Role of the Level of Expression of Striatal Dopamine Transporter Relative to Striatal Dopaminergic Terminals in the Pathogenesis of Levodopa Induced Motor Fluctuations in Parkinson's Disease

By

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Abstract:

Motor complications in response to levodopa therapy are major problems in the treatment of Parkinson's disease (PD). In this study we investigated the possible relationship between the level of dopamine transporter expressed on surviving striatal dopaminergic terminals and variations in extracellular dopamine levels in PD patients with stable response to levodopa and those with motor fluctuations. We assessed the changes of endogenous and exogenous dopamine levels over time in response to oral methylphenidate and levodopa respectively. 3D PET was performed with the D2 receptor antagonist \[^{11}\text{C}]\text{raclopride (RAC)} at baseline, 1 and 4 hours following the administration of oral methylphenidate (0.8 mg/kg) or oral levodopa (250/25 mg) to two groups of PD patients consisting of fluctuators and stable responders to levodopa. In parallel, we measured the ratio of dopamine transporters to the number of dopaminergic terminals in the striatum. For this purpose we used \[^{11}\text{C}]\text{MP and }[^{11}\text{C}]\text{DTBZ PET scans to estimate the levels of dopamine transporter expression and the vesicular monoamine transporter 2 (VMAT2) respectively. At the dose used, oral methylphenidate produced no significant change in extracellular dopamine levels, as estimated by comparing changes in putaminal RAC binding at baseline and one and four hours following its administration. This could be the result of severe degeneration of dopaminergic terminals with subsequent reductions in the levels of endogenous dopamine and DAT. Although the putaminal RAC binding significantly changed after administration of levodopa, the regression of RAC binding potentials at the three times to log (MP/DTBZ) was not significant implying that the ratio of dopamine transporters to dopaminergic terminals did not affect exogenous dopamine release and clearance.
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Abbreviations:

3-MT, 3-methoxytyramine
3-OMD, 3-0-methyldopa
7TM, seven transmembrane domain
ADHD, attention deficit hyperactive disorder
AIR, Automated Image Registration
ALAAD, aromatic L-amino acid decarboxylase
AP-1, activator protein -1
BBB, blood brain barrier
BP, binding potential
Ca, calcium
cAMP, cyclic adenosine mono-phosphate
CAT, choline acetyltransferase
COMT, cathecol-o-methyltransferase
D1, type 1 dopamine receptor
D2, type 2 dopamine receptor
DA, dopamine
DAT, dopamine transporter
dV, distribution volume
dVR, distribution volume ratio
DOPAC, 3,4-dihydroxyphenylacetic acid
Enk, enkephalin
FD, fluorodopa
GABA, gamma-aminobutyric acid
GAD, glutamic acid decarboxylase
GPe, globus pallidus externus
GPi, globus pallidus internus
HVA, homovanillic acid
Kp, K (plasma) input
Kocc, K occipital
L-AADC, L-aromatic amino acid decarboxylase
LDR, long duration response
LNNA, large neutral amino acid
MAO, monoamine oxidase
Mg, magnesium
MMSE, mini-mental state exam
MPTP, 1,2,3,6-methyl-phenyl-tetrahydropyridine
mRNA, messenger ribonucleic acid
NMDA, N-methyl-D-aspartate
PAC, premature atrial contraction
PD, Parkinson’s disease
PET, positron emission tomography
PPN, pedunculopontine nucleus
PVC, premature ventricular
ROI, region of interest
SAM, S-adenosyl-L-methionine
SC, superior colliculus
SD, standard deviation
SDR, short duration response
SN, substantia nigra
SNC, substantia nigra pars compacta
SNr, substantia nigra pars reticulata
SP, substance P
STN, subthalamic nucleus
TH, tyrosine hydroxylase
UPDRS, unified Parkinson’s disease rating scale
VMAT2, vesicular monoamine transporter 2
Acknowledgement

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Chapter 1, Parkinson’s disease and motor complications

1.1 Introduction:

Parkinson’s disease (PD) is a neurodegenerative disease, which affects 100,000 Canadians. The cost of the disease for society is estimated as 2.5 to 5 billion annually in Canada. As the major mechanism for signs and symptoms of the disease is dopamine deficiency in the nigrostriatal pathway, common therapies are restoration of striatal dopamine level by administration of levodopa or using a direct dopamine receptor agonist. Dopamine receptor agonists are not as effective as the dopamine precursor levodopa and levodopa is still the drug of choice for most patients with PD. Unfortunately, after about 3 years of therapy 50% of patients develop motor complications (Chase et al., 1993). These motor complications at first usually appear as shortening in the duration of the action of anti-Parkinson medications, which are called “wearing off” fluctuations. Later, other types of motor complications appear that are independent of the timing of anti-Parkinson medications such as “on-off” fluctuations, and later dyskinesias develop. Although it seems that the mechanisms involved in “on-off” fluctuations and dyskinesia may be different from “wearing off” motor fluctuations, these phenomenon are related to the pathogenesis of “wearing off” fluctuations (de la Fuente-Fernandez et al., 2001). Motor complications are delayed with the administration of dopamine receptor agonists but they are more common with levodopa itself (Kumar et al., 2003). The exact mechanisms involved in the development of these motor complications remain elusive but it has been suggested that both presynaptic and postsynaptic sides of dopaminergic synapses in the striatum can be involved in the pathogenesis of motor complications (de la Fuente-Fernandez et al., 2001). It is already known that a rapid increase in synaptic dopamine levels in response to exogenous levodopa precedes the development of motor fluctuations in those tested while they still show a stable response to levodopa (de la
The disability and discomfort resulting from these complications necessitate more investigation to be carried out on the pathogenesis of the problem. In this study we aim to investigate whether the time course of dopamine change of endogenous dopamine and that derived from exogenous levodopa are related to the level of membrane dopamine transporter (DAT) expression on the dopaminergic terminals.

1.2 Parkinson’s disease:

Parkinsonism or Parkinsonian syndrome is a clinical syndrome diagnosed by its motor symptoms. According to Fahn, Parkinsonism is known by six symptoms and for a diagnosis of Parkinsonism two symptoms should be present while one of them ought to be tremor at rest or bradykinesia and the others could be rigidity, loss of postural reflexes, flexed posture, and the freezing phenomenon (Fahn, 2003). More commonly Parkinsonism is defined as a clinical syndrome comprised of resting tremor, rigidity, bradykinesia and impairment of postural reflexes (Calne et al., 1992). Parkinsonism can be further divided as primary and secondary. Most cases of primary Parkinsonism that are more commonly called Parkinson’s disease are sporadic or idiopathic. Therefore, idiopathic or sporadic PD is defined as a syndrome of Parkinsonism without any known genetic or environmental causes. Other cases of primary Parkinsonism have a familial pattern and are genetically inherited diseases. In contrast secondary Parkinsonism has a known environmental cause. The presence of the following criteria can be used as a guide for the diagnosis of PD versus similar diseases:

(1) PD has an asymmetrical onset and usually affects one side of the body sooner than the other.
(2) Rest tremors can often be the first sign of PD while it is almost always absent in similar syndromes (Parkinson-plus syndromes).
PD signs and symptoms usually respond to adequate dose of levodopa. This is not true in Parkinson-plus syndromes (Table 1) (Fahn, 2003).

Idiopathic Parkinson’s disease (PD) is a progressive neurodegenerative disease that affects the dopaminergic projections of midbrain. The dopaminergic neurons in the midbrain consist of neurons of the substantia nigra (SN) and those of the ventral tegmental area. In Idiopathic PD, those dopaminergic neurons of substantia nigra pars compacta (SNC) which project to the putaminal portion of striatum are most severely affected by the degenerative process. This projection is called the nigrostriatal pathway. The putamen is one of the basal ganglia, which through neuronal circuits involving the motor cortex and cerebellum is responsible for control of movements. Thus, the degeneration of these neurons leads to a deficiency of striatal dopamine and the motor manifestations of Parkinsonism (Young, 1999) (Mink, 1999).

The prevalence of PD is estimated as 1% of the population over 50 years old (Mitchell et al., 1996). There is a gender preference in this disease as the disease reportedly is more common in men rather than women (3:2) (Schoenberg, 1987). At present there is no confirmed study to show ethnic preference.

1.3 Basal Ganglia in Parkinson’s disease:

Table 1: Clinical features of Parkinsonism (adapted from Fahn, 2003).

<table>
<thead>
<tr>
<th>Tremor at rest</th>
<th>Flexed posture of neck, trunk, and limbs</th>
</tr>
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<tbody>
<tr>
<td>Rigidity</td>
<td>Loss of postural reflexes</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>Freezing phenomenon</td>
</tr>
</tbody>
</table>
The basal ganglia are composed of 5 nuclei situated in deep brain tissue in the forebrain that are mainly involved in control of movements (Nauta and Mehler, 1966). These nuclei are classified into different groups: The dorsal striatum which consists of caudate nucleus and putamen, the ventral striatum (nucleus acumbens), the globus pallidus (divided into internal and external segments or GPi and GPe respectively), the subthalamic nucleus (STN), and the substantia nigra (consisting of substantia nigra pars reticulata and compacta or SNr and SNC). The striatum and STN receive most of the cortical and thalamic inputs to these ganglia. Through intrinsic circuits inhibitory projections are sent from these input ganglia to the output ganglia, mainly GPi and SNr and from there to thalamus. The motor information is projected from thalamus to frontal (motor) cortex and some regions of brain stem involved in motor behavior (Wichmann and DeLong, 2003). It is the SNC projections to putamen that preferentially degenerate in PD. Functionally, the intrinsic connections of striatum to the output ganglia can be divided into two pathways: the direct and indirect pathways. The direct pathway starts from a group of striatal neurons that express the D1 dopamine receptor. These neurons project directly to GPi and SNr and express the neuropeptides substance P and dynorphin together with GABA, resulting in inhibition of the output ganglia. The indirect pathway starts from D2 expressing striatal neurons and project to GPe. These inhibitory neurons release enkephalin and GABA (Smith et al., 1998).

The most common dopamine receptors in the striatum (D1 and D2 receptors) have excitatory and inhibitory functions in response to dopamine respectively. Deficiency of dopamine leads to an increase in the activity of STN and GPi in both pathways and as a result to an inhibition of the thalamus and motor cortex (Fahn, 2003; Wichmann and DeLong, 2003). The imbalance between
the direct and indirect pathways is suggested as the mechanism underlying motor complications in the course of therapy in PD (Figure 1) (Baas, 2000).

Fig. 1: The circuitry of basal ganglia in primates. Inhibitory projections are demonstrated in black arrows. The excitatory projections are shown in gray arrows. The output basal ganglia receive cortical information via two pathways: a direct inhibitory projection from the striatum to GPi/SNr and an indirect pathway consisting of an inhibitory projection from GPe to STN and an excitatory projection from STN to GPi/SNr. The two pathways start from two groups of striatal medium spiny neurons. In the direct pathway these neurons express D1 receptors and contain GABA (G) and substance P (SP). In the indirect pathway these neurons express D2 receptors and contain GABA and enkephalin (Enk). In the direct pathway the dopaminergic neurons of SNc have an excitatory effect on the D1 expressing striatal neurons and conversely in the indirect pathway these neurons have an inhibitory effect on D2 expressing striatal neurons. The information from the output ganglia is relayed via thalamus to the cortex or is conveyed to the spinal cord. Abbreviations: PPN, pedunculopontine nucleus; SC, superior colliculus (Adapted from Smith et al., 1998).
1.4 Dopamine and dopamine receptors

1.4.1 Dopamine synthesis and metabolism in the brain:

Dopamine (dihydroxyphenylethylamine) is one of the neurotransmitters known as catecholamines. It is the most abundant catecholamine in the brain. These catecholamines are organic compounds characterized by the presence of a benzene ring with two adjacent hydroxyl substitutions known as catechol nucleus. The enzymatic pathway for dopamine synthesis is in part common with other cathecholamines (Vallone et al., 2000).

The synthesis of dopamine starts from the precursor amino acids tyrosine and phenylalanine. Tyrosine can itself be produced from dietary phenylalanine. Tyrosine in the dopaminergic neurons is first converted to \( L \)-dopa by the function of the enzyme tyrosine hydroxylase (TH). This enzyme is the rate-limiting factor in the synthesis of dopamine and is regulated by phosphorylation as well as the negative feedback of dopamine on TH. The enzyme \( L \)-aromatic amino acid decarboxylase (\( L \)-AADC or dopa decarboxylase) then immediately converts \( L \)-dopa (levodopa) to dopamine (DA). Dopamine is then stored in vesicles. The process of storage is dependent on the function of the vesicular monoamine transporter 2 (VMAT2), a protein located on the membrane of the vesicles. The function of VMAT2 is \( Mg^{2+} \) dependent and can be inhibited by administration of reserpine or tetrabenazine. Dopamine synthesis and storage not only take place in the dopaminergic terminals but also in the dendrites of these neurons (Elsworth and Roth, 1997). Dopamine release from the synaptic terminal to the synaptic cleft is mainly through \( Ca^{2+} \) dependent exocytosis. TH is not involved in the metabolic pathway of exogenous levodopa and levodopa metabolism starts with \( L \)-AADC function. In Parkinson’s disease most of the striatal dopaminergic terminals are degenerated and levodopa is converted to dopamine by other \( L \)-AADC
containing cells like serotoninergic neurons and enters the extracellular space without being stored in vesicles (Figure 2) (Chase et al., 2001).

1.4.2 Dopamine inactivation:

Three major mechanisms are involved in the termination of action of dopamine in the extracellular space.

1- Uptake by the dopamine transporter (DAT): This is the main mechanism for termination of dopamine function in the extracellular fluid in the striatum (Gainetdinov et al., 1998; Jones et al., 1998)

2- Diffusion in the extracellular fluid.

3- Degradation by enzymatic pathways: In the striatal extracellular fluid monoamine oxidase subtype B (MAO_B) and cathecol-o-methyltransferase (COMT) are responsible for enzymatic degradation of the dopamine (Kopin, 1985). Each enzyme can metabolize products of the action of the other enzyme (Deleu et al., 2002). The resulting final dopamine metabolites are homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) (Figure 2) (Elsworth and Roth, 1997). In the neurons of SN and their striatal projections, MAO_A located in the mitochondrial membrane is involved in the enzymatic degradation of dopamine (Burke et al., 2004). The enzymatic pathway has a minor role in removal of dopamine from extracellular space in normal conditions. Thus re-uptake by DAT remains the major mechanism of dopamine inactivation in the synaptic cleft (Figure 2).

1.4.3 Dopamine receptors:

Dopamine receptors are a member of the seven transmembrane domain (7TM) G-protein coupled receptors (Vallone et al., 2000). Dopamine receptors are divided into two main subgroups, D1 like receptors and D2 like receptors. The D1 like subgroup consists of D1 and D5 receptors.
The D2 like subgroup is composed of D2, D3 and D4 receptors (Deleu et al., 2002). Dopamine receptor ligands can distinguish between D1 and D2 like receptors but they cannot fully distinguish the members of each subgroup. Both subgroups act through upregulation or down-regulation of cyclic AMP (cAMP) and regulation of Ca$^{++}$ signaling. D1 like receptors are positively coupled to adenylyl cyclase (the enzyme responsible for cAMP production) through interaction with G proteins. In contrast D2 like receptors are negatively coupled to adenylyl cyclase (Vallone et al., 2000). D1 and D2 receptors are the most abundant types of dopamine receptors in brain tissue. D1 and D2 receptors are both postsynaptic on striatal neurons. D2 receptors are also presynaptic autoreceptors, whereas D1 receptors are expressed presynaptically on non-dopamine neurons only (Figure 3). The presynaptically expressed D2 receptor is functionally an autoreceptor that on the soma and dendrites of midbrain dopaminergic neurons

![Diagram](image-url)

**Fig. 2:** The synthesis and metabolism of DA in brain. DA is mainly metabolized to DOPAC and HVA. Abbreviations: TH, Tyrosine hydroxylase; ALAAD, aromatic L-amino acid decarboxylase; DA, dopamine; COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; SAM, S-adenosyl-L-methionine; 3-MT, 3-methoxytyramine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid (Adapted from Deleu et al., 2002).
modulates the rate of signal activity and on the nerve terminals of these neurons modulates both dopamine synthesis and release. There are some reports on the role of D3 receptors as an autoreceptor capable of modulating dopaminergic function (Joyce, 2001). Simultaneous stimulation of both D1 and D2 receptors has synergistic effects on motor behavior and is necessary for maximal response (Robertson and Robertson, 1986).

Fig. 3: The two isoforms of D2 receptor. Dopamine receptors are one of the seven transmembrane G-protein coupled receptors. Abbreviations: D2S, D2 short; D2L, D2 long (Adapted from Vallone et al., 2000).
1.5 Pathogenesis and etiology:

The exact cause of Parkinson's disease is unknown. Epidemiological studies have suggested some environmental factors to be involved in the pathogenesis of PD. These factors include exposure to pesticides, herbicides and also exogenous toxins such as trace metals, cyanide, organic solvents, carbon monoxide, and carbon disulfide and some industrial chemicals. Living in a rural environment has specifically been related to the development of PD. Other factors associated with Parkinson's syndrome are head trauma and viral infections (Tanner and Langston, 1990; Lees, 1997; Olanow and Tatton, 1999; Takahashi and Yamada, 2001). None of these environmental factors have proved to be the real cause of PD in pathological studies. The only environmental toxin proved to be a causative agent in the pathogenesis of PD is 1,2,3,6-methyl-phenyl-tetrahydropyridine (MPTP). This toxin leads to a syndrome which is very similar to idiopathic PD (Langston et al., 1983). Apart from environmental factors that are believed to have a causative role in PD, there are some familial forms of PD which suggest the involvement of genetic factors in the pathogenesis of the disease. The search for these genetic factors has led to the discovery of eight gene loci that are involved in the juvenile and adult onset familial PD (Huang et al., 2003).

Pathological studies of brain tissue from PD patients have shown the depigmentation of SN, which is the result of loss of neuromelanin-containing cells (dopaminergic cell bodies) and their striatal terminals. These studies have also led to the discovery of protein aggregates in the cytoplasm of surviving dopaminergic neurons of substantia nigra pars compacta (SNc). These aggregates are called "Lewy bodies". A major component of Lewy body is a protein known as 𝛼-synuclein. It is hypothesized that mutations of 𝛼-synuclein or oxidative damage to this protein lead to folding of this protein and the formation of the Lewy bodies. Lewy bodies are present in both
idiopathic and familial types of PD and seems to be an important component of pathogenesis of PD (Olanow and Tatton, 1999).

As most PD patients do not have a definite toxic exposure or familial history of the disease, it is believed that genetic susceptibility, environmental factors and a combination of gene mutations and environmental factors combine to lead to the development of PD. The process of aging has a contributory role in the pathogenesis of PD as mean age of PD patients at the onset of the disease is 55 years (Olanow and Tatton, 1999; Huang et al., 2003).

1.6 Presentations of PD:

There are three characteristic signs in Parkinson’s disease:

1- Tremor: the tremors in PD are usually of resting type but postural tremors may also be present. This sign is usually the first sign in the course of the disease.

2- Rigidity: the rigidity of Parkinson’s disease is known as “cogwheel rigidity” because of its characteristic stiffness of motion on physical examination.

3- Bradykinesia: Bradykinesia or slowed movement in PD is responsible for the poor balance control or poor postural reflexes observed in this disease (Young, 1999).

These signs and symptoms are mostly caused by dopamine deficiency and usually respond to levodopa therapy at the early stages of the disease. With progress of the disease the above presentations of disease increase in severity and also some other signs and symptoms like flexed posture, freezing, and loss of postural reflexes appear that do not respond to levodopa therapy. At this stage, bradykinesia may also not respond to usual doses of levodopa. These levodopa non-responsive representations of the disease are the major cause of disability in PD patients. Other signs and symptoms may present themselves in the course of the disease such as fatigue, depression, anxiety, sleep disturbances, disorders of bowel and bladder motility, sexual disorders.
and sensory symptoms. In some cases, PD at first may present with sleep problems and anxiety instead of movement disorders (Fahn, 2003).

1.7 Common drugs used in treatment of Parkinson’s disease:

There is no standard regimen for all PD patients as not all patients respond similarly to treatments. Currently there are several medications used as monotherapy or combination therapy. The purpose of treating PD patients is alleviation of movement disabilities and improving the quality of life. In this regard anti-Parkinson medications should be started only when PD symptoms interfere with patient’s routine life (Young, 1999). Most common medications used in Parkinson’s disease can be categorized into three groups based on the mechanisms of action (Table 2).

(1) Drugs that increase dopamine concentration in the striatum: Several neurotransmitters are reportedly deficient in Parkinson’s disease but dopamine concentration loss of 80% or more is the most significant change observed in the striatum of PD patients (Bernheimer et al., 1973). It was first in 1961 when Birkmayer et al. reported that intravenous administration of levodopa (LD) could improve movement deficits in PD patients (Birkmayer and Hornykiewicz, 1961). Later other investigators using oral levodopa approved this finding (Cotzias et al., 1967). Up to now levodopa has remained the gold standard of therapy in Parkinson’s disease. The combination of levodopa and carbidopa (a dopa decarboxylase inhibitor which prevents peripheral conversion of levodopa to dopamine, allowing more levodopa to cross the blood brain barrier) is currently used for dopamine replacement therapy (Young, 1999).

Amantadine, an antiviral agent, is a dopamine re-uptake inhibitor and also a NMDA receptor antagonist (Blanchet et al., 2003) currently used synergistically with levodopa in PD or as
monotherapy in mild PD and is shown to be effective in the control of some levodopa induced motor complications (Adler et al., 1997).

(2) Dopamine receptor agonists: These anti-Parkinson drugs are used as monotherapy at the early stages of Parkinson’s disease and also in combination with levodopa in later stages. Although the use of dopamine receptor agonists as monotherapy is not as effective as levodopa therapy in control of PD symptoms, there is some evidence that justifies their use at the early stages of the disease (Schrag and Quinn, 2000). Dopamine receptor agonists can delay motor complications of levodopa therapy, which are more common in young PD patients. Some authors have suggested that these drugs can have a protective role for dopaminergic neurons and can delay the course of disease progression (Schwarz, 2003); this is, however, quite controversial. Furthermore, the use of dopamine receptor agonists may be accompanied by side effects that restrict their use in some PD patients.

(3) Inhibitors of dopamine metabolism: This group prevents enzymatic degradation of dopamine in striatum. These medications are inhibitors of monoamine oxidase subtype B (MAO-B) and cathecol-o-methyltransferase (COMT), the two enzymes responsible for enzymatic degradation of dopamine in the extracellular space (Kopin, 1985). COMT inhibitors are usually used with levodopa for control of end-of-dose deterioration as they increase the bioavailability of levodopa. MAO-B inhibitors not only reduce the clearance of dopamine but in theory also have a neuroprotective role through reduction of neurotoxic free radicals (Deleu et al., 2002).

Other than the three major groups there are some medications with anticholinergic characteristics that are used as an adjunctive therapy with levodopa especially in the management of tremors (Young, 1999).
Table 2: The common medications used in the treatment of PD (Adapted from Fahn, 2003).

<table>
<thead>
<tr>
<th>Common Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine precursor: levodopa</td>
</tr>
<tr>
<td>Dopamine agonists: bromocriptine, pergolide, Pramipexole, ropinirole, apomorphine, and carbergoline</td>
</tr>
<tr>
<td>Peripheral decarboxylase inhibitors: Carbidopa, benserazide</td>
</tr>
<tr>
<td>Dopamine releaser: amantadine</td>
</tr>
<tr>
<td>Catechol-O-methyltransferase inhibitors: Tolcapone, entacapone</td>
</tr>
<tr>
<td>MAO type B inhibitor: selegiline, rasagiline</td>
</tr>
</tbody>
</table>

1.8 Motor complications in response to levodopa therapy in PD:

Motor complications in response to levodopa are a major problem in the course of therapy in PD. After 5 years 50% of PD patients experience deterioration in motor response to levodopa (Dupont et al., 1996; Kumar et al., 2003; van Laar, 2003). In another study it is shown that each year the number of PD patients who develop motor fluctuations increases by 10% (Schrag and Quinn, 2000). In other words, after 10 years most PD patients develop motor fluctuations. When motor complications start, they are difficult to treat. At present most efforts are made to control these motor complications or delay them.

There are two major forms of motor complications: motor fluctuations in response to levodopa therapy and dyskinesia. Current evidence suggests that the mechanisms responsible for motor fluctuations are different from those of dyskinesias (Baas, 2000). However, all motor complications require two things: these are levodopa therapy and neuron loss in the nigrostriatal pathway. Thus, motor complications can be related to the duration of disease (Schneider, 1989; Kumar et al., 2003).

Motor complications clinically may have various presentations. Usually the first presentations of motor complications appear after a few years of levodopa therapy as “end of dose deterioration” of PD signs and symptoms. This phenomenon is also known as “wearing off”
fluctuations. “Wearing off” fluctuators have a shorter period of levodopa effect and the period of single dose effectiveness decreases with the progress of the disease (van Laar, 2003). Later with the progress of the disease other forms of motor complications appear which are even more disabling. These are “on-off” motor fluctuations and dyskinesia. Generally motor fluctuations can be classified to three types:

1- “Wearing off” fluctuations, which are characterized by predictable end-of-dose deterioration.

2- “On-off” fluctuations, which present as sudden unpredictable changes in motor response to dopaminergic treatment.

3- The “inhibitory response”, defined as worsening of symptoms at the beginning or end of dose.

Due to the relationship of motor complications with levodopa therapy, first the clinical response to levodopa will be discussed.

1.9 Clinical response to levodopa:

Clinical response to levodopa can be categorized based on the timing and the quality of response:

(1) Short duration response (SDR): This response starts after the administration of a single dose of levodopa and lasts for a few minutes or hours depending on the dose of levodopa during which the PD patient experiences some improvement in motor function (Nutt and Holford, 1996). This temporary response correlates with plasma levodopa concentration and thus depends on pharmacokinetics of levodopa (Nutt and Carter, 2000; Nutt, 2001).

(2) Long duration response (LDR): This response is a constant response that appears after a few days of taking levodopa. This effect of levodopa is responsible for the sustained improvement in motor function between doses of levodopa that persist even when the patient forgets to take a single dose of levodopa (Nutt and Holford, 1996). As this response is detectable after
administration of dopamine agonists as well as levodopa, the mechanism involved in this response may not involve levodopa pharmacokinetics and instead may be on the postsynaptic terminals where dopamine receptors exist (Nutt and Carter, 2000).

It seems the short duration response forms the peaks of motor function improvement observed during daily levodopa therapy while the long-term response forms the nadirs. In long-term levodopa therapy the effect of levodopa changes and these changes are responsible for motor complications.

1.10 Mechanisms of motor complications

The mechanisms responsible for motor complications including motor fluctuations are currently under investigation. These mechanisms can be categorized as follows:

1.10.1 Peripheral pharmacokinetics of levodopa:

It is estimated that only a small fraction of an oral dose of levodopa combined with a peripheral dopa-decarboxylase inhibitor (carbidopa) reaches the brain (about 10%) (Nutt et al., 1994). The peripheral pharmacokinetics of levodopa can be affected at the level of proximal small intestine and also at the level of the blood brain barrier (BBB) where it is transported to plasma and brain respectively by the function of the large neutral amino acid (LNAA) system (Deleu et al., 2002). In a high protein diet large neutral amino acids can compete with levodopa for the transporter, interfering with levodopa absorption (Pincus and Barry, 1987; Frankel et al., 1989; Nutt et al., 1989). Rescheduling and decreasing daily protein intake can increase levodopa absorption and also transport to the brain (Karstaedt and Pincus, 1992). The rate of gastric emptying is also a determinant of levodopa absorption. Gastric emptying may be delayed by food (especially fat) and drugs with anticholinergic properties resulting in delayed absorption of levodopa. In this regard, levodopa should be administered at least half an hour before meals and
the diet should be reduced for its fat content. Domperidone (a peripheral antagonist of dopamine receptors) can facilitate gastric emptying and thus levodopa absorption (van Laar, 2003). Studies on the role of 3-O-methyldopa (3-OMD) competition with levodopa for LNAA system in “wearing off” fluctuations have failed to confirm an important role for this mechanism (Deleu et al., 2002).

Plasma pharmacokinetics of levodopa have been studied in relation to the pathogenesis of the motor fluctuations. It was shown that clearance of levodopa from peripheral circulation was not different between fluctuators and PD patients with a stable response to levodopa. Besides, motor complications do not usually occur until after several years of levodopa therapy and are related to the progress of the disease. Therefore, although peripheral pharmacokinetics of levodopa may affect motor fluctuations, this cannot explain the basis for motor fluctuations in Parkinson’s disease (Fabbrini et al., 1987; Mouradian et al., 1987; Nutt and Holford, 1996). Other studies have investigated the role of central mechanisms in the pathogenesis of motor fluctuations.

1.10.2 Central actions of levodopa:

Changes in central actions of levodopa motor complications can be discussed at the presynaptic and postsynaptic levels:

(1) Presynaptic mechanisms: Fabbrini et al. compared the efficacy half-life of levodopa in several groups of PD patients who were in different stages of the disease and also with a control group of normal subjects. The results of this study showed that the efficacy half-time of levodopa was 48% lower in those with a stable response to the drug in comparison to drug naive patients and it was even lower than this in PD patients with motor fluctuations. In other words, with the progress of the disease the efficacy half-life of levodopa was reduced (Fabbrini et al., 1988). In the same study it was shown that after abrupt discontinuation of intravenous levodopa, the response
decay graph was composed of an initial rapid decline and a later slow decline. This is the rapid
decay phase that corresponds with the short duration response to levodopa (SDR) (Nutt and
Holford, 1996). While the later decay phase slope was equal between patients in different stages of
the disease, the slope of the early decay phase was greater in "wearing off" fluctuators in
comparison to stable responders and in stable responders in comparison to drug naive PD patients.
In this study the efficacy half-life of levodopa was shown to be greater than plasma half-life of
levodopa in PD patients with a stable response to levodopa but changed to less than plasma half-
life in patients with motor complications. This implied the role of buffering mechanisms in
dopaminergic terminals and an increase in levodopa response decay rate could be the result of loss
of buffering mechanisms in the striatum due to the progress of the disease and dopaminergic
neuron degeneration. Degeneration of presynaptic terminals is accompanied by loss of the
capacity for decarboxylation of levodopa and storage of the resulting amine. Therefore this loss
can expose postsynaptic sites to the oscillatory pattern of oral levodopa administration leading to
clinical phenomenon of "wearing off" fluctuations (Fabbbrini et al., 1988).

[18F]6-fluorodopa (FD) PET scanning is a reliable method for assessing the extent of
neurodegeneration of nigrostriatal dopaminergic neurons in vivo (Snow et al., 1993). De la
Fuente-Fernandez et al. reported a differential putaminal FD uptake between fluctuators and stable
responders to levodopa. The 2 hour FD uptake was significantly lower in fluctuators than those
with a stable response to levodopa, which is in keeping with a more severe degeneration in
fluctuators than stable responders (de la Fuente-Fernandez et al., 2000). As decarboxylation
capacity is not considered to be a limiting factor with the progress of PD, the changes in FD
uptake should be the result of difference in storage capacity between the two groups of PD patients
(Fig 4). This result supports the role of presynaptic buffering in the pathogenesis of "wearing off"
fluctuations (de la Fuente-Fernandez et al., 2000). However, the substantial overlap observed in the FD Ki of the fluctuators and stable responders suggested that the extent of nigrostriatal damage cannot be the only determinant of the quality of response to levodopa and other factors are also involved in the pathogenesis of motor fluctuations.

**Fig. 4:** The process of DA synthesis, release and inactivation in a dopaminergic terminal. $[^{18}F]6$-fluorodopa (FD) has the same metabolic pathway as levodopa. Two hour FD PET scan can be used for estimation of levodopa uptake, decarboxylation and storage in synaptic vesicles ($K_i$ or $K_{occ}$). Four hour FD PET scan is used for the estimation of DA enzymatic degradation ($K_{loss}$). Dopamine turnover can be determined from the ratio of $K_{loss}/K_i$. In normal conditions most of the extracellular DA is taken back up into the dopaminergic terminals via DAT.

In another study conducted on asymmetric cases of PD treated with levodopa, patients did not show a differential clinical motor response in two sides of the body and the efficacy half-life of levodopa was equal between the two sides (Kempster et al., 1989). As neurodegeneration of nigrostriatal pathway correlates with the severity of Parkinson’s disease, asymmetric Parkinson’s disease is the result of asymmetric neurodegeneration of striatal dopaminergic neurons, which implies the presence of different levels of buffering capacity between the two sides. This equal response suggests that nigrostriatal degeneration and loss of buffering cannot explain motor
fluctuations and is in favor of involvement of other mechanisms in the pathogenesis of motor fluctuations.

Other suggested mechanisms for motor fluctuations in PD are postsynaptic changes and increased turnover of dopamine in the striatum. An increase in dopamine turnover can be the result of upregulation of dopamine degrading enzymes or altered uptake of dopamine. It is known that before the signs and symptoms of PD appear, the dopamine content of the nigrostriatal pathway has already decreased by about 80%. Therefore before the overt Parkinsonian syndrome begins, striatal dopaminergic system function should remain almost constant (Chase et al., 2001). During this period it is believed that compensatory mechanisms are responsible for maintaining balanced interactions in the basal ganglia (Bernheimer et al., 1973). The idea of the role of presynaptic mechanisms in the preclinical period was first proposed by Hornykiewicz et al. (Hornykiewicz, 1966) (Figure 5). A variety of compensatory mechanisms have been proposed for this stage of the disease. Studies on the 6-hydroxydopamine animal model of PD have shown that the dopaminergic neuron loss is compensated at least in part by increased dopamine synthesis and release from surviving dopaminergic neurons. While the total amount of dopamine in the striatum decreases, the fractional release of dopamine (that is per neuron) can increase 5 times. These changes occur along with a decrease in inactivation of dopamine through down regulation of DAT expression. It has also been suggested that the efficacy of dopamine function may be increased at the postsynaptic level by upregulation of dopamine receptors (Calne and Zigmond, 1991; Bezard and Gross, 1998; Widner, 2003).

In animal studies it is demonstrated that an increase in the ratio of dopamine metabolites to dopamine occurs early in the course of changes observed after lesioning of the nigrostriatal pathway (Zigmond, 1997). This ratio, which is known as dopamine turnover, is suggested as
another compensatory mechanism at the preclinical stage of PD. In human studies this ratio can only be assessed in postmortem experiments. However, prolonged (four hour) \([^{18}\text{F}]\)fluorodopa PET scans can be used to estimate effective dopamine turnover in vivo in human subjects (Figure 4). An increase in dopamine turnover was demonstrated in the early stages of PD by estimating an index of effective dopamine turnover in PD patients (Doudet et al., 1998; Sossi et al., 2002). This finding also supported the role of increased dopamine turnover as a compensatory mechanism at early PD (Sossi et al., 2002). Leenders et al. conducted a study on a group of PD patients using four hour \([^{18}\text{F}]\)fluorodopa PET scanning. The results from this study showed that the striatal

**Fig. 5:** The comparison between the pattern of dopaminergic neuron loss in SNC in aging and Parkinson’s disease. In the process of aging the number of dopaminergic neurons of SNC decreases but the decrease never reaches the symptomatic level. PD is caused by severe degeneration of dopaminergic neurons. The symptoms of PD appear when at least 60% of SNC dopaminergic perikarya are lost. Compensatory mechanisms are involved in the asymptomatic period (Adapted from Bezard et al., 1997).
radioactivity was lower in fluctuators than stable responders after four hours of tracer injection which implied a more rapid egress of $[^{18}\text{F}]$fluorodopa from the striatum of fluctuators than stable PD patients (Leenders et al., 1986).

In a study by de la Fuente-Fernandez et al., a group of PD patients was scanned with $[^{11}\text{C}]$RAC at an early stage of the disease when all patients still showed a stable response to levodopa. These patients had three scans, one at baseline, the second scan one hour after administration of an oral dose of standard release levodopa (250/25 mg) and the third scan 4 hours after the dose of levodopa. All patients had an assessment of motor function in response to levodopa three years later. This study showed that those patients who had a rapid decline in RAC binding during the one hour scan with a rapid return toward baseline later became fluctuators and those patients who showed a moderate decline in RAC binding with a further decrease in RAC binding in the four hour scan still remained stable. As RAC binding has a negative correlation with extracellular dopamine levels, it was deduced that oscillations in dopamine levels preceded the “wearing off” motor fluctuations (Figure 6) (de la Fuente-Fernandez et al., 2001). The last two studies are in favor of the role of dopamine turnover in the pathogenesis of motor fluctuations.

In DAT knockdown mice, expressing only 10% of the wild-type expression of DAT, postmortem studies demonstrated a higher extracellular level of dopamine and also a higher ratio of dopamine metabolites to dopamine in the striatum. The results from this study suggest that the expression of DAT may affect the degree of dopamine turnover, and may thereby play a role in the pathogenesis of “wearing off” fluctuations (Zhuang et al., 2001).

(2) Postsynaptic changes: Changes of D1 and D2 receptors and their downstream pathways have been under investigation as a cause for motor fluctuations. In a study by Bravi et al., after infusion of the D1 and D2 agonist apomorphine to two groups of levodopa naive PD patients and those
with advanced PD with motor fluctuations the mean efficacy half-life of apomorphine in patients with motor fluctuations was reduced to almost half of that of levodopa naive patients (Bravi et al., 1994). As apomorphine is active at the postsynaptic level only, this result implies the role of postsynaptic mechanisms in producing motor fluctuations. Guttman et al. measured postmortem brain tissue from PD patients for D2 receptor density in caudate and putamen regions and the

![Graph](image)

**Fig. 6:** Estimated levodopa-induced changes in synaptic dopamine after levodopa administration, expressed as % decline in RAC binding. (Adapted from de la Fuente-Fernandez et al., 2001).

results showed that D2 receptor density is not prone to change with age, disease duration and duration of levodopa therapy (Guttman et al., 1986). PET studies have shown that an initial increase in D2 expression in PD patients returns to normal after levodopa therapy (Antonini et al., 1997). Another postmortem study showed only minor changes in D1 receptor density in distinct regions of postmortem brain tissue from PD patients (Hurley et al., 2001). D1 oligonucleotide anti-sense could inhibit dyskinesia in an animal model of Parkinson’s disease produced by chronic pulsatile levodopa, while PET studies did not show significant changes in D1 receptor densities between stable and dyskinetic patients (Turjanski et al., 1997; Van Kampen and Stoessl, 2000). So
it is believed that the pathways down stream to D1 receptor can be responsible for motor complications in advanced PD, rather than changes in the receptors themselves. Changes in receptors signaling pathways can be the result of exposure to oscillating levels of dopamine in the striatal extracellular space. These changes may be more involved in the pathogenesis of dyskinesia and "on-off" fluctuations rather than "wearing off" motor fluctuations and may explain the temporal sequence of motor complications beginning from "wearing off" and evolving to dyskinesia (Kumar et al., 2003).

A study by Andersson et al. showed a relationship between striatal fosB (a member of the fos family of immediate early genes) induction and levodopa induced dyskinesia (Andersson et al., 1999). In this regard, Vallone et al. showed that in 6-hydroxydopamine lesioned rats the "priming" phenomenon was related to an increase in the expression of the pre-existing AP-1 complex and the new synthesis of AP-1 complexes (activator protein-1, a transcription factor) formed by FosB and JunD-related proteins. While nigral denervation alone leads to an increase in FosB, the first stimulation of dopamine receptors by a dopamine agonist in a lesioned animal model of PD leads to an increase in JunD-related proteins. The concurrence of both events is necessary for the formation of AP-1 complexes which are thought to be involved in the phenomenon of priming in PD (Vallone et al., 1997).

Intermittent levodopa therapy in a 6-hydroxydopamine lesioned rat model of PD can alter glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT) enzymatic activities and neuropeptide content in the striatum and these changes are different from those seen following continuous levodopa administration (Engber et al., 1991). In another study it was shown that selective D1 and D2 dopamine receptor agonists (direct and indirect pathways respectively) can
differentially affect neuropeptide levels in the striatum (Engber et al., 1992). These results may be a clue to the involvement of neuropeptides in the pathogenesis of motor complications in PD.

It is also shown that in the 6-hydroxydopamine lesioned rat with response complications to levodopa, there is an increase in the level of glutamate receptor phosphorylation. The role of glutamate receptors in the pathogenesis of motor complications is supported by the control of dyskinesia when levodopa is co-administered with the NMDA antagonist amantadine (Baas, 2000; Kumar et al., 2003). The involvement of opioid receptor signaling pathways is also suggested as a mechanism involved in motor complications (Kumar et al., 2003).

Finally the fact that past studies could not relate changes of D2 or D1 receptors to motor complications may imply the involvement of D3 receptors which cannot easily be selectively detected with common techniques used for D1 and D2 receptor binding (Bordet et al., 1997).

1.11 Hypothesis:

Regarding the results from the past studies and based on the presynaptic mechanisms of motor fluctuations, we hypothesized that the turnover of endogenous dopamine and that derived from exogenous levodopa was dictated by the expression of the membrane dopamine transporter (DAT), relative to the number of surviving dopamine terminals (Figure 7).

This study was designed in two parts. In part 1 we conducted a study with oral methylphenidate intervention to estimate the time course of endogenous dopamine release and clearance. In part 2, the study was conducted with the administration of an oral dose of levodopa to estimate the time course of exogenously derived dopamine release.
Chapter 2, Methodology

This study was conducted using positron emission tomography (PET) in human subjects.

2.1 PET:

Positron emission tomography (PET) is an imaging technique that can provide us with information on the biochemical processes of neurons in vivo. It has been known since the invention of PET that receptor availability in brain tissue of a living subject can be measured from estimation of receptor binding with a radioactive ligand. Detection of the radioactivity in a specific region of the brain usually makes this possible. The source of radioactivity is the decay of radioisotopes bound to molecules with known biological properties. In the case of receptor studies these molecules are receptor ligands, usually an antagonist. The radioactive material decay results in 2 photons that travel at 180° from one another that can be detected from outside the body by a series of crystals linked to photomultiplier tubes. In PET, an event is recorded when 2 detectors 180° apart are activated simultaneously (coincidence detection). The data are reconstructed to create the PET images after correction for attenuation, scatter and normalization (Ollinger and Jeffrey, 1997).

The in vivo character of this technique makes it a convenient tool for diagnosis and research in the field of neurology. In Parkinson’s disease PET may occasionally be used as a diagnostic tool for differentiating PD from cases of Parkinson’s syndrome. More commonly however, it is used as a research tool for achieving a better understanding of the mechanisms underlying the disease, monitoring progression of the disease, drug action in PD, pathogenesis of complications of treatment and compensatory changes to progression of the disease (de la Fuente-Fernandez et al., 2001).

2.2 Tracers:
Fig. 7: The proposed mechanism for extracellular variations in DA levels in PD. The number of dopaminergic terminals and as a result the number of storage vesicles do not differ between the two conditions (a and b). However “a” has a higher level of DAT expression versus “b”. This may lead to a higher DA uptake (gray arrows) and lower enzymatic degradation (black arrows) in “a” rather than “b”.

2.2.1 Definition:

A tracer in PET is a radioactively labeled biologically active compound that, when injected, behaves identically, or similarly, to its naturally occurring compound. Tracers can be detected in the body by measuring their radioisotope decay, which results in gamma rays.

2.2.2 Tracers in a PD study:

In PD the radiotracers are synthesized for binding sites on neurons of basal ganglia. The radiotracers used in our study are $[^{11}\text{C}]{\text{raclopride}}$ ($[^{11}\text{C}]\text{RAC}$), $[^{11}\text{C}]-d$-threo-$[^{11}\text{C}]-\text{methylphenidate}$ ($[^{11}\text{C}]\text{MP}$) and $[^{11}\text{C}](\pm)\text{dihydrotetabenazine}$ ($[^{11}\text{C}]\text{DTBZ}$). These tracers bind to D2/D3 receptors, the dopamine transporter (DAT) and the vesicular membrane amine transporter 2 (VMAT2) respectively. $[^{11}\text{C}]\text{RAC}$ is a reversible dopamine receptor antagonist that competes with dopamine for binding to the receptors (Volkow et al., 1994). $[^{11}\text{C}]\text{RAC}$ binding to dopamine receptors inversely correlates with the dopamine levels in the synaptic cleft. This character of $[^{11}\text{C}]\text{RAC}$ makes it possible to measure the changes in dopamine release by measuring RAC displacement.
after administration of drugs that can affect dopamine levels (de la Fuente-Fernandez and Stoessl, 2002). \[^{[1]}C\]MP, an active enantiomer of methylphenidate (ritalin), is a radiotracer that selectively binds to the dopamine transporter (DAT) and is used for measuring DAT availability in human brain (Volkow et al., 1995). \[^{[1]}C\]DTBZ is the tracer used in binding assays of the vesicular monoamine transporter 2 (VMAT2) which is responsible for the removal of monoamine neurotransmitters including dopamine from the cytosol to the storage vesicles (Kilbourn et al., 1995). MP, DTBZ and RAC are used in the treatment of psychiatric and neurologic disorders. These drugs may have some side-effects at therapeutic doses, but the low doses of these drugs used as radiotracers in this study are not expected to lead to any significant clinical effect or side-effect.

### 2.2.3 Subjects:

The participants were recruited from the Movement Disorders Clinic at the University of British Columbia Hospital. All subjects were diagnosed with idiopathic PD (Calne et al., 1992). We recruited idiopathic PD patients with similar severity of disease. The severity of the disease was assessed by unified Parkinson's disease rating scale scores (UPDRS) off treatment. In order to check for the differences between fluctuators and PD patients with stable response to levodopa in the time course of dopamine release and the ratio of DAT/VMAT2 we recruited both PD patients with stable response and those with response fluctuations. We estimated a requirement of at least 17 subjects in each group of stable responders and fluctuators studied with levodopa or methylphenidate intervention, in order to detect differences between the 2 patient groups. To minimize the confounding factors all PD medications were withdrawn 18 hours before scanning. Patients taking amantadine and selegiline were excluded. We also excluded patients with dementia, which was evaluated by the Mini-Mental State Examination and patients taking other
psychoactive medications. All participants gave written consent after they were given information on the nature of the experiments and possible hazards. These studies were approved by the Research Ethics Board of the University of British Columbia.

2.2.4 Scans:

We used an ECAT 953B/31 tomograph in 3D mode (Siemens Canada/ CTI, Knoxville, TN) for scanning. Dopamine release and clearance were estimated by measuring the changes of $[^{11}\text{C}]$RAC over time in response to oral methylphenidate or levodopa. In both parts of the study the patients had three RAC scans within a single day, following intravenous administration of $[^{11}\text{C}]$raclopride (RAC). The first scan was done as a baseline, while the next 2 scans were performed one hour and four hours after drug interventions (de la Fuente-Fernandez et al., 2001; Volkow et al., 2001). $[^{11}\text{C}]$raclopride is a specific D2-like receptor antagonist whose binding is subject to competition from endogenous dopamine (Seeman et al., 1989; Volkow et al., 1994). $[^{11}\text{C}]$Raclopride PET is highly reproducible (Volkow et al., 1993). Within 3 months of the RAC scans the patients were scanned with $[^{11}\text{C}]$MP and $[^{11}\text{C}]$DTBZ tracers in one day to measure for DAT and VMAT2 binding in the striatum. For each study 16 sequential scans were performed over 60 minutes immediately after intravenous injection of the tracers. In order to correct for attenuation, we performed a 15-minute transmission scan using $^{68}$Ge rods at the beginning of each scan. In all scans the subjects were positioned supine on the scanner bed with the head positioned in the center of the field of view. For increasing the consistency between scans, a thermoplastic mask was made for each subject to minimize head movements and the same mask was used for all scans of each patient. Using a previously described method we reconstructed the 3D data and corrected for scatter and detector normalization (Oakes et al., 1998; Sossi et al., 1998). In order to measure the average activity in each specific region the five axial planes in which the striatum was
best visualized were summed on temporally integrated images (derived from 30 to 60 minutes after scan initiation). One circular region of interest (ROI) of 61.2 mm$^2$ was positioned on each caudate nucleus and three circular ROIs of 61.2 mm$^2$ were placed without overlap along the axis of each putamen. All ROIs were adjusted to obtain the highest average ROI activity. In RAC scans the average background activity was measured from one elliptical ROI (1918.5 mm$^2$) placed over the center of the cerebellum. In MP and DTBZ scans this average was measured from 6 circular regions of interest (296.95 mm$^2$), three on the occipital cortex on each side. Each of the ROIs was then transposed onto images obtained from each time frame, in order to obtain a time activity curve. We applied a graphical model for reversible tracers using a tissue input function to determine the effective distribution volume ratio and from this, the tracer BP ($B_{\text{max}}/K_d$) as described by Logan et al. (BP=DVR-1) (Logan et al., 1996). As putamen is more involved in motor function while caudate has a role in cognition and also degeneration of dopaminergic neurons in PD mostly takes place in putamen, in this study the average of binding in 3 putaminal regions on each side were used for comparisons (Kish et al., 1988; Brooks et al., 1990).

2.2.5 Data analysis:

The data from the RAC studies were analyzed by repeated measures analysis of variance using baseline binding potential as a covariate. Also, the ratio of MP/DTBZ was correlated with RAC change using linear regression, following logarithmic transformation of the ratio data in order to provide a more normalized distribution. A p value of <0.05 was considered significant. Age was included as covariate in analysis of the data.
Chapter 3, RAC study with oral methylphenidate intervention

3.1- Introduction:

In this study, we assessed the changes of endogenous dopamine (DA) levels over time in response to oral methylphenidate and from this to estimate endogenous dopamine turnover. Methylphenidate (MP) is commonly used in the treatment of attention deficit hyperactivity disorder (ADHD) in children. Its therapeutic efficacy is thought to be related to an increase in the levels of norepinephrine and dopamine in brain through blockade of the related transporter proteins (Volkow et al., 2001). It is also thought that the major mechanism for terminating the action of extracellular dopamine in the normal brain is reuptake by the dopamine transporter (DAT) (Giros et al., 1996; Piccini, 2003).

A previous study has shown that oral methylphenidate, at therapeutic doses used in the treatment of ADHD, can block more than fifty percent of the dopamine transporters in the brain tissue (Volkow et al., 1998). A more recent study with oral methylphenidate has provided in vivo evidence for the capacity of oral methylphenidate to increase extracellular dopamine concentrations in the brains of normal human subjects using PET (Volkow et al., 2001).

It is suggested that many of the motor complications of PD may arise from increased turnover of dopamine (de la Fuente-Fernandez et al., 2000; de la Fuente-Fernandez et al., 2001). Thus, in patients who go on to develop fluctuations, there is a larger and earlier increase in the extracellular levels of dopamine following oral levodopa than is seen in patients who maintain a stable response to medication (de la Fuente-Fernandez et al., 2001). However, changes in RAC binding following levodopa reflect changes in release of exogenously derived dopamine. In contrast, because methylphenidate blocks the DAT, but (unlike amphetamine) does not release dopamine itself (Piccini et al., 2003), changes in RAC binding following methylphenidate should provide insight
into the role of the DAT in the handling of synaptic DA in pathological conditions and its contribution to the changes in DA turnover consistently reported in Parkinsonian patients and animal models of Parkinsonism.

3.2 Materials and methods:

Five patients with idiopathic PD (one female and four males) with a mean age of 63.8 ±11.6 years and mean disease duration of 12 ±6.6 years participated in this study. All subjects met criteria for clinically definite PD (Calne et al., 1992) and all suffered from disease of similar severity as assessed by unified Parkinson’s disease rating scale scores (UPDRS) off treatment (mean UPDRS score = 35.6 ±12.8; range =24-54). All patients were on levodopa/carbidopa; two patients also took bromocriptine and one took pergolide. All PD medications were withdrawn 18 hours prior to PET. Patients taking amantadine were excluded as it could mask the expression of dyskinesia as were patients with dementia (Mini-Mental State Examination score<26) and patients taking other psychoactive medications. Because of the long half-life of selegiline that could affect the time course of dopamine release and also dopamine turnover those patients who took this medication were not included in this study.

The patients had three sequential RAC scans in 3 dimensional mode in one day, following intravenous administration of $[^{11}C]$raclopride (RAC) as tracer. After the baseline scan, the next 2 scans were performed one hour and four hours after taking an oral dose of methylphenidate (0.8mg/kg) on an empty stomach, as reported by Volkow et al. (Volkow et al., 2001). The PET protocol for all scans included 4 x 60-seconds, 3 x 120-seconds, 8 x 300-seconds, 1 x 600-seconds consecutive emission scans starting at the time of tracer injection for a total scanning time of 60 minutes. The tracer was injected using a Harvard pump in 60 seconds (5 mCi, mean ±SD specific activity: 4164 ±880). For increasing the consistency between the three RAC scans, the 1 hour and
4 hour scans were realigned to the baseline scan using the Automated Image Registration (AIR) algorithm (Woods et al., 1993). Within 3 months of the RAC scans the patients underwent MP and DTBZ scans. We used the same PET protocol used in RAC scans for MP and DTBZ scans. As a routine patients were first scanned with $^{11}$C)DTBZ and after an interval of 2.5 hours from the first tracer injection the patients were infused with $^{11}$C)MP for the MP scan. The interval was for allowing the radioactive material to decay (more than 7 half-lives for $^{11}$C) (Fig 8 and 9). The data from the scans were reconstructed and corrected for attenuation, scatter and detector normalization (Oakes et al., 1998; Sossi et al., 1998). The average activity of the tracers in the striatum was measured with the method described above. In RAC scans the cerebellum was used as the reference region and the background activity was measured by one elliptical ROI put on each of two contiguous axial planes for each scan. In MP and DTBZ scans the occipital cortex was used as the reference region and the background activity was calculated by averaging the activity in 6 circular ROIs placed on the occipital cortex on 5 adjacent planes containing the best striatal images on each frame of the dynamic sequence. We applied a graphical model for reversible tracers using a tissue input function to determine the average putaminal binding potential for each scan as mentioned before (Logan et al., 1996). Heart rate and blood pressure were monitored and recorded for each patient throughout the study, as were subjective symptoms following oral administration of methylphenidate. Heart rate and blood pressure were recorded 15 minutes before the administration of oral methylphenidate as baseline and every 15 minutes during the 1 hour RAC scan. Open end questioning was used to obtain subjective feelings of the patients.

The data were analyzed by repeated measures analysis of variance using baseline binding potential as a covariate. A p value of <0.05 was considered significant.

3.3- Results:
3.3.1- The effect of oral methylphenidate on dopamine levels:

There was no significant change in RAC binding potential at 1 or 4 hours following oral methylphenidate (p = 0.70; Fig. 9). The measurements at 1 and 4 hours were correlated with the baseline in each subject (p=0.011). No correlation was made between the RAC data and MP/DTBZ ratio, as oral methylphenidate did not significantly affect endogenous dopamine release.

Fig. 8: The timeline of a RAC study with oral methylphenidate intervention.

Fig. 9: The timeline of MP and DTBZ PET studies for each subject.
3.3.2- Behavioral and physical effects of oral methylphenidate:

Following administration of methylphenidate, two patients felt “high” or “widely awake”. In one patient (patient 1; Fig. 9) the subjective feeling of “high” was prominent. This patient is a female and also younger than our other subjects (age: 47 years). All patients experienced minor asymptomatic cardiovascular effects such as an increase in heart rate and blood pressure (Table 3). Only the increase in heart rate reached a significant level in comparison to the baseline (p=0.018). In one patient, extremely low frequency PVCs (premature ventricular contraction) and PACs (premature atrial contraction) were recorded in the electrocardiogram. Three patients developed 3+ tremors after receiving the oral dose of methylphenidate in the same limbs where they usually experienced the resting tremor of PD (They had not received their usual anti-Parkinson medications). Although motor function was not formally assessed during the scans in order to avoid a potential confounding effect on dopamine release, there was no obvious improvement in PD symptoms following methylphenidate (Table 3).

RAC Displacement Studies with MP Intervention

Fig. 10: Comparison of putaminal RAC binding potentials across baseline, one and four hours after methylphenidate. No significant change was observed in RAC binding. Abbreviations: RAC, raclopride; MP, methylphenidate; 1h, 1 hour; 4h, 4 hour.
Table 3: Behavioral and physical effects of MP in PD patients (SD, standard deviation; MP, methylphenidate; PVC, premature ventricular contraction; PAC, premature atrial contraction; N, number of subjects).

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<th>Baseline (Mean±SD)</th>
<th>After MP (Mean±SD)</th>
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<td><strong>Cardiovascular effects:</strong></td>
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<td></td>
</tr>
<tr>
<td>Heart rate (beat/min):</td>
<td>66.5±10.5</td>
<td>75.7±10.4</td>
<td>0.018</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg):</td>
<td>127.5±15</td>
<td>144±28.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg):</td>
<td>76.2±7.5</td>
<td>84±11.3</td>
<td>0.17</td>
</tr>
<tr>
<td>Arrhythmia:</td>
<td>-</td>
<td>very low frequency (N=1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PVCs and PACs in one patient.</td>
<td></td>
</tr>
<tr>
<td><strong>Motor side effects:</strong></td>
<td>-</td>
<td>3+ tremor (N=3)</td>
<td></td>
</tr>
<tr>
<td><strong>Other signs and symptoms:</strong></td>
<td>-</td>
<td>Feeling “too much caffeine” (N=2) and becoming chatty, feeling “widely awake”, feeling “energetic”.</td>
<td></td>
</tr>
</tbody>
</table>
A previous study provided in vivo evidence for the ability of oral methylphenidate to increase extracellular dopamine in the striatum of normal human subjects (Volkow et al., 2001). In our study, the estimation of extracellular dopamine levels and degree of D2 receptor occupancy using \([^{11}C]\text{raclopride PET}\) in response to oral methylphenidate depend on both the level of DAT blockade by methylphenidate and also the basal level of dopamine release from presynaptic terminals (as opposed to methamphetamine, which not only blocks the DAT, but also stimulates dopamine release). As the mean disease duration of our subjects was 12 ±6.6 years significant degeneration of presynaptic dopaminergic terminals had occurred. Thus, our negative result can in part be attributed to the degeneration of dopaminergic presynaptic terminals in the striatum, with subsequent reductions in both endogenous dopamine release as well as the availability of DAT in PD patients. Our negative result is in keeping with a recent report of reduced dopamine release in the striatum of PD patients in response to methamphetamine compared to normal subjects (Piccini et al., 2003). Furthermore, a recent PET study, using raclopride to measure endogenous dopamine release, showed the close dependency between the integrity of dopamine terminals and dopamine release with decreased or no change in raclopride BP in monkeys with varying degrees of MPTP-induced Parkinsonism (Doudet and Holden, 2003).

The rise in endogenous dopamine in response to oral methylphenidate has also been found to decrease sharply with increased age in normal human subjects and this effect is attributed to the effect of age on the expression (and possibly function) of DAT on presynaptic terminals and also dopamine release (Gerhardt and Maloney, 1999; Stanford et al., 2001; Volkow et al., 2001; Piccini, 2003). Our PD subjects had a mean age of 63.8 ±11.6 (range 47-79) years and this may have been an additional factor contributing to the lack of effect of methylphenidate in this group of PD patients. In another study conducted in our center, 6 normal subjects (one female and five
males) with a mean age of 38.3 ±15 (range 21-56) years underwent scans before and after oral methylphenidate (60 mg). In these control subjects, there was no consistent change in RAC binding following oral methylphenidate (P= 0.37). Three out of 6 (aged 21, 23 and 56) demonstrated reductions of 23%, 10% and 15% respectively (Yatham et al., unpublished observations) while the other three subjects (aged 35, 40 and 55 years) showed slight increases in RAC binding following methylphenidate challenge. This result is in contrast with those reported by Volkow et al., suggesting that response to methylphenidate challenge varies even in normal healthy subjects and may depend on various factors including age (Volkow et al., 2001).

A recent clinical study showed no improvement in signs and symptoms of PD patients after oral methylphenidate alone (Nutt et al., 2004). Our results are in keeping with the lack of clinical benefit following oral methylphenidate in advanced idiopathic PD. However, when given in conjunction with levodopa, oral methylphenidate was shown to potentiate the effects of intravenous levodopa, suggesting that while blockade of DAT alone may be insufficient to result in therapeutic benefit in PD, the DAT nonetheless plays an important role in determining the effects of exogenously derived dopamine in this disorder (Camicioli et al., 2001).

Indeed, despite the lack of obvious therapeutic benefit in our PD patients, we did see a variety of responses after administration of methylphenidate, similar to those reported by Volkow et al. (Volkow et al., 2001). Our results also correlate with the blunted subjective effects observed by Persico et al. after administration of oral methylphenidate in PD patients (Persico et al., 1998). A female gender and a young age might explain the more prominent behavioral effect of oral methylphenidate in one of the patients (patient 1; Fig. 9), as both factors might contribute to higher expression of DAT (Gerhardt and Maloney, 1999; Stanford et al., 2001; Piccini, 2003). Those patients who experienced behavioral changes may have had methylphenidate-induced
increases in extracellular dopamine levels in the ventral striatum, where dopamine levels are relatively preserved in PD. Patient 1 was the youngest subject among our PD patients and had the lowest UPDRS score which suggests less severe degeneration of the nigrostriatal and meso-accumbens projections (Figure 9). This relative preservation of dopaminergic neurons compared to the other subjects may have led to a more prominent subjective feeling of “high” (Persico et al., 1998). Three patients developed 3+ tremors during the 1-hour scan which could have resulted from abstinence from their usual PD medications, or anxiety during the study.

Alterations in dopamine turnover are thought to play a major role in the emergence of treatment complications in PD (de la Fuente-Fernandez, 1999; de la Fuente-Fernandez et al., 2001). They may be a very early manifestation of disease and even occur in the pre-clinical period (Doudet et al., 1998; Sossi et al., 2002). Down-regulation of DAT has been reported in early Parkinson’s disease (Lee et al., 2000). It is not clear to what extent the DAT serves to regulate dopamine turnover in PD patients, but while our preliminary results suggest that oral methylphenidate is not a useful probe for assessing dopamine release in this population, they do not exclude a role for manipulation of DAT function in the long-term treatment of PD.
Chapter 4, RAC study with levodopa administration:

4.1- Introduction:

Levodopa, a precursor of dopamine is currently the gold standard of therapy in PD. Medication induced motor complications in PD are closely related to levodopa therapy and are usually delayed in those PD patients who receive direct dopamine agonists as monotherapy or in conjunction with levodopa. A past study has shown that the time course of exogenous dopamine release is different between fluctuators and stable responders to oral levodopa in such that in fluctuators there is a rapid increase in extracellular dopamine level with fast return to baseline (de la Fuente-Fernandez et al., 2001). These results imply the role of dopamine turnover in motor fluctuations. It is also known that in DAT knockdown mice an increase in dopamine turnover occurs, suggesting the involvement of DAT expression in the pathogenesis of motor fluctuations (Zhuang et al., 2001). Different studies have shown down-regulation of DAT or DAT mRNA in the nigral dopaminergic neurons of animal models of PD and also PD patients (Ito et al., 1999; Miller and Abercrombie, 1999; Lee et al., 2000). There is evidences for down-regulation of DAT in PD subjects after receiving short-term levodopa therapy and to a lesser degree in those who receive a direct dopamine agonist as therapy (Guttman et al., 2001). These findings are suggestive of a possible role of DAT expression in determining exogenous dopamine turnover in PD and also in the pathogenesis of “wearing off” motor fluctuations in response to therapy. Here we investigated if the estimation of the time course of exogenously derived dopamine release and clearance in the striatum of PD patients correlated with the ratio of DAT expression on dopaminergic terminals.

4.2 Materials and methods:
Eleven patients diagnosed with idiopathic PD were recruited to this study (Calne et al., 1992). The subjects (3 females and 8 males) had a mean age of 64.82 ± 9.74 years (range: 47-79 years) and mean disease duration of 9.10 ± 1.64 years (range: 6-11 years) and suffered from similar disease severity measured after discontinuation of PD medications by unified Parkinson’s disease rating scale scores (UPDRS) (mean UPDRS score: 27.27 ± 7.28; range: 17-44). Those patients taking amantadine, selegiline and other psychoactive medications were excluded. Also patients suffering from dementia (MMSE≤26) were excluded. The subjects took a variety of PD medications. All subjects were taking levodopa, four were taking bromocriptine, three were taking pergolide, three were taking amantadine and one was taking trihexyphenidyl (artane). All PD medications were discontinued 18 hours before scanning. The patients had three $^{[11]}$C]RAC scans during one day and within three months of the RAC scans the patients had two scans with $^{[11]}$C]MP and $^{[11]}$C]DTBZ. We used the same method as described by de la Fuente-Fernandez for $^{[11]}$C]RAC PET scans (de la Fuente-Fernandez et al., 2001). The PET protocol for all scans included 4 × 60-seconds, 3 × 120-seconds, 8 × 300-seconds, 1 × 600-seconds consecutive emission scans starting at the time of tracer injection for a total scanning time of 60 minutes. All patients received an oral dose of domperidone (20 mg) before the baseline scan and the same dose of this medication half an hour before the oral dose of levodopa. All scans were in three-dimensional mode. In the RAC studies the first scan was performed as a baseline, the second scan was performed 1 hour after administration of an oral dose of standard release levodopa (250/25 mg) on an empty stomach and the third scan was performed 4 hours after administration of levodopa (Figure 11). For attenuation correction, we performed an initial 15 minute transmission scan using $^{68}$Germanium as a source of radioactivity. Then the RAC scans were performed by obtaining 16 sequential scans over 60 minutes for each of the three times, starting at the injection
of $[^{11}\text{C}]$RAC (5mCi; mean ± SD specific activity: 5050 ± 3363 Ci/mmol at ligand injection). After correction for attenuation and scatter and detector normalization, the data were reconstructed to PET images (Oakes et al., 1998; Sossi et al., 1998). The 1 hour and 4 hour scans were realigned to the baseline scan using the AIR algorithm as described by Woods et al. (Woods et al., 1993). Likewise, $[^{11}\text{C}]$DTBZ and $[^{11}\text{C}]$MP scans each were performed by obtaining 16 sequential scans over 60 minutes, starting at the time of tracer injection. The tracers were infused by a Harvard pump in 60 seconds (5mCi; ligand mean ± SD specific activity: 3131± 983 Ci/mmol and 3580± 2340 Ci/mmol for $[^{11}\text{C}]$DTBZ and $[^{11}\text{C}]$MP respectively) (Figure 9). Here, we used the same PET protocol as for RAC scans. The average putaminal activity of the three tracers was measured with the same method, reference regions and ROIs as described above. We used a graphical model for reversible tracers using a tissue input function to calculate the average putaminal binding potential ($B_{\text{max}}/K_a$) for each scan (Logan et al., 1996). The data from RAC bindings were analysed using a comprehensive repeated measures ANCOVA model with time (at baseline, 1 hour and 4 hour) as the within subjects factor and log (MP/DTBZ) as the covariate [Time × Log (MP/DTBZ)]. A p value of less than 0.05 was considered significant.

Fig. 11: The timeline of a RAC study with levodopa intervention.
4.3 Results:

Adjusting $[^{11}]C$MP binding for age did not change the results significantly. Because MP/DTBZ ratio is not normally distributed, in this analysis log (MP/DTBZ) which is closer to a normal distribution, was used (Figure 13 and 14).

(1) **The time course of exogenous dopamine release after administration of levodopa:** The difference between RAC binding potentials at baseline minus 1 hour and baseline minus 4 hour were significant ($p=0.0009$ and $p=0.013$ respectively) but the difference between 1 hour minus 4 hour was not significant ($p=0.78$) (Figure 12).

(2) **Contrasts on time by covariate:** Slopes of RAC binding against log (MP/DTBZ) were not different at the different times (for baseline minus 1 hour: $p=0.58$, baseline minus 4 hour: $p=0.36$, 1 hour minus 4 hour: $p=0.46$). This result implies that log (MP/DTBZ) did not differentially affect the pattern of either dopamine release or its clearance (Figure 15).

![RAC Displacement Studies with Levodopa](image-url)
MP and DTBZ PET studies

Fig. 13: The putaminal binding potentials of MP and DTBZ studies for each subject.

Fig. 14: The ratio of MP to DTBZ putaminal binding potentials for each subject.
Fig. 15: Regression of RAC bindings at baseline, 1 hour and 4 hour on log (MP/DTBZ) did not show any significant differences between the slopes.

4.4 Discussion:

PET scanning using $[^{11}\text{C}]$RAC can be used for determining levodopa induced changes in extracellular dopamine levels in PD (Tedroff et al., 1996). Our group has already shown that subcutaneous injection of apomorphine (0.06 mg/kg) results in significant decrease in $[^{11}\text{C}]$RAC binding (16%) that returns to baseline within 2 hours, implying the rapid recovery of D2 receptors after dopamine agonist injection. Furthermore, in patients with fluctuations RAC binding returns to baseline level within 4 hours of levodopa, despite significant decreases 1 hour after medication (de la Fuente-Fernandez et al., 2001). Therefore decreased $[^{11}\text{C}]$RAC binding potential from baseline measured at the 4 hour scan likely reflects persistent synaptic dopamine resulting from exogenous levodopa. In this study the changes in dopamine levels at 1 and 4 hours were
significantly different from baseline, in keeping with previous results (Volkow et al., 1993; de la Fuente-Fernandez et al., 2001).

It is already known that the pattern of extracellular dopamine changes in response to levodopa is different between stable PD patients and those with motor fluctuations. While fluctuators show a rapid decline in $[^{11}\text{C}]$RAC with a fast return to baseline or even an overshoot above baseline, stable responders to levodopa show a slow but constant decrease in $[^{11}\text{C}]$RAC binding from 1 to 4 hours after levodopa administration. These results along with other studies were suggestive of increased dopamine turnover in fluctuators in comparison to stable responders (de la Fuente-Fernandez et al., 2001). The demonstration of increased dopamine turnover in DAT knockdown mice, further suggested a relationship between DAT expression on the striatal dopaminergic terminals and dopamine turnover as well as the pathogenesis of "wearing off" motor fluctuations (Zhuang et al., 2001).

In our study the 1 hour scan was representative of exogenous dopamine release while the 4 hour scan was representative of clearance of dopamine from the striatal extracellular space. The changes of striatal extracellular dopamine levels over time were used as an estimate of dopamine turnover, although this term is more typically based on postmortem measures of the ratio of metabolites to dopamine, and can also be assessed in vivo by prolonged 6-$[^{18}\text{F}]$fluorodopa PET scans (Doudet et al., 1998; Sossi et al., 2002).

We estimated that 34 PD patients would be required for this study, based on an effect size of 1 with 80% power. Our present result is based on the recruitment of 11 PD patients, which is less than one third of the required number. So although regression of $[^{11}\text{C}]$RAC binding potentials at different times on log (MP/DTBZ) was not significant, this negative result does not necessarily
reject our hypothesis. Definitive results will require completion of the study with an adequate sample size.

In this study we measured the changes of $[^{11}C]$RAC binding potential over time ($\Delta$) and also the ratio of $[^{11}C]$MP binding potential over $[^{11}C]$DTBZ binding potential. The dynamic range of such data is limited and it may therefore be difficult to detect a statistically significant correlation. In order to improve the consistency between scans we used thermoplastic masks to reduce head movements and also realigned the RAC scans to the baseline scans. Still the analysis of PET scans is an analyst dependent method that can affect the small values obtained in this study. In addition, as we did not measure plasma levodopa levels, subjects were required to have a low protein breakfast in the morning of scanning. Any variations in protein intake between subjects and other factors that could affect the pharmacokinetics of levodopa should be considered. Of course reaching the sample size estimated for the study will reduce any uncontrolled confounding factors between subjects.
Chapter 5, Discussion and conclusion:

5.1 Discussion of the results:

The results from part 1 and part 2 of this study did not achieve definite positive results. In both parts we tried to use the changes of $[^{11}\text{C}]\text{RAC}$ binding over time as an estimation of dopamine turnover. In part one of this study estimates of the time course of endogenous dopamine release following oral methylphenidate failed to show significant results, which made the correlation of the resulting data with MP/DTBZ ratio impossible. Although this part of study had negative results, nevertheless it provided an in vivo correlate for lack of therapeutic benefit from oral methylphenidate in advanced PD and confirmed the negative result from a previous clinical study on the effects of oral methylphenidate alone in PD patients (Nutt et al., 2004). Regarding the changes in $[^{11}\text{C}]\text{RAC}$ binding and dopamine levels in PD, we failed to reproduce the findings of Volkow et al., who showed changes in dopamine levels over time in normal subjects. However, we found similar effects of methylphenidate on the behavioral and physical measures (Volkow et al., 2001). The effects of methylphenidate depend on both DAT density and extracellular dopamine levels, thereby explaining the lack of benefit from oral methylphenidate in PD. Although administration of methylphenidate alone proved to be ineffective in PD, administration of this medication together with intravenous levodopa could potentiate the motor responses to levodopa in PD patients (Camicioli et al., 2001).

In the second part of this study we measured the time course of exogenous dopamine release after administration of oral levodopa as an estimate of exogenous dopamine turnover. Our results were in keeping with the variations in extracellular dopamine levels reported by de la Fuente-Fernandez et al. (de la Fuente-Fernandez et al., 2001). However the regression of $[^{11}\text{C}]\text{RAC}$ bindings at different times against log (MP/DTBZ) failed to demonstrate any consistent effect of
relative DAT density on exogenous dopamine levels at the stage of dopamine release or clearance from the striatal extracellular space. This may indicate a lack of effect of DAT density on exogenously derived dopamine turnover in the striatum. This finding may also be seen as contradicting the results from a previous study that reported increased dopamine turnover in DAT knockdown mice (Zhuang et al., 2001). However, our negative result does not definitively exclude an effect of DAT density on exogenous dopamine turnover, as further experiments on more subjects will be needed to resolve this question.

5.2 Future directions:

In general our findings are in agreement with some results of previous studies. In order to investigate the effects of density of DAT on the time course of dopamine release and dopamine turnover, recruitment of new subjects should be considered until the estimated sample size is reached (34 PD patients). In parallel, dopamine turnover should additionally be measured using a previously established in vivo method with [18F]6-fluoro-L-DOPA PET scans and then regressed on the MP/DTBZ ratio (Doudet et al., 1998; Sossi et al., 2001; Sossi et al., 2002). Using this method not only provides us with an alternate approach to testing our hypothesis, but also allows for a comparison between the two methods.

While animal studies have shown the contribution of DAT expression on striatal dopaminergic neurons in determining dopamine turnover, it may be interesting to test different methods of manipulating the expression of DAT in vivo and to check for the effects of manipulation on dopamine turnover and levodopa induced motor complication (Zhuang et al., 2001). Several different intracellular signaling pathways and molecules have been demonstrated to be involved in the regulation of DAT (such as protein kinase C, protein kinase A and tyrosine
kinases). Manipulation of these pathways and molecules can regulate the dopamine transporter (Mortensen and Amara, 2003). This may provide us with another way of testing our hypothesis.

Further investigation of the clinical benefit from oral methylphenidate alone in early PD may be of interest in future as in the current study we investigated the effect of this medication on advanced PD. At the earlier stages of PD we can expect a more prominent motor response from oral methylphenidate as we have less degeneration and higher levels of DAT expression and dopamine release.

As co-administration of oral methylphenidate and intravenous levodopa in PD patients has proved to increase the motor response to levodopa, another experiment in future would be to test the motor response from co-administration of oral methylphenidate and oral levodopa and the effect of this regimen on controlling “wearing off” motor fluctuations (Camicioli et al., 2001).

5.3 Conclusion:

To date there is no effective way of treating motor complications in response to levodopa therapy in PD. Although direct dopamine receptor agonists are thought to delay levodopa induced motor complications in PD these drugs are not a satisfactory solution with regard to motor complications as they are not as effective as the dopamine precursor levodopa. Amantadine and COMT inhibitors are also used as adjuncts to levodopa therapy for controlling motor complications but none of these drugs can control motor complications perfectly. Therefore this problem will finally affect most PD patients in the course of treatment. Many hypotheses are proposed and tested in this regard. Our hypothesis is a continuum of past studies suggesting the role of striatal presynaptic DAT expression and dopamine turnover in the pathogenesis of motor complications in PD (de la Fuente-Fernandez et al., 2001; Zhuang et al., 2001). The results from our study whether negative or positive will answer some of the questions raised on the
pathogenesis of these motor complications. Testing our hypothesis will guide us to possible treatments for this side effect of levodopa and also will provide us with information for designing further experiments in this field.
References


