

**FROM GENE TO BRAIN AND BEHAVIOR: DOPAMINERGIC EFFECTS  
ON MOTOR SEQUENCE LEARNING IN PARKINSON'S DISEASE**

by

Youngbin Kwak

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Doctoral Committee:

Associate Professor Rachael D. Seidler, Chair  
Professor Thad A. Polk  
Associate Professor J. Wayne Aldridge  
Associate Professor Nicolaas I. Bohnen  
Research Investigator Martijn L.T.M. Müller

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## LIST OF ABBREVIATIONS

- ADHD:** Attention deficit hyperactive disorder
- ANCOVA:** Analysis of covariance
- ANOVA:** Analysis of variance
- BA:** Brodmann's area
- BOLD:** Blood oxygen level dependent
- BP:** Binding potential
- BPND:** Non-displaceable binding potential
- COMT:** Catechol-O-methyl transferase
- DA:** Dopamine
- DAT1:** Dopamine transporter
- DNA:** Deoxyribonucleic acid
- DRD2:** Dopamine D2 receptor
- <sup>11</sup>C-DTBZ:** <sup>11</sup>C-dihydrotetrabenazine
- fMRI:** Functional magnetic resonance imaging
- FOV:** Field of view
- GRE:** Gradient-echo
- H & Y:** Hoehn and Yahr
- ITI:** Inter-trial interval
- LA:** Less affected
- L-DOPA:** Levodopa
- LM\_error:** Learning magnitude calculated by error rate
- LM\_RT:** Learning magnitude calculated by response time
- MA:** More affected

**MDRS:** Mattis Dementia Rating Scale  
**MMSE:** Mini-Mental State Exam  
**MNI:** Montreal Neurological Institute  
**MOCA:** Montreal Cognitive Assessment  
**NC:** Normal Control  
**PCR:** Polymerase chain reaction  
**PD:** Parkinson's Disease  
**PD OFF:** PD patients OFF medication  
**PD ON:** PD patients ON medication  
**PET:** Positron Emission tomography  
**PFC:** Prefrontal cortex  
**R:** Random block  
**ROI:** Region of interest  
**RSI:** Response to stimulus interval  
**S:** Sequence block  
**SNP:** Single-nucleotide polymorphism  
**SPGR:** Spoiled gradient-recalled  
**S-R:** Stimulus-response  
**TE:** Echo time  
**TR:** Repetition time  
**UPDRS:** Unified Parkinson's Disease Rating Scale  
**VMAT2:** Presynaptic monoamine vesicular transporter  
**VOI:** Volume of interest

## **ABSTRACT**

Parkinson's disease (PD) is a neurodegenerative disorder affecting the dopamine neurotransmitter system which is crucial for motor control and cognitive function. Dopaminergic medications alleviate Parkinsonian motor symptoms however evidence shows that they also interfere with normal functioning in other domains. The current dissertation aimed to determine the effect of dopaminergic medication on motor sequence learning which underlies daily motor adaptability. In order to gain a thorough understanding of the nature of medication effects on sequence learning behavior, I performed four separate studies using behavioral, pharmacological, functional brain imaging and genetics approaches.

The first study determined the behavioral effects of medication over the time course of motor sequence learning. I report a selective medication-associated learning impairment in the early acquisition phase of sequence learning. The second study addressed the neural underpinnings of L-DOPA medication effects during motor sequence learning. The results showed an L-DOPA associated decrease in ventral putamen activation which correlated with the degree of L-DOPA associated motor sequence learning impairment at the early phase. The last two studies aimed to identify how factors contributing to the endogenous level of dopamine transmission interact with L-DOPA associated sequence learning changes. In the third study, the level of nigrostriatal dopaminergic denervation (assessed with  $^{11}\text{C}$ -DTBZ PET) was used as an

index of endogenous dopamine availability whereas in the fourth study, COMT and DRD2 polymorphisms were studied as genetic factors contributing to dopamine availability and D2 receptor expression. The results of the last two studies showed that the deleterious effect of L-DOPA on sequence learning is more significant in individuals with higher endogenous dopamine transmission.

Collectively, the results of the current dissertation have significant implications for understanding and treating the pathophysiology of PD suggesting careful use of dopaminergic medication taking into account factors such as one's disease progression and genotype.

## **CHAPTER I**

### **General Introduction**

#### **Motivation**

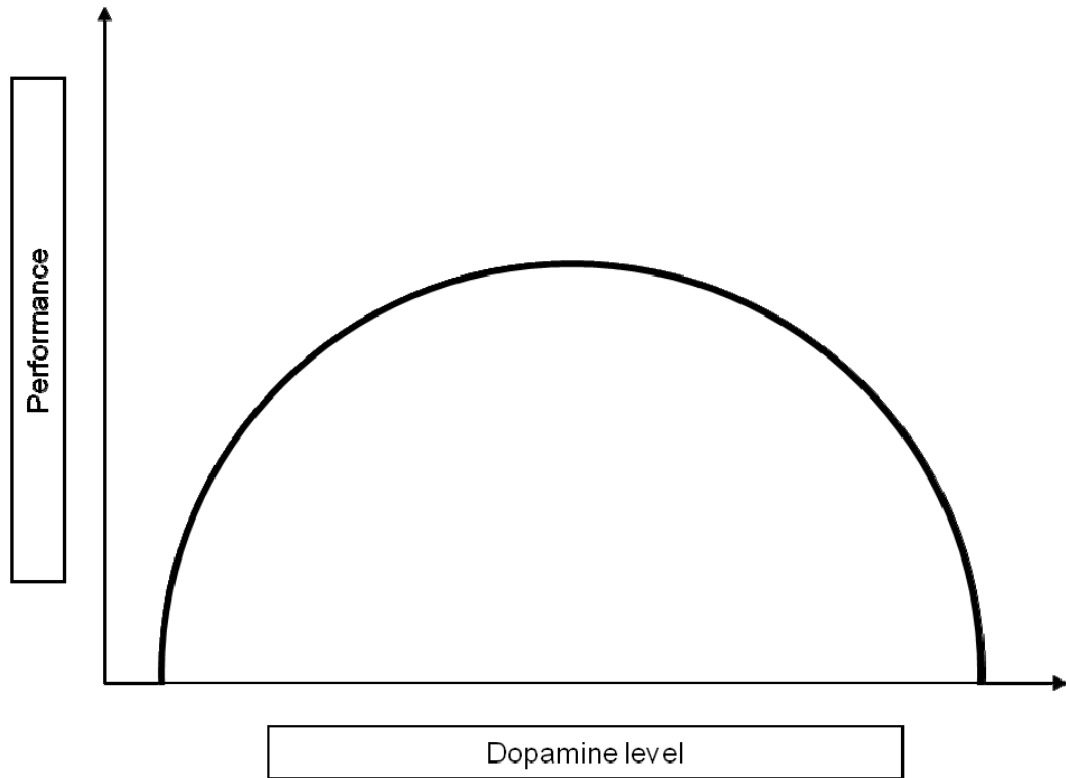
Parkinson's disease (PD) is one of the major movement disorders, affecting over 1 million people in the United States. The cost per patient per year in the US is around \$10,000 adding up to the total burden of approximately 23 billion dollars (<http://www.parkinson.org>). It is a neurodegenerative disorder affecting the dopamine neurotransmitter system, which is crucial for motor control. The prominent motor disability in PD is shown by Muhammad Ali when he tried to light the Olympic torch with his shaky and rigid hands. Dopaminergic medications alleviate these typical motor disabilities, however due to the wide range of involvement that dopamine has in human behavior these medications can also interfere with normal functioning of other domains. In my dissertation, I aim to investigate the effect that dopamine has on motor sequence learning, a form of skill learning, in PD patients. This chapter will address the background and rationale for the current investigations.



## Dopamine

The neurotransmitter dopamine (DA) is involved in a broad array of human behaviors including motor control, learning, working memory and executive function, motivation and reward processing (Arnsten and Robbins, 2002, Schultz, 2007, Frank and Fossella, 2011). DA acts across multiple basal ganglia circuitries supporting these functional behaviors through an intricate interplay of facilitative and inhibitory regulation via both tonic and phasic neural activity (Alexander and Crutcher, 1990, Schultz, 2007, Hauber, 2010).

Both human and animal studies investigating the role of DA transmission during cognitive tasks such as working memory have shown that either too little or too much DA transmission can be detrimental to performance (Arnsten and Robbins, 2002). That is, depletion of DA or blocking of DA receptors impairs performance, as does administration of high doses of DA agonists (Brozoski et al., 1979, Sawaguchi and Goldman-Rakic, 1991, Zahrt et al., 1997, Arnsten, 1998). This relationship between the level of DA and performance has been described as following the ‘inverted-U’ curve (Arnsten and Robbins, 2002) (Fig 1.1.). The ‘inverted-U’ relationship between DA level and behavior has important implications for understanding the behaviors of PD patients who are actively taking dopaminergic medications. Although dopaminergic medications are effectively treating the disease’s motor symptoms, there are complications with other behaviors such as compulsive gambling associated with medication (Frank et al., 2007, Wiecki and Frank, 2010, Maia and Frank, 2011).



**Figure 1.1.** Inverted-U relationship between DA level and performance. The relationship shows that too little and too much DA are both deleterious to performance.

### **Parkinson's disease**

It is crucial to understand the neuropathology of PD when considering the action of dopaminergic medications in PD. PD is a neurodegenerative disorder of the DA neurotransmitter system. The disease shows a conglomerate of symptoms including motor disability such as bradykinesia, tremor, gait and balance problems as well as cognitive and affective symptoms due to the wide involvement of DA in human behavior. The Braak staging system describes PD as a schema of ascending pathology, beginning in the lower brainstem and anterior olfactory structures, progressing to the basal mid- and forebrain nuclei, and then to the cortex (Braak et al., 2003, Braak et al., 2006).

At the stage of the disease when motor symptoms first begin to appear (i.e. stage 3 of the Braak system), the neuropathology of PD is demonstrated by the loss of dopaminergic neurons in the substantia nigra pars compacta and the ventral tegmental area with degeneration of the striatal nerve terminals (Braak et al., 2006). Dopaminergic denervation is not distributed evenly in the striatum in PD. The denervation begins asymmetrically and then becomes bilateral later in the disease (Hornykiewicz, 1966). Additionally, there is also a dorsal to ventral and posterior to anterior gradient in DA depletion. Specifically, depletion is greater in the dorsal and posterior striatum as opposed to the ventral and anterior striatum early in the disease, with subsequent involvement of the anteroventral striatum with advancing disease progression (Bernheimer et al., 1973, Kish et al., 1988, Rakshi et al., 1999, Frey et al., 2001).

Anti-Parkinsonian dopaminergic medications such as L-DOPA and DA agonists ameliorate motor deficits of PD by compensating for the loss of DA in the dorsal and posterior striatum. However, these medications can have conflicting effects on cognitive performance particularly in the early disease stages (Shohamy et al., 2005, Shohamy et al., 2006, Shohamy et al., 2008). While in some cases medication benefits performance, there are many studies showing deleterious effects of medication. One proposed explanation for these conflicting results is the so-called “DA over-dose hypothesis” (Cools et al., 2001, Frank et al., 2004, Frank, 2005, Cools, 2006, Cools et al., 2006). This hypothesis proposes that in early PD, while dopaminergic medications can improve cognitive performance on tasks associated with the depleted dorsal and posterior striatum such as task switching (Sohn et al., 2000, Brass et al., 2003), they can interfere with the functioning of the ventral and anterior striatum for behaviors such as reversal learning

(Cools et al., 2002) by overdosing this region which is relatively intact in the early stage of the disease (Cools et al., 2002). These findings are consistent with the ‘inverted-U’ relationship between DA and cognitive performance, which demonstrates that both insufficient and excess levels of DA impair normal cognitive functions (Williams and Goldman-Rakic, 1995, Zahrt et al., 1997, Arnsten, 1998).

To date, there is an abundance of evidence showing DA overdose effects (for review see Cools, 2006, Wiecki and Frank, 2010) on cognitive behaviors, particularly those linked to the nucleus accumbens. However, studies have not investigated whether motor behaviors relying on the ventral and anterior putamen are susceptible to DA overdose effects despite the fact that the primary deficits in PD are motor. One potential motor behavior that could be affected by DA overdose in PD is motor sequence learning. This is an important issue given the relevance of action sequences to everyday behaviors.

### **Motor sequence learning**

This section will review the current literature on motor sequence learning and address the necessity of studying this behavior in relation to DA overdose effects in PD. Motor sequence learning underlies many everyday activities such as typing, driving, or playing a musical instrument. It also constitutes a critical component of physical rehabilitation interventions for motor disabilities such as PD. Throughout the time course of motor sequence learning or skill learning in general, one goes through different phases which involve different cognitive and motor resources (Hikosaka et al., 1998, Doyon & Benali, 2005, Seidler et al., in press). During the early acquisition phase, one engages in

diverse cognitive processes including working memory and reward and error processing (Seidler et al., in press). Towards the later phase of learning, involvement of cognitive resources decreases and one can perform the acquired sequence or skill in an automatic motoric fashion. Moreover in the healthy brain, the neural substrates of motor sequence learning exhibit a functional reorganization of cortical and subcortical contributions across the time course of learning (Hikosaka et al., 1998, Toni et al., 1998, Doyon et al., 2002, Muller et al., 2002, Seidler et al., 2002, Doyon et al., 2003, van Mier et al., 2004, Floyer-Lea and Matthews, 2005, Lacourse et al., 2005, Lehericy et al., 2005, Poldrack et al., 2005, Bapi et al., 2006, Duff et al., 2007, Sun et al., 2007, Tamas Kincses et al., 2008, Seidler et al., in press). Such activation shifts are well-manifested in the basal ganglia-thalamocortical circuitry (for review see Doyon and Benali, 2005). Specifically, in the early phase of learning, when subjects acquire sequences of action in a controlled manner by engaging in cognitive processes, the ventral/anterior striatal pathway (i.e. associative striatum) including the anterior cingulate and dorsolateral prefrontal cortex is involved (Berns et al., 1997, Doyon et al., 2003, Lehericy et al., 2005, Bapi et al., 2006, Duff et al., 2007, Tamas Kincses et al., 2008). In the later phase, when subjects perform acquired sequences in an automatic manner, the dorsal/posterior striatal pathway (i.e. sensorimotor striatum) including the primary and secondary motor cortical areas is involved (Hikosaka et al., 2002, Doyon et al., 2003, Lehericy et al., 2005, Bapi et al., 2006, Duff et al., 2007, Tamas Kincses et al., 2008).

Motor sequence learning has also been widely studied in PD patients (Pascual-Leone et al., 1993, Roy et al., 1993, Doyon et al., 1997, Helmuth et al., 2000, Nakamura et al., 2001, Smith et al., 2001, Feigin et al., 2003, Ghilardi et al., 2003, Carbon et al.,

2004, Ghilardi et al., 2007, Muslimovic et al., 2007, Seidler et al., 2007, Doyon, 2008, Price and Shin, 2008). The results of these studies are not consistent however. Some studies show preserved sequence learning whereas others find impaired learning in PD patients. Moreover only a few studies have evaluated sequence learning with patients in both the ON and OFF medication states, and none have approached the problem working from the framework of the DA overdose hypothesis. It is important to delineate medication effects on motor sequence learning considering the significance of this behavior to daily motor activity and the necessity for physical rehabilitation in PD patients. In particular, based on the selective engagement of the ventral/anterior and posterior/dorsal striatal circuitry during motor sequence learning in a time varying fashion, the medication effects should be examined taking into account these distinctive neural circuitries with careful consideration of the time course of learning.

### **The current investigation**

The overarching goal of the current dissertation is to determine dopaminergic medication effects over the time course of motor sequence learning in PD patients. I hypothesize that there will be a selective medication associated learning impairment during the early learning phase when the relatively less denervated ventral/anterior striatum is involved. To gain a thorough understanding of the nature of the medication effect on sequence learning behavior, I also determined the underlying neural mechanisms of medication effects using fMRI. Further, I used an individual differences approach to determine the roles of dopaminergic denervation (assessed with  $^{11}\text{C}$ -DTBZ

PET) and endogenous DA transmission (inferred via COMT and DRD2 genotype, which results in variations in dopamine availability and D2 receptor expression in the striatum) on medication associated effects on sequence learning in PD patients. The dissertation is comprised of four studies addressing the following questions:

***Question 1 (Chapter 2). How does dopaminergic medication affect motor sequence learning at different time points during learning in PD patients?***

I hypothesized that there would be a selective medication associated impairment of motor sequence learning in the early learning phase. I evaluated this hypothesis by having 14 mild to moderate stage PD patients perform a motor sequence learning task both ON and OFF their respective dopaminergic medications. Eleven healthy age matched control participants were also recruited for one study session. Behavioral measures of learning were compared across different time points during the task between groups.

***Question 2 (Chapter 3). How does L-DOPA change neural recruitment during motor sequence learning, and how does this relate to behavioral changes associated with L-DOPA?***

I hypothesized that there would be medication associated neural activation decreases in the ventral / anterior striatum during the early learning phase which will explain behavioral changes. I tested this hypothesis by having a new cohort of 17 mild to moderate stage PD patients perform motor sequence learning inside the MRI scanner both ON and OFF a controlled dose of L-DOPA. Twenty-one

healthy age matched controls were recruited for one study session. Neural recruitment during sequence learning was compared between PD ON and PD OFF within the brain regions that were typically recruited in controls at different time points of sequence learning. I also determined whether the degree of change in neural recruitment due to L-DOPA was correlated with the medication associated behavioral changes.

***Question 3 (Chapter 4). Do individual differences in the level of striatal dopaminergic denervation predict L-DOPA associated changes in sequence learning?***

I hypothesized that the medication associated early learning impairment would be more severe in patients with less dopaminergic denervation. I evaluated this hypothesis by having a new cohort (separate from those reported in Chapters 2 and 3) of 17 mild to moderate stage PD patients perform motor sequence learning both ON and OFF a controlled dose of L-DOPA. The patients also had a <sup>11</sup>C-DTBZ PET scan taken to measure their level of striatal dopaminergic denervation. I determined whether the L-DOPA associated behavioral change is explained by one's current level of striatal dopaminergic denervation shown by <sup>11</sup>C-DTBZ binding potential.

***Question 4 (Chapter 5). How do the COMT and DA D2 receptor polymorphisms play a role in the medication associated changes in sequence learning?***

I hypothesized that the medication associated early learning impairment would be more apparent in genotype groups with higher endogenous DA



transmission. I tested this by genotyping individuals that participated in the studies reported in Chapters 3 and 4 for their COMT and D2 receptor genotype. I compared the degree of L-DOPA associated performance change across genotype groups to determine whether individual differences in endogenous DA transmission predict how patients' sequence learning performance changes with L-DOPA administration.

Collectively, I have used a multifaceted approach combining behavior, pharmacology, fMRI, <sup>11</sup>C-DTBZ PET, and genetics techniques to address my research questions. I obtained comprehensive evidence regarding the effects of dopaminergic medications on motor sequence learning in PD patients. These results will enhance our understanding of the nature of dopaminergic effects on brain function and behavior in PD patients. My findings lay the groundwork for future studies refining motor rehabilitation protocols for PD patients. Moreover, my results suggest the importance of prescribing dopaminergic medications taking into account factors such as individual differences in dopaminergic denervation and genotype. Furthermore these investigations will lead to a deeper understanding of the DA neurotransmitter system and its mediation of behavior.

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## CHAPTER II

### **Effect of dopaminergic medications on the time course of explicit motor sequence learning in Parkinson's disease**

#### **Abstract**

The capacity to learn new motor sequences is fundamental to adaptive motor behavior. The early phase of motor sequence learning relies on the ventral and anterior striatal circuitry whereas the late phase relies on the dorsal and posterior striatal circuitry (Lehericy et al., 2005). Early Parkinson's disease (PD) is mainly characterized by dopaminergic denervation of the dorsal and posterior striatum while sparing anterior and ventral regions. Dopaminergic medication improves dorsal and posterior striatum function by compensating for the loss of dopamine. However, previous work has shown that dopaminergic medication interferes with ventral and anterior striatum function, by overdosing this relatively intact structure in early state PD (Cools, 2006). Here I test whether these effects are also observed over the time course of motor sequence learning. Fourteen PD patients ON and OFF dopaminergic medications and eleven healthy age matched control participants performed an explicit motor sequence learning task. When sequence learning was compared across different learning phases in patients ON and OFF

medication, a significant impairment associated with medication was observed in the early relative to later phases of learning. The rate of learning in the early phase measured trial by trial in patients ON was significantly slower than controls and when patients were OFF. No significant impairment was found in the later learning phases. These results demonstrate that dopaminergic medications may selectively impair early phase motor sequence learning. These results extend and generalize the dopamine overdose effects previously reported for (antero)ventral striatum-mediated cognitive tasks to motor sequence learning.

## **Introduction**

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized primarily by motor symptoms such as tremor, rigidity and bradykinesia. However cognitive executive deficits also occur, even in the early stages of disease diagnosis (Caballol et al., 2007). The Braak staging system describes PD as a schema of ascending pathology, beginning in the lower brainstem and anterior olfactory structures, progressing to the basal mid- and forebrain nuclei, and then to the cortex (Braak et al., 2003, Braak et al., 2006). In stage 3 of the Braak system, when motor symptoms first begin to appear, the neuropathology of PD is demonstrated by the loss of dopaminergic neurons in the substantia nigra pars compacta and the ventral tegmental area with degeneration of the striatal nerve terminals (Braak et al., 2006). Dopaminergic denervation is not distributed evenly in the striatum in PD. Dopaminergic denervation begins asymmetrically and then becomes bilateral later in the disease (Hornykiewicz, 1966). Additionally, there is also a dorsal to ventral and posterior to anterior gradient in dopamine (DA) depletion. Specifically, depletion is greater in the dorsal and posterior striatum as opposed to the ventral and anterior striatum early in the disease, with subsequent involvement of the anteroventral striatum with disease progression (Bernheimer et al., 1973, Kish et al., 1988, Frey et al., 1996, Rakshi et al., 1999). Anti-Parkinsonian dopaminergic medications such as L-DOPA and dopamine agonists ameliorate motor deficits of PD by compensating for the loss of dopamine. However, these medications can have conflicting effects on cognitive performance particularly in the early stages of the disease (Shohamy et al., 2005, Shohamy et al., 2006, Shohamy et al., 2008). One proposed explanation for these conflicting effects is the so-called



“dopamine over-dose hypothesis” (Gotham et al., 1988, Swainson et al., 2000, Cools et al., 2001, Frank et al., 2004, Frank, 2005, Cools, 2006, Cools et al., 2006). This hypothesis proposes that in early PD, while dopaminergic medications can improve cognitive performance on tasks associated with the depleted dorsal and caudal striatum such as task switching (Sohn et al., 2000, Brass et al., 2003), they can interfere with the normal cognitive performance associated with the ventral and rostral striatum such as reversal learning (Cools et al., 2002) by overdosing this region which is relatively intact in the early stage of the disease (Cools et al., 2001, Cools, 2006). These findings that support the “dopamine-overdose hypothesis” are consistent with the ‘inverted U’ relationship between dopamine and cognition identified in experimental animal studies, which demonstrates that both insufficient and excess levels of dopamine impair normal cognitive functions (Williams and Goldman-Rakic, 1995, Zahrt et al., 1997, Arnsten, 1998).

Another behavior that could plausibly be affected by dopamine overdose in Parkinson’s patients is motor sequence learning. Motor sequence learning underlies many everyday activities such as typing, driving, or playing a musical instrument. It also constitutes a critical component of physical rehabilitation interventions. In healthy controls motor sequence learning relies on the basal ganglia thalamocortical loops, including the dorsal and posterior striatal pathways involving the supplementary motor area and the premotor cortex, as well as the ventral and anterior striatal pathways involving the anterior cingulate cortex (Berns et al., 1997, Miyachi et al., 1997, Hikosaka et al., 2002, Seidler et al., 2002, Lehericy et al., 2005, Seidler et al., 2005). The neural recruitment of these pathways changes across the time course of learning. Specifically in

the early phase of learning, when subjects acquire sequences of action in a controlled manner by engaging in cognitive processes, the ventral and anterior striatal pathway (i.e. associative striatum) is involved (Hikosaka et al., 2002, Doyon et al., 2003, Lehericy et al., 2005, Duff et al., 2007, Tamas Kincses et al., 2008). In the later phase of learning, when subjects perform acquired sequences in a more automatic fashion, the dorsal and posterior striatal pathway (i.e. sensorimotor striatum) becomes involved (Hikosaka et al., 2002, Doyon et al., 2003, Lehericy et al., 2005, Duff et al., 2007, Tamas Kincses et al., 2008). Due to the participation of basal ganglia thalamocortical circuits in motor sequence learning, this behavior has been frequently studied in PD patients (Pascual-Leone et al., 1993, Roy et al., 1993, Doyon et al., 1997, Helmuth et al., 2000, Nakamura et al., 2001, Smith et al., 2001, Feigin et al., 2003, Ghilardi et al., 2003, Carbon et al., 2004, Ghilardi et al., 2007, Muslimovic et al., 2007, Seidler et al., 2007, Doyon, 2008, Price and Shin, 2008). These studies show that some aspects of sequence learning are preserved in PD patients whereas others are impaired (Smith et al., 2001, Muslimovic et al., 2007, Seidler et al., 2007). Not many of these studies have looked at the effects of medication status on sequence learning in PD patients, however, which would be required to determine whether a dopamine overdose effect plays a role in this behavior.

Furthermore, the studies that have tested whether sequence learning performance changes with medication status in PD patients have found mixed results (Feigin et al., 2003, Ghilardi et al., 2007, Argyelan et al., 2008). Feigin et al. (2003) found that L-dopa administration led to reduced sequence learning in patients with PD, but this was only observed using a subjective measure of self-reported learning (i.e. the number of accurately performed sequence elements self-reported by each participant). When

comparing objective measures such as reaction time and accuracy, investigators have not observed significant differences between performance in the ON versus OFF medication states (Ghilardi et al., 2007). In addition, a recent functional neuroimaging study found that the level of learning-related brain *deactivation* was reduced with dopamine, but there was no significant behavioral evidence for ON versus OFF performance differences (Argyelan et al., 2008). I believe that such mixed findings are due to the fact that prior studies did not compare patients ON and OFF medication specifically at different phases of sequence learning, while the dopamine overdose hypothesis predicts varying effects of medication across different learning phases. That is, since early sequence learning relies on the relatively intact ventral and anterior striatum (Lehericy et al., 2005), administration of dopaminergic medication should reduce performance selectively in the early phase of sequence learning for PD patients. In contrast, the late phases of sequence learning rely on the dorsal and posterior striatum (Lehericy et al., 2005), which are impacted by early stage PD. Given this, administration of dopaminergic medication should improve performance in the late phase of sequence learning for PD patients. Thus, a selective deficit in the ON state in early sequence learning may have been missed by previous studies which have evaluated performance averaged across the time course of learning.

In the current study I tested whether there is an interaction between the time course of sequence learning and the presence or absence of dopaminergic medication in early stage PD patients. To test the “over-dose hypothesis”, I compared motor sequence learning performance across different phases of learning in PD patients ON and OFF medication and healthy age-matched control participants. Previous literature has used

multiple tasks to assess motor sequence learning, including the serial reaction time task, trial and error learning, and probabilistic motor sequence learning. All of these have the component of learning a sequential movement either implicitly or by using an explicit strategy. Due to the common nature of learning sequential movements, studies have found similar underlying neural systems including corticostriatal pathways during different sequence learning paradigms. For example probabilistic motor sequence learning involves the primary motor cortex (Wilkinson et al., 2009) and striatum (Wilkinson and Jahanshahi, 2007), and trial and error sequence learning involves prefrontal cortex (Sakai et al., 1998) and the pre-motor and supplementary motor areas (Mentis et al., 2003). Many studies have either just focused on sequence learning itself not taking into consideration the different phases of learning or did not find involvement of different striatal regions across the phases of learning. The motor sequence learning paradigm that has shown a clear striatal involvement in different phases of learning has been an explicitly learned sequence of finger actions (Lehericy et al., 2005). Thus in my study, I used the classic serial reaction time task with explicit instructions. The time course of learning in the current task (30 – 40 minutes) was comparable to the period over which a shift in basal ganglia activation was observed by Lehericy et al. (2005; 10 – 50 minutes). The early phase of learning was approximately 10 – 15 minutes in duration, corresponding with time T2 in Lehericy et al. (10 minute time point).

## **Materials and Methods**

### Participants

14 PD patients ( $65 \pm 10$  yrs, 2 females) in the mild to moderate stages of disease (Hoehn and Yahr stages 1 – 2.5, Hoehn and Yahr, 1967) and 11 healthy participants ( $64 \pm 9$  yrs, 3 females) in the same age range participated in this study. Patients were excluded for any neurological or psychiatric disease other than PD. Patients were included as long as they were on a stable dosage of dopaminergic medications for the previous 6 months, and were evaluated using the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS, Fahn et al., 1987) by a neurologist. All study participants underwent the Mini-Mental State Exam (MMSE, Folstein et al., 1975) and the Mattis Dementia rating scale (MDRS, Mattis, 1988). The demographic and clinical characteristics of the patients are listed in Table 2.1. Participants were compensated for their participation, which included two testing days for PD patients. All participants signed a consent form approved by the Institutional Review Board of the University of Michigan.

**Table 2.1.** Demographic and clinical variables.

Patient	Age (yrs)	M/F	Disease duration (yrs)	H&Y	UPDRS*		DRS		MMSE		List of dopaminergic medications
					ON	OFF	ON	OFF	ON	OFF	
PD_1	70	M	12	2	15	21	143	144	27	30	Levodopa <sup>1</sup> , Ropinirole <sup>2</sup>
PD_2	59	M	1	1	8	11	144	143	30	30	Rotigotine <sup>2</sup>
PD_3	78	M	20	2.5	21	27	142	141	27	28	Levodopa <sup>1</sup> , Pramipexole <sup>2</sup> ,
PD_4	65	M	6	2	21	27	143	144	28	30	Levodopa <sup>1</sup> , Pramipexole <sup>2</sup>
PD_5	70	M	5	2	13	16	136	138	25	25	Levodopa <sup>1</sup> , Pramipexole <sup>2</sup>
PD_6	70	M	1	2.5	28	28	142	142	27	27	Pramipexole <sup>2</sup>
PD_7	68	M	6	2	22	27	142	134	24	23	Pramipexole <sup>2</sup>
PD_8	57	F	1	2.5	11	17	144	140	27	27	Pramipexole <sup>2</sup>
PD_9	82	F	10	2.5	4	14	144	144	28	28	Levodopa <sup>1</sup> , Pramipexole <sup>2</sup>
PD_10	51	M	5	2	8	13	142	142	27	29	Levodopa <sup>1</sup> , Ropinirole <sup>2</sup>
PD_11	48	M	3	2	26	30	137	135	27	27	Levodopa <sup>1</sup>
PD_12	61	F	12	1.5	15	18	143	141	30	28	Pramipexole <sup>2</sup>
PD_13	58	M	4	2	11	25	144	142	27	27	Levodopa <sup>1</sup>
PD_14	71	M	1	1	5	5	144	143	26	28	Levodopa <sup>1</sup>
Mean ± S.D.	65±10		6±6	2	15±8	20±8	142±3	141±3	27±2	28±2	

H&Y: Hoehn and Yahr ratings (Hoehn and Yahr, 1967). \*p < 0.05 difference ON and OFF medications. <sup>1</sup> L-Dopa, <sup>2</sup> Dopamine agonist

## Procedure

PD patients underwent two testing days corresponding to the ON and OFF medication states, separated by no more than two weeks. Seven patients were tested ON

first and seven OFF first. Patients withdrew from their regular dose of anti-parkinsonian dopaminergic medication 12 - 18 hours prior to testing for the OFF state. Control participants (NC) underwent one testing session. On each testing session, participants performed the explicit motor sequence learning task. Additionally, the Purdue Pegboard Test (Tiffin and Asher, 1948) and the Grooved Pegboard Test (Lafayette Instruments, Lafayette, IN) were used to measure motor abilities.

### Explicit Motor Sequence Learning

Participants were instructed to press a key-press device with their fingers in response to stimuli presented on a computer screen. The index and middle finger from each hand were assigned to the four response buttons in the following fashion: left middle – first button, left index – second button, right index – third button, and right middle – fourth button. There were four visual stimulus boxes corresponding to each of the four response buttons. Participants were instructed to press the appropriate button as fast as possible when an “X” appeared in one of the stimulus boxes. The trial blocks were either a “sequence” block, in which stimuli were presented in a sequential order, or a “random” block, in which stimuli were presented in a pseudorandom fashion. Participants were presented with a 6-element sequence (i.e. 1, 3, 2, 3, 4, 2) repeatedly in the sequence block. Participants were explicitly told that there was a sequential pattern to the stimuli presentation, and they were shown the 6 element sequence prior to the test session. They were instructed to learn the sequence. They were informed which of the two blocks (i.e. random or sequence) they would be performing at the beginning of each block. If

participants failed to respond by pushing the correct button, the same stimulus location was presented again on the next trial. Each block consisted of 96 trials spaced by a constant response-to-stimulus interval (RSI) of 500 ms, and there were 11 blocks in total including both sequence and random blocks. The order of sequence and random blocks were as follows: R1-R2-S3-S4-R5-S6-S7-R8-S9-S10-R11 (S: sequence, R: random). The first two sequence blocks (S3 and S4) were referred to as the early phase and the last two sequence blocks (S9 and S10) as the late phase of learning. Patients were presented with different sequences on the two testing days.

### Data Analysis

Sequence learning performance was measured by reaction time and number of errors. The random blocks in the sequence learning paradigm were positioned in between sequence blocks across the duration of the task to account for baseline motor function and the possible learning of general visuomotor behavior across the sequence learning paradigm in each group (NC, PD ON and PD OFF). The first trial in each block, error trials, and trials immediately following an error were excluded from RT analysis. I computed the median RT and total number of errors in each block for analysis. Repeated measures ANOVA and paired or independent sample t-tests were performed. The Huynh-Feldt epsilon (Huynh and Feldt, 1970) was used to determine whether the repeated measures data met the assumption of sphericity ( $\epsilon > 0.75$ ). In cases where the sphericity assumption was not met, the F statistic was evaluated for significance using the Huynh-Feldt adjusted degrees of freedom.



## Results

### Neuropsychological Assessments

Paired t-test showed no significant difference between PD ON and PD OFF for the MDRS ( $t_{13} = 0.096$ ,  $p = 0.925$ , PD ON:  $142 \pm 3$ , PD OFF:  $141 \pm 3$ ) or MMSE ( $t_{13} = -0.836$ ,  $p = 0.418$ , PD ON:  $27 \pm 2$ , PD OFF:  $28 \pm 2$ ). Independent sample t-tests showed no significant difference between NC and PD ON for MDRS ( $t_{23} = 1.269$ ,  $p = 0.217$ , NC:  $143 \pm 2$ ) or MMSE ( $t_{23} = 1.257$ ,  $p = 0.221$ , NC:  $28 \pm 1$ ). There was also no significant difference between control and PD OFF for MDRS ( $t_{23} = 1.661$ ,  $p = 0.11$ ) or MMSE ( $t_{23} = 0.455$ ,  $p = 0.645$ ). These results demonstrate that patients' general cognitive abilities (MMSE, MDRS) were not significantly different ON and OFF medication or compared to NC. In patients, performance on the Purdue pegboard and the Grooved pegboard was analyzed separately for the more affected and less affected sides. Paired t-tests showed no significant performance differences in the Purdue pegboard test across medication states in both the more affected ( $t_{13} = 1.57$ ,  $p = 0.142$ ) and less affected ( $t_{13} = 0.74$ ,  $p = 0.472$ ) sides. Similarly in the Grooved pegboard test, no significant difference was found for either the more affected ( $t_{13} = 1.31$ ,  $p = 0.212$ ) or less affected ( $t_{13} = -0.14$ ,  $p = 0.891$ ) sides. When compared to NC, both PD ON (right:  $t_{23} = 2.265$ ,  $p = 0.033$ , left:  $t_{23} = 2.158$ ,  $p = 0.042$ ) and PD OFF (right:  $t_{23} = 2.485$ ,  $p = 0.021$ , left:  $t_{23} = 2.277$ ,  $p = 0.032$ ) performed worse in Purdue pegboard in both the left and right hands. Performance on the Grooved pegboard was marginally different between NC and PD ON in the right hand ( $t_{23} = -2.01$ ,  $p = 0.056$ ) and between NC and PD OFF in the right ( $t_{23} = -1.899$ ,  $p = 0.07$ ) and left ( $t_{23} = -2.004$ ,  $p = 0.057$ ) hands. The UPDRS motor component showed a significant difference between ON and OFF medication ( $t_{13} = -5.2$ ,  $p < 0.00001$ ),

reflecting that motor symptoms were more severe when patients were OFF (mean  $\pm$  S.D:  $20 \pm 7.6$ ) versus ON ( $15 \pm 7.7$ ) medication. These results demonstrate that patients' manual motor performances (Purdue pegboard, grooved pegboard) were worse compared to controls in either the ON or OFF state. However they were not significantly different ON and OFF medication, whereas patients' motor symptoms (UPDRS) were more severe in the OFF medication state.

### Explicit Motor Sequence Learning

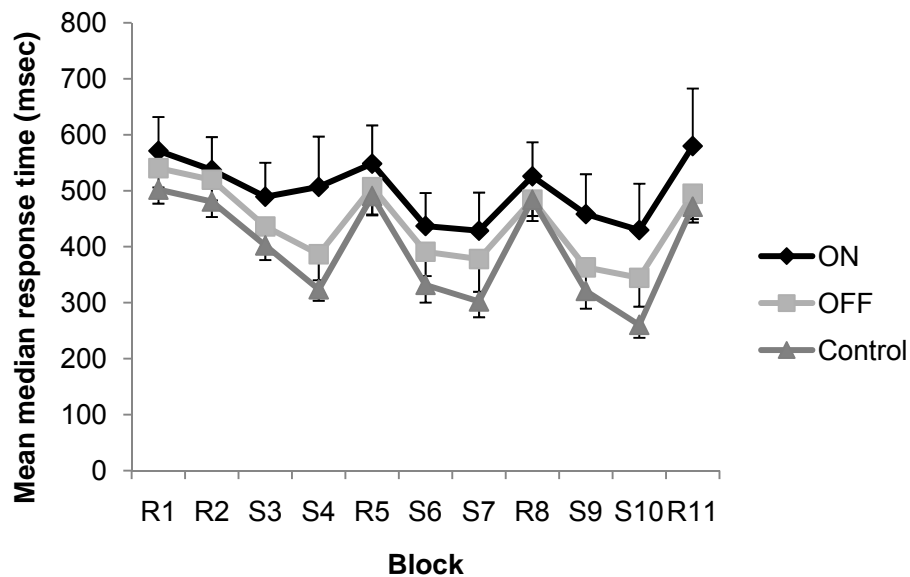
I hypothesized that medication status would affect sequence learning in a phase-dependent fashion. To test this hypothesis I first performed a medication status (PD ON, PD OFF) by learning phase (early, intermediate, late) by block series (first, second, third block) repeated measures ANOVA on RT with medication status, learning phase and block series as within subject factors. R2, S3, S4 were considered as the first, second, and third block series of the early phase whereas R5, S6, S7 and R8, S9, S10 were considered as the intermediate and late phases, respectively. I considered sequence learning as the change in RT across the three block series starting from a random block within each phase. I found a significant main effect of phase ( $F_{1,397, 18,164} = 11.033$ ,  $p = 0.002$ ) and a main effect of block series ( $F_{2, 26} = 24.334$ ,  $p < .00001$ ) (Fig. 2.1.). This indicates that RT was different across the three learning phases and that sequence specific learning, represented as change in RT across block series within each phase, was present throughout the task. Importantly, I also found a significant medication status by phase by block series interaction ( $F_{2,971, 38,619} = 3.052$ ,  $p = 0.04$ ). This indicates that medication

status affected sequence specific learning in a phase-dependent fashion. I also found a marginally significant medication status by block series interaction ( $F_{2, 26} = 3.2, p = 0.057$ ). No significant medication status main effect or other two way interactions were found. In light of the significant medication status by phase by block series interaction, I followed up with a medication status by block series repeated measures ANOVA with medication status and block series as repeated measures in each learning phase separately. A significant medication status by block series interaction was found selectively in the early phase ( $F_{1, 489, 19.352} = 7.7, p = 0.006$ ) and not in the intermediate ( $F_{1, 464, 19.029} = 0.047, p = 0.909$ ) or late phases ( $F_{2, 26} = 2.151, p = 0.137$ ). This indicates that medication status affected sequence learning specifically in the early phase. In order to test whether performance on the last sequence block was significantly changed from random, I proceeded with paired t-tests to test for RT differences between the first random block (R2) and the last sequence block (S4) in the early phase in PD ON and PD OFF separately. There was a significant RT decrease across the two blocks in PD OFF ( $t_{13} = 5.676, p < .00001$ ). However no significant RT difference was found in patients ON medication ( $t_{13} = 0.738, p = 0.474$ ). This indicates that sequence learning in the early phase was impaired in patients ON medication. I also compared the last sequence block (S4) of the early phase to the following random block (R5) using paired t-tests in order to confirm that the medication-associated impairment was specific to sequence learning and not merely representing changes in stimulus-response (S-R) mapping over time. RT was significantly different between S4 and R5 ( $t_{13} = -6.235, p < 0.00001$ ) in PD OFF, but not significantly different in PD ON ( $t_{13} = -1.343, p = 0.202$ ). This confirms that RT improvements exhibited by PD OFF represent sequence-specific learning.

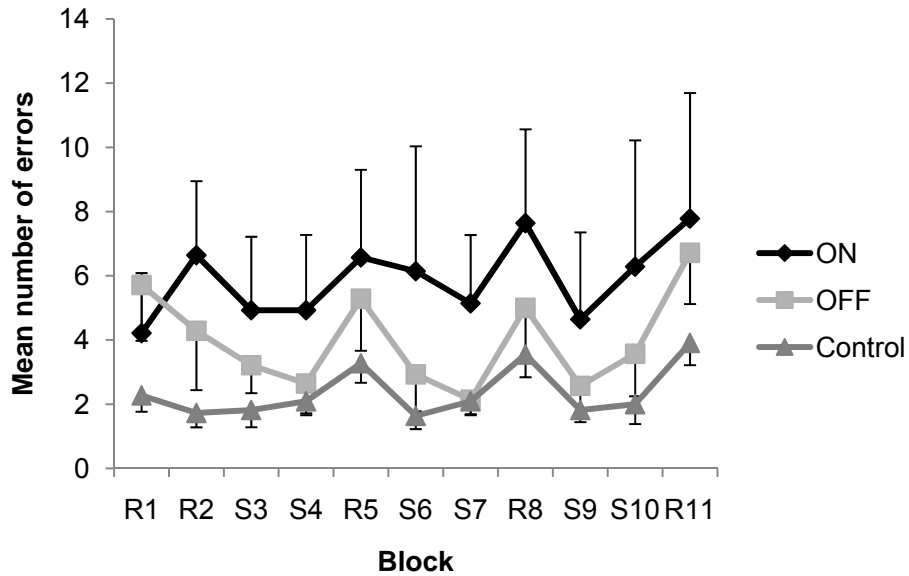
To further evaluate whether PD ON and PD OFF differed in the late phase of learning, I performed an additional medication status by block analysis including data for the patients tested ON and OFF medication for the final sequence and subsequent random block (S10 and R11). This comparison is the standard assessment of the amount of learning that has occurred for studies employing the serial reaction time paradigm. As suggested by the parallel slopes of the lines connecting these two blocks for the two medication status groups in Figure 2.1., this ANOVA resulted in a medication status by block effect of  $F_{1,13} = 0.000$ ,  $P = .999$ , indicating a lack of medication status effects on sequence learning magnitude in the late phase of learning.

I also compared patients' performance to controls separately for the two medication states. I performed a group (NC, PD) by learning phase (early, intermediate, late) by block series (first, second, third block) repeated measures ANOVA with learning phase and block series as within subject factors and group as between subject factor including only PD ON or PD OFF separately. When PD ON was compared to NC, a significant main effect of phase ( $F_{2,46} = 8.824$ ,  $p = 0.001$ ) and block series ( $F_{2,46} = 46.049$ ,  $p < 0.00001$ ) and significant block series by group ( $F_{2,46} = 6.585$ ,  $p = 0.003$ ) and phase by block series ( $F_{2,46} = 4.348$ ,  $p = 0.003$ ) interactions were found. However no significant group by phase by block series interaction was found ( $F_{4,92} = 1.472$ ,  $p = 0.217$ ). When PD OFF was compared to NC, a significant main effect of phase ( $F_{1,473,18.736} = 18.726$ ,  $p < 0.00001$ ) and block series ( $F_{1,473,33.058} = 84.387$ ,  $p < 0.00001$ ) and a significant phase by series interaction ( $F_{4,92} = 5.038$ ,  $p = 0.001$ ) were found. No significant group by phase by block series interaction was found ( $F_{4,92} = 1.174$ ,  $p = 0.327$ ).

I performed the same set of data analyses on the number of errors (Fig. 2.2.). Error data were analyzed in order to confirm that the RT results were not due to differences in speed-accuracy trade-off. As in the RT data, I first performed a medication status (PD ON, PD OFF) by learning phase (early, intermediate, late) by block series (first, second, third block) repeated measures ANOVA with medication status, learning phase and block series as within subject factors using number of errors in each block. I only found a significant main effect of block series ( $F_{2, 26} = 8.881, p = 0.001$ ). No other main effects, two-way or three-way interactions were found to be significant. This confirms that the results with the RT data were not due to speed-accuracy trade-off differences across the groups. The same set of analyses was performed comparing NC to PD ON or PD OFF. When PD ON was compared to NC, I only found a significant main effect of block series ( $F_{1, 285, 29, 548} = 6.825, p = 0.009$ ). The same was true for PD OFF ( $F_{1, 214, 27, 926} = 5.422, p = 0.022$ ).



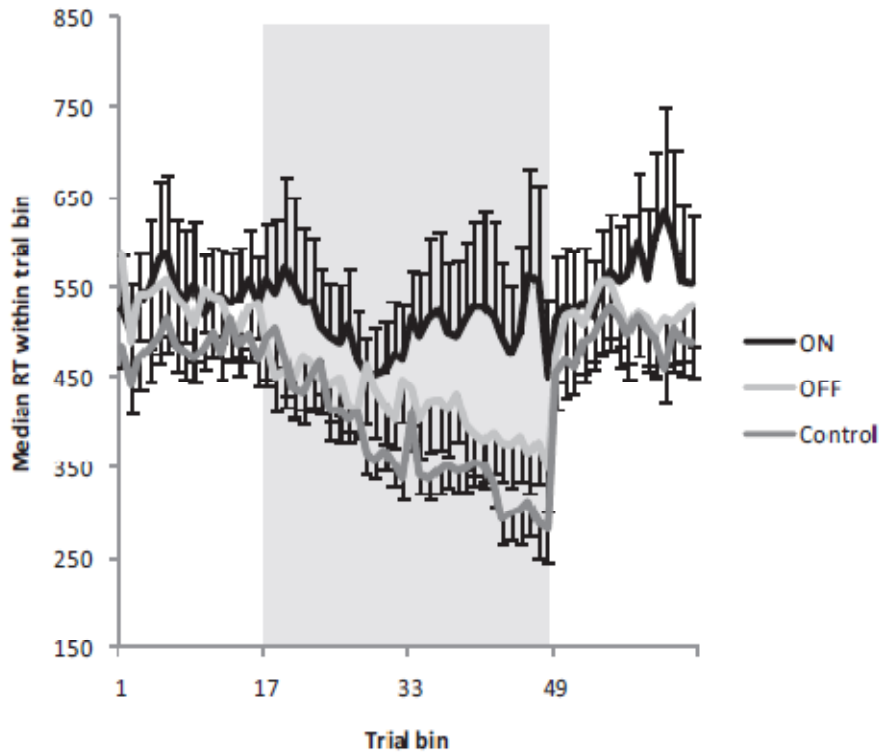
**Figure 2.1.** Group mean of the median response time across 11 blocks in controls, patients ON and OFF medication. Error bars indicate standard error.



**Figure 2.2.** Group mean of the number of errors across 11 blocks in controls, patients ON and OFF medication. Error bars indicate standard error.

To illustrate the evolution of early phase learning, I plotted the median RT within shorter bins of trials across the first two sequence blocks (Fig. 2.3.). Median RT from 6 sequence elements was combined as a bin. The first trial of the block, the error trials and the trials immediately after error were excluded. The two bounding random blocks were included for better visualization of RT change in the sequence blocks. The same strategy for computing median RT across 6 elements was used. If there were no errors in a block, there would be 16 bins in total per block. Since participants made different numbers of errors in each block, there weren't always 16 bins per block. In case of missing values, linear interpolation was used to compute 16 values within the block. I examined how the

learning rate (slope of the evolution of memory) changed across trial bins in sequence blocks at the early phase. I hypothesized that there would be a linear decrease in RT across trial bins and the slope of RT decrease would be different across medication states. Specifically I predicted that the slope in PD ON would be less steep compared to PD OFF or NC. To test this hypothesis, I performed a medication status (PD ON, PD OFF) by trial bins repeated measures ANOVA using medication status and trial bin as within subject factors. I evaluated the within subject linear contrast across trial bins. The interaction for group by linear contrast was marginally significant ( $F_{1,13} = 4.334, p = 0.058$ ). This indicates that there was a linear decrease in RT across the trial bins in the early phase and the slope of RT decrease or the learning rate was steeper for PD OFF than PD ON. I performed the same analysis comparing PD ON and NC using group as a between subject factor. The group by trial bin linear fit interaction was significant ( $F_{1,23} = 6.256, p = 0.02$ ), which demonstrates that the RT across trial bins changed linearly and the slope differed in the two groups. However, when comparing PD OFF and NC, the linear contrast for the interaction effect was not significant ( $F_{1,23} = 2.754, p = 0.111$ ). These results demonstrate that compared to controls, patients ON medication were impaired in sequence learning as characterized by the evolution of RT change trial by trial in the early phase, whereas patients OFF medication were not impaired compared to controls.



**Figure 2.3.** Group mean of the median response time for each trial bin across the early phase of learning. Trials 1 through 16 and 49 through 64 were from the two bounding random blocks. Trials 17 through 32 and 33 through 48 were from the first two sequence blocks (S3 and S4). Error bars indicate standard error.

To provide further support for the claim that the difference in learning rate between PD ON and PD OFF was specific to the early phase of learning, I evaluated whether there were significant group x linear fit interactions in the middle and late phases of learning. In addition, because the two groups might be at different stages in the learning process such that more advanced learning phases in PD ON would be equivalent to the early learning phase in PD OFF (due to the early phase advantage for PD OFF), I also evaluated whether rate of learning differed between PD ON from the intermediate phase or the late phase of learning with PD OFF from the early phase of learning. There were no significant effects for any of these analyses ( $P > .05$  for all comparisons).



## **Discussion**

### Effect of Dopaminergic Medications over the Time Course of Motor Sequence Learning

I found a significant sequence learning impairment associated with dopaminergic medication that was dependent on the phase of learning. Compared to patients OFF medication, patients ON medication showed significantly less RT reduction across the early learning phase. This was also confirmed by the steeper trial by trial RT decrease during the first two sequence blocks for patients OFF medication compared to when they were ON medication. The rate of trial by trial RT decrease for PD OFF was similar to that of healthy controls. There was no significant medication effect on the number of errors, providing support that the medication effect for RT change was not due to differences in speed-accuracy trade off. In addition, the ON medication deficit was restricted to sequence learning behavior. That is, performance was equivalent ON and OFF medication for the Purdue Pegboard and the Grooved Pegboard tests. Moreover, participants performed more poorly on the UPDRS when OFF than ON medication. This demonstrates that the baseline motor abilities were matched across medication states and patients' motor symptoms were worse in the OFF state. These behavioral data collectively provide evidence for the dopamine over-dose effect in early motor sequence learning, implicating a detrimental effect of dopaminergic medication confined to the phase of learning that is known to be dependent on the ventral and anterior striatal circuitries (Berns et al., 1997, Miyachi et al., 1997, Hikosaka et al., 2002, Seidler et al., 2002, Lehericy et al., 2005, Seidler et al., 2005).

The data showed equivalent learning for patients ON and OFF medication for the

intermediate and late phase of learning. One possible cause of this may be the fact that the task has now moved away from the early phase of learning where the medication shows the most deleterious effect. Another possibility is that medication benefitted sequence learning behavior in the later phase which is known to rely on the posterior and dorsal striatal circuitry (Miyachi et al., 1997, Hikosaka et al., 2002, Seidler et al., 2002, Lehericy et al., 2005, Seidler et al., 2005). There was not a sufficient number of practice trials in the current study to fully evaluate this, however. In order to reach the late phase during which automatized sequential movements rely purely on dorsal and posterior striatum (i.e. sensorimotor striatum), previous studies have used multiple days of practice (Lehericy et al., 2005). In the current task subjects were exposed to only a limited number of trials across 11 blocks, which might not have been enough for them to completely reach the late automatic phase of learning, purely supported by the sensorimotor striatum. In other words, subjects might be in the interim phase relying on both the associative and sensorimotor striatum.

It is of note that the designation of early, intermediate and late phases was in respect to the current task design. That is, I arbitrarily defined the three learning phases respective to the time course of the task in the current study. Since I did not acquire neuroimaging data, I was not able to determine whether there was indeed a shift of neural recruitment across the three arbitrarily defined learning phases in the current task. However, in the Lehericy et al. (2005) study, the extent of area activated in the anterior putamen decreased even after 50 mins of motor sequence training. There was also a significant decrease in anterior putamen activation from 10 mins of training to 50 mins of training. By contrast they found an increase in the extent of posterior putamen

recruitment even after 10 min of practice. This shows that the shift from anterior to posterior putamen recruitment happened within 10 min to 50 min of practice. The sequence learning task used in the current study spanned a comparable period (30-40 min in total depending on the individual). Thus I think that the arbitrary designation of the early, intermediate and late phase in the current task was sufficient for capturing the shift in neural recruitment of basal ganglia sub-regions.

A potential limitation to the current study is the large variation seen in the patients' performance, as evidenced by the relatively large error bars in the plots. This may be due to a small sample size and large variability in the patients' performance. Despite these limitations, I found a significant medication effect supporting my hypothesis. That is, I found a medication-associated impairment selective to the early phase and not the later two phases of learning. I believe this is not due to learning being saturated in the later two phases since there was a main effect of block series showing that sequence learning was present throughout the task. While it is more difficult to detect group differences later in learning when the learning curve starts to plateau, the current analyses clearly demonstrated a lack of medication status on sequence learning magnitude in the late phase of learning. It is also noteworthy that large variability in patients' performance is more apparent in the ON medication state. This supports my hypothesis of the medication effect as such effects can be quite significant but variable at the same time depending on the type and dosage of medication. The variability may also depend on the individual degree of striatal denervation in each patient.

## Motor Sequence Learning in Parkinson's Patients & Dopaminergic Medications

Previous studies of motor sequence learning in Parkinson's patients have found conflicting results with some showing impaired learning (Helmuth et al., 2000, Stefanova et al., 2000, Nakamura et al., 2001, Muslimovic et al., 2007) and others showing intact learning (Smith et al., 2001, Werheid et al., 2003, Seidler et al., 2007). My data suggest that relatively intact basal ganglia subregions in the mild to moderate stage of PD, such as the ventral and anterior striatum, can function properly as shown by comparable sequence learning for patients OFF medication and controls.

The effect of dopaminergic medication on motor sequence learning of PD patients has not been clearly identified in previous studies (Feigin et al., 2003, Ghilardi et al., 2007, Argyelan et al., 2008). These studies have either found no significant difference in performance between the two medication states (Ghilardi et al., 2007, Argyelan et al., 2008) or only found a difference for a subjective measure of self-reported learning (Feigin et al., 2003). My results show a clear detrimental effect of dopaminergic medication that is confined specifically to the early phase of motor sequence learning for PD patients. Whether the nature of this negative medication effect on early motor sequence learning is similar to that of other demonstrations of dopamine overdose such as reversal learning is not clear at this point. In probabilistic reversal learning, there is explicit positive or negative feedback that will invoke reward and error processing by the nucleus accumbens (Cools et al., 2001, Cools, 2006). In contrast, the current sequence learning paradigm does not include explicit feedback, with the exception of repeated trials in the case of incorrect responses. It is plausible that this indirect feedback might have partially driven learning. For example, the repeating trial might have acted as

negative feedback. A previous study has shown that PD patients OFF medication are more sensitive to negative than positive feedback whereas patients ON medication become more sensitive to positive feedback (Frank et al., 2004). My data corresponds to this finding showing better performance in the OFF state in a task that potentially involves negative feedback. However the specific brain regions involved in early sequence learning that may be sensitive to dopaminergic medication are not clear at this point. Areas including the medial prefrontal cortex and ventral putamen, which receive dopaminergic projections from the ventral tegmental area similar to the nucleus accumbens (Hu et al., 2004) may be involved.

Collectively my data showed that the dopamine overdose effect impacts motor sequence learning in a time varying fashion for PD patients. Specifically, I found that early learning relative to late learning was impaired by dopaminergic medication. These findings underscore the importance of taking into account the time varying nature of the contributions of different striatal loops to motor sequence learning when investigating the effect of dopaminergic medication on sequence learning in PD patients. These data also provide further evidence that ventral and anterior striatal loops play a greater role in early sequence learning.

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## **CHAPTER III**

### **L-DOPA impairs ventral striatum recruitment during motor sequence learning in Parkinson's disease**

#### **Abstract**

In Chapter 2, I have shown that the effect of dopaminergic medication on motor sequence learning in early stage Parkinson's (PD) patients varies across the time course of learning. I found a medication-associated impairment that was specific to the early phase of learning. In the current study, I investigated the neural substrate of this deleterious effect of L-DOPA on motor sequence learning. I hypothesized that L-DOPA would negatively affect recruitment of the ventral striatal circuitry during the early phase of motor sequence learning. Seventeen early stage Parkinson's patients ON and OFF L-DOPA and 21 healthy control participants (separate from the cohort presented in Chapter 2) performed an explicit motor sequence learning task inside the MRI scanner. Behavioral evidence of a dopamine overdose was not consistent at the group level when performance was measured by response time and error rate. Results from the neural activation data showed a sequence learning-specific activation during the early phase in the ventral putamen for controls and PD OFF but not for PD ON L-DOPA. A comparison

of activation between PD OFF and PD ON within the ventral putamen showed that activation was decreased in PD ON compared to PD OFF. The extent of the L-DOPA associated activation decrease was positively correlated with the degree of sequence learning impairment in the early phase of learning. These findings provide evidence for the selective negative effects of L-DOPA on the ventral cortico-striatal loops involved in motor sequence learning in Parkinson's disease.

## **Introduction**

The capacity to learn new motor sequences is critical to adaptive motor behavior. The neural substrates of motor sequence learning exhibit a functional reorganization of cortical and subcortical contributions across the time course of learning (Hikosaka et al., 1998, Toni et al., 1998, Doyon et al., 2002, Muller et al., 2002, Seidler et al., 2002, Doyon et al., 2003, van Mier et al., 2004, Floyer-Lea and Matthews, 2005, Lacourse et al., 2005, Lehericy et al., 2005, Poldrack et al., 2005, Bapi et al., 2006, Duff et al., 2007, Sun et al., 2007, Tamas Kincses et al., 2008). Such activation shifts are well-manifested in the basal ganglia-thalamocortical circuitry (for review see Doyon and Benali, 2005). Specifically, in the early phase of learning, when subjects acquire sequences of action in a controlled manner by engaging in cognitive processes, the ventral/anterior striatal pathway (i.e. associative striatum) including the anterior cingulate and dorsolateral prefrontal cortex is involved (Berns et al., 1997, Doyon et al., 2003, Lehericy et al., 2005, Bapi et al., 2006, Duff et al., 2007, Tamas Kincses et al., 2008). In the later phase, when subjects perform acquired sequences in an automatic manner, the dorsal/posterior striatal pathway (i.e. sensorimotor striatum) including the primary and secondary motor areas is involved (Hikosaka et al., 2002, Doyon et al., 2003, Lehericy et al., 2005, Bapi et al., 2006, Duff et al., 2007, Tamas Kincses et al., 2008).

Deficits in motor sequence learning have been reported in PD patients both ON and OFF dopaminergic medications, which is not surprising considering the significant involvement of the basal ganglia-thalamocortical circuitry in sequence learning (for review see Doyon, 2008). In Chapter 2, I reported that the effect of dopaminergic medication varies across the time course of motor sequence learning in early stage PD

patients. Specifically, I found that dopaminergic medication affected the early learning phase, and I ascribed these results to a “dopamine-overdose” effect. Cools (2006) hypothesized that, while medications can improve performance on tasks relying on the depleted dorsal striatum for PD patients, they can interfere with performance on tasks relying on the ventral striatum such as reversal learning (Cools et al., 2002) by overdosing this region which is relatively intact in the early stage of PD (Cools et al., 2001, Cools et al., 2006). Results from Chapter 2 provide behavioral evidence in line with the dopamine-overdose hypothesis, but whether the medication actually interferes with ventral striatal activity remains an open question.

The neural substrates of motor sequence learning have also been much studied in PD patients (for review see Doyon, 2008). Previous research has reported higher activation in the premotor and parietal cortex in PD patients compared to controls during performance of sequential finger movements (Samuel et al., 1997, Catalan et al., 1999, Mallol et al., 2007). A series of PET studies by Eidelberg and colleagues found that the pattern of sequence learning brain activation differed between healthy controls and PD patients, with striatal activation only found in controls (Nakamura et al., 2001, Carbon et al., 2003, Carbon and Eidelberg, 2006). Using a voxel-based principal component analysis, they also found an irregular pattern of learning-associated networks in PD patients on therapy including deep brain stimulation and levodopa compared to controls (Carbon et al., 2003). A more recent study by the same group found that L-DOPA disrupted sequence learning related prefrontal cortex (PFC) *deactivation* in PD patients (Argyelan et al., 2008). This study demonstrated that not only activation but also the level of deactivation associated with learning can be affected by dopaminergic medication. The

series of studies however did not take into consideration the involvement of distinctive corticostriatal circuitries (i.e. ventral / anterior vs. dorsal / posterior) across the time course of sequence learning and the potentially differential effect of dopaminergic medications in these circuitries during motor sequence learning.

In the current study I investigated the neural correlates of the findings from Chapter 2 regarding the effect of dopaminergic medication during sequence learning in PD patients. A previous fMRI study examining the dopamine-overdose effect during probabilistic reversal learning – a task dependent upon the ventral striatum – demonstrated significantly lower activation in the nucleus accumbens for PD patients ON L-DOPA compared to OFF L-DOPA (Cools et al., 2007a). In line with the dopamine overdose hypothesis and based on the results of Chapter 2, I predicted that in the early sequence learning phase, L-DOPA would suppress activation in the ventral striatal circuitry. However, I did not expect to see this in the nucleus accumbens because this structure is not involved in sequence learning. In contrast, I predicted that the effect would be apparent in the associative striatum which includes the ventral / anterior caudate and putamen. In the late learning phase however, I predicted that L-DOPA would increase activation in the dorsal striatal circuitry due to medication compensating for dopaminergic loss in these areas. Additionally, I predicted that the L-DOPA associated difference in neural activity would be associated with sequence learning performance across individual patients.

## **Materials and Methods**

### Participants

Seventeen mild to moderate stage PD patients (Hoehn and Yahr (H & Y) stages 1.5 – 2.5, Hoehn and Yahr, 1967,  $63 \pm 8$  yrs, 2 females) and 21 healthy participants ( $63 \pm 7$  yrs, 5 females) in the same age range participated in this study. Twenty-four patients were initially recruited, however due to excessive head motion and not performing the task properly 7 of them were excluded from the study. This is a new cohort of patients and controls than those reported in Chapter 2. All PD patients were evaluated using the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS, Fahn et al., 1987) by a neurologist (P. D.). The demographic and clinical characteristics of the patients are listed in Table 3.1. Participants were compensated for their participation, which included two testing days for PD patients and one for controls. All participants signed a consent form approved by the Institutional Review Board of the University of Michigan.

**Table 3.1.** Demographic and clinical variables.

	Age	Gender	Disease Duration	UPDRS ON	UPDRS OFF	H & Y	MMSE ON	MMSE OFF	MOCA ON	MOCA OFF
PD_01	52	M	5	16	20	2.5	30	30	26	30
PD_02	60	M	2	10	9	1.5	30	29	30	27
PD_03	69	M	11	20	24	2	30	30	30	27
PD_04	65	M	7	21	27	2.5	29	30	27	28
PD_05	66	M	< 1	9	9	2	29	29	27	26
PD_06	73	M	1	18	14	2.5	29	29	22	24
PD_07	68	M	1	25	27	2	29	28	27	25
PD_08	64	M	6	8	6	2	28	29	18	20
PD_09	59	F	1	5	6	2	29	28	27	25
PD_10	72	M	3	27	26	2.5	27	28	21	25
PD_11	64	M	2	28	30	2	28	28	28	28
PD_12	51	M	2	32	37	2.5	30	28	25	26
PD_13	62	M	1	10	17	2	29	28	26	25
PD_14	80	M	5	11	13	2	27	28	29	27
PD_15	63	M	6	28	28	2.5	30	30	26	28
PD_16	56	F	< 1	11	21	2	29	28	26	26
PD_17	50	M	2	13	13	1.5	29	30	24	22
average_PD	63.2 ± 8		3.7 ± 3	17.2 ± 8	19.2 ± 9		28.9 ± 1	28.8 ± 1	25.8 ± 3	25.8 ± 2
average_Control	63.3 ± 7							29 ± 1		25.7 ± 3



## Procedure

PD patients underwent two testing days corresponding to the ON and OFF medication states. Nine patients were tested OFF first and eight ON first. I used a single blind placebo controlled design with a single dose of L-DOPA (200 mg) across all PD patients. Healthy age-matched control participants underwent one testing day without any medication procedure. PD patients attended both testing days in the OFF state achieved by withdrawal from medication 12 - 18 hours prior to testing. For the ON testing day patients received a 50 mg dose of carbidopa followed by L-DOPA in combination with carbidopa (200 mg of L-DOPA and an additional 50 mg of carbidopa) after half an hour. For the OFF testing day, patients received placebo medications following the same time schedule in combination with the 50 mg of carbidopa. The L-DOPA dosage I used was tolerable to all study participants. Study procedures began one hour after the patient had taken either L-DOPA or the placebo, by which time L-DOPA reaches its peak plasma dose. For PD patients, the UPDRS motor section was assessed in both the ON and OFF session 5 minutes prior to the start of the scan. All study participants underwent the Mini-Mental State Exam (MMSE, Folstein et al., 1975), the Montreal Cognitive Assessment (MOCA, Nasreddine et al., 2005) and the Grooved Pegboard Test (Lafayette Instruments, Lafayette, IN) on each testing day after the whole scan series which took about one hour.

## Explicit Motor Sequence Learning

All study participants performed an explicit motor sequence learning task inside the MRI scanner. I used the same paradigm as in Chapter 2 with slight modifications to

optimize the design for fMRI. Participants were instructed to press an MRI compatible key-press device with the index and middle fingers of each hand in response to the corresponding visual stimuli. The index and middle finger from each hand were assigned to the four response buttons in the following fashion: left middle – first button, left index – second button, right index – third button, and right middle – fourth button. There were four visual stimulus boxes corresponding to each of the four response buttons.

Participants were instructed to press the appropriate button as fast as possible when an “X” appeared in one of the stimulus boxes. Stimuli were presented through a mirror mounted on a set of specialized goggles, reflecting the video projection screen at the rear of the scanner. Participants were presented with a 6-element sequence (e.g. 1, 3, 2, 3, 4, 2, numbers indicating the location of stimuli from the left) repeatedly, for the sequence blocks (S) and with a pseudo-randomized sequence for the random blocks (R).

Participants were explicitly told that there was a sequential pattern to the stimuli presentation during sequence blocks. In order to increase the explicitness of the sequence, the first element of the repeating sequence was always shown as a red “X” whereas the other stimuli were presented in black. Participants were informed of which of the two blocks (i.e. R or S) they would be performing at the beginning of each block with either an “R” or an “S” positioned on the center of the screen. This also served as a resting period (20 secs) and participants were told to fixate on the letter and not make any movements during this period. If participants failed to respond by pushing the correct button, the same stimulus location was presented again on the next trial. In total there were 6 fMRI runs, each including both random (R) and sequence (S) blocks. The order of R and S blocks within a learning run was R-S-S-S-R, with each run preceded by a resting

period of 20 sec. R blocks consisted of 18 trials and S blocks consisted of 36 trials each. The number of sequence trials in each learning run was equivalent to one sequence block in Chapter 2. Each trial was spaced by a constant inter-stimulus interval of 1250 ms with 200 ms stimulus duration. Data from Chapter 2 showed that in most mild to moderate stage PD patients, RT was within 1250 ms from stimulus onset. Patients were presented with different sequences on each testing day. Task presentation and response collection were accomplished with E-prime software ([www.pstnet.com](http://www.pstnet.com)). Prior to the scan all subjects practiced the task with randomly presented stimuli for 5 mins.

### fMR Image acquisition

Images were acquired using a 3.0 Tesla GE MRI scanner (General Electric, Waukesha, WI) using the standard GE head coil. Each imaging session began with a 3D T1 axial overlay (TR=8.9 ms, TE=1.8 ms, flip angle=15°, FOV=260 x 260 mm, slice thickness=1.4 mm, 124 slices; matrix=256 × 160) for anatomical localization. Functional images were then acquired using a custom single-shot gradient-echo (GRE) reverse spiral pulse sequence (Glover and Law, 2001). Pulse sequence parameters were TR/TE/FA/FOV of 2000ms/30ms/ 90/220mm and a voxel size of 3.44 x 3.44 x 3mm. Forty 3.0 mm thick slightly oblique axial slices (no gap) were acquired.

To facilitate spatial normalization, a 110 sliced (sagittal) inversion-prepped T1-weighted anatomical image using spoiled gradient-recalled acquisition in steady state (SPGR) imaging (flip angle=15°, FOV=260 x 260 mm, 1.4 mm slice thickness) was acquired.

## Data analysis

### *Behavioral data*

Sequence learning data for one PD patient in the ON state was not acquired due to system failure. Sequence learning performance in each run was measured by the learning magnitude calculated by RT (LM\_RT) or error rate (LM\_error). LM\_RT was calculated as the difference in response time between R and S blocks. Median RT was computed for each block and was used in the following equation:  $LM\_RT = \text{average median RT of the two R blocks} - \text{average median RT of the three S blocks within a run}$ . The error trials (i.e. errors by commission and omission), and trials immediately following an error were excluded from RT analyses. LM\_error was calculated in a similar fashion. Error rate was computed for each block and was used in the following equation:  $LM\_error = \text{average error rate of the two R blocks} - \text{average error rate of the three S blocks within a run}$ . Average LM\_RT and LM\_error for the first two fMRI runs was considered as the early learning phase magnitude, for the next two as the middle phase, and for the last two runs as the late learning phase magnitude.

### *fMRI data*

The first five volumes per run were discarded to allow for signal equilibration. FSL MCFLIRT was used for motion correction. Head motion was less than 2 mm in the x, y, and z dimensions in all study participants. Slice timing differences were corrected using local sinc interpolation (Oppenheim et al., 1999). SPM5 (Wellcome Department of Cognitive Neurology, London, UK; <http://www.fil.ion.ucl.ac.uk>) was used for subsequent fMRI analyses. The functional scans were normalized to MNI space using the following

procedure: I first registered the 3D T1 axial overlay to the functional images and then registered the high-resolution SPGR image to the T1 overlay. The transformation to align the SPGR image to the MNI template was finally applied to the functional data. Functional images were spatially smoothed using a full width at half-maximum 8mm Gaussian smoothing kernel.

I used a general linear model with a boxcar regressor modeling the duration of R or S blocks separately for the two blocks. Sequence learning related activation was examined by an  $S > R$  contrast in the early (run 1 and 2), middle (run 3 and 4) and late (run 5 and 6) phases of sequence learning. The six head motion parameters were included as nuisance regressors in the general linear model. Applying the general linear model to the imaging data resulted in least square estimates of the regressors on a subject-specific level. Individual contrast maps were carried over to second-level analyses. The following procedures were used for second-level analyses. First, sequence learning specific activation at each learning phase was examined in each group (i.e. controls, PD OFF and PD ON) separately with  $p < 0.001$  uncorrected and an extent threshold of 10 contiguous voxels. Next, ROI analyses were performed using functional ROIs created based on the group statistics results of the control group. Spherical ROIs ( $r = 10$  mm) were created using the peak activation coordinates from the control group results at each learning phase. Two ROIs were chosen for the early and middle phases and one ROI was chosen for the late phase. Sequence learning specific activation was then compared between PD OFF and PD ON within these ROIs at each learning phase. In order to control for the issue of multiple comparisons I performed a correction by dividing the .05 error by the number of ROIs to test for PD OFF and PD ON comparisons in each learning phase.

Thus  $p < 0.025$  for the early and middle phases and for the consistency of the threshold used,  $p < 0.025$  was also applied for the late phase with an extent threshold of 10 contiguous voxels. This corresponds to a per voxel false-positive probability of less than 0.000001 (Forman et al., 1995). This method of dealing with multiple comparisons has been reported elsewhere (Konishi et al., 1998, Konishi et al., 1999, Poldrack et al., 1999, Wagner et al., 2001, Knutson et al., 2004, Knutson et al., 2006, Monk et al., 2009).

I then determined whether differences in neural activation between PD OFF and PD ON modulate differences in behavioral performance between the two medication states. To evaluate this, I performed a correlation analysis using PD OFF – PD ON mean parameter estimates from regions identified from the ROI analysis and PD OFF – PD ON sequence learning magnitude. Correlation analyses were only evaluated in the learning phase where significant ON and OFF differences in neural activation were observed.

## **Results**

### *Neuropsychological assessments*

Performance on the MMSE, MOCA and Grooved Pegboard tests was compared across the three groups (PD OFF, PD ON and controls) and the UPDRS motor assessment was compared between PD OFF and PD ON. There were no significant differences between PD OFF and PD ON or PD OFF / ON and controls for MMSE or MOCA. Pegboard performance from the right and left hands was significantly different between controls and PD OFF ( $F_{1,36} = 16.10$ ,  $p < 0.0001$ ) and between controls and PD ON ( $F_{1,36} = 11.73$ ,  $p < 0.005$ ) with better performance in the controls. I compared pegboard performance in the more and less affected hand separately between PD OFF

and PD ON. Performance was marginally poorer in the more affected hand ( $t_{16} = -2.03$ ,  $p = 0.059$ ) when patients were OFF than ON. Further, the UPDRS motor score was significantly higher for PD OFF than PD ON ( $t_{16} = -2.37$ ,  $p < 0.05$ ). In sum, these results convey that the patients exhibited motor deficits, and that the single controlled L-DOPA dose was sufficient to reduce these motor symptoms.

*Behavioral results: learning magnitude measured in RT (LM\_RT)*

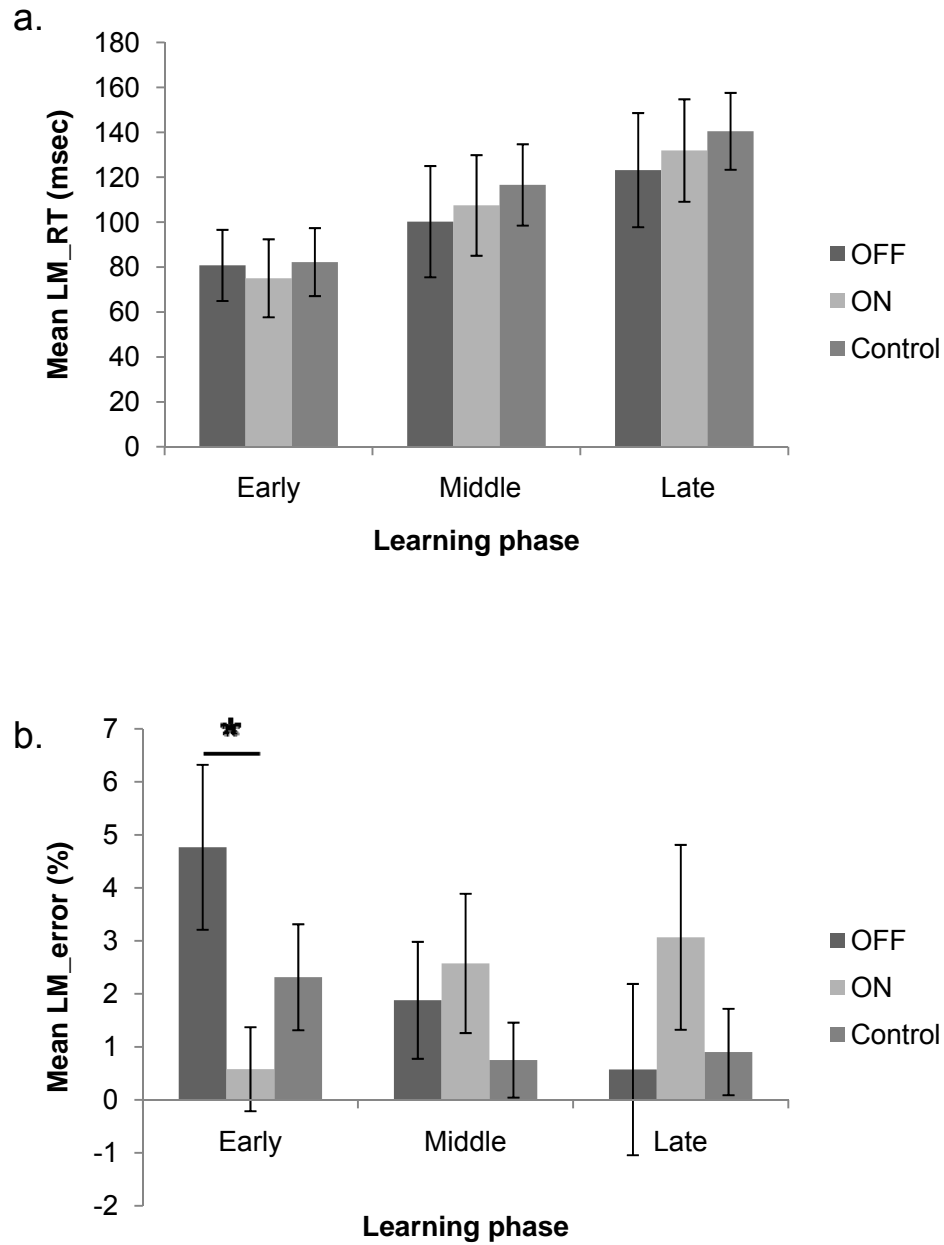
Repeated-measures ANOVAs were used to compare LM\_RT across the three learning phases across the three groups. For the PD OFF versus PD ON comparison, medication status (OFF vs. ON) and learning phase (early vs. middle vs. late) were within subject factors. For the PD OFF versus controls and PD ON versus controls comparisons, group (PD OFF or PD ON vs. controls) was a between subject factor and learning phase was a within subject factor. For all comparisons, I only found a significant main effect of learning phase (PD OFF and PD ON:  $F_{2,30} = 13.51$ ,  $p < 0.0001$ , PD OFF and controls:  $F_{2,70} = 21.97$ ,  $p < 0.0001$ , PD ON and controls:  $F_{2,72} = 35.75$ ,  $p < 0.0001$ ) and no other main effects or interactions (Fig. 3.1a). These results indicate that sequence learning across the three learning phases was present irrespective of group and medication status, and that there was no significant difference across groups in the magnitude of learning-phase specific sequence learning.

*Behavioral results: learning magnitude measured in error rate (LM\_error)*

LM\_error across the three learning phases was compared across groups. A similar analysis approach was followed as for LM\_RT. When comparing PD OFF and PD ON,

we found a significant medication status by learning phase interaction ( $F_{2, 30} = 3.37, p < 0.05$ , Fig 3.1b) and no main effect of medication status or learning phase. A follow up paired t-test comparing PD OFF and PD ON at each learning phase separately showed that there was a significant difference in the early phase ( $t_{15} = 2.30, p < 0.05$ , Fig. 3.1b) with greater LM\_error in PD OFF than PD ON. No significant difference in LM\_error between PD OFF and PD ON was found in either the middle or the late phase. When comparing PD OFF to controls, I only found a main effect of learning phase ( $F_{2, 72} = 5.78, p < 0.001$ , Fig. 3.1b) and no other main effects or interactions. Lastly, when comparing PD ON to controls, I found a significant group by learning phase interaction ( $F_{2, 70} = 3.59, p < 0.05$ , Fig. 3.1b) and no other main effects. A follow up independent sample t-test however, did not show any significant differences between PD ON and controls in any of the learning phases. These results indicate that sequence learning measured by error rate is impaired in PD ON versus PD OFF specifically in the early learning phase. It is also important to note that the behavioral findings are not confounded by a speed-accuracy trade off given that in general, response time and error rate changed in the same direction as shown in Appendix A - Supplementary Figure A.1.





**Figure 3.1.** Mean learning magnitude measure by response time (LM\_RT, a.) and error (LM\_error, b.) at each learning phase in controls, PD OFF and PD ON. Error bars indicate standard error.  $*p < 0.05$ .

### *fMRI results*

I first identified sequence learning-specific (i.e., activation that is greater for sequence than random blocks) neural activation for each learning phase in the three groups (i.e. controls, PD OFF and PD ON) separately. In the control group, I observed

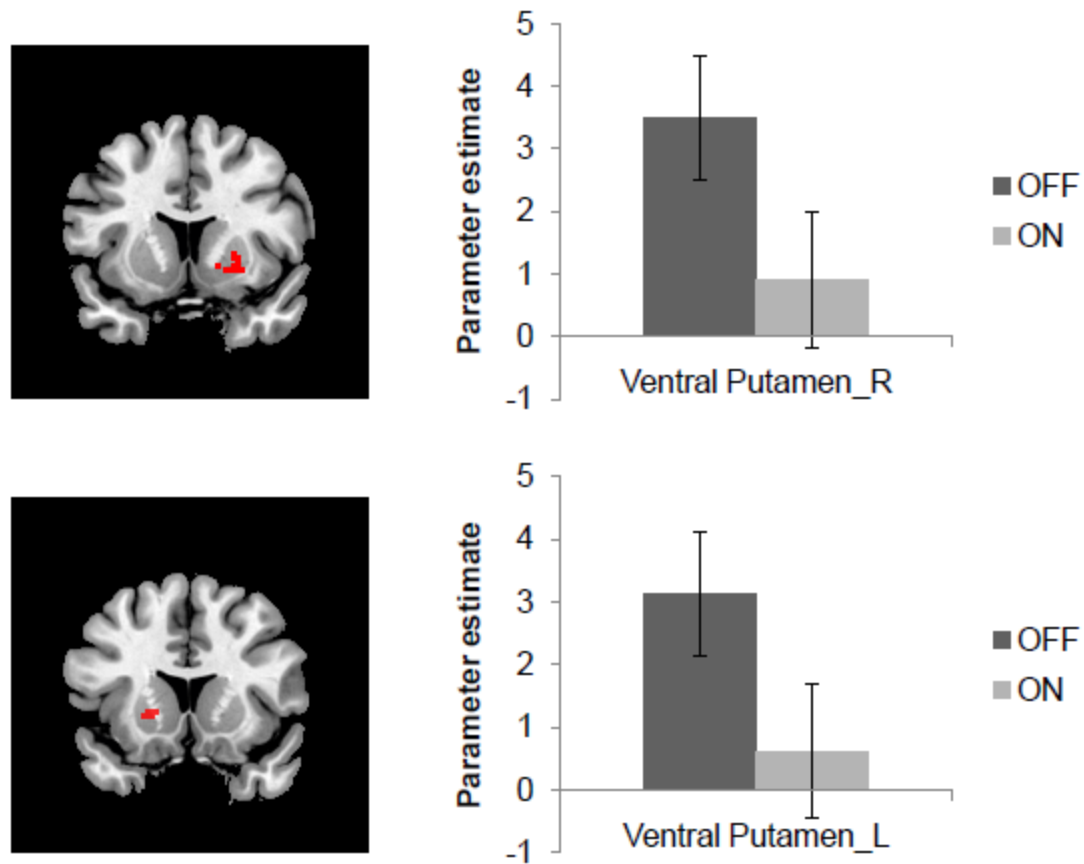
bilateral ventral putamen activation in the early learning phase; left precentral (BA 4) and superior temporal gyri (BA 38) in the middle learning phase; and left precentral gyrus (BA 4) in the late learning phase (Fig. 3.2, Table 3.2). In PD OFF, I observed activation in the bilateral caudate head and the right ventral putamen in the early learning phase and in the bilateral caudate head and the left precentral gyrus (BA 4) for the middle learning phase. For the late phase, there were no regions exhibiting greater activation for the sequence than random blocks above the threshold of  $p < 0.001$ . Since the purpose of this analysis was to confirm whether previously reported sequence learning brain activation patterns are also shown in the subject groups in a exploratory fashion and to qualitatively compare brain activation across the groups, I continued on with a more lenient threshold ( $p < 0.005$ , extent threshold of 10 voxels) for the late learning phase in PD OFF. I observed activation in the left middle frontal gyrus (BA 6) at this threshold in the late learning phase (Fig. 3.2, Table 3.2). For PD ON, I observed activation in the left middle frontal gyrus (BA 6) and precentral gyrus (BA 4/6) during the early learning phase, in the left medial frontal gyrus (BA 11) during the middle learning phase, and in the right precentral gyrus (BA 4) in the late learning phase (Fig. 2, Table 3.2).

**Figure 3.2.** Brain regions showing sequence learning specific neural activation at each learning phase. All clusters are significant at the level of  $p < 0.001$  uncorrected except for the cluster shown for PD OFF in the late phase.

**Table 3.2.** Brain regions showing sequence learning specific neural activation at each learning phase.

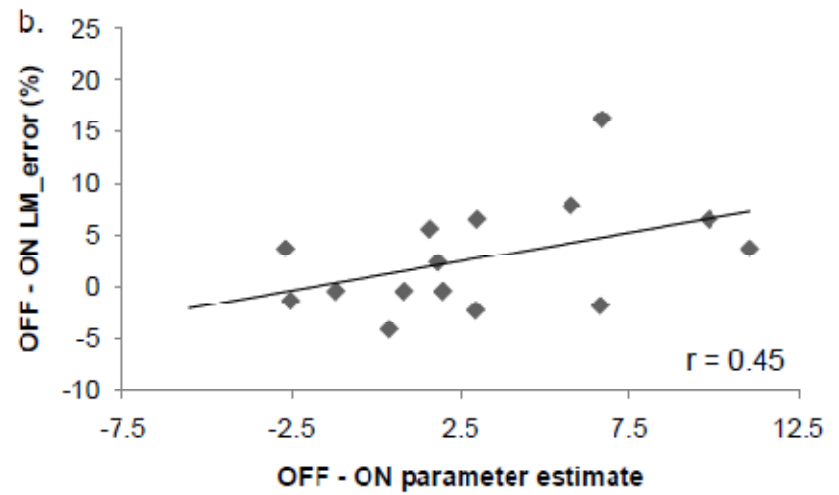
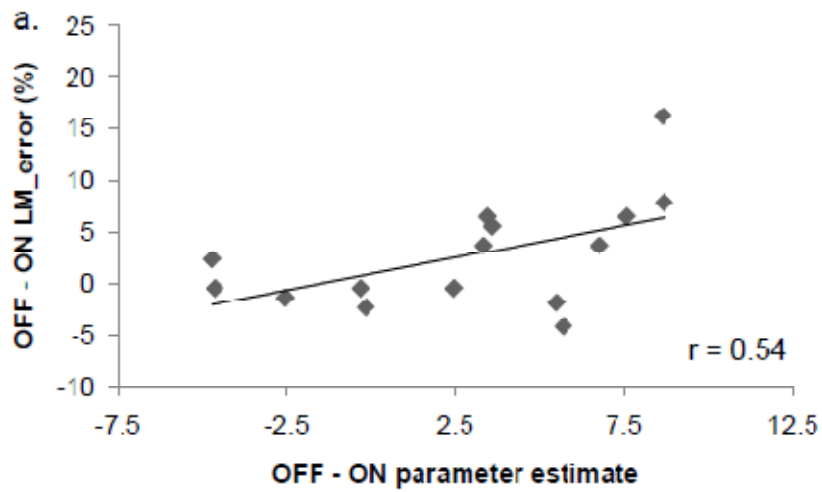
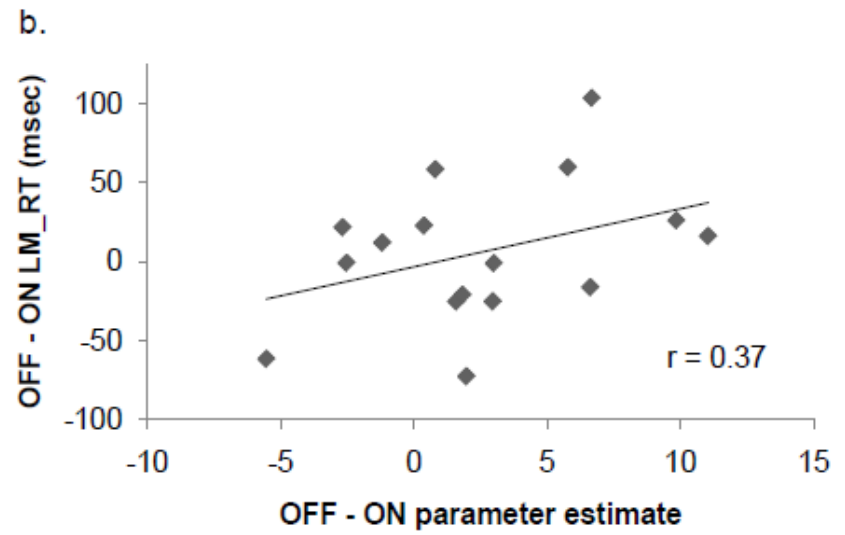
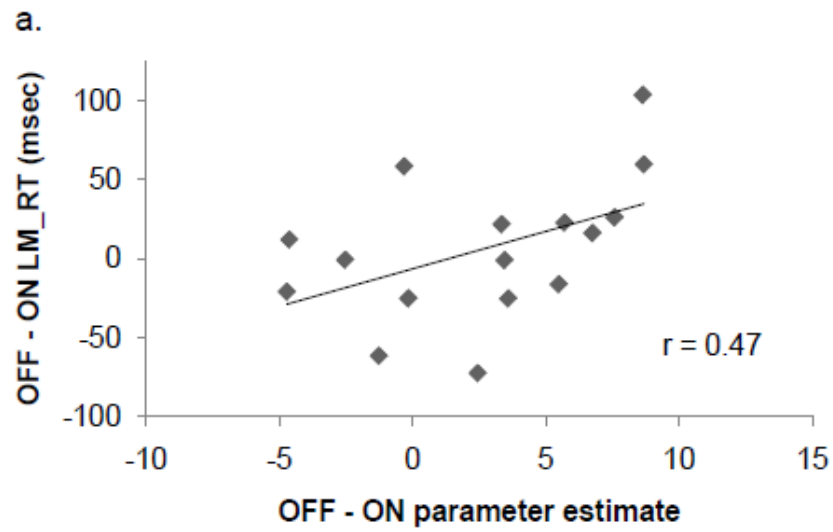
Region	R/L	BA	MNI coordinates			T value
			x	y	z	
<i>Controls</i>						
<i>Early</i>						
Ventral putamen	R		18	10	-6	5.05
Ventral putamen	L		-16	12	-6	3.93
<i>Middle</i>						
Precentral gyrus	L	4	-34	-24	58	5.48
Superior temporal gyrus	L	38	-46	8	-16	4.41
<i>Late</i>						
Precentral gyrus	L	4	-38	-20	56	3.66
<i>PD OFF</i>						
<i>Early</i>						
Caudate head	R		8	12	2	5.38
Caudate head	L		-8	16	2	4.1
Ventral putamen	R		20	10	-6	4.23
<i>Middle</i>						
Caudate head	L		-6	10	4	4.4
Caudate head	R		6	8	2	4.07
Precentral gyrus	L	4	-36	-20	52	4.12
<i>Late</i>						
Middle frontal gyrus	L	6	-28	-4	52	3.32
<i>PD ON</i>						
<i>Early</i>						
Middle frontal gyrus	L	6	-22	-4	56	4.03
Precentral gyrus	L	6	-44	-14	60	3.9
Precentral gyrus	L	4	-42	-22	64	3.97
<i>Middle</i>						
Medial frontal gyrus	L	11	-10	38	-12	5.39
<i>Late</i>						
Precentral gyrus	R	4	22	-16	62	4.25

Next, I compared sequence learning-specific activation in PD OFF versus PD ON using both ROI analyses and whole brain comparisons. I used functional ROIs created using the group statistics results from the control group. This allowed me to compare brain activation between the two medication states within regions that are involved in each sequence learning phase in normal controls. I used spherical ROIs centered on the bilateral ventral putamen (MNI coordinates: 18, 10, -6; -16, 12, -6) for the early learning phase, on the left precentral gyrus (MNI coordinate: -34, -24, 58) and superior temporal gyrus (MNI coordinate: -46, 8, -16) for the middle learning phase, and on the left precentral gyrus (MNI coordinate: -38, -20, 56) for the late learning phase. I found a significant difference in sequence learning related activation between PD OFF and PD ON in the early learning phase within the two ventral putamen ROIs. Specifically, PD OFF exhibited greater activation than PD ON in these ROIs (Fig. 3.3). That is, there was greater activation for PD ON than PD OFF in the early learning phase in the ventral putamen ROIs. The ROI analysis comparing PD OFF and PD ON in the middle and late learning phases did not show any significant differences. In order to confirm whether the greater ventral putamen activation in PD OFF versus PD ON was selective for the early learning phase, I also applied the ventral putamen ROIs to the middle and late learning phases. There were no significant differences between the patient groups for either of these two learning phases.



**Figure 3.3.** Ventral putamen ROI analysis showing decreased activity for PD ON compared to PD OFF in the early learning phase.  $p < 0.025$  was used for comparison within ventral putamen ROIs.

To determine whether the greater ventral putamen activation for PD OFF than PD ON during the early learning phase is related to behavioral differences, I evaluated correlations between the behavioral and neural activation differences. To this end, I used the PD OFF – ON mean beta estimates across all voxels of the ventral putamen clusters and the PD OFF – ON sequence learning magnitude difference from the early learning phase. Both LM\_RT and LM\_error were used in this analysis. I used a one-tailed statistical threshold with the hypothesis that there would be a positive correlation between the two. For LM\_RT, I found a significant positive correlation between these two OFF and ON difference measures for the right ventral striatum ( $r = 0.47$ ,  $p < 0.05$ , Fig. 3.4a) and a trend for a positive correlation for the left ventral striatum ( $r = 0.37$ ,  $p = 0.08$ , Fig. 3.4b). For LM\_error, I also found a significant positive correlation between these two OFF and ON difference measures for the right ventral striatum ( $r = 0.54$ ,  $p < 0.05$ , Fig. 3.5a) and the left ventral striatum ( $r = 0.45$ ,  $p < 0.05$ , Fig. 3.5b), however this was only after removing one outlier.





**Figure 3.4 (top).** Correlation between L-DOPA associated right (a.) and left (b.) ventral putamen activation decrease and sequence learning deficit. The Y axis shows the difference in mean beta estimates in the right/left ventral putamen and the X axis shows the difference in learning magnitude (LM\_RT) between PD OFF and PD ON.

**Figure 3.5 (bottom).** Correlation between L-DOPA associated right (a.) and left (b.) ventral putamen activation decrease and sequence learning deficit. The Y axis shows the difference in mean beta estimates in the right/left ventral putamen and the X axis shows the difference in learning magnitude (LM\_error) between PD OFF and PD ON.

## **Discussion**

The current study examined the effect of L-DOPA on neural activity over the time course of motor sequence learning in early stage PD patients. Based on results from Chapter 2, I predicted a significant negative effect of L-DOPA on the early phase of sequence learning. In particular, I hypothesized that L-DOPA would decrease ventral striatum activation and that the degree of L-DOPA-associated decrease in activation would correlate with the magnitude of the behavioral deficits.

Although the behavioral data showed mixed results in terms of whether L-DOPA impaired early sequence learning, there was an L-DOPA-associated decrease in ventral putamen activation during the early learning phase. Furthermore, the degree of activation decrease correlated with the extent of behavioral deficits shown across the patients. These results support a dopamine overdose effect that is specific to the early phase of sequence learning, which relies on the ventral striatum.

When learning magnitude measured by error rate was compared between PD OFF and PD ON, I found a selective L-DOPA associated decrease in learning in the early phase. In addition, whereas I did not find any learning differences between PD OFF and controls across learning phases, I did find a differential pattern of learning change across phases between PD ON and controls, with reduced learning in PD ON during the early

phase shown by the significant group by phase interaction. One thing to notice here is that the learning magnitude per se is decreasing across phases in both controls and PD OFF but not in PD ON. This could partially be driven by the relatively large error rate in the very first random block. To minimize the error rate in the first block I included a practice session of 5 minutes before the scan. The results might also indicate that performing the task in the early phase involves primarily error-based learning but with more practice this effect decays. The current data indicate that early to moderate stage PD patients are not impaired in sequence learning measured in the form of error-driven learning that is particularly engaged during the early phase. Although there are not many studies investigating sequence learning using error rate, evidence shows that error processing contributes to sequence learning (Russeler et al., 2003, Seidler et al., 2007, Ferdinand et al., 2008). Studies have shown that PD patients are not impaired in learning from negative feedback, and error commission may be considered negative feedback. L-DOPA may interfere with this form of learning, however (Frank et al., 2004, Rutledge et al., 2009, Seo et al., 2010). These results are in line with the ‘dopamine-overdose’ hypothesis (Cools, 2006). In mild to moderate stage PD patients the ventral striatal system involved in error-based learning (McClure et al., 2003, O’Doherty et al., 2003, Haruno and Kawato, 2006, Schonberg et al., 2007, Glascher et al., 2010) is relatively intact (Bernheimer et al., 1973, Kish et al., 1988, Frey et al., 1996, Rakshi et al., 1999). Thus when medication selectively overdoses the ventral striatum performance decreases.

The behavioral data showed inconsistent results regarding the effects of L-DOPA on sequence learning. Firstly, I failed to replicate my finding of an L-DOPA associated decrease in early sequence learning as measured with reaction time, reported in Chapter

2. There are a few methodological differences between the current chapter and Chapter 2 which may have led to this discrepancy. One possible explanation is that I used a constant inter-trial interval (ITI) in the current design as opposed to a constant response-to-stimulus interval (RSI). The reason that I used the constant ITI instead of the RSI in the current study is because it results in a steady movement frequency which might itself affect BOLD signal change. In the case of a constant ITI, the subject's response is paced which might interfere with response time changes. That is, initially when subjects are not used to the pace of the stimuli presentation the task might feel more challenging. However as they learn the sequence they might also not try to maximize their speed as much as they could since the next stimulus appears after a constant interval regardless of how fast they respond in the current trial. A combination of these issues could have blurred the L-DOPA associated learning changes in the current data.

Another difference between the two studies is that I used a single dose of L-DOPA (200 mg) across patients in the current study, rather than patient's individual dose of dopaminergic drugs titrated in the clinic based on their response as in Chapter 2. Although the dosage we used improved their motor symptoms measured by UPDRS overall, the effects were small. Additionally, the use of L-DOPA alone in the current study as opposed to the combination of L-DOPA and dopamine agonists used in Chapter 2 as part of the patients' regular treatment, may have reduced the medication-associated early learning impairment. Studies have shown that the effect of dopaminergic agents (either improving or impairing performance) is particularly significant with D2 receptor agonists and less apparent with L-DOPA (Kimberg et al., 1997, Roesch-Ely et al., 2005, Frank and O'Reilly, 2006, Cools et al., 2007a, Cools et al., 2007b, Cools et al., 2009,

Frank and Fossella, 2011).

The explicit sequence learning task in the current study involved neural structures that are typically shown in motor sequence learning paradigms. A dynamic shift in neural recruitment across the time course of learning was also present as demonstrated in previous studies (Hikosaka et al., 1998, Toni et al., 1998, Doyon et al., 2002, Muller et al., 2002, Seidler et al., 2002, Doyon et al., 2003, van Mier et al., 2004, Floyer-Lea and Matthews, 2005, Lacourse et al., 2005, Lehericy et al., 2005, Poldrack et al., 2005, Bapi et al., 2006, Duff et al., 2007, Sun et al., 2007, Tamas Kincses et al., 2008). Sequence learning related activation was prominent in the ventral/anterior striatum during the early phase and towards the late phase activation was found in the cortical motor areas. These results are in line with prior evidence regarding the shift from the associative striatum pathway during the early phase to the sensorimotor striatum pathway in the late phase (Berns et al., 1997, Hikosaka et al., 2002, Doyon et al., 2003, Lehericy et al., 2005, Bapi et al., 2006, Duff et al., 2007, Tamas Kincses et al., 2008). The involvement of the anterior / ventral putamen in the early phase may also be due to the engagement of movement planning in this structure which includes predicting and selecting movements (Vaillancourt et al., 2007, Prodoehl et al., 2009).

Despite the inconsistent behavioral results, there were also characteristic differences in the pattern of neural recruitment across the three groups. While in controls and PD OFF a prominent ventral striatum activation was observed in the early phase, I did not observe this in PD ON. Rather, activation for PD patients on L-DOPA was confined to the cortical motor areas. These results demonstrate that L-DOPA disrupts the normal recruitment of the ventral striatum during the early phase of motor sequence

learning. To determine whether L-DOPA actually changes the ventral striatum recruitment I compared the activation between PD OFF and PD ON within the ventral striatum ROIs defined based on the control group's pattern of early learning phase activation. In accordance with our hypothesis, I found a significant decrease in bilateral ventral striatal activation in PD ON compared to PD OFF. Moreover the degree of reduction in ventral striatum activation due to L-DOPA correlated with the degree of the L-DOPA associated decrease in sequence learning performance. This pattern was more consistent with learning magnitude measured by response time. The pattern was only significant after removing an outlier with learning magnitude measured by error rate. Considering that response time is a more widely used behavioral metric for motor sequence learning (Rhodes et al., 2004) and that the correlation between the L-DOPA associated behavioral change BOLD signal was not strong, future studies need to be done in order to replicate these results. These results are in line with a previous study examining the effect of L-DOPA on probabilistic reversal learning, which has been shown to be a task relying primarily on the ventral striatum, more specifically the nucleus accumbens (Cools et al., 2007a). In this study, reversal learning-associated activation in the nucleus accumbens was compared across PD OFF and PD ON L-DOPA. Despite the lack of group level behavioral differences, relatively lower nucleus accumbens activation was found in PD ON compared to OFF L-DOPA during reversal learning.

The changes in neural activation associated with PD and L-DOPA were not apparent in the late phase of motor sequence learning. The cortical motor areas were involved in all three groups, but the precise loci of activation differed. Whereas in controls, the activation was shown in the primary motor cortex of the left hemisphere

which is in line with previous findings in motor sequence learning (for review see Ashe et al., 2006, Serrien et al., 2006), in PD ON the activation was found in the right hemisphere. In PD OFF although activation was found in the dorsal premotor area (BA 6) of the left hemisphere, this was only when using a more lenient threshold. These results indicate that the recruitment of the sensorimotor striatal circuitry encompassing the motor cortical regions is disturbed in PD OFF. This may be due to the specific loss of dopaminergic activity in the dorsal/posterior (i.e. sensorimotor) striatum in PD patients. ROI analysis comparing PD OFF and PD ON within the left motor cortex ROI did not reveal any differences in activation. Despite the lack of activation differences, it should be noted that I found activation in the right motor cortex for PD ON, whereas I did not find any motor cortical activation for PD OFF using the same threshold ( $p < 0.001$ , uncorrected). This suggests that L-DOPA restores the activation of the sensorimotor cortico-striatal circuitry which is in line with previous studies (Jubault et al., 2009).

Collectively the current data showed that L-DOPA impacts motor sequence learning and the affiliated neural circuitries in a time varying fashion. Specifically, I found evidence that L-DOPA impaired sequence learning and sequence learning associated ventral striatum activation in the early phase of learning. Moreover the degree of L-DOPA associated learning changes correlated with the degree of L-DOPA associated ventral striatum activation change. These findings provide evidence for the differential effects of L-DOPA across multiple cortico-striatal loops involved during motor sequence learning in PD.

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## CHAPTER IV

### **Individual differences in degree of nigrostriatal dopaminergic denervation correlate with L-DOPA effects on motor sequence learning in Parkinson's disease**

#### **Abstract**

Striatal dopaminergic denervation is not uniform in Parkinson's disease (PD). There is a gradient with more severe denervation in the dorsal and posterior striatum and relative sparing of the anterior and ventral regions in the early stages of the disease. It has been shown that dopaminergic medication can interfere with ventral and anterior striatum function by overdosing this relatively intact structure (Cools, 2006). I have reported a "dopamine-overdose" effect during motor sequence learning in patients with PD in Chapter 2. Specifically, I found that dopaminergic medications selectively interfere with performance in the early phase of motor sequence learning which relies on the ventral and anterior striatum. In the current study, I investigated whether the degree of medication-associated sequence learning impairment is explained by individual differences in the level of dopaminergic denervation in striatum. Eighteen mild to moderate stage PD patients performed an explicit motor sequence learning task ON and OFF L-DOPA in a single blind placebo control procedure. The patients also underwent a

<sup>11</sup>C-dihydrotetrabenazine (<sup>11</sup>C-DTBZ) PET scan to measure striatal dopaminergic denervation. The percent decrease in <sup>11</sup>C-DTBZ binding potential was computed in six different striatal subregions. I found greater impairment in the level of medication-associated sequence learning in patients with more preserved <sup>11</sup>C-DTBZ binding. Furthermore the ratio of denervation in the anterior to posterior dorsal putamen predicted the level of dopamine overdose. These results indicate that L-DOPA is more deleterious to early motor sequence learning for patients with less striatal denervation and more intact anterior versus posterior striatum. In contrast, L-DOPA is beneficial to performance for patients with relatively greater denervation in the anterior versus posterior striatum. Collectively the results demonstrate that individual differences in the spatial pattern of dopaminergic denervation are predictive of medication responsiveness.

## **Introduction**

Progressive denervation of nigrostriatal dopaminergic neurons is a key pathobiological mechanism in Parkinson's disease (PD). Striatal dopaminergic denervation is not uniform in PD. First, dopaminergic denervation begins asymmetrically and then becomes bilateral later in the disease (Hornykiewicz, 1966). Second, there is a dorsal to ventral and caudal to rostral gradient in dopamine (DA) depletion. Specifically, depletion is greater in the dorsal and posterior striatum as opposed to the ventral and anterior striatum early in the disease, with the ventral and anterior striatum becoming more involved in later disease stages (Bernheimer et al., 1973, Kish et al., 1988, Frey et al., 1996, Rakshi et al., 1999).

Anti-Parkinsonian dopaminergic medications such as L-DOPA and dopamine agonists have been shown to result in a so-called “dopamine over-dose” effect for certain cognitive tasks (Gotham et al., 1988, Swainson et al., 2000, Cools et al., 2001, Frank et al., 2004, Frank, 2005, Cools, 2006, Cools et al., 2006). The presumed basis for this hypothesis is that dopaminergic medications can interfere with the normal cognitive performance associated with the ventral and rostral striatum by overdosing this region which is relatively intact in the early disease stage (Cools et al., 2001, Cools, 2006).

I reported similar effects on the time course of motor sequence learning in PD patients in Chapter 2 that may be reflective of dopamine overdose effects. Specifically, I found that dopaminergic medications selectively interfere with performance in the early phase of motor sequence learning, which is known to rely on the ventral and anterior striatum (Lehericy et al., 2005). However, I also observed a large degree of variance

across individuals in the magnitude of the presumed overdose effect. While some individuals showed a large impairment in early learning with dopaminergic medication, others exhibited only little effects. One possible factor that could explain some of the variance in the degree of overdose effect is individual heterogeneity in regional striatal dopaminergic denervation. That is, the level of early learning impairment for a given dose of medication should be larger for patients with less denervation of the ventral and anterior striatum. Furthermore, based on the preferential contribution of the anterior / ventral striatum in early motor sequence learning, individual differences in the ratio of regional denervation along the anterior to posterior or ventral to dorsal striatal gradient may also contribute to the level of medication associated impairment. Despite the fact that several studies have investigated the dopamine overdose effect for cognitive tasks (Gotham et al., 1988, Swainson et al., 2000, Cools et al., 2001, Frank et al., 2004, Frank, 2005, Cools, 2006, Cools et al., 2006), none have associated the degree of this overdose effect with patients' individual degree of regional striatal dopaminergic denervation or the ratio of regional striatal denervation.

Several previous studies have looked at the association between motor sequence learning performance and the level of dopamine (DA) transmission (Lawrence et al., 1998, Goerendt et al., 2003, Carbon et al., 2004, Carbon and Eidelberg, 2006, Badgaiyan et al., 2007). These studies report a significant correlation between performance of healthy controls (Lawrence et al., 1998, Badgaiyan et al., 2007) or PD patients OFF medication (Goerendt et al., 2003, Carbon et al., 2004, Carbon and Eidelberg, 2006) and DA activity in striatal regions. However they did not separately examine different

sequence learning phases, nor did they compare performance for patients ON and OFF medication, both of which are crucial for evaluating the dopamine-overdose hypothesis.

In the current study I evaluated whether there is a relationship between the level of dopamine overdose effect and levels of regional striatal dopaminergic denervation or the ratio of anterior to posterior or ventral to dorsal striatal denervation. The level of dopaminergic denervation in multiple sub-regions of the striatum was measured by  $^{11}\text{C}$ -dihydrotrabenazine ( $^{11}\text{C}$ -DTBZ) and positron emission tomography (PET). The ratio of denervation between the anterior or ventral versus posterior or dorsal sub-regions was computed from  $^{11}\text{C}$ -DTBZ PET.  $^{11}\text{C}$ -DTBZ is a ligand that binds to the type-2 vesicular monoamine transporter, which is a target for quantitative imaging of nigrostriatal synaptic terminals (Bohnen et al., 2006), where the signal is >95% DA. Thus low binding signals in a  $^{11}\text{C}$ -DTBZ PET scan would imply more severe denervation of nigrostriatal nerve terminals in the striatum, and therefore depletion of the neurotransmitter DA.  $^{11}\text{C}$ -DTBZ PET has been used extensively to measure the integrity of the nigrostriatal dopaminergic nerve terminals in PD (Frey et al., 1996, Lee et al., 2000, Frey et al., 2001, De La Fuente-Fernandez et al., 2003, Gilman et al., 2004, Koeppe et al., 2005, Bohnen et al., 2006, Raffel et al., 2006, Sossi et al., 2007, Collantes et al., 2008, Koeppe et al., 2008, Bohnen et al., 2010).

In order to assess the relationship between the level of dopamine overdose and striatal dopaminergic denervation, I determined the association between the level of striatal denervation and the effect of a single dose of levodopa on motor sequence learning. I also determined the correlation between the two measures using the ratio of anterior to posterior and ventral to dorsal striatal denervation. I hypothesized that there



would be a significant correlation between the two measures, illustrating that the deleterious effect of L-DOPA in sequence learning behavior would be more pronounced for patients with less denervation and relatively more intact anterior / ventral versus dorsal / posterior striatum.

## **Materials and Methods**

### Participants

Eighteen PD patients ( $65 \pm 8$  yrs, 4 females; Hoehn and Yahr (H & Y) stages 1 – 3, Hoehn and Yahr, 1967) participated in this study. This was a separate cohort of patients than those reported in my first study (Chapter 2). Patients were recruited for behavioral testing within 6 months from their  $^{11}\text{C}$ -DTBZ PET scan. Patients were excluded for any neurological or psychiatric disease other than PD. Patients were included as long as they were on a stable dosage of dopaminergic medications for the previous 6 months, and were evaluated using the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS, Fahn et al., 1987) by a neurologist. All study participants underwent the Mini-Mental State Exam (Folstein et al., 1975) and the Montreal Cognitive Assessment (MOCA, Nasreddine et al., 2005). The demographic and clinical characteristics of the patients are listed in Table 4.1. Participants were compensated for their participation, which included two testing days for PD patients. All participants signed a consent form approved by the Institutional Review Board of the University of Michigan.

**Table 4.1.** Demographic and clinical variables

	Age	Gender	Disease Duration	Affected body side	UPDRS ON	UPDRS OFF	H & Y	MMSE ON	MMSE OFF	MOCA ON	MOCA OFF
PD_01	52	M	5	Right	16	20	2.5	30	30	26	30
PD_02	60	M	2	Right	10	9	1.5	30	29	30	27
PD_03	74	M	5	Right	20	17	2	28	29	26	27
PD_04	62	F	6	Left	7	9	1.5	28	30	28	29
PD_05	67	M	10	Left	23	29	2.5	29	29	29	28
PD_06	69	M	3	Right	26	28	3	28	30	25	25
PD_07	67	M	5	Left	20	23	2	30	28	26	26
PD_08	69	M	3	Right	33	34	3	27	29	29	27
PD_09	63	M	3	Right	28	29	2	29	28	28	24
PD_10	64	M	8	Left	33	25	2.5	30	30	30	30
PD_11	57	M	9	Both	7	19	2.5	30	30	29	30
PD_12	60	F	3	Left	8	3	1	30	30	25	27
PD_13	55	M	3	Left	18	18	2	30	30	29	27
PD_14	79	M	4	Right	27	30	2.5	27	27	25	21
PD_15	61	M	2	Right	15	14	2	29	29	26	26
PD_16	65	F	2	Left	13	11	2	29	30	26	29
PD_17	68	M	<1	Right	14	15	2	29	29	30	22
PD_18	84	F	4	Left	25	29	3	30	30	27	21
Average	65±8		5±2		19±8	20±9	2±1	29±1	29±1	27±2	26±3

## Procedure

PD patients underwent two testing days corresponding to the ON and OFF medication states, separated by no more than two weeks. Seven patients were tested OFF first and 11 ON first. I used a single blind placebo controlled design with a single dose of L-DOPA (200 mg) across all PD patients. Using a single dose of L-DOPA allowed us to reduce the variability of the overdose effect (e.g. difference in dosage and type of medication). PD patients attended both testing days in the OFF state achieved by withdrawal from medication 12 - 18 hours prior to testing. For the ON testing day, patients received a 50 mg dose of carbidopa followed after 30 minutes by a single dose of L-DOPA in combination with carbidopa (200 mg of L-DOPA and an additional 50 mg of carbidopa). For the OFF testing day, patients received placebo medications following the same time schedule in combination with the 50 mg of carbidopa. The L-DOPA dosage I used was tolerable to all participants. All study procedures began one hour after the patient had taken either L-DOPA or the placebo, by which time L-DOPA reaches its peak plasma dose. On each testing session, participants performed an explicit motor sequence learning task (see below for details). The UPDRS motor section was assessed in both ON and OFF sessions. Additionally, the Grooved Pegboard Test (Lafayette Instruments, Lafayette, IN) was used to measure upper limb motor abilities.

## Explicit Motor Sequence Learning

I used the same paradigm as in my previous study with slight modifications (Chapter 2). Participants were instructed to press a key-press device with the index and

middle fingers of each hand (bimanual responses) in response to a corresponding visual stimulus presented on a computer screen. They were presented with a 6-element sequence (i.e. 1, 3, 2, 3, 4, 2) repeatedly for the sequence blocks (S) and with pseudo-randomized stimuli presented in a non-repeating fashion, but restricted to keep the same statistical structure as the sequence for the random blocks (R). Participants were explicitly told that there was a sequential pattern to the stimuli presentation during sequence blocks. In order to increase the explicitness of the sequence, the first element of the repeating sequence was shown as a red “X” whereas the other stimuli were presented in black. They were informed of which of the two blocks (i.e. R or S) they would be performing at the beginning of each block. If participants failed to respond by pushing the correct button, the same stimulus location was presented again on the next trial. In total there were 6 learning runs, each including both random (R) and sequence (S) blocks. The order of R and S blocks within a learning run were as follows: R-S-S-S-R. Random blocks consisted of 18 button presses and sequence blocks consisted of 36. The number of sequence trials at each learning run was equivalent to one sequence block in my previous study (Chapter 2). Each trial was spaced by a constant inter-stimulus interval of 1250 ms from stimulus onset with 200 ms of stimulus duration. Patients were presented with different sequences on the two testing days.

### MRI and <sup>11</sup>C-DTBZ PET

MRI and <sup>11</sup>C-DTBZ PET data were acquired in 17 out of the 18 patients. Magnetic resonance imaging was performed on a 3 Tesla Philips Achieva system

(Philips, Best, The Netherlands) utilizing an eight-channel head coil and the 'ISOVOX' exam card protocol primarily designed to yield isotropic spatial resolution. A standard T1-weighted series of a 3D inversion recovery-prepared turbo field echo was performed in the sagittal plane using repetition time/echo time/inversion time = 9.8/4.6/1041 ms; turbo factor = 200; single average; field of view = 240×200×160 mm; acquired matrix = 240×200. One hundred and sixty slices were reconstructed to 1mm isotropic resolution. This sequence maximizes contrast among grey matter, white matter and cerebrospinal fluid and provides high-resolution delineation of cortical and subcortical structures.

<sup>11</sup>C-DTBZ PET imaging was performed in 3D imaging mode using an ECAT HR+ tomograph (Siemens Molecular Imaging, Inc., Knoxville, TN), which acquires 63 transaxial slices (slice thickness = 2.4 mm; intrinsic in-plane resolution = 4.1 mm full width at half maximum over a 15.2 cm axial field of view). A NeuroShield (Scanwell Systems, Montreal, Canada) head-holder/shielding unit was attached to the patient bed to reduce the contribution of detected photon events originating from the body outside the scanner field of view (Thompson et al., 2001). Prior to the <sup>11</sup>C-DTBZ injections, a 5 min transmission scan was acquired using rotating <sup>68</sup>Ge rods for attenuation correction of emission data using the standard vendor-supplied segmentation and re-projection routines. No-carrier-added (+)-<sup>11</sup>C-DTBZ (250–1000 Ci/mmol at the time of injection) was prepared as reported previously (Jewett et al., 1997). Dynamic PET scanning was performed for 60 min immediately following a bolus injection of 55% of 666 mega-Becquerel (18 milli-Curies) of (+)-<sup>11</sup>C-DTBZ dose (containing less than 50 µg of cold <sup>11</sup>C-DTBZ mass) over the first 15–30 s of the study, while the remaining 45% of the dose was continuously infused over the next 60 min, resulting in stable arterial tracer levels

and equilibrium with brain tracer levels after 30 min (Koeppel et al., 1997). A series of 15 frame sequence of scans over 60 min were obtained as following: four×30 s; three×1 min; two×2.5 min; two×5 min; and four×10 min. All subjects were studied supine, with eyes and ears unoccluded, resting quietly in a dimly lit room. <sup>11</sup>C-DTBZ images were analyzed using equilibrium modeling to estimate the non-displaceable binding potential (BPND), which is equivalent to the ratio of specific (VS) to non-displaceable (VND) binding in each imaged voxel or target volume of interest (Koeppel et al., 1997). Specific (+)-<sup>11</sup>C-DTBZ binding was estimated by subtraction of the global neocortex value, a reference region very low in VMAT2 binding sites, with the assumption that the non-displaceable distribution is uniform across the brain at equilibrium (Koeppel et al., 1999).

#### VOI Implementation and Data Extraction

T1-weighted MR and <sup>11</sup>C-DTBZ PET datasets were spatially co-registered within subjects with a rigid-body transformation (Minoshima et al., 1993). Volumes of interest (VOI) were manually traced on the MR image for each individual participant to include the striatum of each hemisphere by a trained technologist. The level of dopaminergic denervation from the PET scans was determined in the following regions: anteroventral striatum, middle caudate, caudate head, ventral, dorsal anterior and dorsal posterior putamen (Fig. 4.2.a.). Across the number of axial slices spanning the caudate, the dorsal half of the slices was defined as the caudate head. The ventral half was divided into two subregions, the upper half of which was defined as the middle caudate and the bottom half as the anteroventral striatum. The dorsal half of the axial slices of the putamen was

defined as the dorsal putamen and the ventral half as the ventral putamen. The dorsal putamen was again divided into dorsal anterior and posterior across the midline spanning the anterior-posterior axis. All MR-drawn VOIs were transferred to the PET data for regional sampling of the  $^{11}\text{C}$ -DTBZ binding potential (BP) from the radioactive activity. The more and less disease affected hemisphere was defined as being contralateral to the clinically more and less affected body side. The clinically more and less affected body side was determined by patient report and confirmed by the neurologist (N.B.). Percent decrease of  $^{11}\text{C}$ -DTBZ binding potential from the average binding potential of a group of healthy age-matched control subjects was computed for each VOI in each patient using the following equation:  $(\text{BP}_{\text{control}} - \text{BP}_{\text{PD}}) / \text{BP}_{\text{control}} \times 100$ .

### Data Analysis

Sequence learning performance in each learning run was measured by the learning magnitude calculated by RT (LM\_RT) or error rate (LM\_error). LM\_RT was calculated as the difference in response time between R and S blocks. Median RT was computed for each block and was used in the following equation (Appendix B - Supplementary Figure B.1.a.):  $\text{LM\_RT} = \text{average median RT of the two R blocks} - \text{average median RT of the three S blocks within a run}$ . The error trials (i.e., errors by commission and omission), and trials immediately following an error were excluded from RT analyses. LM\_error was calculated in a similar fashion. Error rate was computed for each block and was used in the following equation (Appendix B - Supplementary Figure B.2.b.):  $\text{LM\_error} = \text{average error rate of the two R blocks} - \text{average error rate of the}$

three S blocks within a run. The first two runs were considered as the early phase, the second two runs as the middle phase, and the last two runs as the late phase. Higher LM\_RT and LM\_error reflect a stronger learning effect.

In order to confirm the characteristic spatial distribution of striatal denervation, I compared % BP decrease across the 6 striatal VOIs in the more and less affected hemispheres using repeated measures ANOVA. The Huynh-Feldt epsilon (Huynh and Feldt, 1970) was used to determine whether the repeated measures data met the assumption of sphericity ( $\epsilon > 0.75$ ).

The association between the effect of a single dose of L-DOPA on learning performance and the level of striatal dopaminergic denervation was explored by correlating sequence learning performance difference ON and OFF L-DOPA and the level of striatal denervation. I used the following two approaches to systematically look at striatal denervation in PD patients. First, I used factor analysis using principal component as extraction method to determine latent variable that explains the variance across the 12 striatal VOIs. The factor scores derived from component factor weightings were used in a linear regression model to predict sequence learning performance change with L-DOPA. Secondly, the within-subject ratios of the anterior to posterior and ventral to dorsal striatal denervation were used in a linear regression model to predict L-DOPA associated learning changes. The within-subject ratios of the anterior to posterior and ventral to dorsal striatal denervation were computed using the following metrics: % BP decrease in dorsal anterior putamen / dorsal posterior putamen, ventral putamen / (averaged dorsal anterior and posterior putamen) and anteroventral striatum / (averaged middle caudate



and caudate head). For each VOI, I averaged across the more and less affected hemisphere for computing the ratio. All statistical analyses were performed using SPSS.

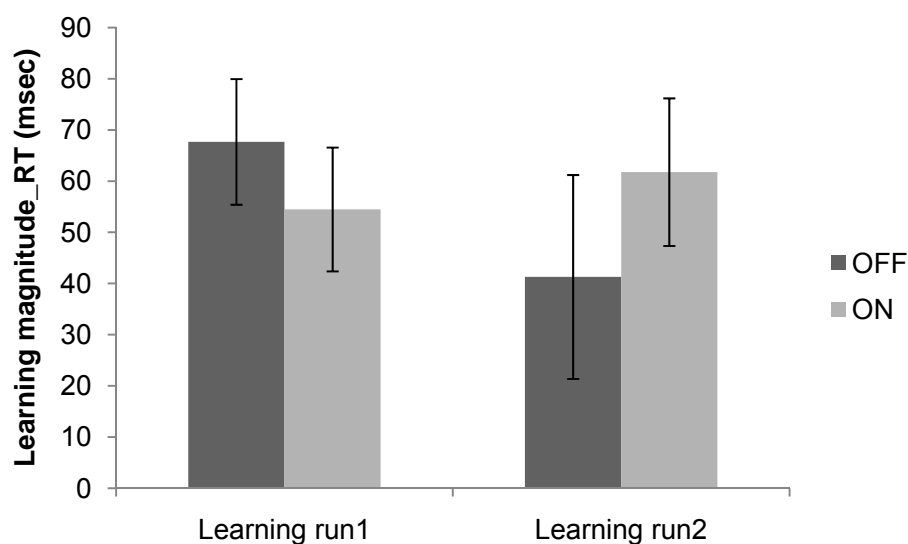
## **Results**

### Behavioral Results

Grooved pegboard could not be assessed in one of the patients due to fatigue. No significant ON and OFF L-DOPA performance difference was found in any of the neuropsychological and quantitative motor assessments including the UPDRS.

Based on my previous report of a selective L-DOPA associated learning impairment in the early phase of learning (Chapter 2), I focused my analysis on the first two learning runs which constitute the early phase. The number of sequence trials within the early phase in the current study was equivalent to that of the early phase in my previous study (Chapter 2). Sequence learning measured by LM\_RT and LM\_error ON and OFF L-DOPA was compared across the two learning runs in the early phase. For LM\_RT, I found no significant main effect of medication or learning run. There was only a trend of medication by learning run interaction ( $F_{1,17} = 3.97$ ,  $p = 0.06$ ). A follow up paired t-test comparing LM\_RT ON and OFF L-DOPA at each learning run showed a trend of ON and OFF difference in the first run ( $t_{17} = 2.08$ ,  $p = 0.05$ ) but no significant difference in the second run. Mean LM\_RT was greater in the OFF ( $67.68 \pm 52.10$ ) than ON ( $54.48 \pm 51.36$ ) L-DOPA state (Fig. 4.1.). The same analysis was performed on the early phase LM\_error. There were no main effects or interaction across the two learning runs in LM\_error. These results indicate that the deleterious effect of L-DOPA on

sequence learning was not present throughout the early phase but only marginally present in the very first learning run of the early learning phase as shown by LM\_RT and not by LM\_error at the group level. Because my main interest here was whether individual differences in the magnitude of this effect relate to the extent of dopaminergic denervation in striatal regions, I performed the subsequent correlation analyses using ON and OFF LM\_RT difference in the first learning run.

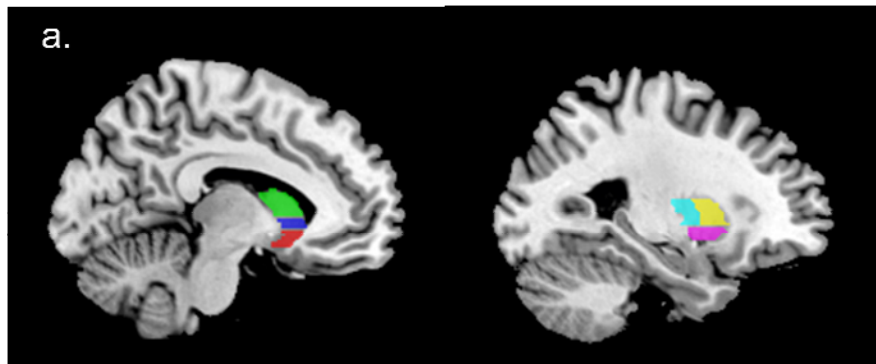


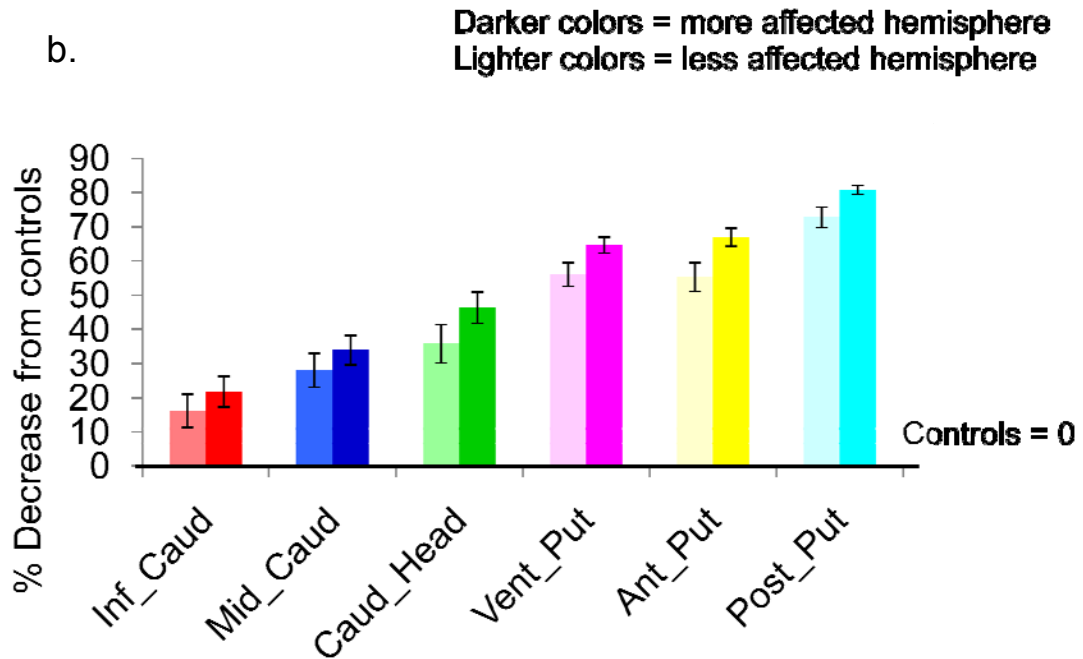
**Figure 4.1.** Learning magnitude measured by RT across the two learning runs of the early learning phase in PD OFF and PD ON.

### <sup>11</sup>C-DTBZ PET

Percent BP decrease relative to controls was compared across the 6 striatal VOIs and the two hemispheres (i.e., more and less affected) using repeated measures ANOVA. VOI and affected hemisphere were used as within subject factors. I found main effects of affected hemisphere ( $F_{1, 16} = 19.06, p < 0.001$ ) and VOI ( $F_{2.79, 44.68} = 118.45, p < 0.001$ ) and no interaction between the two (Fig. 4.2.b.). Average % BP decrease was greater in

the MA hemisphere (mean  $\pm$  S.E.:  $52.44 \pm 2.95$ ) than the LA hemisphere ( $44.08 \pm 3.95$ ). In order to test for the ventral to dorsal and anterior to posterior gradient in striatal denervation, I performed pair-wise comparisons of caudate VOIs (i.e., anteroventral striatum vs. middle caudate vs. caudate head) and putamen VOIs (i.e., ventral putamen vs. dorsal anterior putamen vs. dorsal posterior putamen) averaged across MA and LA hemispheres. Average % BP decrease in the anteroventral striatum was significantly different from middle caudate ( $t_{16} = -5.11$ ,  $p < 0.001$ ) and caudate head ( $t_{16} = -6.22$ ,  $p < 0.001$ ). There was also a significant difference between middle caudate and caudate head ( $t_{16} = -4.92$ ,  $p < 0.001$ ). Putamen VOIs were also significantly different from each other except for between ventral putamen and dorsal anterior putamen. There were significant differences between ventral putamen and dorsal posterior putamen ( $t_{16} = -12.19$ ,  $p < 0.001$ ) and between dorsal anterior and dorsal posterior putamen ( $t_{16} = -10.10$ ,  $p < 0.001$ ). These results indicate that the patients in our group showed the typical pattern of nigrostriatal denervation in PD, i.e. asymmetrical with a posterior to anterior gradient.





**Figure 4.2.** Locations of striatal VOIs overlaid on an MNI template. Anteroventral striatum (red), middle caudate (blue), caudate head (green), ventral putamen (pink), dorsal anterior putamen (yellow) and dorsal posterior putamen (skyblue) (a.). Percent decrease in binding potential from controls in striatal VOIs across the more and less affected hemispheres (b.).

Factor analysis of the % decrease in binding potential of the 12 VOIs revealed the first two components that together explained 88.9 % of the total variance. Component factor loadings for each of the 12 striatal VOIs showed a division of caudate and putamen VOIs into component 1 and component 2 (Table 4.2.). That is all of the caudate VOIs except for caudate head of the more affected hemisphere was better explained by component 1 and all of the putamen VOIs except for ventral putamen of the less affected hemisphere was better explained by component 2.

**Table 4.2.** Component factor loadings for each of the 12 striatal VOIs

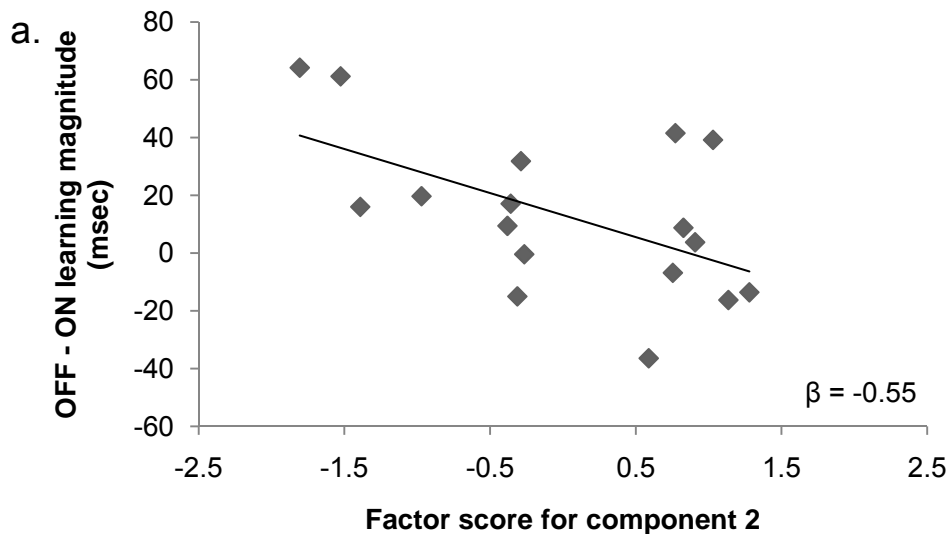
	Component	
	1	2
LA_INF_CAUD	0.97	-0.01
MA_INF_CAUD	0.87	0.30
LA_MID_CAUD	0.86	0.43
MA_MID_CAUD	0.67	0.66
LA_CAUD_HEAD	0.76	0.53
MA_CAUD_HEAD	0.62	0.71
LA_VENT_PUT	0.72	0.61
MA_VENT_PUT	0.68	0.68
LA_ANT_PUT	0.50	0.80
MA_ANT_PUT	0.19	0.91
LA_POST_PUT	0.51	0.76
MA_POST_PUT	0.08	0.94

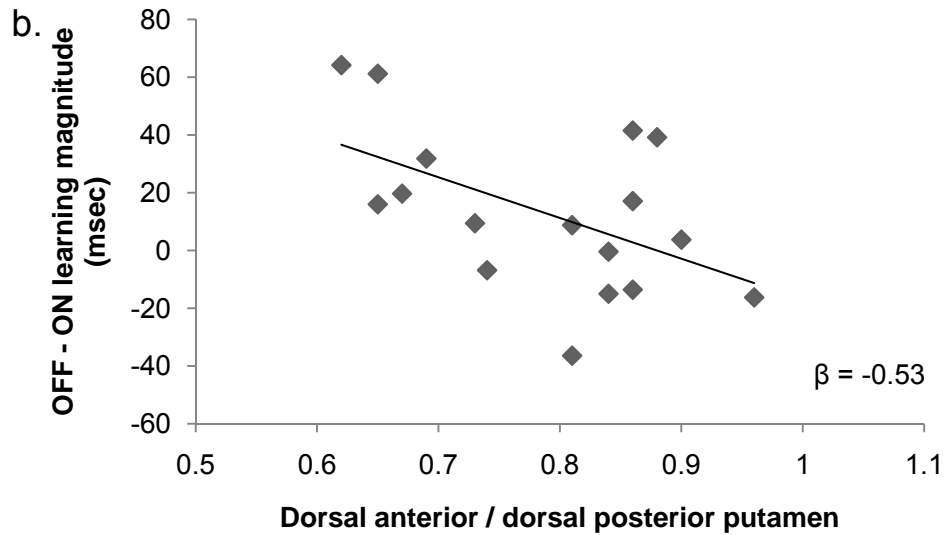
LA = Less affected hemisphere, MA = More affected hemisphere.

#### Association between L-DOPA Induced Sequence Learning Change and <sup>11</sup>C-DTBZ PET

To determine whether the level of striatal dopaminergic denervation was predictive of the degree of L-DOPA associated sequence learning impairment, I tested two linear regression models. In the first model, I used the factor scores of the two components identified in the factor analysis as predictors of L-DOPA associated early sequence learning performance change. The ON OFF difference in LM\_RT of the first learning run in the early phase (i.e. LM\_RT OFF – LM\_RT ON) was used as an index of performance change. I only focused on LM\_RT and not LM\_error since I did not find any evidence of significant L-DOPA effects on LM\_error in the current study. In the second model, the ratio of anterior to posterior putamen, ventral to dorsal caudate and putamen were used as predictors of L-DOPA induced sequence learning change.

In the first regression model, factor scores for component 2 which captured most of putamen variance was a significant predictor for the degree of sequence learning change with L-DOPA ( $\beta = -0.55$ ,  $t_{14} = -2.45$ ,  $p < 0.05$ , Fig. 4.3a.) whereas component 1 was not. Less denervation as indexed by a lower factor score for component 2 was predictive of greater L-DOPA induced learning deficit. In the second model, the within-subject ratios of dorsal anterior putamen / dorsal posterior putamen was a significant predictor for the degree of sequence learning change with L-DOPA ( $\beta = -0.54$ ,  $t_{13} = -2.42$ ,  $p < 0.05$ , Fig. 4.3b.). The ratio of ventral to dorsal putamen or caudate was not a significant predictor. This result shows that the less the dorsal anterior putamen was denervated relative to the dorsal posterior putamen, the more L-DOPA impaired sequence learning.





**Figure 4.3.** Correlation between the level of striatal denervation indexed by component 2 factor score (a.) and the denervation ratio of anterior to posterior dorsal putamen (b.) and the level of dopamine overdose effect.

## Discussion

I aimed to determine whether the effects of L-DOPA on early motor sequence learning are explained by individual differences in the level of dopaminergic denervation in PD patients as established by  $^{11}\text{C}$ -DTBZ PET. I found that the extent of striatal denervation correlated with the extent that a single dose of L-DOPA impaired (or improved) early motor sequence learning. A greater L-DOPA associated impairment was found in patients with less dopaminergic denervation as indexed by a latent factor explaining putamen denervation. Moreover, the ratio of anterior to posterior denervation in the dorsal putamen predicted the degree of L-DOPA associated learning impairment. These results indicate that a given dosage of L-DOPA is more detrimental to early motor sequence learning if a patient has relatively more preserved putamen. Moreover,

relatively more preserved anterior relative to posterior denervation is associated with more deleterious effects.

The behavioral results in the current study did not show a clear dopamine overdose effect of L-DOPA at the group level on early motor sequence learning. There was only a trend showing negative effects of L-DOPA measured by RT in the first learning run of the early phases. The loss of clear overdose effect relative to Chapter 2 findings may be due to the fact that a single dose (200 mg) of L-DOPA was used instead of the medication regimens titrated in the clinic based on individual patients' responses (i.e. patient's own dosage) as in Chapter 2. The fact that L-DOPA did not significantly improve UPDRS score or Pegboard performance demonstrates that the L-DOPA dosage used was not efficient to have clinical improvement. The overdose effect refers to the phenomenon that a clinically efficient use of dopaminergic medication interferes with the relatively intact ventral / anterior striatum in PD. The failure to replicate my findings of Chapter 2 does not necessarily negate the existence of a dopamine overdose effect shown in Chapter 2 since we did not use a clinically most efficient dose of L-DOPA. Moreover L-DOPA is known to particularly restore synaptic dopamine transmission in the more denervated striatal subregions (Tedroff et al., 1996). Depending on one's level of disease progression, it is possible that for the relatively more advanced stage patients, the 200 mg of L-DOPA was all consumed by the more denervated dorsal / posterior striatum and has little effect on the ventral / posterior striatum. The correlation between the extent of L-DOPA associated sequence learning impairment and the anterior to posterior denervation ratio as shown in the current study also supports this prediction.



The comparison of % BP decrease across the striatal subregions in the more and less affected hemispheres showed that overall there was more striatal denervation in the hemisphere contralateral to the side exhibiting the most clinical symptoms. There was also a distinctive pattern of striatal denervation showing a ventral to dorsal and anterior to posterior gradient of denervation. Overall dopaminergic denervation was more severe in the dorsal and posterior subregions of striatum. These results are in line with the characteristic spatial distribution of striatal denervation in PD patients that has been previously reported (Bernheimer et al., 1973, Kish et al., 1988, Frey et al., 1996, Rakshi et al., 1999).

The results of the factor analysis revealed two latent factors that explain the variance across 12 striatal VOIs. Interestingly the two factors each selectively captured most of caudate and putamen denervation. This may represent that the caudate VOIs are more correlated with themselves than the putamen VOIs and vice versa. When the two component factor scores were used as predictors of the extent of deleterious L-DOPA effects on sequence learning in linear regression, I found component 2, which explains the level of overall putamen denervation, as a significant predictor. The largest L-DOPA associated learning impairment was explained with less denervation. Because I used a single dose of L-DOPA across patients, the difference in the degree of overdose effect across patients is not due to differences in the type of medications or the dosage, but rather due to each patient's level of dopaminergic denervation.

The overdose hypothesis is in line with the 'inverted U' relationship between dopamine and cognitive performance identified in experimental animal studies, which demonstrates that both insufficient and excess levels of dopamine impair normal

cognitive functions (Williams and Goldman-Rakic, 1995, Zahrt et al., 1997, Arnsten, 1998). For early PD patients with relatively intact ventral and anterior striatum, dopaminergic medications interfere with normal functioning as shown by previous studies (Gotham et al., 1988, Swainson et al., 2000, Cools et al., 2001, Frank et al., 2004, Frank, 2005, Cools, 2006, Cools et al., 2006). Furthermore the same dosage of L-DOPA will be more deleterious to a less denervated striatum if the nature of the overdose effect is the relative harmfulness of dopamine for more intact striatal subregions. I found that the same dosage of L-DOPA impaired early sequence learning more when there was less decrease in  $^{11}\text{C}$ -DTBZ binding potential as indexed by a latent factor explaining putamen variance. This is in line with a previous study showing an association between the effect of D2 agonist bromocriptine on reversal learning and the level of striatal dopamine synthesis in healthy young adults (Cools et al., 2009). This study showed that subjects benefitted more from bromocriptine when their overall striatal dopamine synthesis level was low. The fact that only the factor associated with putamen and not caudate variance was a significant predictor is in line with my results in chapter 3 where I show that it is the putamen that is significantly recruited during early motor sequence learning which is also affected by L-DOPA.

The results also show that relatively higher preservation of the anterior versus posterior dorsal putamen is associated with greater deleterious L-DOPA effects. This is in line with the hypothesis that L-DOPA interferes with the more intact anterior than the posterior sub-region of the striatum. As early motor sequence learning primarily involves the anterior and ventral striatum, the overdose effect may be more significant with relatively more intact anterior versus posterior striatum, which is what I found. For the

underlying neural mechanism of these effects, I can speculate that for the less denervated patients and for patients with more intact anterior versus posterior dorsal putamen, the same dose of L-DOPA decreases ventral striatum recruitment during early motor sequence learning more so, which results in sequence learning deficits as shown in Chapter 3.

Collectively the results of this study show that the degree of deleterious L-DOPA effects in early motor sequence learning is predicted by individual differences in striatal denervation across PD patients with the same dose of L-DOPA being more deleterious in less disease-affected patients. To my knowledge, this is the first study to relate the level of individual differences in negative L-DOPA effects to differences in the extent of regional striatal dopaminergic denervation and the ratio of anterior to posterior striatal denervation in PD patients. The results give additional support for the nature of dopamine overdose effects by showing that the same dosage of L-DOPA is parametrically more deleterious to patients with less denervation and also relatively more intact anterior versus posterior putamen.

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## CHAPTER V

### COMT and Dopamine D2 receptor polymorphisms predict L-DOPA effects on motor sequence learning in Parkinson's patients

#### Abstract

Variants in genes that regulate dopamine transmission have been shown to affect behaviors including working memory and executive function as well as movement timing and implicit sequence learning. Several reports also demonstrate that these genes affect the pattern of response to exogenous administration of dopaminergic agents. The current study examined whether COMT and dopamine D2 receptor polymorphisms affect the response to L-DOPA in Parkinson's patients in terms of motor sequence learning and manual motor control measured by the grooved pegboard test. Forty-five PD patients were genotyped for the COMT val158met and DRD2 polymorphisms (rs 1076560, G > T). Patients performed an explicit motor sequence learning task and the grooved pegboard task in both ON and OFF L-DOPA states. Task performance change associated with L-DOPA was compared across genotype groups for the two dopamine regulating genes. There was a significant COMT gene effect on the grooved pegboard task, such that performance was worse in individuals homozygous for the met allele. There was also a significant COMT genotype by L-DOPA interaction for pegboard performance showing



significant improvements with L-DOPA only in the val/met group (there was no val/val group). COMT genotype also mediated L-DOPA effects on motor sequence learning measured by learning rate, with the met/met group showing showing greater L-DOPA associated improvement than the val/met group. For the DRD2 genotype, grooved pegboard task performance improved with L-DOPA regardless of patients' genotype. In contrast, there was a significant DRD2 genotype by L-DOPA interaction on early motor sequence learning measured by both learning magnitude and learning rate. In the minor T allele carriers (associated with lower D2 receptor availability), L-DOPA significantly improved sequence learning. In contrast, G homozygotes showed no performance change with L-DOPA. Collectively these results demonstrate a dissociable gene by medication interaction for two motor tasks that rely on distinct cortico-striatal circuitries: motor sequence learning and grooved pegboard.

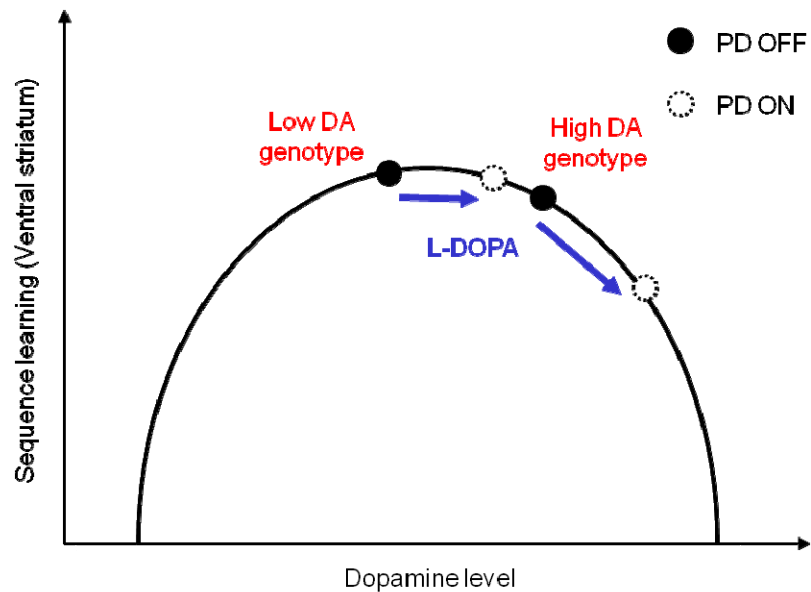
## **Introduction**

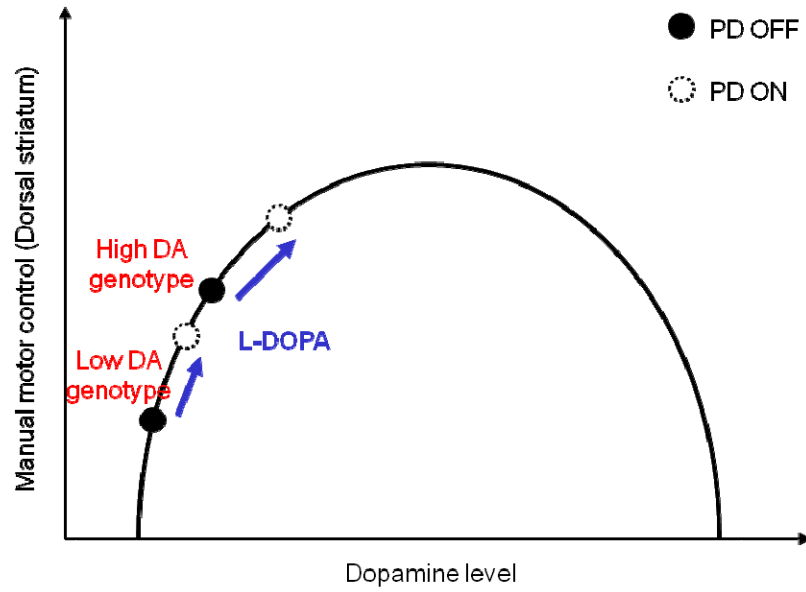
Recent studies have demonstrated the effects of genetic polymorphisms on brain and cognitive function. One example is the COMT gene which contains a common functional polymorphism (a valine (val) substitution for methionine (met)), with the met allele having one fourth the COMT enzymatic activity of the val allele. Individuals homozygous for the met allele have reduced COMT activity and therefore increased DA levels in the prefrontal cortex. It has been shown that COMT genotype mediates working memory, attention, and executive control as well as temporal processing (Egan et al., 2001, Mattay and Goldberg, 2004, de Frias et al., 2005, Galderisi et al., 2005, Harris et al., 2005, O'Hara et al., 2006, Frank et al., 2007, Starr et al., 2007, Liu et al., 2008, Bertolino et al., 2010, Holtzer et al., 2010, Wiener et al., 2011). These studies have shown that individuals with a genotype which leads to higher endogenous dopaminergic activity (i.e. via decreased expression of dopamine metabolates) exhibit superior performance on assessments of cognitive function. Likewise the dopamine D2 receptor (DRD2) polymorphism has been shown to mediate both cognitive and motor abilities including working memory, automatic temporal processing and movement time (Frank et al., 2007, Zhang et al., 2007, Bertolino et al., 2009a, Bertolino et al., 2009b, Frank and Hutchison, 2009, Bertolino et al., 2010, Fazio et al., 2011, Wiener et al., 2011). The DRD2 genotype which leads to greater D2 receptor expression has been shown to be associated with superior performance on these tasks. Similarly, genetic variants in the dopamine transporter (DAT1) gene (SLC6A3) have been shown to mediate implicit motor sequence learning performance (Simon et al., 2011). The genotype that results in increased density of DAT1 (10/10 homozygotes) and thus decreased dopamine levels in the striatum was

associated with lower implicit motor sequence learning. Neuroimaging studies have supported these results by showing genotype associated neural activation effects which are consistently correlated with performance differences (Egan et al., 2001, Mattay and Goldberg, 2004, Bertolino et al., 2006, Schott et al., 2006, Winterer et al., 2006, Caldu et al., 2007, Tan et al., 2007, Zhang et al., 2007, Ettinger et al., 2008, Williams-Gray et al., 2008, Bertolino et al., 2009a, Bertolino et al., 2010, Fazio et al., 2011).

The effects that genetic polymorphisms have on performance and brain activation patterns have also been investigated in Parkinson's disease (Oliveri et al., 1999, Wang et al., 2001, Foltynie et al., 2004, Bartres-Faz et al., 2007, Williams-Gray et al., 2007, Argyelan et al., 2008, Williams-Gray et al., 2008, Hoogland et al., 2010). For example, Williams-Gray et al., (2008) have found that depending on one's COMT genotype, dopaminergic medication can differentially affect attentional control ability and the associated neural recruitment. Specifically, exogenous dopaminergic medications impaired performance on attentional set shifting for the COMT genotype that leads to lower prefrontal cortex (PFC) dopamine level (i.e. val/val). In contrast, medication had no effect on those with high PFC endogenous dopamine levels (i.e. met/met). The authors suggested that COMT genotype determines one's location along the so called 'inverted-U' shaped curve. This function describes the relationship between performance and dopamine level showing that both insufficient and excess levels of dopamine impair normal cognitive functions (Williams and Goldman-Rakic, 1995, Zahrt et al., 1997, Arnsten, 1998) (Fig 5.1.). For example, the high endogenous dopamine group (i.e. met/met for COMT) will be situated more towards the right side of the inverted-U curve than the low endogenous dopamine group (i.e. val/val for COMT). The authors

interpreted that due to a hyperdopaminergic state of PFC in PD, both genotype groups are situated quite low on the right hand side of the curve and the medication associated impairment is more significant in val/val whereas in met/met the effect is minimal due to “floor effects”. In a study of verbal reasoning, higher dopamine availability as indexed by COMT genotype (i.e. met/met) was associated with higher verbal reasoning performance when patients were OFF dopaminergic medications, however these same patients exhibited decreased performance when ON medication (Hoogland et al., 2010).





**Figure 5.1.** Hypothesized ‘inverted – U’ shaped relationship between performance and dopamine level in sequence learning (top) and manual motor control (bottom). For sequence learning which relies on the relatively intact ventral striatum, exogenous L-DOPA administration (arrow pointing toward right) will potentially result in worsening of sequence learning performance more in the high DA genotype group compared to the low DA genotype group. For manual motor control which relies on the denervated dorsal striatum, L-DOPA administration will result in improvement for both the high and low DA genotype groups.

In the current study, I focused on the contribution of COMT and DRD2 genetic polymorphisms on L-DOPA response in Parkinson’s disease. The COMT gene is involved in directly regulating prefrontal dopamine level (Lachman et al., 1996) however it also regulates striatal dopamine level (Akil et al., 2003). Whereas the met allele increases dopamine level in PFC, the *val* allele is associated with increased dopamine level in the striatum in the human brain (Akil et al., 2003). In line with increased striatal dopamine associated with the *val* allele, *val* allele carriers have shown better performance in tasks of cognitive flexibility such as task switching, which relies on the striatum (Colzato et al., 2010, Solís-Ortiz et al., 2010). The DRD2 polymorphism is thought to

affect striatal dopamine transmission based on its expression in the striatum (Missale et al., 1998, Piggott et al., 1999). Despite potentially more direct effects on striatal dopamine level than COMT, the contribution of DRD2 polymorphisms to medication effects on performance in PD has not been widely studied. An intronic DRD2 polymorphism (rs 1076560, G > T) has been shown to be associated with mRNA splicing of the short isoform of the D2 receptor (D2S) (Zhang et al., 2007). The minor T allele is associated with reduced D2S expression and results in declines in working memory performance and altered brain activation patterns (Zhang et al., 2007, Bertolino et al., 2009a, Bertolino et al., 2009b, Bertolino et al., 2010). A PET imaging study has shown reduced D2 receptor binding for T allele carriers confirming the initial finding in Zhang et al., (2007) (Bertolino et al., 2010). The DRD2 polymorphism has also been shown to be associated with increased BOLD response in the cortico-basal ganglia motor circuitry which negatively correlated with reaction time in a motor task (Fazio et al., 2011).

I determined the effects of COMT (val158met) and DRD2 polymorphisms (rs 1076560, G > T) on two different motor behaviors which differ in their reliance on the striatal circuitry: early motor sequence learning and the grooved pegboard, a simple manual motor task. I have previously shown a selective medication associated impairment in the early phase of sequence learning (Chapter 2). However there was a large variance across patients in the magnitude of this effect. One potential factor that might contribute to this large variance may be one's genotype for dopamine regulating genes such as COMT or DRD2. One recent study has reported COMT genotype effects on neural recruitment during motor sequence learning in PD patients ON and OFF L-DOPA (Argyelan et al., 2008). This study demonstrated that COMT genotype was related

to the level of learning-related PFC deactivation. Specifically, L-DOPA reduced the magnitude of learning related deactivation in val allele carriers, but enhanced this response in those homozygous for the met allele. Although this study demonstrated a genotype effect on PFC deactivation level, there were no genotype effects on sequence learning behavior nor was there a genotype by medication interaction. Furthermore, this study only tested 9 patients for the COMT genotype (1 val/val, 4 val/met, 4 met/met) which likely did not provide sufficient power for group comparisons.

I predicted that for early phase sequence learning, which depends on the relatively intact ventral striatal circuitry, genotypes that result in lower striatal dopamine transmission (i.e. met allele carriers for COMT and T allele carriers for DRD2) will be situated near the peak of the curve while higher dopamine genotypes (i.e. val allele carriers for COMT and G allele carriers for DRD2) will be towards the right hand side of the curve. When L-DOPA is administered in PD, the overdose effect manifested by the worsening of performance will be more significant in high dopamine genotype patients than in low dopamine genotype patients. This is predicted due to their relatively closer location to the right hand side of the curve. For the simple manual motor task that relies on the more denervated dorsal striatum, both genotype groups will be situated on the left hand side of the curve. However the low dopamine genotype will be relatively lower towards the left than the high dopamine genotype. Thus, when L-DOPA is administered in PD, both genotype groups will improve their performance although the degree of improvement in the two groups will depend on their actual location on the curve. In addition to these motor tasks I also assessed gene by L-DOPA interactions in general cognitive ability measured by standardized neuropsychological tests.

## Material and methods

### Participants

45 mild to moderate stage PD patients ( $65 \pm 8$  yrs, 9 females) falling within Hoehn and Yahr (H & Y) stages 1 – 3 (Hoehn and Yahr, 1967) participated in this study. Subsets of these patients participated in studies 2 and 3. Their behavioral data were combined and re-analyzed in association with genotype in the current study. Patients were excluded for any neurological or psychiatric disease other than PD. Patients were included as long as they were on a stable dosage of dopaminergic medications for the previous 6 months, and were evaluated using the motor section of the Unified Parkinson’s Disease Rating Scale (UPDRS, Fahn et al., 1987) by a neurologist. All study participants underwent the Mini-Mental State Exam (Folstein et al., 1975) and the Montreal Cognitive Assessment (MOCA, (Nasreddine et al., 2005)). Participants were compensated for their participation, which included two testing days for PD patients. All participants signed a consent form approved by the Institutional Review Board of the University of Michigan. See Table 1 for additional demographics.

**Table 5.1.** Demographic and clinical variables

	Total N (♀)	Age	UPDRS ON	UPDRS OFF	MMSE ON	MMSE OFF	MOCA ON	MOCA OFF
<b>COMT</b>								
val/met	34 (7)	$64 \pm 8$	$17 \pm 7$	$19 \pm 8$	$28.9 \pm 1$	$29.1 \pm 1$	$27 \pm 2$	$26 \pm 3$
met/met	11 (2)	$68 \pm 8$	$22 \pm 7$	$23 \pm 7$	$29.2 \pm 1$	$28.5 \pm 1$	$26 \pm 3$	$26 \pm 3$
<b>DRD2</b>								
TT / GT	11 (4)	$67 \pm 8$	$20 \pm 8$	$21 \pm 8$	$28 \pm 1$	$29 \pm 1$	$25 \pm 3$	$25 \pm 2$
GG	34 (5)	$65 \pm 8$	$18 \pm 7$	$20 \pm 8$	$29 \pm 1$	$29 \pm 1$	$27 \pm 2$	$26 \pm 3$

Mean  $\pm$  S.D.



### Genotype assessments

A saliva sample was collected from each study participant using an ORAGENE kit (DNA Genotek, Inc.). SNP rs4860 of COMT genotype and SNP rs1076560 of DRD2 genotypes were determined using the following procedures: 1) sample purification and DNA extraction, 2) PCR amplification of allelic locus, 3) protease digestion of DNA polymerase, and 4) detection of SNP variants by fluorescent-labeled ligation oligonucleotides.

Genotype groups were divided based on COMT and DRD2 genotype. The number of subjects in each genotype group was as follows for COMT and DRD2: val/met = 34, met/met = 11 (COMT); TT = 1, GT = 10, GG = 34 (DRD2). The COMT genotype distribution is a bit atypical given that there were no val homozygotes, however the DRD2 genotype distribution is consistent with that reported in earlier studies (Bertolino et al., 2009a, Frank and Hutchison, 2009, Bertolino et al., 2010). Since there was only one subject homozygous for the T allele of the DRD2 gene I grouped all subjects carrying at least one T allele (associated with reduced D2 receptor availability). There was no significant age difference across the two genotype groups for both genes. For the COMT gene, approximately 21 % of the val/met group was females and 18 % of the met/met group was females. For the DRD2 gene, there was a noticeable difference in sex ratio between the two groups such that 36 % of the TT / GT group were females while 15 % of the GG were females.

### Procedure

PD patients underwent two testing days corresponding to the ON and OFF

medication states, separated by no more than two weeks. Twenty one patients were tested OFF first and 24 ON first. I used a single blind placebo controlled design using a single dose of L-DOPA (200 mg) across all PD patients following the same procedure to achieve the respective ON and OFF states as in Chapters 3 and 4 (see Methods sections of Chapters 3 and 4 for details). The L-DOPA dosage used was tolerable to all participants. All study procedures began one hour after the patient had taken either L-DOPA or the placebo, by which time L-DOPA reaches its peak plasma dose. On each testing session, participants performed an explicit motor sequence learning task. The UPDRS motor section was assessed in both the ON and OFF sessions. Additionally, the grooved pegboard Test (Lafayette Instruments, Lafayette, IN) was used to measure motor abilities.

#### Explicit Motor Sequence Learning

I used the same explicit motor sequence learning paradigm as in Chapters 3 and 4 (see Methods sections of Chapters 3 and 4 for details).

#### Data Analysis

Sequence learning performance for each learning run was measured by the learning magnitude calculated with response time (LM\_RT) and the progression of RT change across the random and sequence blocks. LM\_RT was calculated as the difference in response time between R and S blocks. Median RT was computed for each block and was used in the following equation (Fig. 5.4.):  $LM\_RT = \text{average median RT of the two R blocks} - \text{average median RT of the three S blocks within a run}$ . The error trials (i.e.

errors by commission and omission), and trials immediately following an error were excluded from RT analyses. The average LM\_RT of the first two learning runs at each medication state was used as the early learning magnitude in the analysis.

The effect of COMT and DRD2 genetic variants on L-DOPA-associated changes in early motor sequence learning magnitude (LM\_RT) was determined by a repeated measures ANOVA using genotype as a between subject factor and L-DOPA status as a within subject factor. The potential interactive effect of genotype and medication status on sequence learning was examined using a repeated measure ANOVA with genotype as a between subject factor and L-DOPA status, run (run 1 and 2) and block (R1/R6 as the first, S2/S7 as the second, S3/S8 as the third, S4/S9 as the fourth block within a run) as within subject factors, focusing on the linear contrast of block.

Similar analyses to learning magnitude (LM\_RT) were conducted for the clinical variables including UPDRS, MMSE, MOCA and pegboard. To account for the difference in sex ratio across the two genotype groups, I additionally performed an ANCOVA factoring out the effect of sex in case there was an effect associated with genotype (i.e. main effect of genotype or genotype by L-DOPA status interaction).

## **Results**

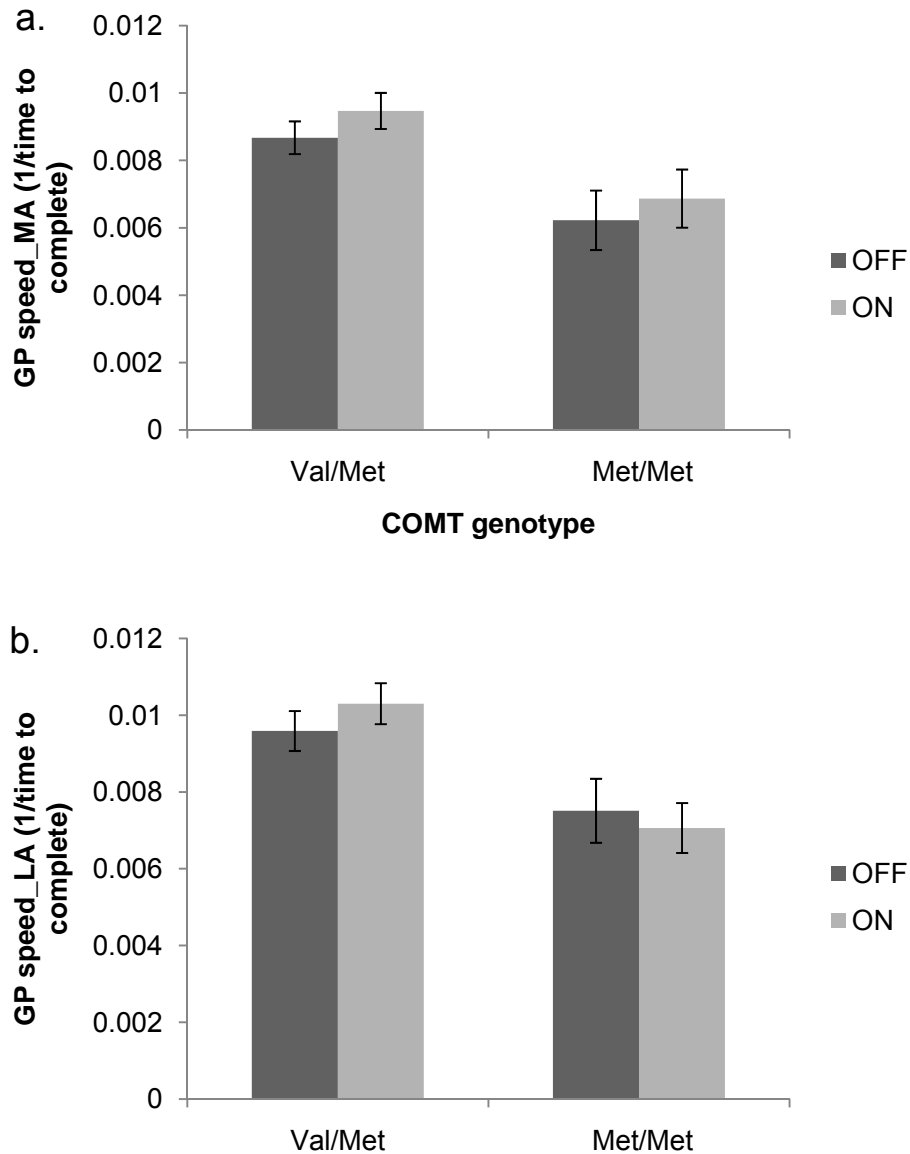
### COMT and DRD2 effects on response to L-DOPA in neuropsychological assessments

Pegboard performance data could not be acquired from two patients (one val/met and one met/met for COMT; both were in the GG group for DRD2) due to fatigue.

COMT genotype by L-DOPA status ANOVAs showed a trend for a main effect of L-DOPA status ( $F_{1,43} = 3.46$ ,  $p = 0.07$ ) and COMT genotype ( $F_{1,43} = 3.75$ ,  $p = 0.06$ ) on

UPDRS with worse performance in the OFF state and in the met/met group, respectively (see Table 1. for average UPDRS scores in each genotype group for each L-DOPA state). The main effect of COMT genotype on UPDRS was still marginally significant after controlling for sex ( $F_{1,42} = 4.03$ ,  $p = 0.05$ ). There was also a main effect of L-DOPA ( $F_{1,41} = 12.69$ ,  $p < 0.005$ ), COMT genotype ( $F_{1,41} = 6.01$ ,  $p < 0.05$ ) and a marginally significant genotype by L-DOPA status interaction ( $F_{1,41} = 4.08$ ,  $p = 0.05$ ) on grooved pegboard performance for the more disease affected hand (Fig. 5.2.a.). The interaction was only marginally significant after controlling for sex ( $F_{1,40} = 3.77$ ,  $p = 0.06$ ) although the main effect of genotype was still significant ( $F_{1,40} = 5.26$ ,  $p < 0.05$ ). Performance was worse in the met/met group, and a follow up paired t-test comparing performance ON and OFF L-DOPA in each genotype group showed that there was a significant L-DOPA associated improvement in the val/met group ( $t_{32} = -3.06$ ,  $p < 0.005$ ) but not in the met/met group. Pegboard performance for the less affected hand showed a main effect of COMT genotype ( $F_{1,41} = 6.78$ ,  $p < 0.05$ ) and a genotype by L-DOPA status interaction ( $F_{1,41} = 4.37$ ,  $p < 0.05$ ) (Fig. 5.2.a.). These effects were both significant after controlling for sex (main effect of COMT:  $F_{1,40} = 5.93$ ,  $p < 0.05$ , interaction effect:  $F_{1,41} = 4.87$ ,  $p < 0.05$ ). Performance was worse in the met/met group and a follow up paired t-test comparing performance ON and OFF L-DOPA in each genotype group showed that there was a significant L-DOPA associated improvement in the val/met group ( $t_{32} = -2.23$ ,  $p < 0.05$ ) but not in the met/met group. I also found a COMT genotype by L-DOPA status interaction for MMSE ( $F_{1,43} = 5.50$ ,  $p < 0.05$ , see Table 1. for average MMSE scores in each genotype group for each L-DOPA state), which was significant after controlling for sex ( $F_{1,42} = 5.44$ ,  $p < 0.05$ ). A follow up paired t-test comparing performance ON and

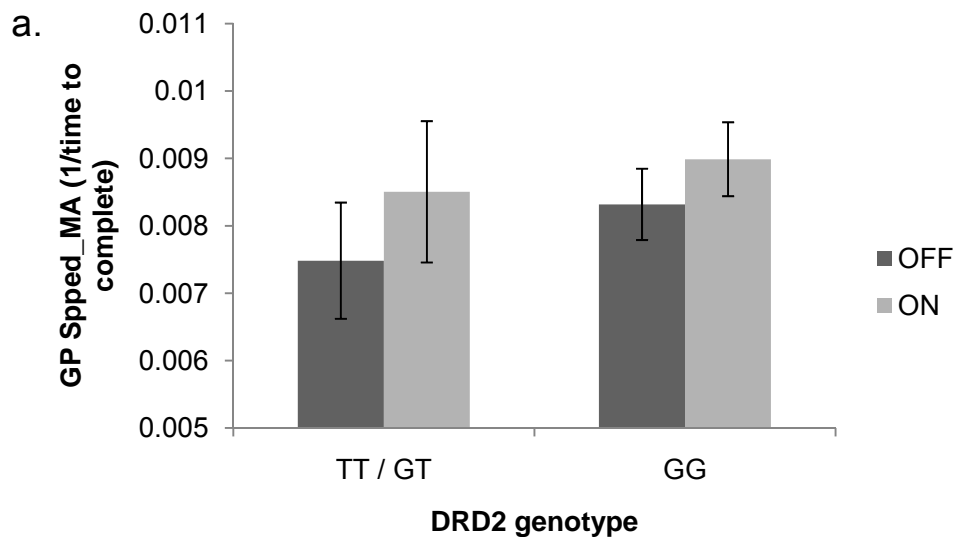
OFF L-DOPA did not show any significant difference in either group. No significant COMT or L-DOPA main effects or interaction was found for MOCA.

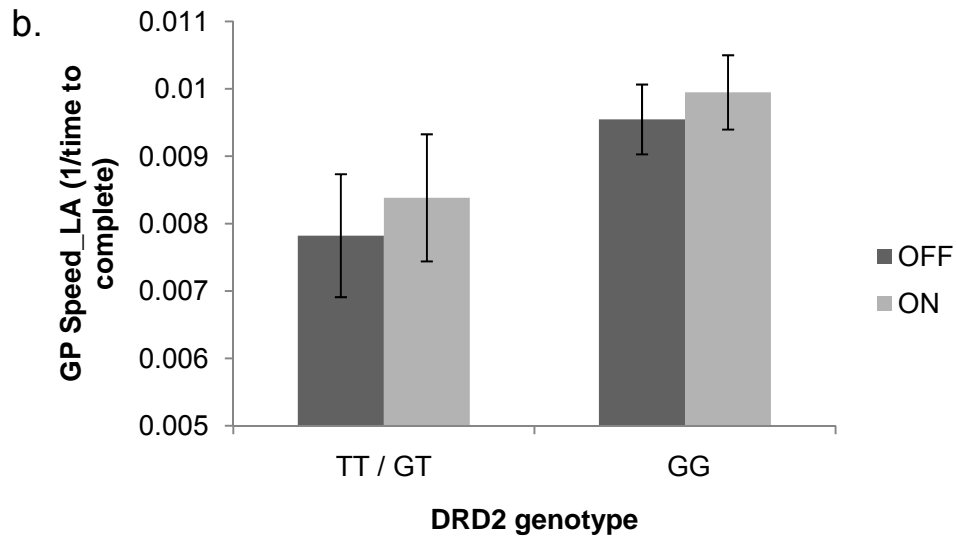


**Figure 5.2.** Performance on the grooved pegboard task (GP) in the more (a.) and less (b.) disease affected hand as measured by speed ON and OFF L-DOPA in each COMT genotype group. Error bars indicate standard error.

The DRD2 genotype by L-DOPA status ANOVAs only showed significant main effects of L-DOPA status for UPDRS ( $F_{1,43} = 4.99, p < 0.05$ ) and pegboard performance

for the more disease affected hand ( $F_{1,41} = 6.08$ ,  $p < 0.05$ ) (Fig. 5.3.a). Pegboard performance of the less affected hand also showed a marginally significant L-DOPA main effect ( $F_{1,41} = 3.37$ ,  $p = 0.07$ ) (Fig. 5.3.b.). No significant main effect of genotype or genotype by L-DOPA interaction was found for UPDRS or pegboard performances. Thus motor symptoms and pegboard performance improved with L-DOPA (see Table 5.1. and Fig. 5.2). There was a main effect of DRD2 genotype for MMSE ( $F_{1,43} = 6.12$ ,  $p < 0.05$ ) and MOCA ( $F_{1,43} = 6.05$ ,  $p < 0.05$ ) scores, with better performance for the GG group than the TT / GT group (see Table 5.1. for mean values in each genotype group). The effect of genotype was significant after controlling for sex in both cases (MMSE:  $F_{1,42} = 9.37$ ,  $p < 0.005$ , MOCA:  $F_{1,42} = 5.26$ ,  $p < 0.05$ ). No significant main effect of L-DOPA or genotype by L-DOPA interaction was found. The statistical test results for the neuropsychological assessments are also shown in Table 5.2.





**Figure 5.3.** Performance on the grooved pegboard task (GP) in the more (a.) and less (b.) disease affected hand as measured by speed ON and OFF L-DOPA in each DRD2 genotype group. Error bars indicate standard error.

**Table 5.2.** Statistical results for neuropsychological assessments

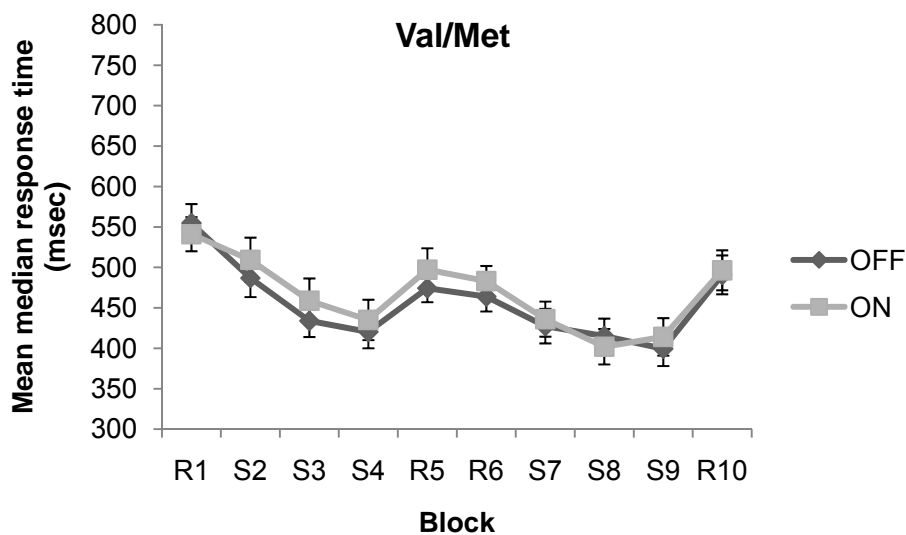
		UPDRS		MMSE		MOCA		Pegboard (MA)		Pegboard (LA)	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
COMT	Genotype	3.75	0.06	N.S.	N.S.	N.S.	N.S.	6.01	< 0.05	6.78	< 0.05
	Genotype (controlling for sex)	4.03	0.05	N.S.	N.S.	N.S.	N.S.	5.26	< 0.05	5.93	< 0.05
	L-DOPA	3.46	0.07	N.S.	N.S.	N.S.	N.S.	12.69	< 0.005	N.S.	N.S.
	Genotype by L-DOPA	N.S.	N.S.	5.5	< 0.05	N.S.	N.S.	4.08	0.05	4.37	< 0.05
	Genotype by L-DOPA (controlling for sex)	N.S.	N.S.	5.44	< 0.05	N.S.	N.S.	3.77	0.06	4.87	< 0.05
	<hr/>										
DRD2	Genotype	N.S.	N.S.	6.12	< 0.05	6.05	< 0.05	N.S.	N.S.	N.S.	N.S.
	Genotype (controlling for sex)	N.S.	N.S.	9.37	< 0.005	5.26	< 0.05	N.S.	N.S.	N.S.	N.S.
	L-DOPA	4.99	< 0.05	N.S.	N.S.	N.S.	N.S.	6.08	< 0.05	3.37	0.07
	Genotype by L-DOPA	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Genotype by L-DOPA (controlling for sex)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

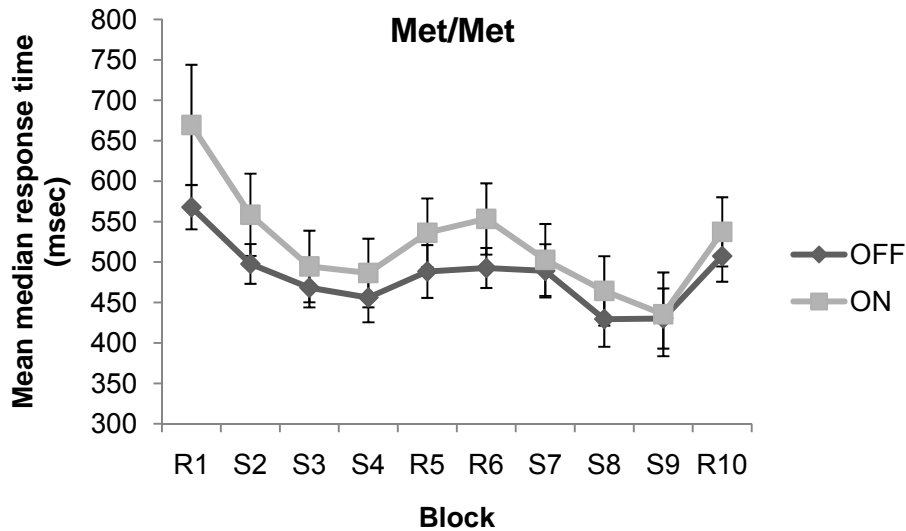
N.S.: not significant



### COMT and DRD2 effects on response to L-DOPA in early motor sequence learning

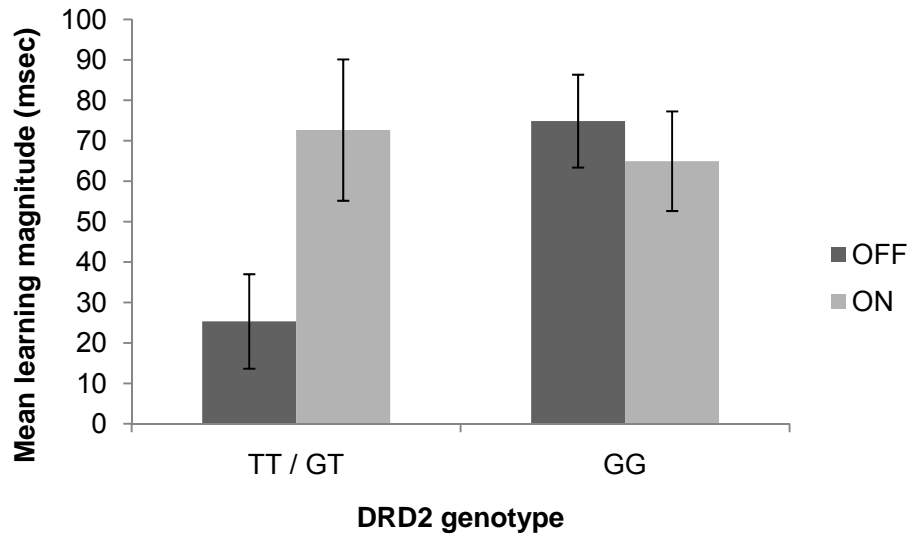
Sequence learning data in the ON L-DOPA state could not be acquired in one patient (met/met for COMT and GG for DRD2 genotype) due to technical failure. The COMT genotype by L-DOPA status ANOVA comparing LM\_RT of the early phase did not show any main effect or interaction effects. Sequence learning measured by the progression of RT across the random and sequence blocks showed a significant COMT genotype by medication by block interaction using a linear contrast for block ( $F_{1,42} = 4.14$ ,  $p < 0.05$  Fig.5.4.). This three way interaction was marginally significant after controlling for sex ( $F_{1,41} = 4.06$ ,  $p = 0.05$ ). A follow up medication by block repeated measure ANOVA in the two genotype groups separately showed a trend for medication by block interaction with the linear contrast of block ( $F_{1,9} = 4.09$ ,  $p = 0.07$ ) in the met/met group but not in the val/met group. That is, there was a trend for an L-DOPA effect on the slope of RT decrease across the random and sequence blocks in the met/met group but not in the val/met group. There were no main effects of L-DOPA in either the val/met or the met/met group.





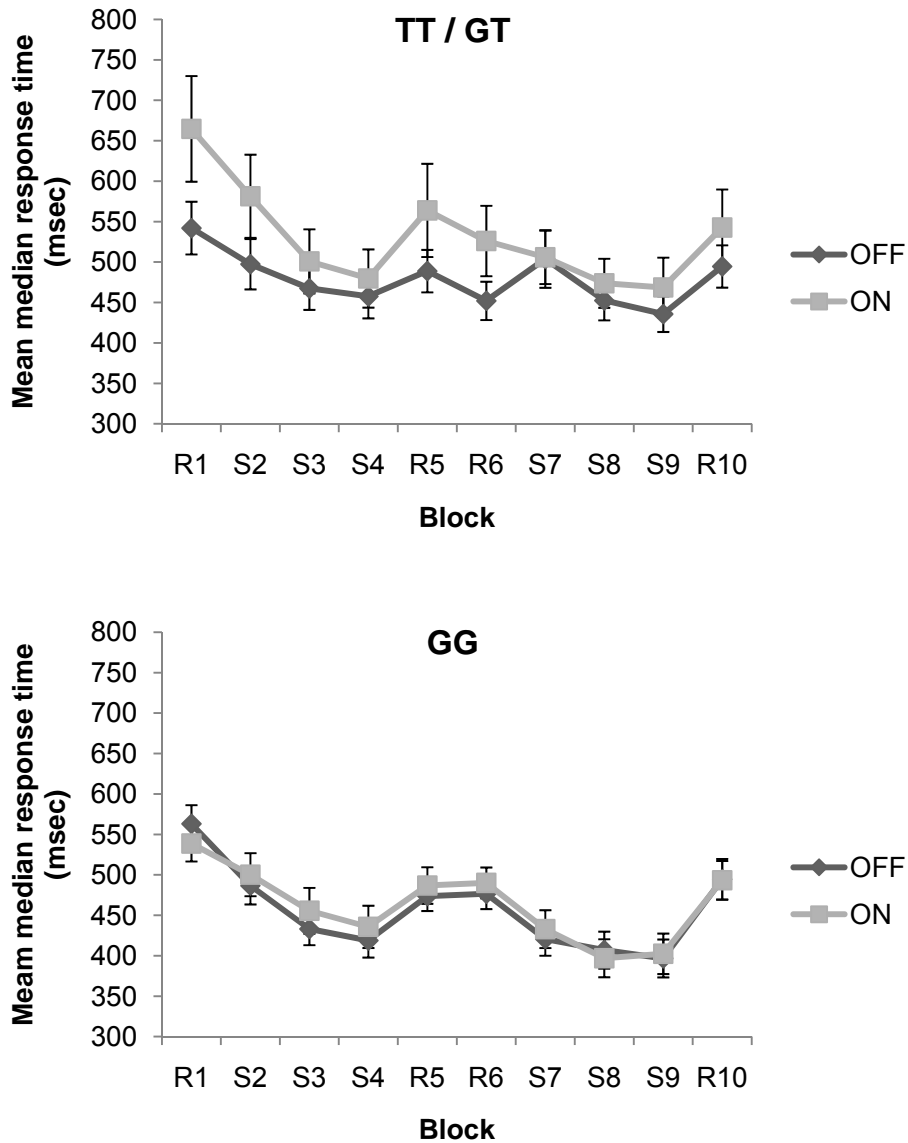
**Figure 5.4.** Mean median response time ON and OFF L-DOPA across the random and sequence blocks comprising the early sequence learning phase for the two COMT genotype groups. The top graph shows the results from val/met group and the bottom graph shows the results from met/met group. Error bars indicate standard error.

The DRD2 genotype by L-DOPA status ANOVA comparing LM\_RT of the early phase showed a significant interaction effect ( $F_{1,42} = 6.90, p < 0.05$ , Fig. 5.5.). No main effect of medication or genotype was found. The genotype by L-DOPA status interaction remained significant after controlling for sex ( $F_{1,41} = 7.71, p < 0.01$ ). A follow up paired t-test comparing performance ON and OFF L-DOPA in each genotype group showed a significant performance benefit from L-DOPA in the TT / GT group ( $t_{10} = -2.71, p < 0.05$ ), but not in the GG group.



**Figure 5.5.** Mean learning magnitude of early phase ON and OFF L-DOPA in two DRD2 genotype groups. L-DOPA significantly improved sequence learning for T carriers whereas it did not for G homozygotes. Error bars indicate standard error.

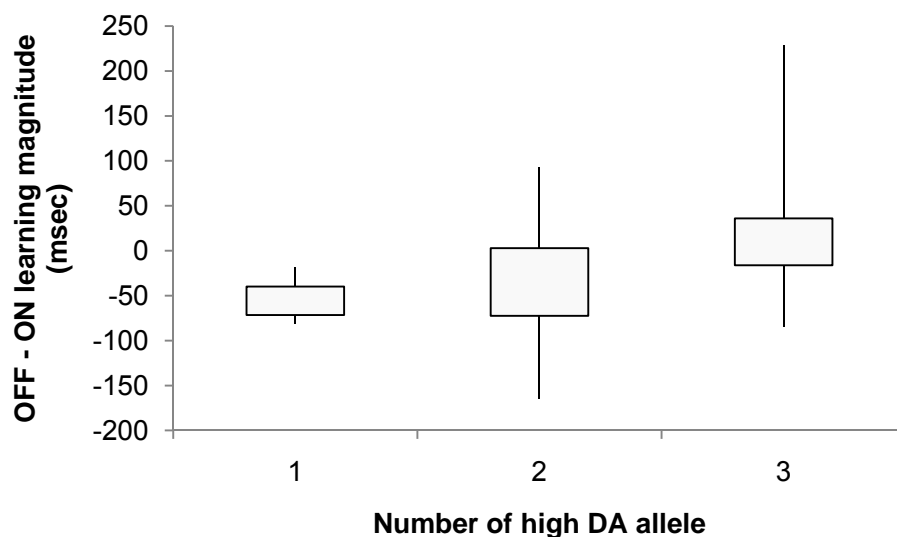
Sequence learning measured by the progression of RT across the random and sequence blocks showed a significant genotype by medication by block interaction using a linear contrast of block ( $F_{1,42} = 6.96, p < 0.05$ ). This three way interaction was significant after controlling for sex ( $F_{1,41} = 6.757, p < 0.05$ ). A follow up medication by block repeated measure ANOVA showed a significant medication by block interaction with the linear contrast of block in the TT / GT group ( $F_{1,10} = 6.03, p < 0.05$ , Fig. 5.6.) but not in the GG group. That is L-DOPA affected the slope of RT decrease across the random and sequence blocks in the TT/GT group but not in the GG group. There were no main effects of L-DOPA in either the TT / GT or the GG group.



**Figure 5.6.** Mean median response time ON and OFF L-DOPA across the random and sequence blocks comprising the early sequence learning phase for the two DRD2 genotype groups. The top graph shows the results from T carriers (i.e. TT / GT) and the bottom graph shows the results from homozygotes for the G allele. Error bars indicate standard error.

Based on the observation that both the COMT and DRD2 genes showed gene by medication interactions in early motor sequence learning I also determined whether there was an effect of the number of alleles across the two genes that results in higher striatal

dopamine transmission (high DA). For this analysis I divided the patient groups in terms of the number of val or G alleles they carry. Those having met/met (for COMT) and TT/GT (for DRD2) were considered as carrying 1 high DA allele ( $n = 3$ ) whereas those having val/met and GG were considered as carrying 3 high DA alleles ( $n = 26$ ). For those who have either val/met and TT/GT or met/met and GG are considered as carrying 2 high DA alleles ( $n = 16$ ). One patient in this group was excluded from analysis due to loss of sequence learning data in the ON L-DOPA state. Thus there were 15 patients in this group. I compared the degree to which L-DOPA interfered with learning in the three groups using one-way ANOVA with a linear contrast (i.e. -1 0 1). There was a significant linear effect showing that the degree of L-DOPA associated learning impairment increased as the number of high DA alleles increased ( $t_{4,21} = 3.07$ , degrees of freedom adjusted for not assuming equal variance,  $p < 0.05$ ) (Fig. 5.7.). There was no quadratic effect (i.e. contrast -1 2 -1).



**Figure 5.7.** L-DOPA associated change in motor sequence learning in three groups differing in their number of high DA alleles. The box indicates the interquartile range. The error bars indicate the minimum and maximum values.

## **Discussion**

In the current study I determined whether COMT and DRD2 genetic polymorphisms interact with L-DOPA during learning of new sequences in Parkinson's patients. In my previous study (Chapter 2), I have shown a selective medication associated sequence learning impairment suggesting the presence of a “dopamine overdose” effect on the ventral and anterior striatum, a structure that is relied upon for early phase sequence learning. I predicted that there would be a significant involvement of COMT and DRD2 genetic variation on this medication effect, such that the effect would be more pronounced with the genotype (i.e. val/met or GG) associated with higher striatal endogenous dopamine transmission as shown in Figure 5.1.

The results showed a significant COMT genotype by L-DOPA interaction in sequence learning measured by the progression of RT change. A follow-up analysis showed a trend for L-DOPA associated learning improvement in the met/met group (lower striatal dopamine availability). L-DOPA did not affect learning in the val/met group. I also found a significant DRD2 genotype by L-DOPA interaction for sequence learning measured by both learning magnitude and RT progression. Follow up analysis revealed that whereas the T allele carriers (low receptor availability) showed significant improvements in sequence learning with L-DOPA, the G homozygotes showed no significant performance change with L-DOPA. Thus my results show that for both COMT and DRD2 genotypes, L-DOPA improved sequence learning in the genotype group with low striatal dopamine transmission whereas in the group with high striatal

dopamine transmission there was no effect of L-DOPA. I initially hypothesized that there would be a significant L-DOPA associated *impairment* in the high striatal dopamine genotype groups (i.e. val/met or G homozygotes) which would be less significant in the low striatal dopamine genotype groups (i.e. met/met or T allele carriers) (see Fig.5.1.).

A potential explanation of the pattern of results that I obtained could be that the group of PD patients in this study may have been more denervated than I initially predicted in my hypothesis. That is, for both genotype groups, the position on the inverted-U curve may be shifted leftward from the locations depicted in Figure 5.1. This may result in the low genotype group being located on the left hand side of the curve and the high genotype group being closer to the peak of the curve. In this case, L-DOPA administration will significantly move the low dopamine group upward and towards the right, whereas the rightward shift with L-DOPA for the high dopamine group may not have significant effects on performance. I also compared L-DOPA associated sequence learning performance change across groups differing in the number of alleles that result in higher dopamine transmission spanning both COMT and DRD2 genes. I found a linear increase in L-DOPA associated interference in learning as the number of high DA alleles increased. This result is in line with what is shown in Chapter IV, where I found great L-DOPA associated learning impairment in less denervated patients. These results together indicate that a single dose L-DOPA is more detrimental to individuals with high endogenous striatal dopamine transmission whereas it may be more beneficial to individuals with low endogenous dopamine transmission.

For grooved pegboard performance there was a dissociable finding between COMT and DRD2 genes. Grooved pegboard was used as an index of manual motor

function primarily relying on the posterior and dorsal striatum. The task has been shown to be a sensitive marker of the level of striatal dopaminergic denervation in PD patients (Bohnen et al., 2007). I predicted that for pegboard performance, the two genotype groups would both be situated on the left hand side of the inverted U curve due to the involvement of posterior and dorsal striatum for this task, which is more depleted in early stage PD patients. I expected that L-DOPA would shift both genotype groups rightward on the curve, and depending on the actual initial position of the two groups and the magnitude of the L-DOPA effects, there may or may not be a genotype by L-DOPA interaction. There was a COMT genotype by L-DOPA interaction showing significant performance improvement with L-DOPA only in the val/met and not in the met/met group. This may be interpreted as a 'floor effect' (Williams-Gray et al., 2008) such that the effect of L-DOPA is not apparent in the met/met group where striatal dopamine availability is low.

Interestingly, there was no significant DRD2 genotype by L-DOPA interaction for grooved pegboard performance, only a main effect of L-DOPA. That is there was a significant performance improvement with L-DOPA across the two DRD2 genotype groups. The dissociable genetic effects on response to L-DOPA may be due to the difference in how the two genes contribute to dopamine transmission. Whereas the COMT gene regulates the availability of dopamine per se in the striatum, the DRD2 gene regulates the expression of D2 receptors. Post-synaptic dopamine receptors in the striatum are up-regulated in PD, particularly in the more denervated subregions (Sawle et al., 1993, Sun et al., 2010). This may be one explanation for why there is no DRD2 genotype effect on pegboard performance. Because the D2 receptors will be up-regulated



particularly in the dorsal/posterior striatum which is the subregion that manual motor control tasks such as the pegboard relies upon, the degree that DRD2 genotype may regulate receptor expression might have been washed out.

The presence of DRD2 effects in sequence learning, MMSE and MOCA suggests that these tasks rely on more intact striatal subregions including ventral/anterior striatum and the caudate which is relatively spared from receptor up-regulation. In contrast to DRD2, COMT has a more direct effect on the availability of the neurotransmitter dopamine and showed genotype effects on all of our neuropsychological measures (except MOCA) as well as sequence learning. Although I did not find significant ON versus OFF L-DOPA differences in MMSE, there still was a COMT by L-DOPA interaction showing that L-DOPA was more beneficial to met/met than val/met individuals.

Collectively, these results demonstrate a significant and selective genetic contribution to medication effects on motor sequence learning in PD. These results also provide support for the nature of the dopamine overdose as a non-beneficiary, deleterious effect of L-DOPA, being more pronounced in individuals with higher endogenous dopamine transmission. Additionally the data provide evidence for the distinctiveness of neural structures that the two tasks (i.e. sequence learning vs. pegboard) rely on, particularly providing evidence for the involvement of the more intact anterior and ventral striatum during sequence learning.

Although there have been several studies looking at COMT effects on response to dopaminergic medication in PD (Foltynie et al., 2004, Williams-Gray et al., 2007, Williams-Gray et al., 2008, Hoogland et al., 2010), DRD2 effects have not been widely

investigated. Several studies have shown an effect of D2 receptor polymorphism on response patterns of dopaminergic medications for PD, such as an increased risk in developing L-DOPA induced dyskinesia (Oliveri et al., 1999, Wang et al., 2001, Paus et al., 2008), however the results have not been consistent. These previous studies have either focused on the DRD2 TaqIA polymorphism (Wang et al., 2001, Paus et al., 2008) or the intronic short tandem repeat (Oliveri et al., 1999). The results of these studies are not consistent as to whether there is an association between DRD2 genotype and increased risk of L-DOPA associated motor fluctuations. There have not been any studies looking at the effect of the intronic DRD2 polymorphism (rs 1076560, G > T) on L-DOPA response patterns in PD (Zhang et al., 2007). Evidence shows that this polymorphism is associated with working memory performance with the minor T allele resulting in decreased performance or maladaptive neural recruitment in healthy young adults and schizophrenic patients (Zhang et al., 2007, Bertolino et al., 2009a, Bertolino et al., 2009b, Bertolino et al., 2010). A recent study has also shown that T allele carriers showed increased recruitment of the motor circuitries including basal ganglia, thalamus, primary and supplementary motor area which negatively correlated with reaction time during a motor task (Fazio et al., 2011). Similar results with the DRD2 TaqIA A1 allele, which is known to be the minor allele, were found in PD patients during a complex sequential motor task (Bartres-Faz et al., 2007). These studies suggest that the DRD2 polymorphism has effects on the motor system as well as for cognitive functions such as working memory.

To my knowledge, the current study is the first to clearly identify the effect of the COMT (val15met) and DRD2 polymorphisms (rs 1076560, G > T) on L-DOPA

associated performance change in PD patients. Although several studies have investigated the effects of the COMT gene and dopaminergic medication on prefrontally-mediated behaviors such as executive function in PD (Foltynie et al., 2004, Williams-Gray et al., 2007, Williams-Gray et al., 2008, Hoogland et al., 2010), few have investigated dopaminergic gene effects on motor behaviors, let alone gene by medication interactions in Parkinson's patients. Importantly I've focused on the contribution of COMT gene on striatal dopamine availability whereas in most studies, COMT gene is considered to regulate prefrontal dopamine level (Egan et al., 2001, Mattay and Goldberg, 2004, de Frias et al., 2005, Galderisi et al., 2005, Harris et al., 2005, O'Hara et al., 2006, Frank et al., 2007, Starr et al., 2007, Liu et al., 2008, Bertolino et al., 2010, Holtzer et al., 2010, Wiener et al., 2011). Whereas sequence learning involves the whole corticostriatal circuitry including both prefrontal cortex and striatum (Doyon et al., 1997, Hikosaka et al., 1998, Duff et al., 2007, Tamas Kincses et al., 2008), L-DOPA binds more directly to the denervated striatum than the prefrontal cortex in Parkinson's disease (Tedroff et al., 1996). Considering that the focus of this study was on genetic contributions to the medication effects, I've hypothesized that the COMT gene should be interpreted as a factor contributing to striatal dopamine availability where L-DOPA would have the most effect. The fact that I found a linear relationship between the number of high DA alleles and L-DOPA response assuming COMT gene contributing to striatal dopamine level also demonstrates that COMT and DRD2 genes work in an additive fashion. This implies a contribution of COMT in striatal dopamine levels which in turn influence medication effects in Parkinson's disease.

It is of note however that the COMT genotype results should be interpreted with caution. Because the level of disease stage measured by UPDRS showed a trend for a difference between the two COMT genotype groups, the results may have been due to the difference in disease stage or an interaction between disease stage and COMT genotype. Additionally I acknowledge that the distribution of genotypes for COMT was not consistent with previous studies.

In conclusion the current study demonstrated a dissociable gene by medication interaction in two motor tasks that rely on distinct cortico-striatal circuitries: motor sequence learning and grooved pegboard. The results suggest that L-DOPA acts differently during motor tasks depending on one's COMT and DRD2 polymorphism. The results furthermore suggest the importance of taking into account one's genotype when prescribing dopaminergic medications for PD patients.

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## **CHAPTER VI**

### **General discussion and conclusions**

#### **Overview**

The goal of this dissertation was to elucidate the mechanism of dopaminergic action on motor sequence learning. I have employed a multifaceted approach in a Parkinson's disease model to manipulate dopamine level and characterize the nature of dopaminergic effects on behavior, including identification of its underlying neural mechanisms. I have shown that dopaminergic medications are particularly deleterious to the early phase of motor sequence learning in mild to moderate PD patients (Chapter 2). In a follow up fMRI study I have shown that there was an L-DOPA associated reduction in ventral / anterior putmen recruitment during the early phase of learning and that the level of reduction in ventral / anterior putamen recruitment explained the level of L-DOPA associated performance change in sequence learning (Chapter 3). I have also reported that an individual's current level of striatal denervation and the ratio of anterior to posterior denervation explain the level of performance change associated with L-DOPA. Specifically, I have shown that the less denervation and the greater the gradient of anterior to posterior denervation the more interference that occurs due to L-DOPA

during motor sequence learning (Chapter 4). Finally, I have demonstrated that the genetic polymorphisms of COMT and dopamine D2 receptor genes determine how L-DOPA influences motor sequence learning, such that patients with the genotype that results in low dopamine transmission availability benefit from L-DOPA, but patients with the genotype that results in high dopamine transmission do not (Chapter 5). Collectively, these findings contribute to a comprehensive understanding of dopaminergic action on brain and behavior with complimentary evidence from behavioral, pharmacological fMRI, dopaminergic PET and genetics approaches.

### **General discussion**

In the first two studies I aimed to identify the behavioral and neural signatures of dopaminergic action over the time course of motor sequence learning in PD patients. My overall hypothesis was that with relatively more intact ventral / anterior than dorsal / posterior striatum in mild to moderate stage PD patients (Bernheimer et al., 1973, Kish et al., 1988, Frey et al., 1996, Rakshi et al., 1999), dopaminergic medications would selectively overdose the ventral / anterior sub-region. This would result in interfering with early phase motor sequence learning which relies on this region (Berns et al., 1997, Miyachi et al., 1997, Hikosaka et al., 2002, Seidler et al., 2002, Lehericy et al., 2005, Seidler et al., 2005).

I indeed found a selective interference of medication during the early phase of learning (Chapter 2). The ability to learn a sequence could be assessed repeatedly with the explicit motor sequence learning paradigm that I used because a new sequence can be

presented at each test session. This allowed me to selectively tease apart dopaminergic effects on sequence learning using a within subject design. The fMRI study was performed to identify the pattern of neural recruitment during motor sequence learning and to determine whether there is a parallel neural change associated with dopaminergic medications in PD patients. My data confirmed the involvement of the ventral / anterior striatum (i.e. associative striatum), particularly the ventral putamen, during the early phase of learning. Moreover, there was increasing involvement of sensorimotor cortical regions towards the late phase of learning. L-DOPA decreased activation of the ventral putamen during early sequence learning, with the degree of this reduction explaining performance changes in motor sequence learning.

The first two studies serve as behavioral and neural evidence showing dopaminergic action particularly during the early phase of motor sequence learning in PD patients. The two studies collectively suggest the existence of a “dopamine overdose” effect acting on the ventral / anterior striatum, a subregion that is relatively intact in early stage PD patients. Importantly, these two studies give additional support to a growing literature reporting behavioral side effects of dopaminergic medications in PD patients (for review see Cools, 2006, Wiecki and Frank, 2010) and the “inverted-U” relationship between dopamine level and performance (Arnsten and Robbins, 2002). Furthermore, my findings make a unique contribution to this literature by demonstrating that it is not just the nucleus accumbens and cognitive behaviors that are susceptible to these effects, but also the ventral putamen and motor behaviors. Although the fMRI results demonstrated primarily striatal involvement during early learning at least in the control group, it is likely that the whole ventral / anterior striatal circuitry including the prefrontal

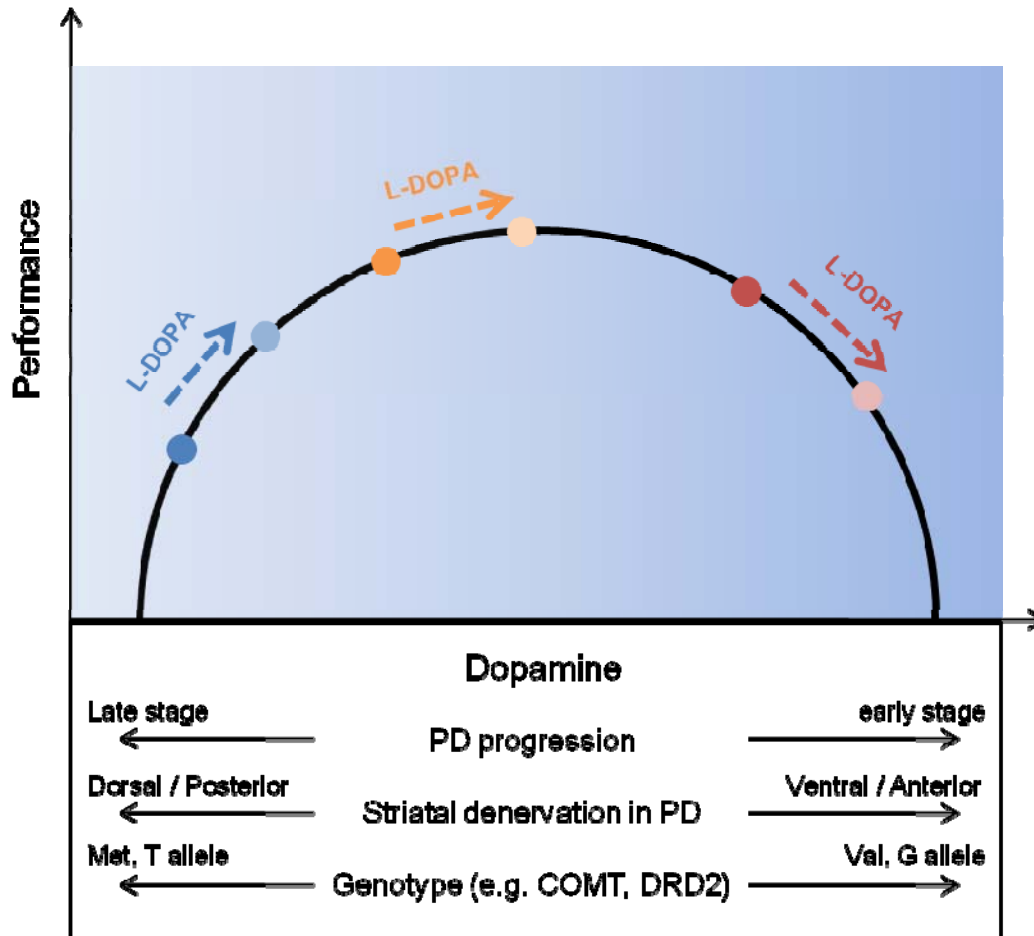
cortical target regions of striatum is involved in early learning and thus susceptible to L-DOPA effects indirectly via the striatum.

My next two studies examined how individual differences in patients' endogenous dopamine levels interact with exogenous dopaminergic administration. Specifically, the individual's current level of endogenous dopaminergic activity was indexed by  $^{11}\text{C}$ -DTBZ PET in Chapter 4 as a measure of dopaminergic denervation and the genetic variant in COMT and dopamine D2 receptor genes in Chapter 5. While the level of striatal denervation varies mostly due to disease progression, one's genotype determines the phenotype which is either dopamine availability or D2 receptor expression in striatum, irrespective of the disease. Thus the two measures give complementary information on one's current level of endogenous dopamine activity. In these two studies, I found that the extent to which L-DOPA impairs early motor sequence learning was inversely correlated with individual levels of striatal denervation (Chapter 4). Likewise the effect of L-DOPA on early motor sequence learning was significantly different between the two COMT and DRD2 genotype groups, who differ in terms of the resulting dopamine transmission (Chapter 5).

The results of these two studies demonstrate the nature of dopaminergic action on sequence learning behavior in a manner that is compatible with the "inverted-U" framework introduced in Chapter 1 (Arnsten and Robbins, 2002). In this framework, the endogenous level of one's dopaminergic activity as shown by striatal denervation or genotype will determine one's starting location along the inverted-U curve. For an equivalent dose of L-DOPA each individual will be shifted rightwards along the curve and the degree of performance change associated with L-DOPA will differ based on

one's starting point. That is, the further to the left one's initial location is (i.e. lower endogenous dopaminergic activity) the more they will benefit and the further to the right their initial location (i.e. greater endogenous dopaminergic activity) the more medication will interfere with performance. Results from Chapters 4 and 5 are in line with these explanations. The equivalent dose of L-DOPA was more beneficial to individuals with greater striatal denervation or a gene that results in less dopamine availability and D2 receptor expression and more deleterious to individuals with less denervation or a gene with greater dopamine and D2 expression level.

The series of studies in the current dissertation collectively demonstrates the nature of dopaminergic action on motor sequence learning in PD patients. These investigations led to a comprehensive understanding of the mechanisms of dopaminergic effects on brain and behavior relying on dopaminergic circuitries (Figure 6.1.).



**Figure 6.1.** Factors contributing to dopaminergic effects on performance. The severity of dopaminergic denervation and genotype can determine endogenous levels of dopamine. The differential denervation of striatal subregions can also contribute to the gradient of dopamine level in PD. These factors determine the starting location on the inverted-U as shown by the darker colors. Depending on their initial location, the equivalent dose of L-DOPA may have differential effects on performance as depicted by the degree of rightward shift from darker to lighter colors.

### Limitations

A few limitations should be taken into account when interpreting the findings of my experiments. First of all, the designation of learning phases (i.e. early and late phase) was arbitrary with respect to the duration of the task paradigm that I used. While many studies arbitrarily divide the duration of learning into early and late phases (Stephan et

al., 2009, Yin et al., 2009, Coynel et al., 2010, Park et al., 2010), they inevitably do not take into account the fact that late learning is dependent on how one does in the early phase of learning. This has little impact on my investigation of early phase learning, but since the late phase was determined in this arbitrary fashion it should not be treated independently of the early phase. The lack of medication effect on the late phase as shown in Chapter 2 may thus be due to this arbitrary designation of the late phase period. I predicted that late learning would benefit from L-DOPA but it did not. One potential way of resolving this issue may be to define the late phase as when subjects reach a plateau in performance change. For example, the late phase can be defined as when there is no longer a decrease in response time when performing sequences. I did not provide sufficient practice for this however; indeed several studies have shown that sequence performance continues to improve across several days of practice (Lehericy et al., 2005 for example).

Another complication that affects interpretation of the data across the four studies is that the deleterious effect of dopaminergic medications on the early phase of learning was not consistent across the four studies. There was a clear early learning impairment as measured by response time in Chapter 2, but this was not replicated in the following studies. Although this is not critical to the interpretation of each study, the fact that the behavioral findings are not consistent requires a consideration of methodological differences across the studies. The overdose effect refers to the phenomenon that a clinically efficient use of dopaminergic medication interferes with the relatively intact ventral / anterior striatum in PD. The lack of a clear overdose effect at the group level in terms of reaction time for Chapters 3, 4 and 5 may be due to the fact that we used a single

dose (200 mg) of L-DOPA instead of using the most clinically efficient medication for each patient (i.e. patient's own dosage) as in Chapter 2. The fact that the change in UPDRS score with L-DOPA was only minimal in Chapter 3 and not even significant in Chapter 4 demonstrates that the L-DOPA dosage we used was not sufficient to result in clinical improvement for all of the patients. Thus the failure to replicate my findings in Chapter 2 does not necessarily negate the existence of a dopamine overdose effect in Chapter 2. Moreover L-DOPA is known to particularly restore synaptic dopamine transmission in the more denervated striatal subregions (Tedroff et al., 1996). Depending on one's level of disease progression, it is possible that for the relatively more advanced stage patients, the 200 mg of L-DOPA we used was all "consumed" by the more denervated dorsal / posterior striatum and thus has little effect on the ventral / anterior striatum. The correlation between the extent of L-DOPA associated sequence learning impairment and the anterior to posterior denervation ratio as shown in Chapter 4 also supports this contention.

Additionally, the use of L-DOPA alone in the current study as opposed to the combination of L-DOPA and dopamine agonists used in Chapter 2 as part of the patients' regular treatment, may have reduced the medication-associated early learning impairment. Studies have shown that the effect of dopaminergic agents (either improving or impairing performance) is particularly significant with D2 receptor agonists and less apparent with L-DOPA (Kimberg et al., 1997, Roesch-Ely et al., 2005, Frank and O'Reilly, 2006, Cools et al., 2007a, Cools et al., 2007b, Cools et al., 2009, Frank and Fossella, 2011). Clinically, medication-associated behavioral complications in PD including impulse control disorders and compulsive gambling has been most prevalent



with dopamine agonists (Fenu et al., 2009). These clinical manifestations of agonist specific interference in normal reward and feedback processing cortico-striatal circuitry propose a greater role agonist may have in deleterious effect to sequence learning. Thus although my results in Chapters 2, 3 and 4 do not sufficiently determine the dopamine over-dose effect itself, it allows for the prediction of which patient is in the higher risk for dopamine overdose.

Despite the loss of clear behavioral overdose effects, using a single dose of L-DOPA allowed me to interpret the results in Chapters 4 and 5 without other confounding factors. That is, the variability in L-DOPA-associated sequence learning performance is explained by the level of striatal denervation as shown in Chapter 4 because the performance change was induced using a single dose of L-DOPA across all patients. If the patients' own medications were used, the variability in performance change which also includes the inter-individual difference in medication regimens may not be explained by the level of denervation. Also the genotype by medication interaction observed in Chapter 5 can also be clearly explained as a significant genetic contribution since across the two genotype groups a consistent amount of exogenous dopamine was administered.

### **Implications and future directions**

The current studies have significant implications for understanding and treating the pathophysiology of PD. First of all, the results suggest that PD patients learn new motor skills differently depending on their relative dopaminergic state. The fact that they learn better OFF medication, at least during the initial acquisition period, implies that the

first few sessions of physical rehabilitation for learning new strategies of movement control might benefit from being performed when patients are OFF meds. However in order to generalize these findings, the validity of these medication effects should be tested using other forms of motor skill learning, such as sensorimotor adaptation. In addition, considering that there are both implicit and explicit forms of learning (Seidler et al., in press), whether or not dopaminergic medications affect both forms of learning should be investigated as well.

Secondly, the current dissertation proposes a more careful use of dopaminergic treatments in PD. There have been numerous reports of medication associated maladaptive behaviors associated with treatments in PD such as compulsive gambling (Moustafa et al., 2008, Rossi et al., 2010, Ambermoon et al., 2011, Hassan et al., 2011, Zahodne et al., 2011). In line with these effects, I have documented a medication-associated impairment in a form of motor learning which is critical for daily motor adaptability. Furthermore I have shown that the degree that patients will be affected by medications depends on their individual level of striatal denervation and their genotype for genes that regulate dopaminergic activity. It is of note that the neural activation changes associated with L-DOPA were detected even in the absence of behavioral effects at the group level and that the neural changes were able to predict the behavioral changes as shown in Chapter 3. Although I haven't tested whether the neural activation changes are also determined by one's level of striatal denervation or genotype, my results suggest the potential use of the neural activation measures for determining the degree of medication associated side effects. It is also of note that the neurotransmitter dopamine interacts with other neurotransmitter systems such as the cholinergic system (Lester et al.,

2010). Recently there has been an emerging view of PD as a multisystem disease including the cholinergic system (Zahodne et al., 2008, Bohnen & Albin, 2009, 2010). It is therefore important to consider the interaction between dopaminergic treatments and the neuropathology of PD associated with the cholinergic system.

These results collectively suggest a careful prescription of dopaminergic agents titrated for one's current state of denervation and even one's genotype (i.e., individualized medicine). It is crucial to take into account that the neurotransmitter dopamine plays a significant role in a number of behavioral domains such as learning, reward and affective processing as well as motor control (Arnsten and Robbins, 2002, Schultz, 2007, Frank and Fossella, 2011). Thus treating PD patients with dopamine may inevitably interfere with all of these other behavioral domains apart from treating the patients' motor symptoms. It will be critical to come up with a medication regimen that achieves an optimal balance between restoring the depleted dopamine in the dorsal and posterior striatum without interfering with other functional domains. In a similar vein, the current findings also have potential for interpreting side effects associated with dopaminergic treatments in other neuropsychiatric diseases, such as Schizophrenia, ADHD and Tourette's syndrome.

## **Conclusions**

The current dissertation provides a mechanistic account of dopaminergic action on motor sequence learning in PD patients. By combinatorial approaches of behavioral, pharmacological, fMRI, dopaminergic PET and genetics I have shown the following: 1)

dopaminergic medications are deleterious to early phase motor sequence learning in mild to moderate stage PD patients, 2) L-DOPA reduces ventral putamen activation during early motor sequence learning, 3) the current level of an individual's dopaminergic denervation and one's COMT and D2 receptor genotype explain the extent of L-DOPA-associated sequence learning performance change. These studies have significant implications for the design of effective physical rehabilitation protocols and suggest a careful use of dopaminergic treatment in PD considering multiple factors including one's level of dopaminergic denervation and genotype. In a broader perspective these studies also provide a more profound comprehension of how the neurotransmitter dopamine contributes to human behavior.

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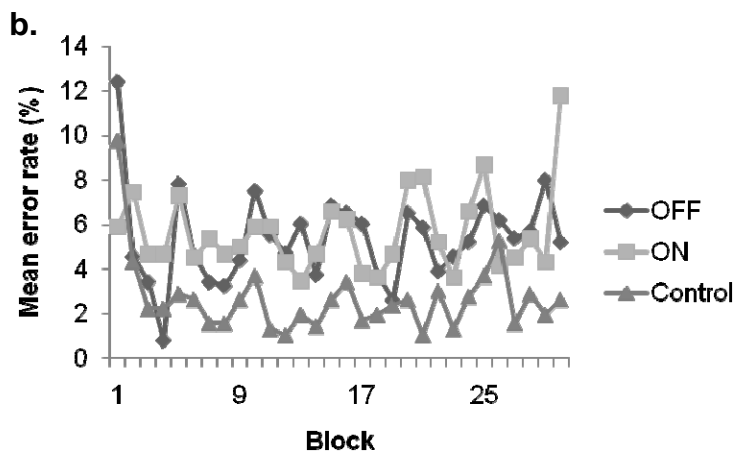
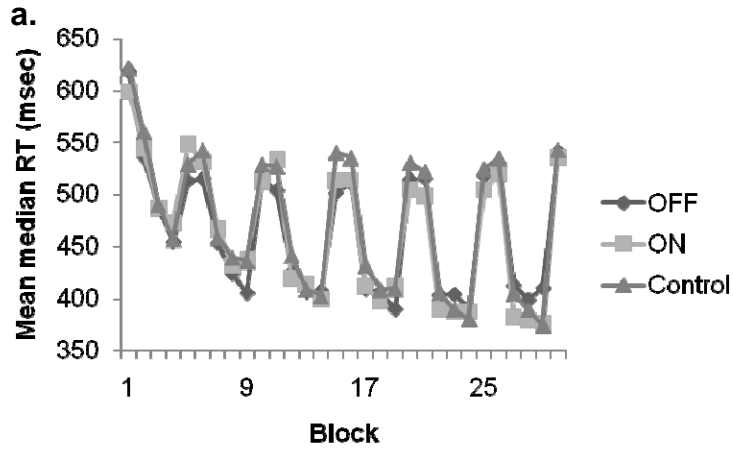
## **Appendix A - Supplementary Material for Chapter III**

I also performed whole brain comparisons of sequence learning specific activation at each learning phase across the three groups using  $p < 0.001$  uncorrected and an extent threshold of 10 contiguous voxels. Whole brain comparisons between the PD groups showed greater left thalamus activation in PD OFF than PD ON in the early phase of sequence learning. In the middle phase, greater right thalamus, left precentral (BA 6) and middle temporal gyrus (BA 39) activation was found in PD OFF compared to PD ON. Finally, in the late learning phase, PD ON showed greater activation in the left insula (BA 13) than PD OFF. No significant clusters were found with the reverse contrast in each phase. Comparisons between each PD group and controls showed a decrease in sequence learning related activation in the PD groups. This pattern was only present for the early learning phase with both PD OFF and PD ON exhibiting decreased activation in the left putamen compared to controls. Additionally PD OFF showed decreased activation in the left inferior frontal gyrus (BA 47) (Supplementary Table A.1.).



**Supplementary Table A.1.** Whole brain group level comparisons of sequence learning specific neural activation at each learning phase.

Region	R/L	BA	MNI coordinates			T value
			x	y	z	
<i>Early</i>						
<i>OFF &gt; ON</i>						
Thalamus (Pulvinar)	L		-26	-24	8	4.53
<i>OFF &lt; Controls</i>						
Inferior frontal gyrus	L	47	-44	34	-12	3.91
Putamen	L		-32	-8	-6	3.89
<i>ON &lt; Controls</i>						
Putamen	L		-24	-24	4	4.15
<i>Middle</i>						
<i>OFF &gt; ON</i>						
Thalamus (Ventrolateral nucleus)	R		14	-10	6	4.4
Precentral gyrus	L	6	-60	2	6	4.39
Middle temporal gyrus	L	39	-50	-68	10	4.32
<i>Late</i>						
<i>ON &gt; OFF</i>						
Insula	L	13	-34	-36	20	5.24

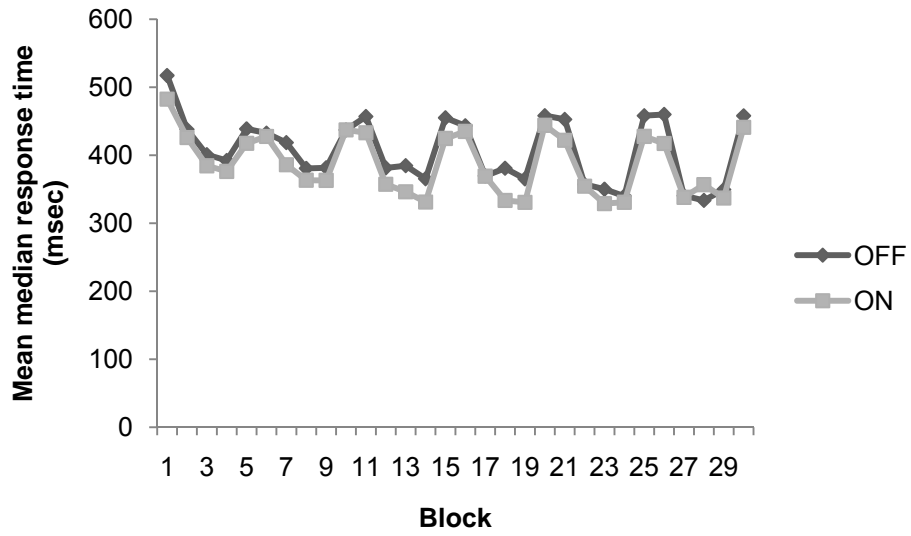


**Supplementary Figure A.1.** Mean median response time (a.) and mean error rate (b.) across 30 blocks of the explicit motor sequence learning task including both random and sequence blocks for patients in ON and OFF L-DOPA states. Blocks 1, 5, 6, 10, 11, 15, 16, 20, 21, 25, 26, and 30 comprised randomly presented stimuli while blocks 2-4, 7-9, 12-14, 17-19, 22-24 and 27-29 consisted of sequentially presented stimuli.

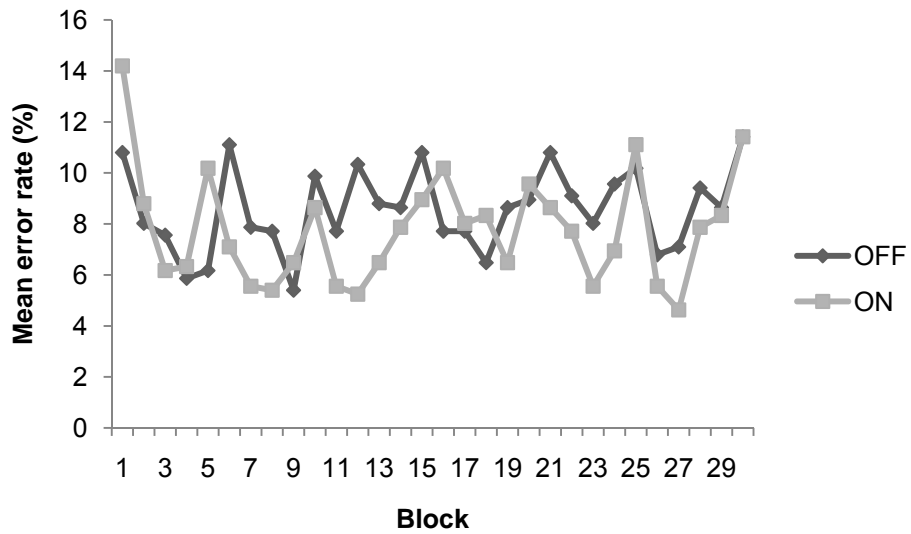
## **Appendix B - Supplementary Material for Chapter IV**

I also compared LM\_RT and LM\_error ON and OFF L-DOPA across the six learning runs. A medication status by learning run repeated measures ANOVA on LM\_RT showed no main effect of medication status and a significant main effect of learning run ( $F_{5,85} = 4.62$ ,  $p < 0.005$ , Supplementary Figure B.1.). There was also a nonsignificant trend for a medication status by learning run interaction ( $F_{5,85} = 1.96$ ,  $p = 0.09$ , Supplementary Figure B.2.). A medication status by learning run repeated measures ANOVA on LM\_error showed no significant main effects nor interactions (Supplementary Figure B.3.).

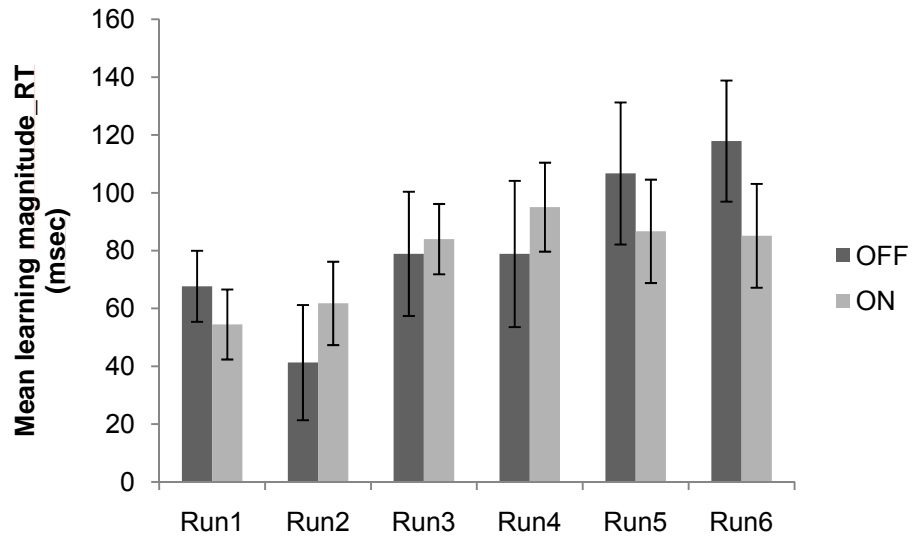
a.



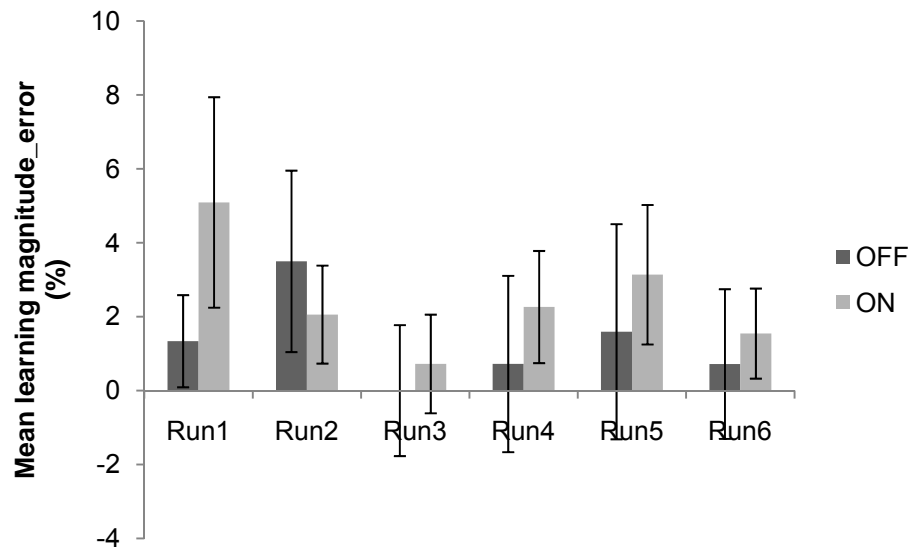
b.



**Supplementary Figure B.1.** Mean median response time (a.) and mean error rate (b.) across 30 blocks of the explicit motor sequence learning task including both random and sequence blocks for patients in ON and OFF L-DOPA states. Blocks 1, 5, 6, 10, 11, 15, 16, 20, 21, 25, 26, and 30 comprised randomly presented stimuli while blocks 2-4, 7-9, 12-14, 17-19, 22-24 and 27-29 consisted of sequentially presented stimuli.



Supplementary Figure B.2. Mean LM\_RT across 6 learning runs in ON and OFF L-DOPA states.



Supplementary Figure B.3. Mean LM\_error across 6 learning runs in ON and OFF L-DOPA states.