Abstract

L-dopa remains the most effective drug for improving motor symptoms of Parkinson's disease (PD). However, following long-term chronic treatment, the therapeutic effects of L-dopa are often accompanied by debilitating peak-dose dyskinesia.

The mechanisms underlying L-dopa induced dyskinesia remain unknown. Rapid increases of dopamine (DA) in the severely DA denervated striatum are associated with L-dopa induced dyskinesia (Miller and Abercrombie, 1999). This DA efflux is believed to cause many post-synaptic changes that are associated with L-dopa induced dyskinesia (Olanow et al., 2000). Therefore, it is of interest to examine the underlying mechanisms of the L-dopa induced DA release.

The objectives of the present experiments were to examine the role for the DA transporter (DAT) in mediating L-dopa-induced DA release. Firstly, systemic injection of a DAT antagonist, methylphenidate (MP) was used to assess the role of the DAT in L-dopa induced dyskinesia in chronically L-dopa treated animals. Results showed a dose-dependent effect of MP in the attenuation of L-dopa induced dyskinesia. Secondly, we investigated the functional mode of the DAT by examining the effects of MP pre-treatment on the L-dopa induced DA efflux and dyskinetic responses in three groups of rats 1) L-dopa-naïve, 2) 1-week L-dopa treated, and 3) 3-week L-dopa treated, rats. MP pretreatment had no effect on L-dopa induced DA efflux in L-dopa naïve, or 1-week L-dopa treated animals. In contrast, systemic pre-treatment of MP significantly attenuated the L-dopa induced DA response in 3-week treated rats, which was correlated with a similar decrease in L-dopa induced dyskinesia.

The results from these experiments lend support to our hypothesis that reversal of the DAT through chronic L-dopa treatment contributes to the pathogenesis of L-dopa induced
dyskinesia. Therefore, these findings suggest that the DAT is an important pharmacological target in the study and treatment of L-dopa induced dyskinesia.
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Abbreviations

6-OHDA  6-hydroxydopamine
AADC  Aromatic amino acid decarboxylase
AIM  Abnormal involuntary movements
Axial  Axial dystonia
ANOVA  Analysis of Variance
DA  Dopamine
DAT  Dopamine transporter
DOPA  Dihydroxyphenylalanine
DOPAC  3,4 dihydroxyphenylacetate
CREB  cAMP response element binding protein
COMT  Catechol-O-methyl transferase
GABA  γ-aminobutyric-acid
GPi  Globus pallidus, internal segment
GPe  Globus pallidus, external segment
HVA  Homovanillic acid
i.p.  Intraperitoneal
L-dopa  Levodopa
Loco  Locomotive rotation
Limb  Limb dyskinesia
LTP  Long-term potentiation
MAO-B  Monoamine oxidase-B
MP  Methylphenidate
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<tr>
<td>MPTP</td>
<td>1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine</td>
</tr>
<tr>
<td>NET</td>
<td>Noradrenalin transporter</td>
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<tr>
<td>PD</td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td>PDyn</td>
<td>Prodynorphin</td>
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<tr>
<td>PPE</td>
<td>Pre-proenkephalin</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein-kinase-A</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td>SNC</td>
<td>Substantia nigra <em>pars compacta</em></td>
</tr>
<tr>
<td>SNR</td>
<td>Substantia nigra <em>pars reticulate</em></td>
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<tr>
<td>STN</td>
<td>Subthalamic nucleus</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>UCH-L1</td>
<td>Ubiquitin C-terminal hydroxylase L1</td>
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<tr>
<td>UPS</td>
<td>Ubiquitin Proteasome System</td>
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INTRODUCTION

Overview

Parkinson's disease (PD) is a progressive neurodegenerative disorder that primarily affects the elderly population. The pathological hallmarks of PD are a relatively selective degeneration of the nigrostriatal dopamine (DA) neurons in the substantial nigra pars compacta (SNc), and cytoplasmic inclusions of ubiquitin-rich aggregates termed Lewy bodies. The loss of dopaminergic neurons subsequently leads to DA depletion in the striatum, producing a constellation of motor deficits, such as bradykinesia, rigidity, and resting tremor, which are the cardinal features of PD. Additional symptoms such as depression, anxiety, sleep disturbances and cognitive impairment often compound the motor deficits in PD. Together, these symptoms lead to a significantly compromised quality of life in the majority of PD patients. While genetic predisposition is a known contributor to PD, the majority of cases are idiopathic. The exact mechanisms underlying spontaneous neurodegeneration are still unclear, although recent advances in genetic research of PD, combined with improved experimental models, have provided new insight into the cellular and molecular mechanisms that may be involved in the pathogenesis of both familial and sporadic forms of PD.

Without a clear understanding the etiology of PD, development of neuroprotective or neurodegenerative treatments for this disorder remains a challenge. Therefore, most patients currently rely on symptomatic treatments alone. These treatments typically involve the use of DA agonists, which act to supplement the diminishing dopaminergic stimulation in the striatum. Developed in the 1960s, levodopa (L-dopa) is one such drug that provides efficacy in the treatment of parkinsonism. Unfortunately, while most patients respond well in the first few years of L-dopa therapy, benefits may be overshadowed by the emergence of response
fluctuations or dyskinesia that are associated with prolonged L-dopa treatment. Despite these setbacks however, few other treatments have shown such consistent success in improving parkinsonian motor symptoms. As such, L-dopa remains the most commonly used treatment for PD. It is thus critical to elucidate the underlying mechanisms involved in L-dopa-induced motor fluctuations and dyskinesia.

The key focus of the studies described in this thesis is to investigate a novel mechanism by which chronic L-dopa therapy can lead to the induction of dyskinesia. Specifically, the dynamic role of the dopamine transporter (DAT) in the development of L-dopa-induced dyskinesia is assessed using a unilateral 6-hydroxydopamine (6-OHDA) lesioned rat model of L-dopa-induced dyskinesia (Lee, 2000). The remainder of this chapter will present, 1) a more in-depth description of PD, 2) a review of the neuropathophysiology of PD, 3) the current hypotheses which have been put forward to explain L-dopa-induced dyskinesia, and finally, 4) the experimental rationale for the present experiments presented in this thesis.

**Parkinson's Disease**

**Epidemiology and Etiology**

PD affects approximately 0.3% of the general population, and 1% of people over the age of 60. It has a slightly higher incidence in men, and is evident across all ethnic groups. The average age of onset is in the mid-50s to 60s for most patients, but can be as early as the mid 20s for patients with young-onset PD. Familial PD accounts for only a small proportion of all PD cases. This suggests that, although genetic inheritance may play a role in the etiology of PD, environmental factors, if not primarily involved, are strongly implicated as well. Recent data from a large-scale study examining the concordance of PD in monozygotic twins suggest that heredity is not a major etiological factor in PD, especially in cases involving onset after 50 yrs of
age. However, further analysis shows that genetic components of PD are much more prevalent in early-onset cases (Tanner et al., 1999).

Genetic predisposition

Although the exact causes of PD are unknown, the discovery of several genes and gene loci associated with familial PD has enhanced our understanding of the molecular pathogenesis of the disease. Mounting evidence suggest that neurodegeneration may be a consequence of dysfunction in the mitochondrial I complex, resulting in oxidative stress and production of misfolded proteins. There is further evidence that failure in the ubiquitin proteasome system may contribute to aggregation of mishandled proteins and hence, to neurodegeneration in PD.

Parkin and ubiquitin C-terminal hydroxylase L1 (UCH-L1) are two proteins that are directly involved in the ubiquitin proteasome system. Genetic mutations on genes (PARK 2 and PARK 5, respectively) that encode for both proteins account for some of the familial cases of PD. Mutations in PARK 1, the gene that codes for α-synuclein, have also been linked to PD. Incidentally, α-synuclein form a major component of the Lewy bodies associated with PD. Overexpression of α-synuclein in flies and rodents reiterate some of the behavioural and pathological aspects of PD, including selective, age-dependent degeneration of dopaminergic neurons and formation of Lewy body-like, α-synuclein positive inclusions (Feany and Bender, 2000; Kirik et al., 2002).

Other genetic mutations that have been implicated in PD impair mitochondrial function and response to oxidative stress. The PINK1 gene encodes a protein that responds protectively to oxidative stress. Overexpression of the mutated gene has been shown to increase cell death in vitro (Valente et al., 2004). Similarly, the protein DJ-1, which has also been shown to modify mitochondrial function in response to oxidative stress, also has a critical role in neuroprotection.
Mutations in both PINK1 and DJ-1 genes have been documented in autosomal recessive PD (Tanner et al., 1999).

**Environmental factors**

In the early 1980's, Langston and colleagues (Langston and Ballard, 1984) reported on a series of patients who developed acute Parkinsonism after being exposed to MPTP, a small molecule which readily crosses the blood brain barrier and subsequently converted into MPP+ by astrocytes. MPP+ enters dopaminergic neurons via the DAT and causes neurotoxicity through inhibition of the mitochondrial complex I. Since MPTP is one of few exogenous substances directly linked to the development of Parkinsonism, the discovery of MPP+ induced neurotoxicity has lent support to the hypothesis that environmental exposure to chemically similar substances may contribute to the development of the disorder. Exposure to pesticides, industrial chemicals, and contaminated drinking water have all been associated with PD. At the same time, while exposure to certain substances increases the risk of PD, some appear to reduce it. For instance, cigarette smoking and caffeine intake have both been associated with neuroprotection and lowered incidence of PD (Morens et al., 1995; Ross et al., 2000).

In summary, it is likely that PD is a heterogeneous disorder with a multifactorial etiology. Nigral degeneration may be a consequence of exposure to environmental toxins, particularly in individuals who are genetically predisposed. Although familial PD is rare, the genetic advances made by studying genetic mutations in these cases have increased our understanding of the pathogenesis underlying both inherited and sporadic forms.

**Treatment for PD**

Treatment for PD can be divided into two categories: one to prevent or retard neurodegeneration, and the other to alleviate the symptoms of the disorder. Since the exact
mechanisms for nigral cell death are not fully understood, effective neuroprotective therapies for PD are currently unavailable. However, several neuroprotective agents, including an anti-inflammatory drug, minocycline, have shown promising preliminary results, and now await clinical testing (Blum et al., 2004; Ravina et al., 2003). Although neuroprotection at any stage of the disorder should prevent further degeneration, the benefits of neuroprotective therapies would be maximized if initiated in the early stages of the disorder. Unfortunately, due to the efficient compensatory mechanisms for striatal DA depletion, Parkinsonian symptoms appear only after approximately 50% of DA cells in the SNc are lost (Bernheimer et al., 1973). Therefore, development of diagnostic measures sensitive to neuronal loss in the presymptomatic stages of the disease is greatly needed to enhance the value of neuroprotective therapies.

Another approach that is receiving increasing attention is cell replacement therapy. To date, several cell replacement strategies have been attempted in animal models with varying success, including intrastriatal transplantation of various stem cells, embryonic mesencephalic tissue, and retinal pigmentary epithelial cells on gelatin beads. Some studies have demonstrated significant repair of DA cells, which was accompanied by behavioural improvement (Storch et al., 2004). However, development of tumors associated with cell transplant is an important concern with this technique. Furthermore, some patients developed dyskinesia, for which the cause is unknown.

While these aforementioned approaches to PD treatment have demonstrated a great deal of promise, many are still at the experimental stage. It may be many years before these treatments can be proven practical and accessible to patients. The most common and effective treatments to date, are symptomatic therapies, the majority of which restores defective dopaminergic neurotransmission. This is accomplished either by enhancing the synthesis of DA
via administration of an exogenous DA precursor (L-dopa), or by employing direct agonists which act on mainly the D2 class of DA receptors (pramipexole, ropinirole) (Hallett and Standaert, 2004). Unfortunately, a vast majority of patients treated with dopaminergic drugs develop unstable responses and side effects after prolonged treatment. Direct DA agonists are less likely to cause dyskinesia than L-dopa, but, they have a weaker anti-parkinsonian efficacy (Brotchie et al., 2005). Therefore, while monotherapy with a DA agonist is usually sufficient for the first few years of treatment, patients eventually turn to L-dopa treatment. The mechanisms of L-dopa treatment and consequences of chronic L-dopa use will be described in greater detail later on in this chapter.

In conclusion, while traditional therapeutic approaches with L-dopa and DA agonists are usually effective for improving the symptoms of PD, shortcomings of these drug treatments related to their chronic use have incited a need for better understanding of pharmacological actions of these drugs at the molecular level, and other alternative treatments aimed towards neuroprotection or replacement of diminishing cells.

Animal Models of PD

The ideal animal model would capture most of the pathological features and behavioural deficits of sporadic PD. Currently, common animal models of PD use 6-OHDA lesion of nigrostriatal fibers in a variety of species, and MPP+ induced toxicity primarily in primates to produce these motor deficits. Both bilateral and unilateral 6-OHDA lesioned animal (mainly rats) are effective in producing DA depletion. As motor deficits are greater in bilaterally lesioned animals, intensive nursing is often required. Therefore, unilaterally lesioned animals are a more practical alternative, and prove to be useful for when a within-subject control design is preferred. All of these models usually entail acute and severe (>95%) depletion of DA cells in
the lesioned striatum. Therefore, these “end stage” models (i.e., modeling the advanced PD with severe DA denervation) are useful for evaluation of new therapies. In recent years, lesion techniques have been refined to allow experimenters better control of lesion size and severity, as well as to obtain a gradual and progressive lesion that is more characteristic to that of PD patients (Bezard et al., 1997; Lee et al., 2000; McNaught et al., 2004). Although animal models do not replicate all the features of PD, they reliably cause DA depletion and Parkinsonism and furthermore, are practical and invaluable tools for evaluation of symptomatic treatments and new emerging therapies such as cell replacement and restoration therapies.

The Neural Substrates of PD

A functional model of the basal ganglia: normal and PD states

Situated in the basal forebrain, the basal ganglia form a complex network of parallel connections involving the cortex, the basal ganglia, and the thalamus (Figure 1). Cortical motor information is conveyed to basal ganglia via entry of the striatum. Information then passes through several basal ganglia nuclei, before arriving at the output nuclei, namely, the substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi). The GPi/SNr then communicates with the brainstem and the thalamo-cortical neurons, both of which are crucially involved with the execution of motor events. Accordingly, the basal ganglia are perfectly situated to act as an integrator and relay station for motor information. Dysfunction of this “motor circuit” commonly underlies the pathophysiology of many movement disorders. The following sections describe the classical model of basal ganglia function, and the proposed functional changes occurring in the PD state.

The normal state
Figure 1. Schematic drawing representing basal ganglia function in the normal brain. The red lines with round ends depict excitatory connections. The blue lines with blunt ends depict inhibitory connections. Abbreviations: \(D1\) = dopamine \(D1\) receptor; \(D2\) = dopamine \(D2\) receptor; \(GPe\) = external segment of globus pallidus; \(GPi\) = internal segment of the globus pallidus; \(SNC\) = substantia nigra pars compacta; \(SNr\) = substantia nigra pars reticulata; \(STN\) = subthalamic nucleus.
Figure 1
The GABA containing medium spiny neurons in the striatum are activated by descending glutamatergic projection originating from various cortical motor areas (Figure 1). In turn, these neurons communicate with the GPi/SNr via two pathways: 1) direct and 2) indirect. Neurons that constitute the ‘direct’ pathway send direct GABAergic projections to the GPi/SNr. These neurons contain dopamine D1 receptors, and in addition to GABA, co-express substance P and dynorphin.

Activation of striatal projection neurons in the direct pathway results in inhibition of the GPi/SNr neurons. In contrast, the striatal projection neurons involved in the indirect pathway express dopamine D2 receptors and enkaphalin, and innervate the GABA-containing cells of the external segment of the globus pallidus (GPe). Activation of the GPe exerts a tonic inhibition over the subthalamic nucleus (STN) and the GPi/SNr. Therefore, activation of the striatal projection neurons in the indirect pathway results in inhibition of the GPe, and consequently the disinhibition of the GPi/SNr. Therefore, these two pathways exert opposing influences over the GPi/SNr, and thus, the balance between the activation of these two processes allows for the facilitation of appropriate movements, and suppression of inappropriate movements (Brotchie et al., 2005).

As the striatal projection neurons in both pathways contain DA receptors, nigrostriatal DA neurons modulate the activity of these two pathways. As D1 receptors are facilitatory, activation of striatal D1 receptors in the direct pathway results suppression of GPi/SNr neurons. In contrast, D2 receptors are inhibitory, and stimulation of striatal D2 receptors disinhibits GPe neurons, which in turn, suppresses GPi/SNr activity. Therefore, stimulation of both D1 and D2 DA receptors in the striatum leads to suppression of the output nuclei in the basal ganglia.
circuitry. In sum, this model illustrates the critical influence of DA over the basal ganglia output activity (DeLong, 1983).

The PD state

This model of basal ganglia circuitry has been a useful, albeit incomplete, tool used to explain the clinical syndrome of PD. It describes the neuromodulatory role of the DA system in motor function, and how deficient DA stimulation in the striatum changes the functional state of the basal ganglia circuitry (DeLong, 1983). In the direct pathway, decreased D1 receptor stimulation leads to disinhibition of the GPi/SNr (Figure 2). In the indirect pathway, reduced D2 receptor stimulation leads to increased activity in the GPe, and which in turn leads to increased activity of the STN neurons and the GPi/SNr. In sum, DA depletion in the striatum, as in PD, results in a greater inhibitory influence over the GPi/SNr, and their target structures, namely the brainstem and the cortex, which are associated with motor behaviour.

Neurochemistry of DA

DA synthesis

Tyrosine molecules cross through the blood brain barrier by carrier-mediated transport, and enter DA nerve terminals (Figure 3), where they are converted into dihydroxyphenylalanine (DOPA) by the rate limiting enzyme tyrosine hydroxylase (TH). Subsequently, DOPA is decarboxylated by the abundant enzyme aromatic amino acid decarboxylase (AADC) to form DA. Since endogenous levels of DOPA are low relative to the amount of AADC in the remaining DA terminals in the denervated striatum, administration of exogenous L-dopa can substantially increase synthesis of DA (Cooper, Bloom, & Roth 1996).
Figure 2.
Schematic drawing representing basal ganglia function in the parkinsonian brain. The red lines with round ends depict excitatory connections. The blue lines with blunt ends depict inhibitory connections. Thick lines represent overactive projections, whereas thin lines represent underactive projections. Consistently, boxes with thick outlines (the STN, GPi/SNr) represent nuclei which are overactive. Dotted Lines or outlines represent projections or nuclei that are eliminated due to DA cell death. Abbreviations: $D1 = dopamine D1$ receptor; $D2 = dopamine D2$ receptor; $GPe = external segment of globus pallidus; GPi = internal segment of the globus pallidus; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus.
Figure 2
Figure 3. Neurochemistry of DA
DA originates from tyrosine molecules entering DA neurons. Tyrosine is then converted into
dopa molecules by the rate limiting enzyme tyrosine hydroxylase (TH). DOPA is then
decarboxylated by aromatic amino acid decarboxylase (AADC), converting it to DA. DA then
forms a transient, cytosolic pool, and then transported into DA vesicles. DA release occurs via
two known mechanisms, namely vesicular and DAT-mediated release. Exogenous L-dopa, on
the other hand, enters DA neurons, and is directly converted into DA by AADC, bypassing the
rate limiting factor, TH.
DA release

Once synthesized, intraterminal DA forms a primary, transient, cytosolic pool from which DA is vesicularized into a secondary pool. It is generally accepted that these two distinct pools of DA that are released through different mechanisms (Leviel, 2001). While vesicular DA release is known to be dependent on the depolarization of the nerve terminal and subsequent increase in intracellular Ca\(^{2+}\). Although the mechanisms mediating cytosolic DA release is less clear, it is known to be independent of neural depolarization. However, considering the established role of DAT in mediating non-vesicular release in amphetamine induced DA efflux (Leviel, 2001; Rothman and Baumann, 2003; Elliott and Beveridge, 2005), and more recently, under physiological conditions (Falkenburger et al., 2001), the DAT may be an important contributor to impulse independent release of cytosolic DA.

Homeostatic mechanisms regulating extracellular DA

Physiological basal striatal DA levels, as measured by in vivo sampling techniques such as microdialysis or voltammetry, are maintained at a relatively stable 4-20nM concentration range (Parsons et al., 1991; Smith and Justice, 1994; Jones et al., 1998; Smith and Weiss, 1999; Chen, 2005). This is in contrast to the \(\mu\)M range of DA levels found in the synaptic cleft after burst firing of DA neurons in response to behaviourally relevant stimuli (Chen, 2005). However, despite these bursts of DA neuronal discharge causing massive release of DA into the synaptic cleft, extracellular DA levels remain relatively stable (Chen, 2005), suggesting the existence of mechanisms regulating the diffusion of synaptic DA into the extrasynaptic extracellular space. Although enzymatic breakdown of DA by extraneuronal catechol-O-methyl transferase (COMT) into HVA and monoamine oxidase-B (MAO-B) into DOPAC contributes to the degradation of released DA, the primary mechanism regulating extracellular DA levels is the DAT, which is
located at the vicinity of the synaptic cleft, and quickly removes released synaptic DA before it diffuses into the extracellular space. These homeostatic mechanisms play a vital role in maintaining constant, tonic stimulation of the extrasynaptic DA receptors. However, with progressive DA denervation in PD, these mechanisms alone can not maintain the same pattern of activity and stimulation in the striatum. Therefore, DA depletion triggers different compensatory mechanisms in attempt to sustain the same level and pattern of DA receptor stimulation.

**Compensatory mechanisms in the striatum with DA terminal loss**

Symptoms of PD begin to appear only after about 50% of DA neuronal loss in the substantia nigra, and about 80% of DA loss in the striatum (Bernheimer et al., 1973; Fearnley and Lees, 1991). As suggested by these pathological observations, experimental studies have shown evidence that highly effective compensatory mechanisms take place both in presynaptic and postsynaptic phases of dopaminergic neurotransmission.

**Presynaptic mechanisms**

It has been well recognized that extracellular DA levels in the striatum remain within a normal range despite a substantial loss of nigrostriatal DA neurons (Abercrombie et al., 1990). Normalization of extracellular DA levels in the striatum with DA terminal loss is an outcome of presynaptic compensatory mechanisms that affect all aspects of DA metabolism in remaining DA terminals, which are working concurrently or in stages: (i) increased DA synthesis in the remaining DA neurons by increased activity of TH protein (Pasinetti et al., 1992; Blanchard et al., 1995) and increased expression of TH mRNA (Blanchard et al., 1995); (ii) increased DA release by increased activity of surviving neurons (Agid et al., 1973; Zigmond et al., 1984), and increased extracellular levels of glutamate, which have a facilitative effect on DA release (Robelet et al., 2004) and (iii) decreased DA uptake by the downregulation of DAT (Bezard et
al., 2000; Lee et al., 2000). These regulatory changes in DA metabolism are expected to increase extracellular DA levels towards the normal range, representing major presynaptic compensatory mechanisms in the striatum of PD patients.

*Altered regulation of post-synaptic dopamine receptors*

While the studies described above relate to the intrinsic properties of presynaptic DA neurons, post-synaptic changes also occur in response to reduced DA stimulation. One of these changes is mediated by “supersensitivity” of DA receptors, which is characterized by enhanced responsiveness, mainly behavioural, to certain dopaminergic agents such as L-dopa or apomorphine (Kostrzewa et al., 2005). This phenomenon may appear late in the development of PD, when DA denervation is severe (Zigmond and Stricker, 1980; Chen, 2005). Receptor “supersensitivity” can be attributed to two main causes: 1) an increase in DA receptor levels, and 2) increased sensitivity of DA receptors (Bezard and Gross, 1998).

DA denervation does not affect D1 and D2 receptors similarly. There is a plethora of evidence which suggests, in humans and in several animal models, that expression of D2 receptors in the untreated striatum of PD is upregulated (Qin et al., 1994; Frohna et al., 1995; Narang and Wamsley, 1995; Morissette et al., 1996; Rioux et al., 1997; Araki et al., 1998; Piggott et al., 1999; Betarbet and Greenamyre, 2004). Effect of DA denervation on D1 expression is more equivocal; upregulated in some studies (Narang and Wamsley, 1995; Morissette et al., 1996), but downregulated in others (Marshall et al., 1989; Qin et al., 1994). In other studies (Gerfen et al., 1990; Qin et al., 1994; Morissette et al., 1996), downregulation of D1 mRNA was also observed. These discrepancies regarding the regulation of D1 receptors remain unexplained. It is possible that regulatory changes of the DA receptors are time dependent, and the time at which these experiments are conducted following DA denervation
may contribute to the differences observed (Narang and Wamsley, 1995). In line with this view, Araki et al (2000) demonstrated a sequential pattern of receptor regulation with D2 changes preceding D1 changes (Araki et al., 2000). In addition, D1 changes are more transient than those of the more sustained D2 receptor changes. In addition, there are also differing patterns of regulation in various parts of the striatum. For instance, D2 receptor increase in the ventromedial striatum is sustained much longer than the D2 increase in the dorsolateral striatum (Araki et al., 2000). Together, these data demonstrate a complex pattern of changes in DA receptor levels after DA denervation.

In conclusion, multiple mechanisms are involved in maintenance of physiological levels of DA stimulation despite the progressing depletion of DA cells. Initially, increased release combined with decreased uptake may be sufficient to uphold extracellular levels. However, with further progression, post synaptic changes take effect. Therefore, at different stages of the disease and DA denervation, different compensatory mechanisms may be essential to regulate DA.

**Neural Mechanisms of L-dopa-induced dyskinesia**

**Pharmacology of L-dopa**

Since its discovery in the early 1960s, L-dopa has been the cornerstone in the treatment of PD. Exogenous L-dopa is converted to DA by AADC in the striatum, bypassing TH, the rate limiting factor in DA synthesis. Therefore, administration of L-dopa may increase striatal DA levels in a dose-dependent way (Henry et al., 1998; Lundblad et al., 2004). It has been proposed that L-dopa is also converted to DA in cells other than DA neurons in the striatum, such as AADC-containing glia (Nakamura et al., 2000), or other monoaminergic neurons, particularly serotonergic (Arai et al., 1994), and GABAergic neurons (Melamed et al., 1980; Hefti et al.,
This view has been used to explain how L-dopa is converted to DA in the striatum with almost complete loss of DA terminals (Poewe and Wenning, 2002; Kostrzewa et al., 2005).

The benefit of L-dopa treatment often erodes after the first few years of treatment due to the emergence of fluctuating response, dyskinesia or both. The anti-parkinsonian effect of L-dopa may change gradually over time: becoming shorter in duration ("wearing-off"), and greater in magnitude, eventually leading to fluctuating responses after each dose of L-dopa. Similarly, chronic treatment with L-dopa may induce dyskinesia, which is involuntary movements appearing after administration of L-dopa, particularly when anti-parkinsonian effects of L-dopa are maximal (hence, peak-dose dyskinesia).

**Risk Factors for L-dopa-induced Dyskinesia**

Although mechanisms of L-dopa-induced dyskinesia are not fully understood, it has been recognized that certain factors related to the drug or the disorder increase the risk of L-dopa-induced dyskinesia.

In regards to the drug-related factors, it appears that not only the duration of treatment, but also the type, dose and preparation of the drug influence the incidence of L-dopa-induced dyskinesia: (i) PD patients treated are less likely to develop dyskinesia with DA agonists than with L-dopa; (ii) incidence tends to be higher in PD patients treated with higher doses. At moderate doses, L-dopa elicits dyskinesia in approximately 50% of treated patients, and rises to 75%-80% with a higher dose (Calon et al., 2000; Nutt, 2001). This dose dependent effect of L-dopa on dyskinesia seen in patients is observed in animals as well (Henry et al., 1998; Lundblad et al., 2004); (iii) Observations in experimental and clinical studies suggest that pulsatile drug treatment, but not continuous drug treatment, causes motor complications including L-dopa-induced dyskinesia in animal models of PD and patients with PD (Olanow et al., 2000).
Although the mechanisms of this phenomenon are not fully understood, it is, at least in part, associated with downstream changes induced by pulsatile stimulation of DA receptors (discussed in detail at a later section).

In regards to PD related factors, cumulated evidence from clinical and experimental studies has shown that DA denervation is necessary for L-dopa treatment to induce dyskinesia. (Mones, 1971; Nutt, 1990; Langston et al., 2000). For instance, in patients with asymmetric PD, L-dopa-induced dyskinesia appears earlier and is more severe on the more affected side than the less affected side (Mones, 1971).

**Current Views on the Mechanisms of L-dopa-induced Dyskinesia**

Although the neural mechanisms of L-dopa-induced dyskinesia are not fully understood, the next section highlights several of the neural changes that have been established as key contributors to this phenomenon.

*Increased Activity in the Basal Ganglia Output Nuclei*

It has been suggested that dyskinesia results from reduced activity in the STN and GPi/SNr neurons (Obeso et al., 2000). We can again use the classical model of basal ganglia to describe the consequences of this DA-induced overstimulation of the direct and indirect pathways on the output nuclei of the basal ganglia, the GPi/SNr (Figure 4). First, overstimulation of striatal GABAergic projections in the direct pathway reduces the activity of the GPi/SNr. Secondly, overstimulation of striatal GABAergic projections in the indirect pathway reduces the activity of GPe neurons, which in turn, disinhibits GPi/SNr activity. Furthermore, it has been proposed that this reduces the tonic inhibitory influence of the GPi/SNr on the thalamo-cortical neurons, along with excess cortical-striatal activity, drive the expression of unwanted movements (Obeso et al., 2000). Data from electrophysiological studies have
Figure 4.
Schematic drawing representing basal ganglia function in the on-state of L-dopa-induced
dyskinesia. The red lines with round ends depict excitatory connections. The blue lines with
blunt ends depict inhibitory connections. Thick lines represent overactive projections, whereas
thin lines represent underactive projections. Consistently, boxes with thick outlines (the GPe,
thalamus) represent nuclei which are overactive. Dotted Lines or outlines represent projections
or nuclei that are eliminated due to DA cell death. Abbreviations: $D1 = \text{dopamine } D1 \text{ receptor};$
$D2 = \text{dopamine } D2 \text{ receptor}; GPe = \text{external segment of globus pallidus;} GPi = \text{internal}
\text{segment of the globus pallidus;} SNc = \text{substantia nigra pars compacta;} SNr = \text{substantia nigra}
pars reticulata; STN = \text{subthalamic nucleus}$
Figure 4
confirmed reduced neuronal firing in the GPe and increased activity in the GPi during apomorphine induced dyskinesia in MPTP treated monkeys (Filion et al., 1991) and as well, in PD patients (Lozano et al., 2000). This model of L-dopa-induced dyskinesia provides a useful theoretical framework to explain many experimental results and to generate effective working hypotheses. However, to say that an underactive GPi/SNr is the sole mechanism underlying L-dopa-induced dyskinesia would be inaccurate. Accordingly, this model is unable to explain why reduction of GPi neuronal firing through pallidotomy, consistently improves, rather than induce, dyskinesia (Lang et al., 1997). Closer investigation of GPi/SNr activity suggested that not only is the net activity decreased, but patterns of firing and synchronization is also affected in L-dopa-induced dyskinetic animals (Matsumura et al., 1995; Vitek and Giroux, 2000; Boraud et al., 2001). Therefore, an interaction of many factors is likely involved in the production of downstream neuronal changes that lead to abnormal firing rate and pattern associated with L-dopa-induced dyskinesia. As regulation of the basal ganglia involves several neurotransmitter systems, the following section will review some evidence of how key systems may be involved in the generation of L-dopa-induced dyskinesia.

Post-synaptic changes due to D1 and D2 pulsatile stimulation

As mentioned earlier, pulsatile, but not continuous, DA receptor stimulation is associated with development of L-dopa-induced dyskinesia (Chase and Oh, 2000). DA receptor stimulation is relatively tonic in the striatum, except for short phasic bursts that occur with expectation of certain behaviours and reward (Schultz and Romo, 1990). Therefore, in the DA denervated striatum, DA receptors are more dependent on exogenous stimulation by DA agents such as L-dopa. Due to the short half-life of L-dopa (60-90 minutes), the peripheral or plasma levels of L-dopa derived DA fluctuates. As the number of DA terminals decreases, the storage capacity of
L-dopa derived DA decreases, and the ability of DA neurons to buffer fluctuating plasma levels become reduced (Chase and Oh, 2000). Therefore, DA receptors in the DA denervated striatum are exposed to an abnormal pattern of high and low stimulation. (Sealfon and Olanow, 2000). Consistent with this concept, chronic treatment of a really high dose of L-dopa in unlesioned monkeys elicit the expression of dyskinesia, suggesting that even with a normal number of DA terminals, the buffering capacity can be overwhelmed if a large enough dose is used (Pearce et al., 1999). It is likely that this abnormal pattern of DA receptor stimulation leads to downstream neuronal changes leading to L-dopa-induced dyskinesia. Data from animal studies showed that DA denervation caused upregulation of pre-proenkephalin (PPE) and downregulation of prodynorphin (PDyn) mRNA expression in animal striatum (Gerfen et al., 1990; Jolkkonen et al., 1995). Enkaphalin levels remain elevated in rats which develop dyskinesia, but are normalized with administration of long-acting DA agonists (Herrero et al., 1995; Jolkkonen et al., 1995). Similar observations were made by Cenci and colleagues (1998), who detected increased mRNA expression of PPE and PDyn in the lesioned striata of dyskinetic rats (Cenci et al., 1998). Furthermore, they found that PDyn mRNA levels had the strongest correlation to severity of dyskinesia. These data suggest a role for stimulus-induced, post-synaptic changes in the gene expression of PPE and PDyn in L-dopa-induced dyskinesia.

Role of D3 receptors in L-dopa-induced dyskinesia

Another receptor subtype that is receiving increasing attention for its role in L-dopa-induced dyskinesia is the D3 receptor. In the normal brain, D3 receptors are typically localized in the shell of the nucleus accumbens, which is associated with emotional and cognitive processes. They are virtually absent in the dorsal striatum (Sokoloff et al., 1990; Levesque et al., 1995) and the core region of the nucleus accumbens, two areas that are involved in the motor
disturbances. However, studies conducted by Bordet and colleagues (1997) in 6-OHDA lesioned rats demonstrated D3 receptor upregulation in the nucleus accumbens core by 680%, and in the dorsal caudate putamen by 130%, following repeated L-dopa treatment (Bordet et al., 1997).

Induction of gene expression was correlated in D3 upregulation. Behavioural sensitization can be elicited by administration of D3 agonists alone, and can be blocked by pretreatment of D3 antagonists, suggesting the critical role for D3 receptors in expression of behavioural sensitization. Furthermore, it appears that D1 receptors are involved in the induction of D3 gene expression following L-DOPA, because D1 agonist SKF38393 produced similar behavioural effects as L-dopa, and D1 antagonist SCH23390 blocked the L-dopa-induced behavioural effect.

In MPTP treated primates and in humans, D3 binding decreases following denervation, a trend that is reversed with repeated treatment of L-dopa (Morissette et al., 1998; Ryoo et al., 1998; Bezard et al., 2003). Coincidently, D3 upregulation correlates with the development of L-dopa-induced dyskinesia and motor fluctuations, and when normalized, is correlated with the attenuation of L-dopa-induced dyskinesia (Bezard et al., 2003).

*Role of glutamate receptors in L-dopa-induced dyskinesia*

As previously mentioned, the excessive drive from the motor cortices may an important contributing factor in the etiology of L-dopa-induced dyskinesia. Indeed, many studies have reported changes in glutamate regulation following DA denervation in animal models, and after chronic L-dopa treatment. Enhancement of corticostriatal glutamate transmission has been noted following DA denervation (Lindfors and Ungerstedt, 1990; Meshul et al., 1999; Calabresi et al., 2000), but is further augmented with L-dopa therapy (Robelet et al., 2004). Therefore, numerous studies have examined the effects of L-dopa treatment on glutamate receptors and their possible roles in L-dopa-induced dyskinesia. Co-administration of NMDA-receptor antagonists in MPTP
lesioned monkeys substantially prevented the dykinesiogenic, but not the anti-parkinsonian
effects of L-dopa (Gomez-Mancilla and Bedard, 1993; Papa and Chase, 1996; Blanchet et al.,
1998). Similarly, administration of AMPA receptor antagonist NBQX produced similar effects
(Marin et al., 2000). Metabotropic glutamate receptors also colocalize with the ionotropic
 glutamate receptors on the dendrites of the medium spiny neurons (Testa et al., 1994). However,
their contribution to L-dopa-induced dyskinesia has yet to be tested.

Faulty learning hypothesis

It can take up to several weeks of chronic L-dopa administration in animal models before
dyskinesia develops. Once the symptoms appear, however, they are difficult to extinguish.
Even weeks after treatment withdrawal, the first dose of L-dopa will trigger the onset of
dyskinesia, indicating that long-term treatment with LD has made lasting modifications in the
response of the basal ganglia to DA (Calon et al., 2000). Recently, new experimental evidence
has been used to support a new hypothesis explaining the irreversibility of L-dopa-induced
dyskinesia, based on a model of synaptic memory (Picconi et al., 2003). Long-lasting synaptic
changes due to non-physiological stimulation of glutamate receptors have been studied
extensively in animal models of learning and memory, especially in relevance to long term
potentiation (LTP). The mechanisms of LTP involve several phosphatase-kinase signaling
pathways, alterations in gene expression and subsequent protein regulation. Remarkably, the
development of L-dopa-induced dyskinesia is associated with similar processes in striatal
neurons, including alterations in the expression of immediate early genes, phosphorylation of
 glutamate receptors, activation of similar signaling cascades, and expression of transcription
factors. Phosphorylation of one such transcription factor, the cAMP-response-element-binding
protein (CREB), is critical in the induction of LTP. PKA-dependent activation of CREB is also
triggered by stimulation of D1 receptors (Dudman et al., 2003), providing further support that mechanisms involved in LTP may underlie the pathology of L-dopa-induced dyskinesia.

Recent studies performed in vitro demonstrated LTP in striatal neurons in response to high frequency stimulation of glutamate which as DA dependent, suggesting that L-dopa treatment may be a substrate of the long term synaptic changes associated with L-dopa-induced dyskinesia (Picconi et al., 2003). Furthermore, striatal slices taken from non-dyskinetic animals, but not dyskinetic animals, demonstrated the ability to depotentiate after 10 minutes of low frequency stimulation. Taken together, these data suggest bidirectional plasticity in striatal neurons may be important for the storage, as well as deletion of appropriate and inappropriate motor information. Therefore, inability to erase nonessential motor information may form a pathological basis for L-dopa-induced dyskinesia.

**Treatment of L-dopa-induced Dyskinesia**

Although currently there is no cure for L-dopa-induced dyskinesia, several approaches have been attempted to reduce the severity of L-dopa-induced dyskinesia. The first measure is typically reduction of drug (L-dopa or DA agonists) dosage. Lower dosage is typically correlated with lessening of dyskinesia severity. Anti-dyskinetic drug (usually anti-glutamatergic) supplements can also be used conjunctively with L-dopa. However, while many of these drugs alleviate dyskinesia, they also reduce the efficacy of L-dopa (Brotchie et al., 2005). Furthermore, tolerability of these drugs is highly variable among patients (Brotchie et al., 2005). Recently, the development of surgical procedures facilitating stimulation of deep brain nuclei has also proved effective.

**A Pathogenic Role for the Dopamine Transporter in L-dopa-induced Dyskinesia**

**Overview**
Previous studies have shown that in 6-OHDA treated rats, an acute injection of high dose of L-dopa induced a surge of DA efflux in the lesioned striatum that showed a similar time course of L-dopa-induced rotational behaviour (Nakazato and Akiyama, 1989; Abercrombie et al., 1990). Increases in striatal DA have also been observed in the denervated striatum of PD patients who display response fluctuations to L-dopa and dyskinesia (de la Fuente-Fernandez et al., 2001). In contrast, DA level increases in the unlesioned striatum are either mild or absent. This L-dopa-induced efflux of extracellular DA in the lesioned striatum may be pathogenic as it exposes the DA receptors to bursts of a fluctuating pattern of stimulation, resulting in downstream, long lasting changes in post-synaptic striatal projection neurons (Calon et al., 2000; Olanow et al., 2000). Furthermore, it seems that once these changes are present, they are very difficult, if not impossible, to reverse (Brotchie et al., 2005). Therefore, in order to better understand the pharmacology of L-dopa and its effect on DA regulation, it is important to understand the mechanisms mediating L-dopa-induced DA efflux in the DA denervated striatum.

To study the mechanism underlying this DA efflux, Miller and Abercrombie (1999) used in vivo microdialysis to examine the effect of TTX, an inhibitor of fast Na\textsuperscript{+} channels, on L-dopa (50mg/kg) induced DA efflux in the DA-denervated striatum (Miller and Abercrombie, 1999). Their results indicate that TTX significantly attenuates the DA surge response, suggesting the mechanisms for release is largely Ca\textsuperscript{2+} mediated. However, TTX administration only resulted in a partial attenuation, suggesting that part of the L-dopa-induced DA efflux may be mediated by a depolarization-dependent mechanism.

Ca\textsuperscript{2+}-mediated vesicular release is one mode of DA release, however, recent evidence suggests that the DAT is also involved in the release of DA under physiological conditions, in particularly under the influence of increased glutamatergic activity, as observed in the DA
dendrites in the SNr (Falkenburger et al., 2001). In addition to DA denervation, chronic treatment with L-dopa appears to be necessary to further enhance glutamate release in the striatum (Robelet et al., 2004). Given this evidence, it is of interest to study the role of reverse transport as a mechanism for L-dopa-induced DA release.

**The Dopamine Transporter**

The DAT is a critical player in the modulation of the expression of movement and in PD. By uptaking released DA molecules back into intracellular stores, it has a key role in regulating the intensity and duration of DA stimulation. The DAT belongs to a family of monoamine uptake transporters which also includes the NET and SERT [for noradrenalin (NA) and serotonin, respectively]. Furthermore, the monoamine transporters are established targets for pharmacological agents such as anti-depressants and psychostimulant drugs of abuse such as cocaine, amphetamines and amphetamine-like drugs. Therefore, an examination of the mechanisms by which these drugs interact with the DAT has greatly contributed to our understanding of the function and regulation of this transporter protein, as well as the interactions between the DAT and DA.

The DAT is localized mainly on the pre-synaptic terminals of dopaminergic neurons (Ciliax et al., 1995), but is also found on axons and dendritic spines, (Falkenburger et al., 2001) along with extraneuronal sites such as glial cells. The abundance of presynaptic DATs on nigrostriatal neurons has led to imaging of the transporter as a common measure of the progressive DA neuronal loss in PD. Decrease in DAT binding in PD patients is widely established in clinical studies. Similarly, animals that have received nigrostriatal lesions also consistently show decreased binding to the DAT.
Uptake of DA molecules is dependent on the sequential binding and co-transport of Na\(^+\) and Cl\(^-\) ions, and is driven by the electrochemical gradient generated by the Na\(^+\)/K\(^+\) ATPase pump. Binding of 2 Na\(^+\) and 1 Cl\(^-\) and 1 DA molecule activates a conformational change in the DAT that allows the translocation of ions and DA into the cell, coupled with the binding and outward transport of K\(^+\). Inward transport of DA involves the net movement of positively charged ions into the cell; therefore, a negative membrane potential facilitates DA influx. Conditions that change membrane potential, ion gradients, or amount of ATP molecules can evidently change the function of the DAT, including the directional mode (Blakely, 2001).

DAT blocking drugs fall into two basic categories. Drugs like cocaine and methylphenidate (MP) are non-selective competitors of DAT which blocks substrate. Amphetamines, on the other hand, are carrier substrates which themselves are transported into the cell, resulting in a block of DA uptake and additionally, simultaneous stimulation of DA efflux from intracellular stores. Therefore, it seems that while the normal function of the DAT is uptake DA, under certain circumstances, the DAT can partake in the release of DA as well via a reversal of its functional mode. Traditionally, reversed transport was considered a drug-induced phenomenon; however, more recent findings suggest that DAT mediated DA release occurs under more physiological circumstances (Falkenburger et al., 2001).

**Evidence for DAT mediated DA release in L-dopa-induced dyskinesia**

Recent findings from our lab demonstrated that in unilaterally lesioned rats chronically exposed to a low, clinical dose of L-dopa (10mg/kg, i.p.), reverse dialysis of specific DAT blocker GBR 12909 significantly attenuated the L-dopa-induced DA efflux (Ahn et al., 2004). Although GBR 12909 also attenuated the DA response in L-dopa naïve rats, the effect was much smaller compared to that in chronically treated animals. Additionally, perfusion of Ca\(^{2+}\) free...
artificial cerebral spinal fluid resulted in a significant decrease of the L-dopa-induced DA response in drug-naïve, but not chronically treated, rats. Taken together, these data indicate that over a course of 3 weeks chronic treatment, L-dopa induces a functional reversal of the DAT, from uptake, to release mode. More importantly, the role of DAT in mediating L-dopa-induced DA release has important implications for L-dopa-induced dyskinesia.

**Experimental rationale**

The rationale for the experiments conducted for this thesis is based in part on the findings, that although reverse dialysis of GBR 12909 diminished the L-dopa-induced DA response, this DAT antagonist failed to attenuate dyskinetic behaviour. It is possible that when administered via reverse dialysis, the amount of drug that diffuses into the immediate column of tissue surrounding the microdialysis probes is insufficient to cause any behavioural effect. The objectives of the studies presented in this thesis are to 1) replicate the effects of intrastriatal GBR 12909 using systemic administration of an alternate DAT blocker, methylphenidate (MP), 2) to assess the effects of MP on L-dopa-induced DA efflux in 6-OHDA lesioned rats receiving different periods of L-dopa treatment, and 3) assess the effect of MP on L-dopa-induced dyskinesia.

**Experimental considerations**

To achieve the aims of these experiments, we selected MP as the drug of chose to investigate the function of the DAT. As well, the unilateral 6-OHDA lesioned rat model was selected for these studies. The following sections provide some background information on these two investigative tools, as well as our rationale for using them.

*Methylphenidate*
Like amphetamine, MP is classified as a DAT-blocking psychostimulant. MP is the active ingredient used in the clinically approved drug Ritalin, which has been established primarily for treatment of ADHD, but has been used as well to treat depression, narcolepsy, and fatigue (Leonard et al., 2004). Although the main mechanism of action of MP is blocking of the DAT, MP shows affinity for the NET as well. Therefore, the therapeutic effects of MP most likely come from increases in both extracellular DA and noradrenalin levels (Leonard et al., 2004).

Although MP has a high pre-systemic metabolism (i.e., metabolized in the mouth and the gut), only a small amount is needed for extensive diffusion in the brain (Leonard et al., 2004). PET imaging shows that the peak uptake of MP in humans was approximately 60 minutes following oral administration (Volkow et al., 2002). Plasma concentrations of MP correlate well with DAT occupancy, suggesting that measuring plasma concentrations is a good indicator of brain levels of MP. In animal studies of MP, animals are typically injected intraperitoneally, allowing for quicker availability in the brain. Microdialysis studies showed that (Huff and Davies, 2002) increases in plasma levels of MP are correlated with extracellular striatal DA levels and drug-induced behaviour, all of which peaked at approximately 20-40 minutes after administration and persisted for about 3 hours.

MP was selected as the DAT blocker used for the experiments in this thesis for several reasons. First, it demonstrates a similar time course as L-dopa, displaying similar peak and duration. Second, as opposed to some other DAT blocking agents, MP is relatively easy to dissolve and inject. Finally, as MP is already a clinically approved drug, any therapeutic value for L-dopa-induced dyskinesia that may be demonstrated from theses studies can be easily tested in pre-clinical trials.
Animal models of L-dopa-induced dyskinesia

6-OHDA and MPTP lesioned animals display L-dopa-induced dyskinesia in a dose-dependent way (Henry et al., 1998; Lundblad et al., 2004). With chronic L-dopa treatment, MPTP-lesioned primates develop dyskinetic movements that resemble very closely to that seen in PD patients (Cenci et al., 2002). Unilaterally 6-OHDA lesioned rats also display dyskinesia in the contralateral forelimb, trunk, and orofacial musculature (Cenci et al., 2002). Both rats and primates show a time course that is similar to peak-dose dyskinesia in humans (Lee et al., 2000). Furthermore, both models show similar molecular changes that include upregulation of dynorphin and enkephalin mRNA and receptor binding (Cenci et al., 1998), and similar responses to pharmacological agents used to treat L-dopa-induced dyskinesia in patients.

Despite this accumulating evidence, the suitability of the 6-OHDA lesioned rat model for L-dopa-induced dyskinesia has been questioned (Cenci et al., 2002). Critics of this model argue that the main behavioural effect elicited by L-dopa in rats is contraversive rotational locomotion, a behaviour that is absent from human dyskinesia. Furthermore, they argue that limb and trunk movements may simply be compensatory responses to the intense rotation (Langston et al., 2000). However, recent findings challenge this view by showing that limb and axial dyskinesia can be produced separate from locomotive rotation, using specific unilateral intrastriatal lesions in the lateral regions of the putamen (Lundblad et al., 2002). Similarly, lesions in the medial putamen elicit L-dopa-induced rotation without forelimb and trunk movements. Furthermore, L-dopa causes dyskinesia without rotation in bilaterally lesioned rats. Taken together, these data suggest that rotation is distinct from the abnormalities in limb, mouth, and trunk movements.
GENERAL METHODS

L-OHDA Lesioning and Probe Implantation

Sprague-Dawley female rats weighing 275-300 grams from Charles River (St. Constant, Quebec, Canada) or University of British Columbia (Animal Care Centre Breeding Unit, South Campus) were used in all experiments. All animals were pair-housed upon arrival and individually house after surgery in plexiglass cages with free access to rat chow and water, in a colony maintained at 19-22 °C, with a 12:12 hour light-dark cycle.

For induction of anaesthesia, 4% isoflurane mixed with oxygen was used, and then subsequently lowered to 1.5 – 2.5% isoflurane (AErrane, Baxter Co., Ontario, Canada) for maintenance throughout the rest of surgery. Animals were positioned in flat skull position (mouth bar at -3.2mm) in a stereotaxic apparatus that was lined with a heating pad to maintain the animals' body temperature. DA-denervating lesions were performed by unilateral injection of 6-OHDA (3ug/uL; 2ug/uL) prepared in 0.02% ascorbic acid solution into 2 locations: the SNc (from bregma, -5.2 mm AP, -2.0 mm ML; from dura, -7.1 mm DV), and the medial forebrain bundle (MFB; -4.2mm AP, -1.7 ML, -7.9 DV); all coordinates were determined according to Paxinos and Watson (1997). Injection of 6-OHDA into the MFB and SNc, the origins of the nigrostriatal pathway, is the lesion procedure used in most studies using the 6-OHDA model (Schwarting & Huston, 1996). Injections were performed at the rate of 1 mL/min, and the injection needle was left at the lesion site for additional 3 minutes to allow for drug diffusion. Additionally, 19 gauge stainless steel guide cannulae (15mm) were implanted directly above the lesioned and unlesioned striata (+1.0 AP, +/-3.1 MP, -1.0 DV). Approximately 30 minutes before rats were taken of isoflurane anaesthesia, rats were given a subcutaneous dose of
analgesic (ketoprofen 0.05mL or buprenorphine 0.1mL) before placed under a heat lamp for recovery.

**Drugs and Preparation**

6-hydroxydopamine hydrobromide (6-OHDA), L-3.4-dihydroxyphenylalanine methyl ester (L-dopa), benzerazide hydrochloride, fluoxetine hydrochloride, and desipramine hydrochloride were all obtained from Sigma Aldrich, Ontario, Canada. L(+)-ascorbic acid was obtained from EM Science, Gibbstown, NJ, USA. Methylphenidate hydrochloride was synthesized from the lab of Dr. Tom Ruth at the University of British Columbia (Vancouver, BC, Canada). 6-OHDA was dissolved in 0.02% L-ascorbic acid solution immediately prior to infusion. L-dopa was prepared fresh daily in a mixed solution with peripheral DOPA-decarboxylase inhibitor, benzerazide hydrochloride (15mg/kg) in a 0.9% saline solution. MP, fluoxetine, and desipramine were all dissolved in 0.9% saline solution.

**Behavioural Analysis: Abnormal Involuntary Movement (AIM) Scale**

A modified version of the AIM scale (Lee et al., 2000) was used to classify and rate three different components of L-induced behaviour according to their topographic distribution: 1) locomotive rotation (loco), 2) axial dystonia (axial), and 3) limb extension (limb). Each component was rated on a scale of 0-4 based on the proportion of time/monitoring period: 0 being absence of behaviour, 1 being intermittence under 50% of the time, 2 being intermittence over 50% of the time, 3 being continuous dyskinesia that can be interrupted by sensory distraction, and 4 being continuous dyskinesia that is oblivious to sensory distraction. A total-AIM score was calculated summing all AIM scores during total observation period (160 minutes after L-dopa administration).

**Microdialysis**
Microdialysis probes consisted of a semipermeable membrane 4.0 mm in length (340 μm outer diameter; 65,000 Dalton molecular weight cutoff; filter 12; Hospal, Neurnberg, Germany), and were flushed continuously at 1 μl/min with artificial cerebrospinal fluid (147.0 mM NaCl, 1.2 mM CaCl2, 3.0 mM KCl, 1.0 mM MgCl2, and a 10.0 mM sodium phosphate buffer) using a 2.5 ml gas-tight syringe (Hamilton, Reno, NV) and a syringe pump (model 22; Harvard Apparatus, South Natick, MA) before implantation into rat striata. Rats then remained in testing chambers overnight with access to food and water. On test day, samples were collected at 10 minute intervals and assayed for DA and its metabolites 3,4 dihydroxyphenylacetate (DOPAC) and homovanillic acid (HVA) until a stable baseline was established (4 samples within 10% variation). Drugs (L-dopa or MP) were administered to rats, and samples continued to be collected every 10 minutes for 165 minutes after L-dopa injection. L-dopa-induced AIM was also monitored and rated using a modified AIM rating scale at 10 minute intervals, and videotaped for later reference.

**High Pressure Liquid Chromatography**

Two HPLC-electrical detection systems were employed for assaying DA and DA metabolite contents in striatal dialysates. The systems consisted of an ESA 582 pump (Chelmsford, MA), a Scientific Systems, Inc. (State College, PA) pulse damper, a Rheodyne manual injector (20 μl injection loop, Rhonert Park, CA), a TOSOH Biosep (Montgomeryville, PA) Super ODS TSK column (2 mm x 10 mm; 2μM particle), an Antec Leyden (Leyden, The Netherlands) Links system and an Antec Intro detector with a VT-03 electrochemical flowcell ($V_{applied} = +0.7$ V). The mobile phase consisting of 70mM sodium acetate, 40mg/L of EDTA, and 50mg/L of sodium octyl sulfate (adjustable), pH 4.0, and 12% methanol, was flowed through
the HPLC system at 0.18mL/min. Chromatographic data was analyzed using EZChrom Elite software.

**Data Analysis and Statistics**

*Neurochemistry*

All neurochemical data are expressed as difference from baseline (average of the last four baselines). Statistical significance was based on analysis of variance (ANOVA) and followed when appropriate by Dunnett’s post hoc test for comparisons (for within-groups comparisons, i.e. time). For between-groups analysis, a 2-way ANOVA was used to compare the effects of MP pretreatment vs. L-dopa only on L-dopa induced neurochemical changes. In addition, a T-test was used to compare L-dopa induced peak neurochemical changes in the lesioned striatum. Peak response was calculated as the average of the three highest consecutive data points following L-dopa injection. Using a T-test, behavioural data (total AIM score) were compared statistically between MP-pretreated vs. L-dopa only groups. In this thesis, only p values were presented for those results that did not reach statistical significance.

**Histology**

After testing, rats were given a minimum of 1-week washout period, before being deeply anaesthetized and decapitated. Brains were promptly removed and flash frozen at 2-methylbutane (Fisher Scientific, New Jersey, USA) cooled in dry ice, and stored at -80°C until being sectioned at 50um for verification of probe placement according to Paxinos and Watson’s rat brain atlas (Paxinos and Watson, 1997).
EXPERIMENT 1 - Dose dependent effect of MP on L-dopa-induced dyskinesia

Introduction

In the first experiment of this study, we investigated whether a systemic dose of MP would attenuate L-dopa-induced AIM, and in addition, whether a dose effect of MP would be observed. In chronically L-dopa treated animals, we hypothesized that MP would result in a dose-dependent attenuation of the L-dopa-induced AIM. In the second experiment, we examined the possible role for other monoaminergic transporter systems in L-dopa-induced dyskinesia, by using pretreatment of fluoxetine and desipramine to inhibit serotonin and noradrenalin transporters, respectively.

Methods

Following a 4 week recovery period after surgery, rats with unilateral 6-OHDA lesions received 3 weeks of daily L-dopa injections (10mg/kg i.p., co-administered with 15mg/kg i.p. of benserazide). Development of dyskinesia on the side contralateral to the lesion was monitored daily using the modified AIM rating scale. L-dopa-induced AIM severity progressed steadily over the first week of treatment, and stabilized after approximately 2 weeks. Using the modified AIM scale, we obtained a pretest baseline measure of L-dopa-induced AIM on each of the two days leading to test day (Days 19 and 20). Scoring began 5 minutes after L-dopa administration, and subsequently every 10 minutes for 160 minutes. Only rats that displayed dyskinetic behaviour on their pre-test baseline were selected for testing. On test day, animals were divided into 5 treatment groups, and were pretreated with MP, fluoxetine, or desipramine 20 minutes between receiving an L-dopa challenge. The animals used in each of the treatment groups are summarized in Table 1.
Table 1. Summary of animals and treatment groups used in Experiment 1.

<table>
<thead>
<tr>
<th>Treatment Group</th>
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<tr>
<td><strong>Experiment #1</strong></td>
<td></td>
</tr>
<tr>
<td>MP 7 mg/kg</td>
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<tr>
<td>MP 14 mg/kg</td>
<td>n=17</td>
</tr>
<tr>
<td>MP 21 mg/kg</td>
<td>n=10</td>
</tr>
<tr>
<td><strong>Experiment #2</strong></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine 25 mg/kg</td>
<td>n=6</td>
</tr>
<tr>
<td>Desipramine 25 mg/kg</td>
<td>n=8</td>
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</tbody>
</table>

Results

*Dosage Effect of Methylphenidate*

With daily repeated L-dopa treatment, the severity of dyskinesia severity increased during the first week, and reached a stable plateau within 2 weeks (Figure 5). Rats in all three groups demonstrated moderate to severe dyskinesia as observed in the pretest baseline measures. After MP administration, rats in all three groups displayed moderate turning behaviour ipsiversive to their lesion due to a greater relative increase of DA in the unlesioned striatum. Direction of turning was reversed soon after L-dopa was administered. This pattern of directional change was observed in all animals that received MP pretreatment in the experiments described in the later sections. MP at all three doses attenuated L-dopa-induced dyskinesia in all rats in comparison to their pre-test baseline (Figure 5). 7mg/kg of MP, resulted in a significant decrease of L-dopa-induced dyskinesia (L-dopa pre-test baseline vs. L-dopa with MP pretreatment: 87.63 ± 3.75 vs. 48.00 ± 6.83, T = 5.266, p < 0.001), as did 14mg/kg (76.20 ± 4.28 vs. 22.94 ± 8.67, T = 5.680, p < 0.001), and 21mg/kg (67.85 ± 7.15 vs. 23.60 ± 5.32, T = 5.231, p < 0.001). While the attenuation produced by 14mg/kg and 21mg/kg were significantly greater than that by 7mg/kg (T = 2.300, p = 0.029; T = 2.681, p = 0.013, respectively), there were
Figure 5.
Dose Effect of Methylphenidate on L-dopa-induced AIM in chronically L-dopa treated rats. Three groups of animals were each tested with a different dose of MP pretreatment (Panel A = 7mg/kg MP, i.p., Panel B = 14 mg/kg MP, i.p., Panel C = 21mg/kg MP, i.p.). The top row of graphs represents L-dopa-induced AIM scores on the day prior to test day. The middle three graphs represent the effect of MP pretreatment on L-dopa-induced AIM scores. The graphs on the bottom row represent a between groups comparison of the total AIM scores on pre-test and test day. The blue bars indicate locomotive turning, either ipsiversive or contraversive to the lesioned hemisphere. The orange bars indicate axial dystonia, and the white bars indicate limb dyskinesia. Significance for experimental group relative to their own control * p< 0.05; ** p< 0.01; *** p< 0.001. Significance effect of treatment between groups # p< 0.05.
no significant differences between 14mg/kg and 21mg/kg ($p = 0.956$). Hence, 14mg/kg MP was the chosen dose for the subsequent experiments for this thesis.

Effect of Fluoxetine and Desipramine

After 3 weeks of daily L-dopa treatment, all animals developed a stable dyskinetic response to L-dopa. On test day, pretreatment of either fluoxetine (Figure 6A) or desipramine (Figure 6B) failed to significantly attenuated AIM scores. (fluoxetine: $p = 0.220$; desipramine: $p = 0.415$). While there is some data that suggest fluoxetine may improve apomorphine-induced dyskinesia (Durif et al., 1995), our data lend support to the present hypothesis that L-dopa-induced dyskinesia is dependent on the DAT system, but not other monoaminergic transporters such as the SERT or the NET.

Discussion

The findings from this experiment demonstrated the dose-dependent attenuation of systemic MP on L-dopa-induced dyskinesia in 3-week L-dopa treated rats. These data support our hypothesis that the DAT may be involved in L-dopa-induced dyskinesia in chronically treated rats, possibly by releasing DA by reversed transport.

Furthermore, blockade of the SERT and NET did not give the same results, indicating that the DAT is the key player in this effect. However, the lack of effect from SERT and NET contradict previous reports in the literature that showed the anti-dyskinetic effects of fluoxetine (Durif et al., 1995). However, these tests were conducted in patients, and much of the improvements shown were for onset- and end-of-dose dyskinesia, which has a different behavioural profile than peak-dose dyskinesia. These differences may help explain the lack of effect of fluoxetine seen in the present study.
Figure 6.

Effect of Fluoxetine and Desipramine on L-dopa-induced AIM. Two groups of animals were each tested with either pretreatment with 25mg/kg i.p of fluoxetine (Panel A) or 25mg/kg i.p. of desipramine (Panel B). The top row of graphs represents L-dopa-induced AIM scores on the day prior to test day. The middle graphs represent the effect of fluoxetine or desipramine pretreatment on L-dopa (10mg/kg) induced AIM scores. The graphs on the bottom row represent a between groups comparison of the total AIM scores on pre-test and test day. The blue bars indicate locomotive turning, either ipsiversive or contraversive to the lesioned hemisphere. The orange bars indicate axial dystonia, and the white bars indicate limb dyskinesia. Significance for experimental group relative to their own control * p< 0.05; ** p< 0.01; *** p< 0.001.
Figure 6
EXPERIMENT 2- Effects of Methylphenidate on L-dopa-induced DA Efflux and Behaviour: a Microdialysis Study

Introduction

After establishing dosage effect with MP, 14mg/kg of MP was chosen for this study. To examine L-dopa-induced functional changes in the DAT, we examined the effects of MP on three groups of rats receiving different durations of L-dopa treatment.

Methods

Following surgery and at least 4 weeks of recovery period, rats were divided into 3 L-dopa treatment groups: 1) no treatment of L-dopa, 2) 6 days of daily L-dopa treatment, or 3) 20 days of daily L-dopa treatment (10mg/kg i.p. L-dopa, coadministered with 15mg/kg i.p. benzerazide): On test days, which was the day following the last day of LD treatment period, rats in each group were administered either with L-dopa only (10mg/kg i.p. coadministered with benzerazide, 15mg/kg i.p.) or LD with a 20 minute pretreatment of MP (14mg/kg, i.p.). Microdialysis and AIM rating were used to assess neurochemical and behavioural responses to test challenge. Only rats that had developed dyskinesia and had complete unilateral lesions (if basal DA levels in the lesion striatum ≤10% of basal DA levels in the unlesioned striatum) were used for analysis in this study. The animals used in each of the treatment groups are summarized in Table 2.

Table 2. A summary of the animals and treatment groups in Experiment 2

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Challenge on Test day</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-dopa-naïve</td>
<td>LD alone</td>
<td>N=7</td>
</tr>
<tr>
<td>L-dopa-naïve</td>
<td>LD + MP</td>
<td>N=6</td>
</tr>
<tr>
<td>L-dopa treatment (1-week)</td>
<td>LD alone</td>
<td>N=7</td>
</tr>
<tr>
<td>L-dopa treatment (1-week)</td>
<td>LD + MP</td>
<td>N=7</td>
</tr>
<tr>
<td>L-dopa treatment (3-week)</td>
<td>LD alone</td>
<td>N=9</td>
</tr>
<tr>
<td>L-dopa treatment (3-week)</td>
<td>LD + MP</td>
<td>N=6</td>
</tr>
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</table>
Results

Effect of L-dopa treatment on basal neurochemical levels

Basal DA levels in the lesioned striatum of were 5% of that in the unlesioned striatum in L-dopa naïve animals [lesioned striatum vs. unlesioned striatum, respectively; (mean ± SEM) 0.44 ± 0.25 vs. 9.63 ± 2.92 nM, T= 2.92; p = 0.008]; 5.5% in 1-week L-dopa treated animals (0.81 ± 0.22 vs. 14.64 ± 4.25 nM, T = 3.38, p = 0.002), and 5% in 3-week L-dopa treated animals (0.50 ± 0.25 vs. 10.28 ± 3.44 nM, T = 3.29, p = 0.003) (Figure 7, top graph). Basal DOPAC levels in the lesioned striatum of were 3% of that in the unlesioned striatum in L-dopa naïve animals [lesioned striatum vs. unlesioned striatum, respectively; (mean ± SEM) 1066.92 ± 138.93 vs. 12.46 ± 6.02 nM, T= 7.83; p < 0.001]; 1.2% in 1-week L-dopa treated animals (1526.85 ± 181.46 vs. 18.87 ± 6.31 nM, T = 8.61, p < 0.001), and 1% in 3-week L-dopa treated animals (1342.02 ± 141.93 vs. 15.54 ± 7.33 nM, T = 11.20, p < 0.001) (Figure 7, middle graph). Furthermore, a comparison between the unlesioned and lesioned striata showed no effect of 6-OHDA on basal 5-HIAA levels (Figure 7, bottom graph). This is consistent with the view that of 6-OHDA targets mainly dopaminergic, but not serotonergic, neurons.

An ANOVA of basal DA and DOPAC levels in the L-dopa-naïve, 1-week, and 3-week treated groups failed to show a significant effect of L-dopa treatment duration in the lesioned (DA: p = 0.699, DOPAC: p = 0.619) and unlesioned (DA: p = 0.499, DOPAC: p = 0.082) striata. While there is some controversy relating to the neurotoxic effects of L-dopa, the present data lend support to the emerging view that it does not (Olanow et al., 2004).

Effect of DAT blockade on L-dopa-naïve rats

In the unlesioned striatum (Figure 8A), A 1-way ANOVA revealed that L-dopa injection alone had no significant effect on extracellular DA levels (p = 0.119), but showed a significant
Figure 7.
Effect of L-dopa treatment on basal levels. The top graph: effect of L-dopa treatment (10mg/kg) duration (0, 1, 3 weeks) on basal levels of DA. The middle graph: effect of treatment of L-dopa (10mg/kg) treatment duration (0, 1, 3 weeks) on basal levels of DOPAC. The bottom graph: effect of L-dopa (10mg/kg) treatment duration (0, 1, 3 weeks) on basal levels of 5-HIAA. The blue bars indicate levels in the unlesioned striatum, and the purple bars indicate levels in the lesioned striatum. Significance of lesioned side relative to unlesioned side; * = p < 0.01; and ** = p < 0.001
Figure 8.
Effect of MP pretreatment on L-dopa-induced responses in the drug-naïve, unlesioned striatum. Panel A illustrates the L-dopa-induced changes in DA, DOPAC, and HVA levels. Panel B illustrates L-dopa induced changes in DA, DOPAC, and HVA levels 20 minutes after treatment with MP. 

A: Following four baseline samples, injection of L-dopa (10 mg/kg, i.p.) was given. Collection of dialysate samples continued for 160 minutes after L-dopa injection. Each diamond represents a dialysate sample collected over a 10-minute bin.

B: Following four baseline samples, injection of MP (14 mg/kg, i.p.) was given. Twenty minutes later, injection of L-dopa (10 mg/kg, i.p.) was administered. Collection of dialysate samples continued for 160 minutes after L-dopa injection. † denotes significance at p < 0.05 relative to the last pre-drug baseline; and ‡ denotes significance at p < 0.001 relative to the last pre-drug baseline.
increased above baseline for DOPAC and HVA levels (DOPAC: $F_{16,96} = 6.89, p = 0.014$; HVA: $F_{16,192} = 7.41, p = 0.008$, Dunnett's test, $p < 0.01$). In the MP pretreated group (Figure 8B), MP significantly increased extracellular DA from baseline, with no further increase following administration of L-dopa ($F_{18,90} = 10.96; p = 0.003$). This MP-induced DA increase peaked approximately 40 minutes after MP injection, and gradually returned to baseline levels after 2 hours. Although MP pretreatment alone did not increase basal DOPAC or HVA levels, a significant delayed rise in HVA levels was observed (HVA: $F_{18,90} = 9.11; p = 0.002$, Dunnett's test, $p < 0.01$). No changes from baseline were observed in DOPAC levels, although a slight, but insignificant decrease was observed in the first 60 minutes after L-dopa. A 2-way ANOVA test showed that DA levels in L-dopa with MP pretreatment group was significantly different compared to that in L-dopa only control group (DA: $F_{16,176} = 9.38, p < 0.001$). In contrast, no effect of MP pretreatment was observed in L-dopa-induced DOPAC, or HVA response (DOPAC: $p = 0.139$; HVA: $p = 0.380$). This pattern of response in the unlesioned striatum was similar in all three groups (drug naïve, 1-week treated, 3-week treated), with only some minor differences.

In the lesioned striatum (Figure 9A), injection of L-dopa caused a significant rise in extracellular DA concentration ($F_{16,96} = 9.00, p = 0.001$, Dunnett's test, $p < 0.01$), which was accompanied by a similar pattern of L-dopa-induced AIM. The DA efflux was followed by a significant delayed increase in DOPAC ($F_{16,96} = 9.54, p = 0.006$, Dunnett's test, $p < 0.01$), and HVA levels ($F_{16,96} = 16.70, p < 0.001$, Dunnett's test, $p < 0.01$). MP pretreatment (Figure 9B), had no effect on basal DA, DOPAC, and HVA levels. L-dopa given 20 minutes following pretreatment of MP resulted in an increase of DA levels (DA: $F_{16,80} = 11.85, p = 0.009$, Dunnett's test, $p < 0.01$), which was accompanied by a similar pattern of AIM. DA efflux was
Lesioned Striatum (L-dopa-naïve Group)

Figure 9.
Effect of MP pretreatment on L-dopa-induced responses in the drug-naïve, lesioned striatum. Panel A illustrates the L-dopa-induced changes in DA, DOPAC, and HVA levels. Panel B illustrates L-dopa-induced changes in DA, DOPAC, and HVA levels 20 minutes after treatment with MP. 9A: Following four baseline samples, injection of L-dopa (10 mg/kg, i.p.) was given, after which dialysate sample collection was continued for 160 minutes. 9B: Following four baseline samples, injection of MP (14mg/kg, i.p.) was given. Twenty minutes later, injection of L-dopa (10 mg/kg, i.p.) was administered. Collection of dialysate samples continued for 160 minutes after L-dopa injection. Each diamond represents a dialysate sample collected, and is drawn over a corresponding measure of AIM (colored bars) observed in a-10 minute bin. The blue bars indicate locomotivc turning, either ipsiversive or contraversive to the lesioned hemisphere. The orange bars indicate axial dystonia, and the white bars indicate limb dyskinesia. † denotes significance at p < 0.05 relative to the last pre-drug baseline; and ‡ denotes significance at p < 0.001 relative to the last pre-drug baseline.
followed by a delayed rise in DOPAC and HVA levels (DOPAC, $F_{16,80} = 42.60, p < 0.001$; HVA: $F_{16,80} = 16.10, p = 0.001$; Dunnett’s test, $p < 0.01$). A 2-way ANOVA test (Figure 9) showed that no significant effect of MP pretreatment on L-dopa-induced DA, DOPAC, or HVA response (DA: $p = 0.857$; DOPAC: $p = 0.547$; HVA: $p = 0.298$). A T-test used to compare L-dopa-induced peak neurochemical responses of DA, DOPAC, or HVA levels showed no significant effect of MP pretreatment (Figure 10A). (DA: $p = 0.971$; DOPAC: $p = 0.874$; HVA: $p = 0.249$).

In comparison to rats which only received L-dopa, a T-test showed that MP pretreatment resulted in a significant reduction in the axial component of L-dopa-induced dyskinesia (Figure 10B; control vs. treatment; Axial: $13.71 \pm 5.25$ vs. $0.33 \pm 0.37$; $T = 2.53; p = 0.028$), but not in locomotor and limb components (Loco: $p = 0.095$; Limb: $p = 0.665$).

**Effect of DAT blockade on 1-week L-dopa treated rats**

In the unlesioned striatum (Figure 11A), a 1-way ANOVA revealed that L-dopa injection alone had no significant effect on extracellular DA levels ($p = 0.201$), but showed an increased for both DOPAC and HVA from baseline levels; however, this was only statistically significant for HVA (HVA: $F_{16,96} = 16.96, p < 0.001$, Dunnett’s test, $p < 0.01$; DOPAC: $p = 0.271$). In the MP pretreated group (Figure 11B), MP significantly increased extracellular DA relative to baseline, with no further increase following administration of L-dopa ($F_{18,108} = 7.95; p < 0.001$). This MP-induced DA increase peaked approximately 40 minutes after MP injection, and gradually returned to baseline levels after 2 hours. Although MP pretreatment alone did not increase basal DOPAC or HVA levels, a delayed rise in HVA levels was observed after L-dopa injection (HVA: $F_{18,108} = 4.70, p = 0.004$, Dunnett’s test, $p < 0.01$). Further analysis showed a significant decrease in the first 60 minutes after L-dopa ($F_{6,36} = 7.26, p < 0.001$, Dunnett’s test, $p$
Figure 10. 

10A. Effect of MP pretreatment on L-dopa-induced peak values in the drug-naïve, lesioned striatum (14mg/kg, i.p. MP; 10mg/kg, i.p. L-dopa). These graphs represent a between-groups comparison of the L-dopa-induced peak response of DA, DOPAC, and HVA (mean ± SEM) without MP pretreatment (green bar), and with MP pretreatment (white bar). 

10B. Effect of MP pretreatment on L-dopa-induced behaviour (14mg/kg, i.p. MP; 10mg/kg, i.p. L-dopa). These graphs represent a between-groups comparison of the L-dopa-induced total AIM scores of each subtype (mean ± SEM). Top graph: Limb dyskinesia; Middle graph: Axial dystonia; Bottom graph: Locomotion. In each graph, the left bar represents L-dopa-induced AIM without MP pretreatment, and right bar represents L-dopa-induced AIM with MP pretreatment. 

Significance relative to L-dopa only control; * p < 0.05
Unlesioned Striatum (1-week L-dopa Treated Group)

**Figure 11.**
Effect of MP pretreatment on L-dopa-induced responses in the 1-week L-dopa treated, unlesioned striatum. *Panel A* illustrates the L-dopa-induced changes in DA, DOPAC, and HVA levels. *Panel B* illustrates L-dopa-induced changes in DA, DOPAC, and HVA levels 20 minutes after treatment with MP. *ILA*: Following four baseline samples, injection of L-dopa (10 mg/kg, i.p.) was given. Collection of dialysate samples continued for 160 minutes after L-dopa injection. Each diamond represents a dialysate sample collected over a 10-minute bin. *ILB*: Following four baseline samples, injection of MP (14mg/kg, i.p.) was given. Twenty minutes later, injection of L-dopa (10 mg/kg, i.p.) was administered. Collection of dialysate samples continued for 160 minutes after L-dopa injection. † denotes significance at p < 0.05 relative to the last pre-drug baseline; and ‡ denotes significance at p < 0.001 relative to the last pre-drug baseline.
A 2-way ANOVA test showed that DA levels in the MP pretreated group showed that L-dopa induced DA levels were significantly different from that of the L-dopa only control group (DA: $F_{16,192} = 10.86, p < 0.001$). In contrast, no effect of MP pretreatment was observed in L-dopa-induced DOPAC, or HVA response (DOPAC: $p = 0.482$; HVA: $p = 0.522$). In the lesioned striatum (Figure 12A), injection of L-dopa resulted in a significant rise in extracellular DA concentration ($F_{16,96} = 17.85, p < 0.001$, Dunnett’s test, $p < 0.01$), which was accompanied by a similar pattern of L-dopa-induced AIM. The DA increase was followed by a significant delayed increase in DOPAC ($F_{16,96} = 26.65, p = 0.001$, Dunnett’s test, $p < 0.01$), and HVA levels ($F_{16,96} = 42.23, p < 0.001$, Dunnett’s test, $p < 0.01$). MP pretreatment (Figure 12B), had no effect on basal DA, DOPAC nor HVA levels. L-dopa given 20 minutes following pretreatment of MP resulted in an increase of DA levels (DA: $F_{16,96} = 7.65, p = 0.013$), which was accompanied by a similar pattern of L-dopa-induced AIM. DA efflux was followed by a delayed rise in DOPAC and HVA levels (DOPAC, $F_{16,96} = 59.175, p < 0.001$; HVA: $F_{16,96} = 22.45, p < 0.001$, Dunnett’s test, $p < 0.01$).

A 2-way ANOVA (Figure 12) test showed no significant effect of MP pretreatment on L-dopa-induced DA nor HVA, but significant effect of MP pretreatment on L-dopa-induced DOPAC response (DA: $p = 0.397$; HVA: $p = 0.184$; for DOPAC : $F_{16,192} = 5.46, p = 0.005$, Dunnett’s test, $p < 0.01$). A T-test used to compare L-dopa-induced peak neurochemical responses of DA, DOPAC, and HVA showed insignificant effect of MP on DA or HVA (Figure 13A), but significantly decreased the peak L-dopa-induced response for DOPAC (DA: $p = 0.572$; HVA: $p = 0.416$; DOPAC: $181.99 \pm 25.11$ vs. $96.52 \pm 8.41$ nM; $T = 3.49; p = 0.004$).

In comparison to rats which only received L-dopa, a T-test showed that MP pretreatment showed significant reduction in the axial and limb components of L-dopa-induced dyskinesia,
Lesioned Striatum (1-week L-dopa Treated Group)

Figure 12.
Effect of MP pretreatment on L-dopa-induced responses in the 1-week L-dopa treated, lesioned striatum. Panel A illustrates the L-dopa-induced changes in DA, DOPAC, and HVA levels. Panel B illustrates L-dopa-induced changes in DA, DOPAC, and HVA levels 20 minutes after treatment with MP. 12A: Following four baseline samples, injection of L-dopa (10 mg/kg, i.p.) was given, after which dialysate sample collection was continued for 160 minutes. 12B: Following four baseline samples, injection of MP (14mg/kg, i.p.) was given. Twenty minutes later, injection of L-dopa (10 mg/kg, i.p.) was administered. Collection of dialysate samples continued for 160 minutes after L-dopa injection. Each diamond represents a dialysate sample collected, and is drawn over a corresponding measure of AIM (colored bars) observed in a 10-minute bin. The blue bars indicate locomotive turning, either ipsiversive or contraversive to the lesioned hemisphere. The orange bars indicate axial dystonia, and the white bars indicate limb dyskinesia. † denotes significance at p < 0.05 relative to the last pre-drug baseline; and ‡ denotes significance at p < 0.001 relative to the last pre-drug baseline.
Figure 13.

13A. Effect of MP pretreatment on L-dopa-induced peak values in the 1-week L-dopa treated, lesioned striatum (14mg/kg, i.p. MP; 10mg/kg, i.p. L-dopa). These graphs represent a between-groups comparison of the L-dopa-induced peak response of DA, DOPAC, and HVA (mean ± SEM) without MP pretreatment (green bar), and with MP pretreatment (white bar).

13B. Effect of MP pretreatment on L-dopa-induced behaviour (14mg/kg, i.p. MP; 10mg/kg, i.p. L-dopa). These graphs represent a between-groups comparison of the L-dopa-induced total AIM scores of each subtype (mean ± SEM). Top graph: Limb dyskinesia; Middle graph: Axial dystonia; Bottom graph: Locomotion. In each graph, the left bar represents L-dopa-induced AIM without MP pretreatment, and right bar represents L-dopa-induced AIM with MP pretreatment.

Significance relative to L-dopa only control; * p < 0.05; ** p < 0.01
but not in locomotor rotation (Figure 13B) (Loco: $p = 0.653$; Axial: $24.14 \pm 7.07$ vs. $5.43 \pm 3.85$, $p = 0.027$; Limb: $34.14 \pm 4.31$ vs. $18.86 \pm 3.24$, $p = 0.010$)

Effect of DAT blockade in 3-week L-dopa treated rats

In the unlesioned striatum (Figure 14A), a 1-way ANOVA revealed that L-dopa injection alone had no significant effect on extracellular DA levels ($p = 0.764$). Both DOPAC and HVA increased from basal levels (DOPAC: $F_{16,112} = 6.79$, $p = 0.007$; HVA: $F_{16,112} = 5.19$, $p = 0.037$, Dunnett’s test, $p < 0.01$). In the MP pretreated group (Figure 14B), MP significantly increased extracellular DA from baseline, with no further increase following administration of L-dopa ($F_{18,90} = 12.81$; $p = 0.001$, Dunnett’s test, $p < 0.01$). This MP-induced DA increase peaked approximately 40 minutes after MP injection, and gradually returned to baseline levels after 2 hours. Although MP pretreatment alone did not increase basal DOPAC or HVA levels, a significant delayed rise in HVA levels was observed about 80 minutes after L-dopa injection (for HVA: $F_{18,90} = 17.40$, $p < 0.001$, Dunnett’s test, $p < 0.01$). In contrast, L-dopa administration following MP pretreatment induced a significant decrease in DOPAC levels in the first 60 minutes after L-dopa ($F_{6,36} = 7.42$, $p = 0.001$, Dunnett’s test, $p < 0.01$). A 2-way ANOVA test showed that L-dopa induced DA levels with MP pretreatment were significantly different from that in L-dopa only control group (DA: $F_{16,176} = 5.18$, $p = 0.011$). In contrast, no effect of MP pretreatment were observed in L-dopa-induced DOPAC, or HVA response (DOPAC: $p = 0.164$; HVA: $p = 0.477$).

In the lesioned striatum (Figure 15A), injection of L-dopa caused in a significant rise in extracellular DA concentration ($F_{16,128} = 24.17$, $p < 0.001$, Dunnett’s test, $p < 0.01$), which was accompanied by a similar pattern of L-dopa-induced AIM. The DA efflux was followed by a delayed increase in DOPAC ($F_{16,128} = 33.71$, $p < 0.001$, Dunnett’s test, $p < 0.01$), and HVA
Figure 14.
Effect of MP pretreatment on L-dopa-induced responses in the 3-week L-dopa treated, unlesioned striatum. Panel A illustrates the L-dopa-induced changes in DA, DOPAC, and HVA levels. Panel B illustrates L-dopa-induced changes in DA, DOPAC, and HVA levels 20 minutes after treatment with MP. 14A: Following four baseline samples, injection of L-dopa (10 mg/kg, i.p.) was given. Collection of dialysate samples continued for 160 minutes after L-dopa injection. Each diamond represents a dialysate sample collected over a 10-minute bin. 14B: Following four baseline samples, injection of MP (14 mg/kg, i.p.) was given. Twenty minutes later, injection of L-dopa (10 mg/kg) was administered. Collection of dialysate samples continued for 160 minutes after L-dopa injection. † denotes significance at p < 0.05 relative to the last pre-drug baseline; and ‡ denotes significance at p < 0.001 relative to the last pre-drug baseline.
Lesioned Striatum (3-week L-dopa Treated Group)

Figure 15.
Effect of MP pretreatment on L-dopa induced responses in the 3-week L-dopa treated, lesioned striatum. Panel A illustrates the L-dopa-induced changes in DA, DOPAC, and HVA levels. Panel B illustrates L-dopa-induced changes in DA, DOPAC, and HVA levels 20 minutes after treatment with MP. 15A: Following four baseline samples, injection of L-dopa (10 mg/kg, i.p.) was given, after which dialysate sample collection was continued for 160 minutes. 15B: Following four baseline samples, injection of MP (14mg/kg, i.p.) was given. Twenty minutes later, injection of L-dopa (10 mg/kg, i.p.) was administered. Collection of dialysate samples continued for 160 minutes after L-dopa injection. Each diamond represents a dialysate sample collected, and is drawn over a corresponding measure of AIM (colored bars) observed in a 10-minute bin. The blue bars indicate locomotive turning, either ipsiversive or contraversive to the lesioned hemisphere. The orange bars indicate axial dystonia, and the white bars indicate limb dyskinesia. † denotes significance at p < 0.05 relative to the last pre-drug baseline; and ‡ denotes significance at p < 0.001 relative to the last pre-drug baseline.
levels ($F_{16,128} = 71.25, p < 0.001$, Dunnett’s test, $p < 0.01$). MP pretreatment (Figure 15B) had no effect on basal DA, DOPAC, and HVA levels. L-dopa given 20 minutes following pretreatment of MP resulted in an increase of DA levels (DA: $F_{16,80} = 9.42, p = 0.009$, Dunnett’s test, $p < 0.01$), accompanied by a similar pattern of L-dopa-induced AIM. DA efflux was followed by a delayed rise in DOPAC and HVA levels (DOPAC, $F_{16,80} = 24.70, p < 0.001$; HVA, Dunnett’s test, $p < 0.01$: $F_{16,80} = 13.47, p = 0.004$, Dunnett’s test, $p < 0.01$).

A 2-way ANOVA (Figure 15) test showed significant effect of MP pretreatment on L-dopa-induced DA, DOPAC and HVA responses (DA: $F_{16,208} = 3.52$, $p = 0.014$, DOPAC: $F_{16,208} = 3.964$, $p = 0.033$; HVA: $F_{16,208} = 19.04$, $p < 0.001$). A T-test used to compare L-dopa-induced peak neurochemical responses of DA, DOPAC, and HVA response showed significant effect of MP pretreatment (Figure 16A) (control vs. treatment; DA: $3.00 \pm 0.33$ vs. $1.66 \pm 0.31$ nM; $T = 3.009, p = 0.010$; DOPAC: $124.79 \pm 14.82$ vs. $64.58 \pm 10.12$ nM; $T = 3.196, p = 0.007$; HVA: $150.17 \pm 9.38$ vs. $48.80 \pm 5.29$ nM; $T = 8.002, p < 0.001$).

In comparison to rats which only received L-dopa, a T-test showed that MP pretreatment resulted in a significant reduction of all components of L-dopa-induced dyskinesia (Figure 16B) (control vs. treatment: Loco: $27.89 \pm 3.31$ vs. $13.67 \pm 3.67$, $p = 0.010$; Axial: $29.00 \pm 4.06$ vs. $0.17 \pm 0.18$, $p < 0.001$; Limb: $32.22 \pm 4.22$ vs. $8.67 \pm 3.83$, $p = 0.001$).

Histology

Bilateral probe tracts were verified in fixed 50μm coronal sections (Figure 17). 15 animals were randomly selected for presentation in this figure.

Discussion

The aim of this experiment was to assess the functional mode in the DAT associated with DA denervation, and with chronic L-dopa administration, in the severely lesioned striatum. The
3-week L-dopa Treated

Figure 16.

16A. Effect of MP pretreatment on L-dopa-induced peak values in the 3-week L-dopa treated, lesioned striatum (14mg/kg, i.p. MP; 10mg/kg, i.p. L-dopa). These graphs represent a between-groups comparison of the L-dopa-induced peak response of DA, DOPAC, and HVA (mean ± SEM) without MP pretreatment (green bar), and with MP pretreatment (white bar). 16B. Effect of MP pretreatment on L-dopa-induced behaviour (14mg/kg, i.p. MP; 10mg/kg, i.p. L-dopa). These graphs represent a between groups comparison of the L-dopa-induced total AIM scores of each subtype (mean ± SEM). Top graph: Limb dyskinesia; Middle graph: Axial dystonia; Bottom graph: Locomotion. In each graph, the left bar represents L-dopa-induced AIM without MP pretreatment, and right bar represents L-dopa-induced AIM with MP pretreatment.

Significance relative to L-dopa only control; ** p < 0.01; *** p < 0.001
Figure 17. Verification of probe placement. The black lines represent the 4mm of exposed membrane area of the probes used for microdialysis.
results of this experiment showed that in the unlesioned striatum, L-dopa alone failed to increase extracellular basal DA levels (Figures 8A, 11A, and 14A). In rats that received MP pretreatment, basal DA levels increased with MP administration, but did not with a subsequent L-dopa injection (Figures 8B, 11B, and 14B). This effect was seen in all three groups (L-dopa naïve, 1-week L-dopa treated, and 3-week L-dopa treated). In contrast, in the lesioned striatum, L-dopa administration resulted in a surge of extracellular DA levels (Figures 9A, 12A, and 15A). In rats that received MP pretreatment, MP alone failed to increase DA basal levels, or attenuate L-dopa induced DA efflux in the lesioned striatum of L-dopa-naïve, or 1-week L-dopa treated animals.

Functional Mode of the DAT in the Striatum: role of DA terminal loss

As previous data have suggested that DAT function may be reversed in the severely DA denervated striatum (Miller and Abercrombie, 1999; Ahn et al., 2004), we examine the role of DA terminal loss by comparing the effects of MP pretreatment on L-dopa induced DA efflux in the unlesioned and lesioned striatum of L-dopa-naïve rats.

DAT uptake in the unlesioned striatum

The absence of DA efflux in the unlesioned striatum following exogenous L-dopa administration may reflect the efficiency of the DAT system by clearing excess DA by reuptake. Consistent with this view, blockade of the DAT with systemic MP caused a significant increase in DA and HVA levels, but a decrease in DOPAC levels (Figure 8). As DA is catabolized into HVA within the extracellular space, the increase in HVA levels is dependent on increased extracellular DA levels. On the other hand, intracellular conversion of DA to DOPAC requires DAT-dependent uptake of DA back into the cytosol, and therefore, following MP pretreatment, further DOPAC synthesis is inhibited and a decrease was observed following L-dopa injection.
Therefore, these observed changes in DA, DOPAC, and HVA indicate that the DAT functions in the uptake mode in the unlesioned striatum.

**DAT function in the lesioned striatum**

In the lesioned striatum, MP administration alone failed to increase basal levels of DA, DOPAC, or HVA. Furthermore, MP pretreatment did not significantly attenuate L-dopa-induced DA efflux (Figures 9, 10A). These data are inconsistent with previous reports (Miller and Abercrombie, 1999; Ahn et al., 2004) which demonstrated, in L-dopa-naïve rats, that (i), the L-dopa-induced DA efflux was partly mediated by a depolarization-independent mechanism (Miller and Abercrombie, 1999); and (ii) continuous reverse-dialysis of GBR 12909 in the denervated striatum attenuated the L-dopa induced DA efflux (Ahn et al., 2004). One explanation for the lack of effect of MP on basal DA levels and L-dopa-induced DA efflux in L-dopa-naïve rats may be due to the dose and method of administration of MP used in the present study. Reverse-dialysis of GBR 12909 may allow for a more continuous and concentrated delivery of the drug into a small amount of tissue, whereas systemic injection of the drug may be subject to peripheral metabolism. Therefore, a higher dose of MP may be required to detect changes in very low basal levels. Similarly, systemic injection of GBR 12909 also failed to affect the L-dopa-induced DA efflux in the DA denervated striatum (Miller and Abercrombie, 1999). Therefore, the role of DA denervation on DAT function is inconclusive in the present results. Together, these data suggest that in L-dopa-naïve animals, at least two mechanisms maybe in involved in L-dopa-induced DA release: first, a predominant impulse-dependent mechanism, possibly through conversion of exogenous L-dopa and release of DA by other AADC-containing neuronal cells, such as the striatal GABAergic interneurons (Melamed et al., 1980; Hefti et al., 1981) and serotonergic afferents (Arai et al., 1994; Mura et al., 1995; Tanaka et
al., 1999); second, a partial impulse-independent mechanism, involving reverse transport of DA via the DAT (Miller and Abercrombie, 1999. Ahn et al., 2004).

**Functional Changes in the DAT Following Chronic L-dopa Treatment**

To investigate how chronic L-dopa treatment changes the mechanism underlying L-dopa-induced DA release in the striatum, the effect MP pretreatment was examined in 3-week L-dopa treated rats. In these rats, systemic MP substantially reduced L-dopa-induced DA, DOPAC and HVA efflux in the lesioned striatum (Figure 15), suggesting that, in contrast to L-dopa naïve, denervated striatum, these responses are predominantly DAT-dependent. Other results from our lab also support this, demonstrating that intrastriatal reverse dialysis of GBR-12909 almost abolishes completely the L-dopa-induced DA, DOPAC, and HVA responses in the lesioned striatum of 3-week L-dopa treated rats (Ahn et al., 2004). Therefore, these data suggest that 3 weeks of chronic L-dopa treatment is sufficient to alter the mode of L-dopa-induced DA release from one that is predominately impulse-dependent, to one that is primarily DAT-dependent.

Although it is unknown when these changes occur during the 3-week treatment period, the present data show that with one week of L-dopa treatment, changes in the functional mode of the DAT have begun to occur. Although the effect of MP on L-dopa-induced DA efflux was not significant in L-dopa naïve or 1-week treated rats, there was a consistent trend for MP-induced attenuation in each of the 1-week treated rats (Figures 12, 13A). These observations may reflect a progressive change in the ratio of DATs in uptake vs. release mode, suggesting that while 1-week treated animals may have a higher proportion of DATs functioning in the release mode than L-dopa naïve animals, longer treatment duration (at least 3 weeks) is required for significant attenuation of DA release by MP blockade of the DAT.

**The Role of Striatal DA in L-dopa-induced Dyskinesia**
One of the principal goals of the present study was to examine the relationship between L-dopa-induced DA levels in the lesioned striatum and the expression of L-dopa-induced dyskinesia. While the L-dopa-induced DA increase was not significantly different between L-dopa naïve, 1-week, and 3-week treated rats, AIM scores increased progressively with longer duration of treatment (Figures 9A, 12A, 15A). These observations suggest that a threshold for triggering dyskinesia was reduced, most likely attributable to sensitization of DA receptors with repetitive exposure to L-dopa. These data are in line with the current view that L-dopa-induced dyskinesia is mediated through downstream gene changes (Calon et al., 2000; Olanow et al., 2000).

It is still unclear exactly how chronic L-dopa treatment alters the relationship between striatal DA levels and expression of L-dopa-induced dyskinesia (kinetic-dynamic association). It could be that DA acts only as a trigger for dyskinesia, or DA may also mediate the magnitude of behaviour. Our data suggest that DA’s role in mediating behaviour may be altered by L-dopa treatment. In animals which were treated for 1 or 3 weeks, changes in DA levels were well correlated with changes in AIM. Consistently, pretreatment with MP in L-dopa treated animals (1- or 3-weeks treated) resulted in a blunted DA response, which was correlated with similar attenuation in AIM scores.

In contrast, this correlation between DA and AIM was not the matched in L-dopa naïve rats. While the rise of DA levels correlated with the appearance of AIM, peak DA levels were earlier compared to the peak of the AIM response. The peak DA response was almost 100 minutes earlier than that of the AIM response. Furthermore, pretreatment with MP did not change the DA response, but selectively attenuated the axial component of AIM. These data suggest that in L-dopa-naïve animals, DA may act simply as a trigger for AIM, which will
express as long as a certain threshold of DA level is reached. However, other non-dopaminergic mechanisms may be involved in determining the intensity of AIM (Cenci et al., 1998; Brotchie et al., 2005). Repeated L-dopa exposure, on the other hand, elicits downstream changes such as increased DA receptor sensitivity and long lasting alterations in gene expression (Olanow et al., 2000), which could change the relationship between DA and AIM. More precisely, in treated animals, DA levels influence the intensity and duration of L-dopa-induced behaviour.
GENERAL DISCUSSION

The aim of the experiments presented in this thesis was to assess functional changes in the DAT associated with chronic L-dopa administration in the severely lesioned striatum, and how these changes contribute to L-dopa-induced striatal efflux of DA and dyskinetic behaviour. These findings support our hypothesis that DAT reversal is a contributing factor in this L-dopa derived DA efflux. While systemic administration of MP failed to attenuate L-dopa-induced DA efflux in the DA-denervated striatum of L-dopa naïve rats, it significantly reduced both L-dopa-induced DA efflux and the corresponding AIM scores in rats treated for 3 weeks. These findings emphasize the importance of chronic L-dopa treatment, as well as DA denervation, as contributing factors for DAT reversal.

DAT Reversal as a Compensatory Mechanism

Extracellular DA levels, and hence motor behaviour, remain normal with up to 80% of DA terminal loss (Abercrombie et al., 1990; Benazzouz et al., 1992; Bezard et al., 1997), indicating that DA denervation causes a sequence of compensatory mechanisms which serve to maintain normal extracellular DA levels. In the initial stages of progressive DA denervation in PD, normal DA levels are maintained by increased DA synthesis by increased TH expression (Pasinetti et al., 1992; Blanchard et al., 1995), increased DA release (Agid et al., 1973; Zigmond et al., 1984), and reduced DA uptake (Bezard et al., 2000; Lee et al., 2000). However, in the severely lesioned striatum, these compensatory mechanism are no longer sufficient in upholding normal levels of DA stimulation. As previously suggested, DA denervation alone may induce reversal of transport in a proportion of the remaining DATs. Therefore, DAT reversal may be an additional compensatory mechanism which appears only with severe DA denervation. As the present data indicate, chronic treatment with L-dopa exaggerates this phenomenon, possibly by
increasing the ratio of striatal DAT in the reverse mode, or by enhancing the reversibility of the DAT (i.e. transient reversal vs. sustained reversal). However, the mechanisms by which L-dopa induces flux reversal of the DAT remain unknown.

**Mechanisms for DAT Reversal**

The concept of reverse transport was first proposed following by the discovery of pharmacological agents, such as amphetamine, that were capable of releasing monoamines in an impulse, Ca\(^{2+}\) independent fashion. During the last two decades, a great deal of research has identified some of the key mechanisms by which these monoamine releasing agents cause the reversal of monoaminergic transporters. Lessons taken from these studies have been useful in determining the causes of reverse transport in pathophysiological conditions.

1) **DAT reversal is facilitated by L-dopa-induced DA synthesis and accumulation of the cytosolic DA pool:** DA is synthesized in the terminal, and is stored in two separate releasable compartments: a primary cytosolic pool, and then vesicularized into a secondary pool. DATs are located preferentially on extrasynaptic sites, therefore it is most likely that DA released by reverse transport originates from the cytosolic pool of the releasable compartment of DA terminals (Leviel, 2001). Thus, increasing the cytosolic DA levels may be an important step in DAT reversal. Support for this suggestion comes from the observation that sustained DA efflux by amphetamine is contingent on amphetamine-induced DA synthesis. However, increasing cytosolic DA levels alone by treatment with reserpine does not induce reverse transport (Jones et al., 1998). These data suggest that while increased cytosolic DA may not be an essential factor in DAT reversal, it may be a facilitative step (Leviel, 2001). Therefore, increasing the concentration of cytosolic DA may be one mechanism through which L-dopa may promote DAT reversal in the lesioned striatum.
2) The directional transport of the DAT is driven by the electrochemical gradient of Na\(^+\):

*the role of glutamate*: DA is co-transported with Na\(^+\), which moves along its concentration gradient, generated by plasma membrane Na\(^+\)-K ATPases. Thus, the relative intracellular/extracellular concentration of Na\(^+\) is a critical determinant of the direction of DA flux. Inversion of the Na\(^+\) gradient by decreasing extracellular Na\(^+\) levels during microdialysis significantly increases DA efflux in the striatum. This increase is attenuated by pretreatment with the DAT blocker nomifensine (Hurd and Ungerstedt, 1989). These data suggest that molecular agents which increase intracellular Na\(^+\) may facilitate the reversed transport of the DAT. Particularly relevant to DAT mediated efflux in the striatum are the corticostriatal glutamatergic afferents, activation of which leads to increased intracellular Na\(^+\) in DA terminals.

Recent experiments have demonstrated that reverse dialysis of NMDA increased DA efflux in the striatum (Andres et al., 1998). Furthermore, under more physiological conditions, both nigral DA neurons and DA terminals in the striatum display DAT mediated DA release, both of which are regulated by glutamate (Lonart and Zigmond, 1991; Falkenburger et al., 2001). Enhanced corticostriatal glutamatergic activity has been long associated with PD (Leviel, 2001). Furthermore, chronic L-dopa treatment in rats has been shown to elevate extracellular glutamate levels (Robelet et al., 2004). It seems very likely, then, that the L-dopa-induced glutamate increase is a mechanism through which chronic L-dopa treatment facilitates DAT reversal.

**DAT Reversal as a Pathogenic Mechanism of L-dopa-induced Dyskinesia**

As mentioned previously, low doses of L-dopa provide therapeutic effects for the motor deficits in PD patients in the first few years of treatment. With extended treatment, neural changes occur that alter the response to L-dopa, including the emergence of L-dopa-induced dyskinesia. While pathogenic mechanisms of dyskinesia are not yet fully understood, many
post-synaptic factors associated with L-dopa-induced dyskinesia have been identified, including alterations in the expression of peptides (PPE, PDyn) and receptors (D3, glutamate). These changes are most likely due to abnormal activation of receptors in the post-synaptic neurons, due to the cycling pattern of high stimulation (when L-dopa is given) and low stimulation (periods without L-dopa), attributable to pulsatile administration of exogenous L-dopa. Therefore, determination of mediating L-dopa-induced DA efflux is critical for understanding the initial events triggering L-dopa-induced dyskinesia. The findings from the present study support a novel hypothesis involving the reversal of the DAT as a mechanism for DA release in the severely denervated striatum, following chronic L-dopa administration. Therefore, DAT reversal may have important implications in the pathogenesis of L-dopa-induced dyskinesia.

In the initial stages of treatment, stable response to L-dopa administration may be explained by the regulated release of DA due to vesicular storage in the remaining DA terminals (Figure 18). Over the course of the disease, the storage capacity decreases due to loss of DA terminals (Chase and Oh, 2000); therefore, non-dopaminergic AADC-containing cells become more involved in the conversion and vesicular release of exogenous L-dopa to DA in the severely DA denervated striatum (Miller and Abercrombie, 1999). However, assuming that non-dopaminergic cells participate in vesicular, and therefore, regulated, release of L-dopa derived DA, this theory does not fully explain why over time, a stable L-dopa response changes to a fluctuating pattern, with chronic L-dopa treatment. Our results, which indicate that DAT reversal is treatment dependent, may offer new insights into how L-dopa-induced DA response becomes unstable over long term administration. As suggested in Figure 18, chronic treatment results in a DAT reversal, which, combined with reduced storage capacity, leads to an
Figure 18. DAT reversal as a pathogenic mechanism of L-dopa-induced dyskinesia
Chronic treatment with L-dopa changes the functional mode of DAT from uptake to release. This mechanism underlies the change from "steady" to "surged" L-dopa induced DA release. This DA surge forms an abnormal and pathogenic pattern of DA receptor stimulation which leads to downstream gene alternations associated with response fluctuations and dyskinesia.
Figure 18
“surged” DA response. Repeated exposure to this short lasting surge of DA stimulation could lead to altered gene expression and ultimately, dyskinesia.

Clinical Studies

The effect of MP pretreatment on dyskinesia has also been studied in PD patients. Preliminary clinical trials by Camicioli and colleagues (2001) demonstrated, in contrast to our data, that MP increased L-dopa-induced dyskinesia in three PD patients (Camicioli et al., 2001). In these patients, MP was administered orally, followed 30 minutes later by an infusion of L-dopa. One possible explanation for these findings could be differences in the degree of DA denervation, as well as the treatment history, in these patients and the animals used in our studies. The patients were all described as “functionally independent” which, in clinical terms, refers to patients who have mild to moderate Parkinsonism. As our data indicate, severe DA denervation and treatment history are both critical factors that can result in differential effects of MP on L-dopa-induced dyskinesia. While our animals received only L-dopa treatment, patients may have received a combination of drugs that could alter DAT regulation/function, and consequently, the effects of MP.

FUTURE DIRECTIONS AND CONCLUSION

In light of the finding of a role for DAT reversal in dyskinesia, it will be of interest to examine further the factors associated with DA denervation and chronic, pulsatile L-dopa treatment that facilitate the reversal of DAT. As mentioned before, one of the possible mechanisms through which the DAT becomes reversed may be elevated glutamate levels in the striatum. In this view, co-administration of anti-glutamatergic and DAT-blocking drugs may offer new perspective regarding the interactions of the glutamate and the DAT in the DA-denervated striatum.
In conclusion, the findings from the experiments in this thesis provide support for our hypothesis that reversal of the DAT may be a pathogenic mechanism for L-dopa-induced dyskinesia. As mentioned before, once dyskinesia appears, it is difficult to distinguish (Brotchie et al., 2005), hence methods of treatment which prevent the emergence of dyskinesia is highly important. Therefore, as our data propose DAT reversal as a novel, presynaptic mechanism for L-dopa induced DA efflux and hence, dyskinesia, they highlight the importance of the DAT as a target for the prevention. Therefore, to conclude, further studies examining the underlying mechanisms of L-dopa-induced DAT reversal will be offer invaluable insights into the treatment, or possibly, the prevention, of L-dopa-induced dyskinesia.
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