), suggests that the PPN may also be involved in the modulation of sensory information to thalamic nuclei. The prominent cholinergic PPN projection to the thalamus and its connections with deep cerebellar nuclei further facilitate the potential role of the PPN as a relay station, providing feedback information important for the modulation of posture and gait initiation (Pahapill and Lozano, 2000).

Apart from this, the PPN have been considered to play a role in the control of sleep and to provide a link between the limbic motivation system and the selection of motor output by the basal ganglia (Winn, 2008).

**A.1.3 - PPN and Parkinson’s disease**
Neuropathological studies on humans have reported nearly 50% degeneration of the cholinergic neurons in the PPNc in akinetic disorders such as PD and progressive supranuclear palsy (PSP) (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989). Moreover, the degree of akinesia in PD has been linked to the extent of loss of the large cholinergic PPN neurons (Zweig et al., 1989). The fact that the neuronal loss within the PPNc is similar in magnitude to that of the SNc suggests that PPN neurons might be susceptible to similar pathogenetic mechanisms as nigral dopaminergic neurons (Pahapill and Lozano, 2000).

According to the direct and indirect pathways model, one would predict that in Parkinson’s disease there would be both increased inhibitory input to PPN from basal ganglia output nuclei and increased excitatory input from STN. Indeed, experimental studies support these predictions. Increased inhibition from the GPi and SNr would lead to suppression of activity in the PPN, which, because the PPN is thought to facilitate movement, would in turn produce a state of motor hypoactivity. In agreement with this, pharmacological blockade of the inhibitory input to PPN with the GABAergic antagonist bicuculline markedly attenuates akinesia in the MPTP monkeys (Nandi et al., 2002a). By contrast, PPN neurons also exhibit increased activity in animal models of PD (Orieux et al., 2000; Breit et al., 2001), which depends on STN (Breit et al., 2001) and thus presumably relates to the hyperactivity of STN neurons after chronic loss of dopamine. In anesthetized rats, 6-OHDA lesion of the SNc not only induces a significant increase in firing rates of PPN neurons (from ~10 Hz to ~18 Hz), but also results in an irregular firing pattern (6% vs. 27% of the neurons) (Breit et al., 2001).

Of relevance to the clinical practice, it was further shown that some of the electrophysiological changes recorded in basal ganglia structures after 6-OHDA nigral injections could be reversed by PPN lesions. These include a decrease in the firing rate of the STN (from 17 to 11 Hz) and SNr (from 26 to 17 Hz), with no remarkable change in the firing patterns (Breit et al., 2006). The reversal of STN and SNr hyperactivity in 6-OHDA-lesioned rats by additional PPN lesion suggests an important modulation of STN activity by the PPN. Another study in anesthetized rats has investigated the response of
PPN cells to STN stimulation (Florio et al., 2007). When applied in single pulses, STN stimulation induced excitatory responses in 20% of the PPN neurons recorded, but when stimulation was provided in 1-5 second trains at 130 Hz, approximately 40% of the recorded PPN neurons responded. Of those, 85% were inhibited and 15% excited by the STN stimulation. This pattern of responsiveness (i.e., approximately 20% to single pulses, and 40% to stimulation trains) was maintained after nigral 6-OHDA injections. In animals that had entopeduncular lesions however, 75% of PPN neurons became responsive to STN stimulation. In addition, a higher proportion of cells responded with excitation (85%) vs. inhibition (15%) following entopeduncular lesions in animals that had nigral saline injections or 6-OHDA. These results suggest that the inhibition recorded in PPN cells after trains of STN stimulation is likely mediated by entopeduncular neurons (Florio et al., 2007).

In MPTP monkeys, microinjection of the GABAergic antagonist bicuculline (but not saline or muscimol) into the PPN, was able to improve parkinsonian symptoms (Nandi et al., 2002a). In addition, when MPTP was systematically injected in monkeys with prior PPN lesioning, the monkeys developed no or very mild parkinsonism relative to MPTP monkeys without a previous PPN lesion (Matsumura and Kojima, 2001). In a later study, Jenkinson and colleagues have studied the effects of unilateral PPN DBS prior to and after inducing MPTP parkinsonism in a macaque monkey (Jenkinson et al., 2004). In the normal monkey, PPN stimulation at 5 Hz increased movement counts (measured per hour), whereas 100-Hz stimulation decreased the same measures. After making the monkey parkinsonian with MPTP, low-frequency PPN stimulation was as effective as levodopa, but high-frequency DBS was not.

Taken together, these findings have suggested an important role for the PPN in generating parkinsonian symptoms and opened up new possibilities in the management of advanced PD.

A.1.4 - PPN as a surgical targeted for PD
The first report on deep brain stimulation in the human PPN involved two PD patients who displayed dominant symptoms of freezing of gait and postural instability before surgery (Plaha and Gill, 2005). Postoperatively short-term clinical improvement was observed with stimulation at 20–25 Hz. Total UPDRS improved 53% and the motor subscore improved 57% when averaged between the two patients. High-frequency stimulation actually worsened gait in one patient. In a different report, Mazzone and colleagues described intraoperative PPN DBS in two PD patients (Mazzone et al., 2005). In this study, low-frequency stimulation at 10 Hz was associated with a feeling of ‘wellbeing’ that correlated with modest improvements in motor function (bradykinesia and rigidity). The effects of posture and gait, however, were not reported in this initial investigation.

In a later study by the same group, the outcomes of bilateral PPN DBS, with or without simultaneous stimulation in the STN, were investigated in 6 patients with severe symptoms including disabling dyskinesia and postural instability (Stefani et al., 2007). PPN DBS was given at 25Hz (60 μsec pulse width) and 1.5-2 volts in a bipolar configuration. At 6 months post-surgery, UPDRS motor scores in the ‘off’ medication condition were improved by 33% with PPN DBS. This improvement, however, was significantly less than the benefits obtained with STN DBS (54%) or STN + PPN DBS (56%). In contrast, improvements in axial symptoms such as rising from a chair, posture, gait, and postural stability were similar with PPN, STN and PPN +STN DBS (60-70%). In the ‘on’ medication condition, UPDRS motor scores were significantly improved by either PPN (44.3%) or STN DBS (51%), as compared to the ‘on’ medication ‘off’ stimulation condition. A significantly better outcome has been achieved with STN + PPN DBS (66.4%), showing that the activation of both electrodes was of value when the patients were taking their medicine. When only the axial symptoms were taken into account, the use of medication and either PPN DBS or PPN+ STN DBS led to a 50-60% improvement, as compared to the ‘on’ medication ‘off’ stimulation condition. STN stimulation alone did not improve axial symptoms when the patients were taking their medication. Importantly, no major complications were described in either series. The only inconvenient side effect reported with PPN stimulation at high frequency was
paresthesia, likely due to the proximity of the target to the medial lemniscus (Stefani et al., 2007).

**A.2 - Introduction to the study**

Although STN deep brain stimulation (DBS) to treat advanced Parkinson’s disease (PD) is a well established procedure and produces striking clinical improvement in motor symptoms such as tremor, rigidity and bradykinesia, it has only a moderate and a short term effect in improving axial symptoms like gait akinesia and postural instability (Kleiner-Fisman et al., 2003). Recently, the pedunculopontine tegmental nucleus (PPN) has been proposed (Pahapill and Lozano, 2000) and examined as an alternative therapeutic target for PD, especially for patients with severe gait and postural impairment (Mazzone et al., 2005; Plaha and Gill, 2005; Stefani et al., 2007).

The PPN is part of the brainstem reticular formation and projects to the thalamus and spinal cord. It is also reciprocally connected to the basal ganglia and is believed to regulate the control of complex central nervous system functions including posture and gait (for review, see Pahapill and Lozano, 2000). In primates, the PPN receives a major projection from the two output structures of the basal ganglia (substantia nigra pars reticulata and internal globus pallidus - GPi) and the subthalamic nucleus (STN). In turn, the PPN projects to the substantia nigra pars compacta and STN (Garcia-Rill, 1991; Pahapill and Lozano, 2000; Winn, 2006). This organization emphasizes the potential role of the PPN in controlling basal ganglia output through subsidiary loops.

It has been shown that neurons in the PPN region degenerate in akinetic disorders such as PD and PSP (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989), and lesions in the PPN can lead to decreased movements (Aziz et al., 1998; Kojima et al., 1997). Therefore, it has been suggested that the PPN may be an alternative target for DBS for the treatment of PD (Jenkinson et al., 2004; Matsumura, 2005; Pahapill and Lozano, 2000; Plaha and Gill, 2005). Indeed, recent studies have shown that low frequency stimulation (<25 Hz) of the PPN increases motor activity in a monkey model of PD (Jenkinson et al., 2004) and
improves clinical motor scores in PD patients (Mazzone et al., 2005; Plaha and Gill, 2005; Stefani et al., 2007).

The PPN occupies an area in the mesopontine tegmentum that contains two populations of neurons, non-cholinergic (likely glutamatergic) and cholinergic (Mesulam et al., 1989). The cholinergic neurons have larger somata, but are less numerous than the non-cholinergic neurons (Honda and Semba, 1995; Rye et al., 1987; Spann and Grofova, 1992). Electrophysiological studies of the PPN in non-human primates have rarely appeared in the literature (Matsumura, 2005). In the PPN of the normal monkey, nearly half of the neurons recorded changed their firing rates in response to voluntary movement of the ipsilateral and/or contralateral arm (Matsumura et al., 1997). In the cat it has been shown that PPN neurons change their firing activity during a conditioned movement of the forelimb (Dormont et al., 1998) and that inactivation of the PPN by injection of muscimol (a γ-aminobutyric acid (GABA) agonist) induces prolonged arrest in the performance of the task (Conde et al., 1998). These findings suggest that the PPN is involved in the initiation and modulation of movements (for review, see Matsumura, 2005).

The aim of the present study was to describe the neurophysiology of the human PPN region and identify neurophysiological landmarks and characteristics that may help in localizing the target for insertion of DBS electrodes in the nucleus for the treatment of PD and PSP.

A.3 - Methods

Patients
We studied seven patients, five with advanced PD and two with PSP, who were undergoing stereotactic surgery for implantation of unilateral PPN-DBS electrodes under local anesthesia. The group consisted of two women and five men who, at the time of operation, had a mean age (±SD) of 64 ± 6.7 years and mean disease duration of 12.4 ± 5.1 years. During surgery the patients were awake and ‘off’ dopaminergic medications for at least 12 h from the last oral dose of medication. Patient #7 had
bilateral STN-DBS electrodes that were implanted one year before the surgery and were
switched off during the recordings. Demographic details of the patients are given in
Table A.T1.

Table A.T1: Demographic and clinical characteristics of the patients

<table>
<thead>
<tr>
<th>Patient # and disease</th>
<th>Age (year)</th>
<th>Disease duration (year)</th>
<th>L-Dopa therapy (year)</th>
<th>Motor UPDRS (off/on)</th>
<th>Gait (off/on)</th>
<th>Postural Instability (off/on)</th>
<th>Freezing (off/on)</th>
<th>Side</th>
<th>Span of record (mm)</th>
<th>Number of neurons sampled (PPN/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - PD</td>
<td>63 M</td>
<td>10</td>
<td>5</td>
<td>34.5 / 20</td>
<td>2.5 / 0.5</td>
<td>3 / 1</td>
<td>yes / yes</td>
<td>L</td>
<td>17</td>
<td>12/34</td>
</tr>
<tr>
<td>2 - PSP</td>
<td>67 F</td>
<td>5</td>
<td>NA</td>
<td>57.5 / NA</td>
<td>3.5 / NA</td>
<td>4 / NA</td>
<td>no / NA</td>
<td>R</td>
<td>13</td>
<td>7/19</td>
</tr>
<tr>
<td>3 - PSP</td>
<td>70 M</td>
<td>10</td>
<td>NA</td>
<td>38.5 / NA</td>
<td>3.5 / NA</td>
<td>3.5 / NA</td>
<td>no / NA</td>
<td>L</td>
<td>13</td>
<td>3/9</td>
</tr>
<tr>
<td>4 - PD</td>
<td>68 M</td>
<td>15</td>
<td>14</td>
<td>41 / 21</td>
<td>2.5 / 0.5</td>
<td>2.5 / 1</td>
<td>yes / no</td>
<td>L</td>
<td>18</td>
<td>12/45</td>
</tr>
<tr>
<td>5 - PD</td>
<td>67 M</td>
<td>21</td>
<td>15</td>
<td>42 / 20</td>
<td>2 / 0.5</td>
<td>2 / 1</td>
<td>yes / no</td>
<td>L</td>
<td>16</td>
<td>12/37</td>
</tr>
<tr>
<td>6 - PD</td>
<td>63 F</td>
<td>11</td>
<td>12</td>
<td>43.5 / 35</td>
<td>2 / 1.5</td>
<td>2 / 2</td>
<td>yes / no</td>
<td>R</td>
<td>13</td>
<td>3/13</td>
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<tr>
<td>7 - PD*</td>
<td>50 M</td>
<td>15</td>
<td>17</td>
<td>25.5 / 14</td>
<td>2 / 0.5</td>
<td>0 / 0</td>
<td>yes / no</td>
<td>R</td>
<td>14</td>
<td>15/43</td>
</tr>
</tbody>
</table>

* Patient had bilateral STN-DBS; NA, not applicable

Surgical procedure

On the morning of the surgery, a stereotactic frame (Leksell G, Elekta, Inc, Atlanta, Ga)
was affixed to the patients’ heads and preoperative magnetic resonance (MR) images
were obtained (Signa, 1.5 T, General Electric, Milwaukee, Wis). Once the MRI based
coordinates of the target were calculated, the patient was brought to the operating room
and a burr hole was drilled 2 cm from the midline in front of the coronal suture.
Microelectrode recordings were then conducted from 15 mm above the target and
extended 2–5 mm below. In each patient except one, only one dual electrode track,
consisting of two microelectrodes, was performed (see General Methods, section 2.2.3
for details). In patient #4 a second electrode track was performed 2 mm medial to the first
since the thresholds for evoking paresthesia in the first track were very low. The
coordinates chosen for electrophysiological recordings were similar across patients.

Microelectrode recordings and stimulation

Neuronal spontaneous firing activity and local field potentials (LFPs) were recorded
simultaneously from two independently driven microelectrodes. For further details of the
procedure see General Methods (section 2.2.3/4). In all patients, we tested the effects of a
1 s microstimulation train (200 Hz, 150 μs pulse width at intensities up to 100 μA) along
the microelectrode trajectory at intervals of 2 mm or less.

*Localization of electrode trajectory*

The approximate location of the microelectrode tracks was estimated post-operatively (by Dr. Clement Hamani) as follows: Preoperative axial stereotactic 3D inversion recovery and T2-weighted MR images were transferred to a StealthStation workstation. Using the FrameLink 4.1 software (Mach 4.1, StealthStation, Medtronic, SNT) these two images were merged, the fiducials of the frame were recognized, and the mean rod marking error was calculated and registered. Coronal and sagittal planes were reconstructed based on axial images. The anterior and posterior commissures (AC and PC) were then targeted in the axial plane and three additional points were plotted in the midline. Thereafter, the images were reformatted parallel to the AC–PC plane and orthogonal to the midline. Pitch, roll, and yaw were corrected in the StealthStation. The coordinate of the microelectrode track and the angles of each trajectory were entered into the neuronavigation system so that they could be reconstructed. As the PPN cannot be clearly visualized on imaging studies, we estimated the location of the region that most likely encompassed the nucleus. We defined this PPN region as the anterolateral portion of the pontine/mesencephalic tegmentum that extended from 2 mm below the inferior colliculus to the transition between the inferior and superior colliculi, according to the Paxinos and Huang atlas (Paxinos G and Huang X.F., 1995).

*Data analysis*

Only sites with good signal-to-noise and stable single unit recordings were analyzed. To characterize the firing activity of the recorded neurons the signals were bandpass filtered at 425–5,000 Hz and single unit activity was discriminated using the template matching tool in Spike2 (CED). As a measure of action potential width we used the duration of the negative phase of the averaged spike waveform (see Figure A.F1A). A similar method was previously used to measure spike duration of PPN neurons (Matsumura et al., 1997; Takakusaki et al., 1997).
Mean firing rates and measurement of firing patterns were performed in MATLAB (version 6.5, The Math Works, Natick, MA) using the Kaneoke and Vitek method (Kaneoke and Vitek, 1996), which uses discharge density to categorize firing patterns into random, bursty or regular. In this method, the discharge density histogram of a neuron is determined by calculating the number of spikes in an interval equal to the mean ISI. The number of occurrences of no spikes, one spike, two spikes, and so on in each time interval is then counted and a discharge density histogram is constructed. This discharge density histogram represents the probability distribution of the neuron’s discharge density and can be compared to a discharge density of a Poisson process with a mean of 1 by using a $\chi^2$ goodness-of-fit test. If the neuronal discharge pattern was random, its discharge density distribution would be statistically similar to that of a Poisson distribution. If the neuron’s discharge pattern was significantly non-random it could be either regular or bursty. In cases of regular firing patterns, the probability of finding one spike per time segment is high. On the other hand, in cases of bursting firing patterns, the probability of finding no spikes or many spikes per time segment is high. Since a Poisson process with a mean of one has a variance equal to one, the former case represents a significantly non-Poisson discharge density distribution with a variance of less than one and the latter case represents a significantly non-Poisson discharge density distribution with a variance of greater than one.

**Figure A.F1.** Width of action potentials. [A] The width of the action potential was measured as the duration of the negative phase of the averaged spike waveform. [B] Distribution histogram of spike durations.
For examining neuronal responses to movement, movement onset was determined from accelerometer recordings and/or electromyograms and a peri-stimulus histogram constructed. Movement-related changes in neuronal firing were initially identified by visual inspection of the histograms. Then, the identified responses were evaluated statistically using nonparametric tests comparing the firing rate during a 200 or 300 ms control period and during 200/300 ms of the visually determined response (P < 0.05, Wilcoxon signed rank test).

For spectral analysis, recordings of ≥11 second duration (mean ± SD: 37.9 ± 19.7 s, range: 11–112 s) were imported into MATLAB. Power spectra and coherence of neuronal discharges and LFP recordings was performed according to Halliday et al. (1995) and Rivlin-Etzion et al. (2006), and is described in detail in the General Methods (section 2.3.1.a/b).

A.4 - Results

A.4.1 - Action potentials and firing properties

A total of 244 neurons was recorded in seven patients. Of these, 235 were recorded while the patient was at rest and were used to characterize the baseline firing activity. Neurons were classified according to the width and polarity of their action potentials. The incidence histogram of spike durations did not reveal a clear bimodal distribution. Nevertheless, we observed a marked drop in the number of neurons that had action potential durations ≥0.65 ms (see Figure A.F1B) which suggests they may have originated from a different population of neurons that are likely to be composed of cholinergic neurons (see Discussion). This population consisted of 38 neurons that comprised 15.6% of the total number of neurons. These neurons were found in all the patients except one (patient #3, PSP) in whom a total of only nine neurons were recorded (see Table A.T1). Their mean (±SD) spike duration was 0.84 ± 0.2 ms compared with 0.47 ± 0.1 ms for the neurons with brief spikes. In addition, these neurons had a significantly lower mean firing rate (medians: 9.3 vs. 17.3 spikes/s, P ≤ 0.001, Mann–
Whitney rank sum test) (Figure A.F2B, D). This provides further support for the assumption that these cells represent a different neuronal population. The majority of these neurons (86.8%) was found within and below the PPN region. In the region of the PPN, these neurons comprised 21% of the neurons (18/85) compared to 19.5% below that region (15/77) and 6% above (5/82).

Interestingly, 16 units had positive-going action potentials and significantly higher mean firing rates (median 67.6 spikes/s, $P \leq 0.001$) (Figure A.F2C, D). These units were found in all seven patients. Six of these neurons were located in the PPN region and nine were located below that region. In addition, most (88%) of these neurons displayed a regular firing pattern as determined by the Kaneoke and Vitek method (Figure A.F3A). This was significantly different from the patterns of activity exhibited by the two types of neurons described above which were primarily random (65 and 68% for the neurons with short and long spike durations respectively) ($P \leq 0.001$, $\chi^2$-test). Comparison of the proportions of both brief-and broad-spike duration neurons exhibiting regular, random or bursty firing
patterns above, within and below the PPN region, revealed a statistically significant difference ($P = 0.016$, $\chi^2$-test) (Figure A.F3B). In the PPN region, the majority (57%) of the neurons fired randomly while about 21% of the neurons exhibited bursty firing. Above and below the PPN region, the proportion of bursting neurons was smaller (11 and 19% respectively) while more neurons exhibited random firing (65 and 75% respectively).

A movement responses

The activity of 103 neurons was recorded during contralateral limb movements. Tasks varied between passive and/or voluntary movements of the wrist, hand, foot, elbow and sometimes shoulder. 36% ($n = 37$) of the neurons responded to at least one type of passive or voluntary movement of the limb. Out of the 41 neurons that were tested only for passive movements, 16 responded (39%) and out of the 50 neurons that were tested only for voluntary movements, 12 responded (24%). Twelve neurons were tested for both passive and voluntary movement, out of which, five neurons responded to passive movement only, two neurons responded to voluntary movement only and two neurons responded to both. Table A.T2 summarizes the results for each of the neuron types.
The changes in firing rates were mostly excitatory (about 80%) and in some cases, were followed by inhibition (see Figure A.4A, C). Within the PPN region 38% of the neurons responded to movement and similar percentages were recorded above and below the PPN region where 37 and 33% of the neurons responded respectively. Movement responsive neurons included all three neuron types. Figure A.F4 shows an example of responses to contralateral movement from each neuron type.

<table>
<thead>
<tr>
<th>Neuron type</th>
<th>Passive movement</th>
<th>Voluntary movement</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase</td>
<td>Decrease</td>
<td>No response</td>
</tr>
<tr>
<td>A</td>
<td>17</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>5</td>
<td>30</td>
</tr>
</tbody>
</table>

Note: 12 neurons that were tested for both passive and voluntary movement were included in both groups.
Figure A.F4. Examples of movement-evoked responses of the three types of neurons. [A] An example of a type A neuron exhibiting an increase in firing rate in response to passive wrist flexion that is followed by a decrease in firing rate (P = 0.001, Wilcoxon signed rank test). [B] Type B neuron exhibiting increased firing rate to passive elbow movement (P ≤ 0.001). [C] Type C neuron exhibiting increased firing followed by decreased firing in response to passive flexion and extension of the foot respectively (P = 0.003). All movements were contralateral to the recording side. Raster plots (middle traces) and stimulus histograms (bottom traces) were aligned to movement onset [indicated by smoothed and rectified EMG (A, B) or accelerometer (C) output] (top traces).
A.4.3 - Microstimulation in the PPN region

In all patients, stimulus trains of 1 second evoked paresthesia at some sites along the microelectrode trajectory. In three patients, the threshold intensities for stimulation-evoked paresthesia varied between 50 and 100 μA (with three exceptions of 20, 25 and 25 μA) (mean ± SD: 79.2 ± 29.6 μA, n = 24). In the remaining four patients, the threshold intensities were lower and varied between 1 and 50 μA (with two exceptions of 100 and 75 μA) (mean ± SD: 18.8 ± 19.9 μA, n = 55). The thresholds were not significantly different when stimulating within or close to the PPN region. These responses were assumed to be due to activation of ascending axons in the medial lemniscus. In addition, eye movements were evoked by microstimulation in two patients and occurred only at the maximum stimulation intensity of 100 μA, and only within and up to 2 mm above the PPN region.

A.4.4 - LFP oscillatory activity in the PPN region

Spectral analyses of the local field potentials revealed oscillatory activity in the beta frequency range (15–30 Hz) in the PPN region of three PD patients (#1, 4 and 7). In all three patients, a significant coherence in the beta range between the two microelectrodes was also observed. It was not possible to adequately assess the existence of LFP oscillatory activity in the other four patients due to suboptimal slow wave recordings in those cases. Figure A.F5A demonstrates LFP beta oscillations recorded from the two microelectrodes (in patient #1), and their significant coherence. In these patients, the LFP power in the beta frequency range varied with depth along the microelectrode track. Beta oscillations were most prominent in the region of the PPN (Figure A.F5B) and up to 4 mm below. Frequencies between 35 and 50 Hz were sometimes encountered in the LFP but the coherence in these frequencies rarely reached significance (this is notable in Figure A.F5A). Similarly, oscillations at frequencies lower than 15 Hz, although occasionally observed, were not a consistent feature in the PPN region. Spectral analyses of the neuronal firing in the PPN region failed to show significant oscillatory activity in the beta frequencies. Moreover, coherence and cross-correlation analyses did not reveal a
significant relationship to the simultaneously recorded LFP.

Figure A.5. Beta oscillatory activity in the PPN region. [A] An example of power spectra of the LFPs recorded from each of the two adjacent microelectrodes (upper and middle traces) and the corresponding coherence function (lower trace). Dotted lines represent 95% confidence limits. [B] An example of LFP power changes along the microelectrode track, above within and below the PPN region. The LFP power was normalized and expressed as the percentage of the total power between 5 to 50 Hz.
A.4.5 - Comparison of PD vs. PSP

No obvious differences were noted in any of the findings between the two groups of patients other than a lower number of neurons encountered in the PSP patients compared to all but one of the PD patients.

A.5 - Discussion

The present study provides new data documenting various electrophysiological characteristics of the PPN region in PD and PSP patients. The lower number of cells recorded from the PPN region in the two PSP patients (see Table A.T1) suggests a lower density of neurons which might reflect the large degree of neuronal degeneration and brainstem atrophy that occurs in PSP (Dickson et al., 2007; Hirsch et al., 1987). We did not observe any obvious neurophysiological differences between the two patient groups. However, in view of the small number of neurons in the 2 PSP patients this comparison may not be very meaningful.

Analysis of spike duration and shape revealed three types of neurons. The existence of short and long duration negative going action potentials in this region agrees with the findings of previous in vitro and in vivo animal studies. In vivo recordings from the PPN in rats (Takakusaki et al., 1997), cats (Dormont et al., 1998) and monkeys (Matsumura et al., 1997) revealed the existence of two types of neurons: ones which fire at low rate and exhibit a long duration spike, and ones which fire at higher rates and exhibit a short duration spike. Yet, the incidence histogram of spike durations in our study did not reveal a clear bimodal distribution as previously demonstrated in the rat PPN (Takakusaki et al., 1997). That study, however, utilized intracellular recordings and therefore cannot be directly compared to our extracellular recordings. Moreover, the incidence histogram in our study is consistent with data from extracellular recordings in the monkey PPN that failed to exhibit a bimodal distribution (Matsumura et al., 1997).

It has been proposed on the basis of in vitro studies that the neurons with broad spikes are
Although the exact borders of the PPN remain indistinct, the cholinergic neurons are a very obvious component of the PPN (Winn, 2006). Indeed, the neurons with broad spikes in our study were found primarily within the region of the PPN and below it. The region below the PPN is likely to be the adjacent laterodorsal tegmental nucleus which is known to contain cholinergic neurons that are interconnected with the cholinergic population in the PPN (Winn, 2006). These findings, together with the low rate of firing, provide further support for the assumption that these neurons are cholinergic. In our study, the majority of the neurons in the PPN region exhibited random firing while about 21% of the neurons had a bursty firing pattern. This finding is supported by earlier studies in rats (Scarnati et al., 1987), cats (Garcia-Rill et al., 2004) and monkeys (Matsumura et al., 1997) which describe a small population of bursty PPN neurons, whereas the majority of the neurons have been reported to fire in an irregular pattern.

The positive-going action potentials may possibly have originated from large diameter axons of passage as extracellularly recorded positive going potentials are generally assumed to be axonal. They had a significantly higher mean firing rate and a regular firing pattern. These electrophysiological properties are different than those of the PPN neurons previously described and are likely to arise from afferents to the PPN region, possibly ascending sensory axons of the medial lemniscus which lies laterally to the PPN (Olszewsky and Baxter, 1982).

Previous electrophysiological studies in cats and monkeys have identified PPN neurons that exhibit changes in firing rate in response to limb movement and include neurons with both brief and broad spikes (Dormont et al., 1998; Matsumura et al., 1997). In monkey, PPN neurons have also been found to respond with either an increase or a decrease in firing rate to voluntary saccades (Kobayashi et al., 2002). Mazzone et al. (2005) reported finding 6 neurons (of a total of 27 studied) in the PPN of two PD patients that responded with a “slight inhibition or inhibition followed by excitation” to a single-joint movement, but no further details were provided. It is important to note that the PPN region targeted in the current study (PPTg according to Paxinos and Huang 1995) may be different from
the region studied by Mazzone et al. (see also (Yelnik, 2007;Zrinzo et al., 2007a;Zrinzo et al., 2007b)). Our study clearly demonstrates the existence of movement-responsive neurons in the human PPN, consistent with the findings of animal studies. Moreover, we found that both short and long spike duration neurons responded to movements. It has been reported that PPN inputs to the basal ganglia are mediated by both cholinergic and glutamatergic synapses (Lavoie and Parent, 1994a). Taken together, these findings suggest that both groups of neurons may contribute to basal ganglia-related motor functions.

In the three PD patients analyzed, the LFPs in the PPN region exhibited oscillatory activity in the beta range (15-30 Hz). Similar oscillations have been previously observed in the STN and GPi of PD patients (Brown et al., 2001;Kuhn et al., 2005;Levy et al., 2002a;Weinberger et al., 2006) and are suggested to play an ‘antikinetic’ role in PD (Brown, 2003;Brown and Williams, 2005). It is important to note, however, that the neuronal firing did not show oscillations in these frequencies. The absence of oscillatory firing may be due to limited sampling or may reflect a lack of coupling between dendritic membrane potential oscillations and the soma. Since the beta oscillations were maximal in the PPN region they are unlikely to reflect far field potentials from distant structures (e.g. STN, cortex). The elevation of the beta frequency band LFP activity around the region of the PPN suggests that this activity may be useful in determining the final positioning of the DBS electrode intra-operatively, but further studies are required to examine the consistency of this feature. In a recent study by Androulidakis et al. (2008), LFP recordings from DBS electrodes in the PPN region in six PD patients revealed 7–11 Hz oscillations that were prominent only after treatment with levodopa, suggesting that these oscillations may represent a physiological pattern of activity in this region (Androulidakis et al., 2008b;Androulidakis et al., 2008c). However, inconsistent with our finding, that study did not reveal beta oscillations in the ‘off’ dopaminergic state. This discrepancy might be due to the difference in the region targeted in that study, which is similar to that reported by Mazzone et al. (see above).

In summary, the electrophysiological characteristics of the PPN region described in this
paper provide further support for the proposed role of this region in motor control. However, in contrast to microelectrode recordings in thalamus, pallidum and STN, we did not find distinctive and definitive neurophysiological characteristics and clear nuclear boundaries for PPN. This is consistent with the absence of precise anatomical borders to the PPN (Winn, 2006), which might reflect its anatomical complexity. Thus microelectrode recordings and microstimulation-induced effects in PPN, although helpful, do not provide the same degree of confirmation of the target as they do in these other regions although future studies may reveal additional features distinctive for the PPN which would be useful in localizing the region and identifying an optimal target for DBS therapy.


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