COMPENSATORY MECHANISMS IN PARKINSON’S DISEASE

by

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ABSTRACT

Parkinson’s disease (PD) is a common movement disorder, affecting 1% of the population over the age of 65. Pathologically, PD results from degeneration of nigral dopaminergic neurons, however symptoms do not appear until an estimated 50% of these cells are lost, suggesting compensatory mechanisms exist which mask disease onset, and may later delay progression of the disease. Compensation may take place over various spatial and temporal scales, from changes in synaptic dopamine release and synthesis that take place over a period of minutes, to recruitment of novel, widespread networks of brain regions for a specific task, which may require formation of new connections over an extended period of time. Neuroimaging techniques have recently allowed the investigation of regional and network changes in activation related to motor performance in PD, however the question of whether such changes represent a downstream effect of basal ganglia degeneration, or a compensatory change, remains difficult to determine. Here, we applied an approach from research into Alzheimer’s Disease, where abnormal activation patterns are studied in the context of tasks of increasing difficulty, such that inferences regarding their compensatory nature can be made. We show that individuals with PD are able to increase the recruitment of normal networks for a motor task (motor reserve) as a form of compensation, in addition to compensatory recruitment of novel networks to accomplish the same task as healthy controls. In particular, we observe a switch from striato-thalamo-cortical (STC) motor loops to cerebello-thalamo-cortical (CTC) loops as a compensatory strategy. This compensatory recruitment involves changes in the amplitude, spatial extent, and connectivity of regions within the CTC pathway. However, this compensation does not come without a price, since we show that compensatory CTC recruitment involving disconnection between the STC and CTC loops occurs in subjects with tremor-dominant PD, but not akinetic-rigidity-dominant
PD, supporting a growing body of evidence that suggests the cerebellum plays an important role in the generation of PD tremor. Together, this body of research has implications for treatments that target the symptom of tremor in PD, as therapies which minimize tremor might also reduce beneficial aspects of compensation.
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<td>ACC</td>
<td>anterior cingulate cortex</td>
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<td>AD</td>
<td>Alzheimer’s disease</td>
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<td>BG</td>
<td>basal ganglia</td>
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<td>BOLD</td>
<td>blood oxygen level-dependent</td>
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<td>CAU</td>
<td>caudate</td>
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<tr>
<td>CER</td>
<td>cerebellum</td>
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<td>COMT</td>
<td>catechol O-methyltransferase</td>
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<td>CTC</td>
<td>cerebello-thalamo-cortical</td>
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<td>DA</td>
<td>dopamine</td>
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<td>DAT</td>
<td>dopamine transporter</td>
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<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
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<td>dihydrotetraphenazine</td>
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<td>electroencephalography</td>
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<td>FDG</td>
<td>fluorodeoxyglucose</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>GLP</td>
<td>globus pallidus</td>
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<td>GPI</td>
<td>globus pallidus internal segment</td>
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<tr>
<td>GPe</td>
<td>globus pallidus external segment</td>
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<td>L-dopa</td>
<td>levodopa</td>
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<td>M1</td>
<td>primary motor cortex</td>
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<td>MAR</td>
<td>multivariate autoregressive modelling</td>
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<td>MPTP</td>
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<td>maximum voluntary contraction</td>
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<td>NAR</td>
<td>novel area recruitment</td>
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<td>posterior cingulate cortex</td>
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<td>PD</td>
<td>Parkinson’s disease</td>
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<td>PDRP</td>
<td>Parkinson’s disease related pattern</td>
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<td>PET</td>
<td>positron emission tomography</td>
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<td>pre-frontal cortex</td>
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<td>pre-proenkephalin-A</td>
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<td>putamen</td>
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<td>rCBF</td>
<td>regional cerebral blood flow</td>
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<td>region-of-interest</td>
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I would like to thank my supervisor, Dr Martin McKeown, for his continued support and guidance throughout my graduate studies. I came to UBC five years ago with little experience of Parkinson’s research, and no neuroimaging experience, and I am grateful to leave with a body of knowledge and an enthusiasm for research that has put me in good stead for my future endeavors and, hopefully, a productive career in research.

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Thanks to my family for always encouraging me through my many years of studies and for making me believe in myself.

And last but by no means least, thank you to Lewis for your love and support, and for always being there. I couldn’t have done it without you.
DEDICATION

For my Dad
CO-AUTHORSHIP STATEMENT

Chapters 2-7 of this thesis involve work performed in collaboration with my research supervisor, fellow graduate students, and colleagues at both the University of British Columbia and Simon Fraser University. Credit has been given to the contributions of others, either in co-authorship of published and submitted manuscripts or within the acknowledgements section. For manuscripts in which I am named as first author, I performed the majority of the work myself, including interpretation of results, all of the data collection and data pre-processing, and all of the writing, save for comments and suggestions from my research supervisor and collaborators, and assistance with technical appendices.

Specifically, for Chapters 2-7 I contributed to the design of the experimental paradigms, and performed all subject recruitment with the exception of Chapter 7, which was recruited for by Dr Edna Ty. I collected all patient and control subject data myself, with the responsibility for training subjects outside of the scanner, running the experimental stimulus while subjects were in the fMRI scanner, and for setting up the EEG cap in Chapter 3. I assisted with assessment of Parkinson’s symptoms from PD subjects while off medication, and obtained written and verbal informed consent from all subjects for Chapters 2-6. Informed consent for Chapter 7 was obtained by Dr Edna Ty. I performed the pre-processing for all functional MRI data and initial pre-processing of EEG data for Chapter 3. I assisted with creation of the figures for all Chapters, and was responsible for interpretation and writing of the manuscripts contained within this thesis. I performed the LLDA analysis for Chapters 2, 5 and 6 using a custom script written in Matlab by Dr Martin McKeown. Similarly, connectivity figures for Chapters 5, 6, and 7 using PCfdr were obtained by running analysis scripts written by Drs Jane Wang and Junning Li.
CHAPTER 1: LITERATURE REVIEW

1.1. INTRODUCTION

It has long been known that the brain can sustain a remarkable amount of damage without any resulting functional impairment (Satz, 1993). The ability of the brain to compensate for such damage and maintain near-normal output requires plasticity. Plasticity has been widely studied at a synaptic level, where both changes in synaptic strength (e.g. via modulation of the excitatory/inhibitory balance) and formation of new synaptic connections contribute to normal development, learning and memory. More recently, it has become apparent that such changes may also occur in response to damage, as the brain tries to maintain an optimum level of performance. Synaptic plasticity following injury is just one form of compensatory mechanism, and often its role is to preserve homeostasis of a particular neurotransmitter system. Other compensatory mechanisms may take place on a larger spatial scale, involving changes in the regions of the brain responsible for controlling a specific function. This may involve increased recruitment of existing resources (neuronal reserve), without the need for structural or functional changes. It is also possible that a region may assume a role in functions it did not previously subserve by utilizing existing connections that were previously inhibited, in a process known as unmasking. Alternatively, plastic changes at the synaptic or microscopic level can allow for network changes at the systems or macroscopic level, resulting in recruitment of novel areas and novel connections to accomplish a given task. An important distinction should be made here between compensatory mechanisms just described, and adaptation, where the behavioural strategy utilized to accomplish a specific endpoint is adjusted to take account of changes in either internal or external factors.
Compensation may also occur over various temporal scales. Increased recruitment of existing neuronal resources may take place immediately, and microscopic synaptic changes may take place within minutes. In contrast, growth of new, replacement connections requires a longer time period. Compensatory recruitment of novel networks may slowly develop over many days, weeks or even months of rehabilitation following damage to a specific region.

_In the following thesis, compensation is defined as any structural or functional change that occurs following damage to the brain which serves to maintain optimal performance._

### 1.2. BACKGROUND TO COMPENSATORY MECHANISMS IN PD

Parkinson’s disease (PD) is the most common form of movement disorder, affecting 1% of the population above the age of 65 (Lang and Lozano, 1998b; Lang and Lozano, 1998a). It is a chronic neurodegenerative disease characterised pathologically by a progressive loss of the dopaminergic neurons of the substantia nigra pars compacta (SNC), and the presence of intraneuronal cytoplasmic inclusion bodies in the substantia nigra, known as Lewy bodies.

The substantia nigra is part of the basal ganglia (BG), a group of interconnected nuclei that together with the cerebral cortex and thalamus comprise the motor loop responsible for control of voluntary movement (Albin et al., 1989). Many of the most common movement disorders can be explained on the basis of our current understanding of the functional anatomy of the basal ganglia (Lange et al., 1997), as shown in Figure 1.1.
In PD, the degeneration of dopaminergic neurons reduces an important modulatory input from the substantia nigra to the corpus striatum, ultimately resulting in a hypokinetic state in which the cardinal symptoms include bradykinesia, resting tremor, rigidity, gait abnormalities and postural disturbances (Lang and Lozano, 1998a; Lang and Lozano, 1998b). Symptoms typically present unilaterally at onset, but become bilateral as the disease progresses. Non-motor symptoms including autonomic disturbances, depression, anxiety, and cognitive impairments may also be present (Clarke, 2007).
The exact etiology of PD is unknown, although various genetic and environmental factors have been identified, and several potential pathological mechanisms have been proposed, including oxidative stress and mitochondrial dysfunction (Olanow and Tatton, 1999). Whatever the underlying cause of nigral cell death, the characteristic symptoms of PD do not appear until an estimated 50% of nigral cells and 60-80% of striatal dopamine levels have been lost (Fearnley and Lees, 1991; Hornykiewicz and Kish, 1987). The length of this presymptomatic stage of PD may vary significantly between individuals, although neuroimaging studies provide estimates of around 5 years (Marek et al., 2001; Morrish et al., 1996). The nature of this presymptomatic phase is not fully understood, but the lack of motor symptoms despite significant cell loss is indicative of the existence of successful compensatory mechanisms (Zigmond et al., 1990), which first mask the existence of PD and later delay the onset and progression of motor abnormalities (Bezard and Gross, 1998).

The existence of compensatory mechanisms in PD may account for the finding that imaging measures of pathological disease progression, such as PET and SPECT, do not appear to correlate well with clinical measures of symptom severity, such as the Unified Parkinson’s Disease Rating Scale (UPDRS) (Marek et al., 2001; Morrish et al., 1996; Nurmi et al., 2001). Evidence from longitudinal studies suggests a non-linear relationship between dopaminergic cell loss and functional impairment (Zigmond et al., 1990), and recent work has indicated that the neurodegeneration of dopamine neurons in PD progresses with a negative exponential course (Hilker et al., 2005). The rate of progression in PD patients is widely variable and is thought to be influenced by factors such as age of onset, dominant symptoms at onset (tremor type vs. akineti-rigidity type), and baseline cognitive and motor impairment (Marras et al., 2002). Patients with similar disease characteristics at onset may still show differing progression,
however, and this can potentially be explained by individual differences in compensatory mechanisms.

Identification of compensatory changes in PD is thus extremely important for the development of sensitive biomarkers that can provide a more accurate prognosis for patients and can better guide the design and interpretation of clinical trials (Marras et al., 2002). Increased knowledge of the preclinical stage of PD may also facilitate earlier detection, which will enable clinicians to optimize the effectiveness of therapeutic strategies.

Despite the many benefits of an increased understanding of compensatory mechanisms, this is an area that remains under-researched in PD, mostly due to the methodological issues involved, namely the problem of differentiating between disease-related changes and adaptive changes, particularly at the macroscopic level. However, in the past decades substantial evidence has emerged in support of both dopaminergic and non-dopaminergic compensatory mechanisms in PD. Prior work typically focused on compensatory biochemical changes at the synaptic level, whilst inherent technical difficulties in investigating functional and structural changes at a systems level meant this area was less accessible to research. Together with recent improvements in neuroimaging and electrophysiological techniques, research has now begun to consider compensatory changes in terms of wider temporal and spatial aspects. Indeed, data from experimental models of PD consistently suggest that the full spectrum of parkinsonian symptoms cannot be attributed solely to a dysfunction of the basal ganglia (Chesselet and Delfs, 1996; Wichmann and DeLong, 1998). This chapter will consider the available evidence for dopaminergic and non-dopaminergic compensatory mechanisms both within and outside of the basal ganglia.
1.3. COMPENSATION AT DOPAMINERGIC TERMINALS

Perhaps the most obvious form of compensatory mechanism in PD is the existence of adaptive neurochemical changes within the nigrostriatal pathway that serve to maintain the homeostasis of dopamine transmission (Zigmond et al., 1990; Bezard and Gross, 1998; Zigmond and Stricker, 1985). Changes in dopamine transmission in PD have been studied for many years, and both pre- and post-synaptic regions have been shown to play a role in the homeostatic regulation of nigrostriatal dopamine in animal models of PD (for reviews see Zigmond et al., 1990; Zigmond et al., 1993; Robinson et al., 1990).

Like other monoamines, dopamine transmission involves 6 processes: synthesis, packaging into synaptic vesicles by type 2 vesicular monoamine transporters (VMAT2), release into the synaptic cleft upon depolarisation of the cell, interaction with pre- and post-synaptic receptors, reuptake of extra-cellular dopamine (DA) via the plasma membrane dopamine transporter, and degradation of remaining DA by catechol O-methyltransferase (COMT).

In PD, presynaptic changes include an increase in the synthesis, release and metabolism of dopamine (e.g. Lee et al., 2000), enabling basal extracellular levels to remain relatively normal. This is achieved through direct somatodendritic release, indirect action of dopaminergic collaterals on the soma, and negative feedback from striatal GABAergic afferents (Groves et al., 1975; Geffen et al., 1976; Llinas et al., 1984; Grace and Bunney, 1985). The DA transporter (DAT) also plays an important role in maintaining normal dopaminergic transmission (Stachowiak et al., 1987; Snyder et al., 1990; Lee et al., 2000). Several studies have shown that DAT activity is down-regulated in the denervated striatum, thus increasing dopamine availability by reducing the amount of reuptake.
Postsynaptic adaptations include increased dopamine receptor sensitivity and long-term changes in the number of postsynaptic receptors (Lee et al., 1978; Morissette et al., 1996). Zigmond and colleagues proposed a 2-step compensatory process in which rapid changes act first to maintain sufficient dopamine concentration in the striatum, followed by slower changes that promote the optimal use of the dopamine supply by surviving neurons (Zigmond et al., 1990; Zigmond et al., 1993). The slower postsynaptic mechanisms are thought to come into effect only when presynaptic mechanisms can no longer maintain striatal dopamine at relatively normal levels.

In addition to these pre- and post-synaptic changes, dopamine homeostasis can be further regulated by so-called volume transmission of dopamine (Zoli and Fuxe, 1996). Specifically, it is suggested that striatal dopamine terminals can undergo changes such that higher concentrations of dopamine diffuse into the extracellular space. Indeed, recent studies in animal models of PD show that in unilaterally lesioned rat striatum, dopamine levels are partially restored by diffusion of dopamine from the unlesioned contralateral region (Bjelke et al., 1994).

1.4. NON-DOPAMINERGIC COMPENSATION IN THE BASAL GANGLIA

The synaptic changes in dopamine transmission just described have been observed in animal models and in individuals with a clinical diagnosis of PD, both cases where nigrostriatal degeneration may already be fairly advanced. However, several reports have questioned whether these same mechanisms act to delay symptom onset in the early, presymptomatic stages of PD, before clinical diagnosis is possible (for review see Bezard et al., 2003). Garris and colleagues (Garris et al., 1997) have shown that with more moderate degeneration, the rate of dopamine release actually decreases in proportion to lesion size, yet dopamine levels in the striatum are
maintained, suggesting that other, non-dopaminergic mechanisms may also act during the presymptomatic period of PD.

Clarification of the true type of compensatory mechanism seen in the presymptomatic stage was difficult in early research due to the absence of animal models that replicated the progressive process of neurodegeneration seen in PD (Bezard et al., 2003). More recently, repeated administration of low doses of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to non-human primates has been used to produce a progressive dopaminergic cell loss over a one month period, more closely resembling the changes seen in human PD. Studies with this progressive model have shown that changes in dopamine metabolism and release occur at a later stage of neurodegeneration (Bezard et al., 2001b). This finding led to investigation of other mechanisms that may compensate for reduced dopamine transmission in the nigrostriatal pathway, turning attention to neurotransmission in other projections of the basal ganglia.

Studies with MPTP-treated monkeys showed an increase in GABAergic and enkephalinergic transmission from the striatum to the globus pallidus pars externalis (GPe) (Asselin et al., 1994; Levy et al., 1995). The resulting increase in inhibition of the GPe is suggested to play a role in development of motor symptoms in PD by modulating activity in the indirect pathway of the basal ganglia, ultimately resulting in overactivity of the inhibitory output regions, the GPi and SNr, leading to a reduction in movement (DeLong, 1990). The role of the co-transmission of GABA and enkephalin in this pathway is not clear, but it has been suggested that an upregulation of the precursor pre-proenkephalin A (PPE-A) represents a compensatory mechanism that attempts to reduce the overactive GABAergic output (Maneuf et al., 1994). Indeed, PPE-A mRNA levels are elevated in asymptomatic monkeys in the progressive MPTP model (Bezard et
al., 2001), but this upregulation eventually disappears once symptoms appear (Schneider et al., 1999).

Another hyperactive pathway in PD which is thought to be compensatory is the glutamatergic pathway from the STN to the substantia nigra (Bezard et al., 1997a). In support of this, Bezard and colleagues (Bezard et al., 1996; Bezard et al., 1997b) showed that temporary blockage of glutamatergic inputs to the SNc in asymptomatic monkeys treated with MPTP induced Parkinsonian motor signs. In monkeys with full MPTP-induced Parkinsonism, blockage of glutamate input to the SNc no longer had any effect (Bezard et al., 1997a).

1.5. REGIONAL CHANGES OUTSIDE THE BASAL GANGLIA

Despite the well established evidence for the aforementioned compensatory mechanisms within the basal ganglia, overactivity of the output regions of the basal ganglia can already be seen in pre-symptomatic primate models of PD, suggesting that structures downstream of the basal ganglia must also provide compensation that prevents the onset of symptoms (Bezard et al., 2001a). Indeed, this would seem likely since no region of the brain works in isolation, and the basal ganglia is only one of a number of structures associated with production of movement. A disruption in transmission through the basal ganglia, then, can potentially disrupt a number of networks within the brain.

Advances in brain imaging technology have enabled regional brain activity to be studied using functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and electroencephalographic or magnetoencephalographic methods (EEG/MEG) that measure brain activity based upon hemodynamic, metabolic and electromagnetic properties respectively. These techniques have enabled changes in brain activity to be assessed in patients both on and off
medication. Determining whether these changes are compensatory as opposed to an effect of the disease-related degeneration is methodologically difficult, and appropriate techniques to disentangle these two types of activation are discussed later in this chapter.

The first neuroimaging technique to shed light on the many regional changes that occur in early PD was positron emission tomography (PET). The main advantage of PET over other imaging techniques is that biological substrates can be labeled with radiotracers to assess their in vivo function. By labeling glucose ([18F]-fluorodeoxyglucose[FDG]) or water ([15O]-H2O), PET can be used to determine local metabolism or blood flow respectively. Eidelberg and colleagues (1994) used 18F-FDG PET to identify changes in resting state regional metabolism in patients with early, unmedicated PD. They identified a specific ‘Parkinson’s Disease related pattern’ (PDRP) characterised by hypometabolism of lateral premotor cortex, supplementary motor area (SMA), dorsolateral prefrontal cortex (DLPFC), and parieto-occipital association areas, together with hypermetabolism of pallidal, thalamic, and pontine regions. This pattern has been consistently identified in several populations of PD patients (Moeller et al., 1999), shows positive correlation with motor disability and disease severity (Eidelberg et al., 1995), and permitted differentiation of PD patients from age-matched controls, as well as between PD and atypical parkinsonism or other forms of nigrostriatal degeneration (Eidelberg et al., 1994; Eckert et al., 2008). Expression of the PDRP is reduced in correlation with clinical improvement following both surgical treatment (Eidelberg et al., 1996; Fukuda et al., 2001) and levodopa administration (Feigin et al., 2001).

Voluntary movement has also been studied in PD with PET - using radiotracers that measure regional cerebral blood flow (rCBF) as a correlate of neuronal activity; and with functional MRI - using the blood-oxygen level dependant (BOLD) signal as an indirect measure of neuronal
activity. Several studies have shown reduced activation in the supplementary motor area (SMA),
dorsolateral prefrontal cortex (DLPFC) and anterior cingulate in PD relative to healthy controls
(Jahanshahi et al., 1995; Playford et al., 1992). Interestingly, these regions represent the main
output projections of the basal ganglia to motor, associative and limbic regions (Thobois et al.,
2004). Primary motor, parietal and lateral premotor cortices were normally activated. Other
studies using either PET or functional MRI have found that activity of the lateral cerebello-
parieto-premotor circuit is increased in PD patients, whilst activity in mesial-SMA-cingulate
circuits is reduced (Rascol et al., 1997; Sabatini et al., 2000; Samuel et al., 1997). The lateral
premotor-posterior parietal network has been implicated in visually guided movements (Wise et
al., 1997), which are relatively unimpaired in PD. A switch to more visually guided motor
networks as a compensatory strategy fits with evidence that PD subjects become increasingly
reliant on external cues to perform movement successfully (Martin, 1967; Dick et al., 1989;
Glickstein & Stein 1991; Cunnington et al., 1995; Praamstra et al., 1998).

Abnormal activation patterns in PD are highly task dependent. For example, SMA activity is
reduced in tasks that require attentional or selection processes (Rowe et al., 2002), but appears
normal in tasks that are externally cued and highly repetitive (Turner et al., 2003). Primary motor
cortex activation has been shown to be normal during joystick movements in a freely chosen
direction cued at a regular pace (Playford et al., 1992; Jenkins et al., 1992), but hyperactive
during a freely chosen joystick movement cued with random inter-trial intervals (Haslinger et al.,
2001). M1 was hyperactive in a study by Sabatini and colleagues in which subjects performed a
learned finger tapping sequence (Sabatini et al., 2000), but hypoactive during self-chosen
regularly paced finger taps in another study (Buhmann et al., 2003).
The fact that cerebral activation is task dependent suggests that PD patients may have difficulty in selecting motor programs that correspond to the type of task they are performing (Thobois et al., 2004). Increased activity may represent an inability to inhibit inappropriate motor circuits and/or select an appropriate circuit (Boecker et al., 1999). Alternatively, increased activity may be a form of compensatory neuronal plasticity for movement in the presence of dysfunctional regions such as the basal ganglia (Rowe et al., 2002).

### 1.6. Changes in Functional and Effective Connectivity

It is becoming increasingly evident that it is insufficient to simply look at discrete regions of activation in relation to neurological disease, since static images of brain regions activated during a particular task do not demonstrate how these regions are interconnected. Consequently, studies of interactions between brain regions are starting to play a more important role in understanding brain function (Jiang et al., 2004), and the concept of neural networks for specific tasks is essential for understanding the organized behaviour of the brain beyond the simple mapping of brain activity (Horwitz, 2003; Lee et al., 2003). Researchers have thus begun to explore inter-regional connectivity during rest or during performance of specific tasks, and this is usually described in terms of functional connectivity or effective connectivity (Friston, 1994).

Functional connectivity refers to the temporal correlation between spatially separate neurophysiological events, whilst effective connectivity refers to the simplest brain circuit that would produce these temporal relationships, providing a connectivity pattern which tells us the strength and directionality of information flow. Several methods have been developed and applied, with functional connectivity commonly estimated by measuring the covariance or coherence of time-locked activation at different sites (Cordes et al., 2000; Biswal et al., 1995).
Additionally, the effect of an experimental manipulation on connectivity within a pre-specified model (usually defined on the basis of prior anatomical knowledge) can be achieved with structural equation modelling (SEM) (McIntosh and Gonzalez-Lima, 1994).

Studies have shown that, like regional activity, functional connectivity between brain regions is largely task-dependent, and can be modulated from a conscious, resting state by varying task demands. For example, Jiang and colleagues identified a large network of regions related to motor function that demonstrates high temporal coherence during rest, and is modulated by initiation, execution, guidance and termination of voluntary movement (Jiang et al., 2004). In addition, there has been much interest in recent years in identification of the so-called “default-mode” of functional connectivity, a series of regions which demonstrated high low-frequency correlation in the resting state (Raichle et al., 2001).

Connectivity patterns from the basal ganglia can be predicted based upon anatomical knowledge. Corticostriatal projections can be organised according to the ‘parallel loop’ model proposed by Alexander et al. (1986), whereby each striatal area receives input from a different area of the cortex and send connections to specific BG nuclei, that in turn project back to the same cortical area via the thalamus. Each loop is suggested to play a role in a specific set of motor or cognitive tasks. A meta-analysis of 126 PET and fMRI studies by Postuma and Dagher confirmed that functional connectivity of the basal ganglia is consistent with the predictions of this model (Postuma and Dagher, 2006).

The relationship between dopaminergic neurotransmission and functional connectivity appears complex (Brown et al., 2001; Rowe et al., 2002; Williams et al., 2002; Honey et al., 2003; Hutchison et al., 2004; Schott et al.; 2007) and depends critically on the balance between D1 and D2/D3 receptor stimulation (Rahman et al., 2001). Honey and colleagues (2003) demonstrated
that decreased dopaminergic transmission induced by sulpiride administration resulted in increased functional and effective connectivity within a cortico-striato-thalamic pathway including the caudate, thalamus and DLPFC.

Few studies have investigated functional and effective connectivity in patients with PD. Wu and colleagues (2009) recently studied functional connectivity of the motor network while at rest in PD subjects off and on levodopa medication. In the off state, PD subjects had decreased connectivity in the SMA, left DLPFC, and left putamen, and increased connectivity in the left cerebellum, left M1, and left parietal cortex compared to healthy controls. Decreased connectivity positively correlated with UPDRS motor scores, and increased connectivity negatively correlated with UPDRS scores. Levodopa normalized the functional connectivity of motor networks during rest. The pattern of results found in this study supports the pattern of regional hypo- and hyperactivation seen in prior imaging studies and suggests that these patterns result from dopaminergic impairment, and can consequently be restored by L-dopa.

However, dopamine does not just influence connectivity within motor networks; Kelly and colleagues (2009) investigated the effect of levodopa on functional connectivity within striatal motor and cognitive networks at rest in healthy adults, and demonstrated that although L-dopa could increase functional connectivity from the putamen to the cerebellum and brainstem, it decreased ventral striatal and dorsal caudate connectivity to posterior cingulate and medial frontal cortex, regions typically associated with a resting or ‘default-mode’ network (Raichle et al., 2001).

Schott and colleagues (2007) have shown that PD subjects show an increased response of anterior cingulate during reward feedback processing, and that this is accompanied by a decrease
in the functional connectivity of the midbrain and ventral striatum, showing that functional connectivity in the mesolimbic dopamine system is also affected in PD.

Like regional activation, functional connectivity is highly task dependent. Sun and colleagues demonstrated that motor learning can influence the patterns of functional connectivity observed during performance of a motor task, with a greater need for sensorimotor, pre-motor and SMA during the early stage of task performance (Sun et al., 2007). Taniwaki and colleagues showed that different functional loops are involved in an externally-guided versus an internally generated motor task (2003), with a stronger role for the BG in tasks that are internally generated.

Rowe and colleagues (2002) demonstrated that PD patients have impaired effective connectivity between prefrontal cortex, pre-motor cortex and supplementary motor area when attending to voluntary movements, such that increasing attention to action does not increase the connectivity between these regions, whilst in healthy controls this network can be modulated by increasing attention.

Given that neurodegeneration can result in compensatory recruitment of additional regions to accomplish a motor task, as shown in prior neuroimaging studies, it is likely that connectivity patterns during a task would also change so that these additional regions become incorporated into a functional network. It is therefore feasible that compensation can be investigated in terms of changes in functional or effective connectivity during task performance, as well as by measuring regional changes. In fact, connectivity changes may tell us more useful information than studying amplitude changes alone, as functional loops involving the basal ganglia and cerebellar systems may project to overlapping areas of cortex, making regional changes difficult to interpret in isolation.
Another prominent feature of neural activity is the existence of neural oscillations; periodic variations in the recordings of neural activity; and the synchronization of these oscillations across spatially separated brain regions is a likely mechanism of neural communication (Schnitzler and Gross, 2005). These oscillations occur on a faster scale than the temporal resolution of current neuroimaging methods, but can be investigated using electrophysiological techniques which have better temporal resolution, making it feasible to investigate changes that take place over millisecond time frames. Electroencephalography (EEG) recordings can be probed for oscillatory activity, which can be divided into distinct frequency bands, each of which may have its own functional significance. Indeed, studies in humans suggest that important information in the EEG is encoded in the frequency domain as opposed to the time domain (Pfurtscheller and Lopes da Silva, 1999; Schnitzler and Gross, 2005).

Alterations in the temporal pattern of neuronal firing appear to play a key role in development of parkinsonian symptoms (e.g. Brown and Marsden, 1999; Obeso et al., 1997; Marsden and Obeso, 1994). Studies of local field potentials in patients undergoing deep-brain stimulation surgery and EEG studies of cortical activity during motor performance have suggested that the motor system involves oscillatory networks that show long-range synchronization in distinct frequency bands between cortical and subcortical structures. Abnormal patterns of synchronisation within this system are thought to be a key pathophysiological mechanism in several neurological disorders including PD (Brown, 2003). Changes occur within specific frequency bands in PD, for example Silberstein and colleagues demonstrated that cortico-cortical synchronisation in the 10-35 Hz band correlated with the disease severity in PD, and restoration
of normal activity in this frequency following levodopa correlated with the clinical improvement (Silberstein et al., 2005).

Research shows that in both human PD and animal models, there is a significant increase in oscillatory activity in the GPe, GPi and STN (Schnitzler and Gross, 2005; Raz et al., 2000; Levy et al., 2000). In patients who are being treated with dopaminergic drugs, synchronization between local field potentials from the STN and GPi resembles the normal state, showing synchronisation at ~60-80 Hz (Brown, 2003). This coherence is increased before and during voluntary movement (Cassidy et al., 2002). Additionally, voluntary movement increases coherence between the STN and the cortex, with STN and GPi oscillations leading cortical activity (Williams et al., 2002). So, the STN, GPi and cortex appear to form a functional network that resonates at 60-80 Hz and supports normal movement. In support of this, studies have shown that stimulating the STN or GPi at frequencies that induce this 60-80 Hz resonance is able to improve akinesia in PD patients (Siegfried and Lippitz, 1994; Limousin et al., 1995).

Other frequencies, specifically those between 3-10 Hz and 11-30 Hz, appear to be antikinetic (Brown, 2003). In a primate model of PD, MPTP-treated monkeys show an increase in oscillatory activity in the beta band (14-30 Hz) and a decrease in the gamma band (30-80 Hz) (Wichmann et al., 1994). Coherence between the STN and GPi and the motor cortex in the beta band has been shown in untreated PD patients, and this decreases with dopaminergic drug treatment (Brown, 2003) and with voluntary movements (Cassidy et al., 2002). It has been suggested that in PD, anti-kinetic beta oscillations from the cortex suppress pro-kinetic gamma oscillations in the basal ganglia resulting in hypokinetic symptoms (Schnitzler & Gross, 2005). In support of this, low frequency stimulation of the STN at between 10 and 15 Hz has been shown to worsen akinesia in PD (Timmerman et al., 2004).
Oscillations in the low frequency (3-10 Hz) range often synchronise with parkinsonian tremor, with tremor-related neurons found in Gpi, STN and thalamus. These tremor-related oscillations are thought to be part of an integrated oscillating network including basal ganglia-thalamo-cortical and cerebello-thalamo-cortical projections (Timmerman et al., 2003). Indeed, parkinsonian tremor can be alleviated not only by deep brain stimulation of the STN and Gpi, but also of the Vim, part of the thalamus that receives input from the cerebellum (Timmerman et al., 2003). Presumably the effect of Vim stimulation alters parkinsonian tremor via its influence on oscillations in the motor cortex, where the basal ganglia-thalamo-cortical and cerebello-thalamo-cortical loops overlap.

Interestingly, the oscillatory motor network identified in parkinsonian tremor is also activated during physiological repetitive movements. When healthy subjects voluntarily imitate resting tremor, the same oscillatory network is activated (Pollok et al., 2004). However, in PD subjects the predominant synchronization is between the thalamus and primary motor cortex, while in the healthy controls, dominant synchronization is between M1 and premotor cortex. Thus it appears that the relative amount of synchronization between component regions of a given network is important to determine the functional state of the network and the subsequent behavioural output. If pathological oscillations exist within a network, it is therefore reasonable to assume that other, beneficial oscillations might also exist that attempt to maintain this important balance of synchronization. While no study to date has specifically investigated compensation using EEG methods, it seems likely that compensatory mechanisms should also exist on this temporal scale.
Many of the regional and connectivity changes seen in PD are normalized or partially normalised by levodopa, suggesting that restoration of dopaminergic transmission within the basal ganglia can correct downstream changes in neurotransmission and restore normal patterns of task-related activation. Interestingly, L-dopa has been shown to normalize both hypo- and hyper-activations of the same brain regions, see for example Haslinger et al. (2001) versus Buhmann et al. (2003)

Evidence suggests that there is an inverse-U shaped relationship between dopamine levels and performance (Williams and Goldman-Rakic, 1995), and indeed it has been shown that while L-dopa can consistently improve motor function in PD, it may have no effect or even worsen the cognitive symptoms associated with PD (Cools, 2006).

The effect of L-dopa on compensation is unclear. On one hand, normalization of dopaminergic transmission may mean that compensatory activation is no longer required and is no longer observed in PD subjects on medication. However, levodopa may fail to fully normalize activation patterns in PD, resulting in continuing need for compensation. Additionally, there may have been compensatory changes in the functional networks for a given task that involved synaptic plasticity and that remain apparent even after administration of levodopa. It is thus not possible to conclude that a particular activation is compensatory based upon its presence or absence following levodopa administration.

Comment should also be made here regarding the metabolic and vascular effects of levodopa, particularly as these effects may contribute to changes in the blood-oxygen level dependent response measured by functional MRI. Studies of regional cerebral blood flow (rCBF) have shown that levodopa can reduce blood flow within specific regions, for example Cools and
colleagues showed that L-dopa reduced the regional cerebral blood flow (rCBF) in the prefrontal cortex during a working memory task (Cools et al., 2002). Similar findings were observed in a working memory study by Mattay et al., (2002) and similar effects were seen in healthy controls given methylphenidate (Mehta et al., 2000). Our group has recently demonstrated that the spatial extent of activation in several motor-related ROIs, as measured by fMRI, is also reduced in PD subjects following levodopa administration (Ng et al., 2009). These observed “focusing” effects of levodopa likely result from the dopamine-induced increase in signal-to-noise within cortical microcircuits (Winterer et al., 2006), which manifests in the BOLD activation as a sharpening or focusing of response. Conversely, a loss of dopamine would result in a larger area of activation. In line with this, Strafella and colleagues demonstrated that in PD subjects, the number of voxels showing TMS-induced dopamine release in the putamen was greater in the symptomatic side than the asymptomatic side (Strafella et al., 2005).

In computational modeling studies, the effects of dopamine at the individual neuronal level are often modeled as a change in shape of the normally sigmoid curve that relates the input to a single neuron to the output (firing rate) of that neuron. Increasing dopamine results in a more non-linear curve, approaching a threshold-like function, while decreasing dopamine results in the input-output curve becoming more linear (Sikstrom, 2007). Neurons with threshold-like functions are less sensitive to small synaptic fluctuations that would be seen with more linear regimes, and thus dopamine can be considered as increasing the signal-to-noise of the neuron. When the effects on individual neuronal units are assembled into a network, the macroscopic effect is that the low-dopamine states, as would be seen in Parkinson's disease, result in indistinct neural representations with many units having similar representations, as opposed to a few selected units being active when the modeled dopamine levels are higher (Sikstrom, 2007; Li and
Sikstrom, 2002). Also, studies of COMT (catechol-O-methyltransferase) genotype (which codes for the dopamine catabolising enzyme COMT) showed relatively diminished BOLD response and increased noise in COMT-Val carriers (which have lower levels of available synaptic dopamine) (Winterer et al., 2006).

Also of importance to imaging studies is the vascular effect of dopamine, which affects BOLD signal changes by altering local blood flow. However, in prior studies L-dopa was found to increase rCBF in some areas and decreased rCBF in others (Cools et al., 2002).

1.9. METHODOLOGICAL APPROACHES TO UNDERSTANDING COMPENSATION

Investigators researching systems-level compensatory mechanisms in other neurological conditions have employed various methods to assess the nature of compensation and deal with the ubiquitous problem of differentiating compensatory changes from direct disease alterations. Of note are two approaches taken from the fields of Multiple Sclerosis (MS) and Alzheimer’s disease (AD) research.

In the first method, subjects can be stratified into groups with mismatch between clinical disability and disease progression using an independent disease marker (e.g. number of MS plaques observed on MRI, or $^{11}$C-DTBZ PET in PD), with the assumption that those with similar clinical disability but significant differences in disease progression have discrepancies in the capacity for compensation. For example, Reddy and colleagues (2000) grouped MS subjects according to both injury (i.e. the extent of pathological damage) and disability (i.e. the severity of clinical symptoms). Patients’ level of injury was estimated using structural MRI and magnetic resonance spectroscopy. By comparing patients matched for injury but with different levels of
disability they were able to identify changes that are seen to a greater extent in patients with less severe disability, and thus interpreted to be compensatory.

PD subjects may be particularly suitable for this approach as, unlike MS, the pathological change in PD is relatively uniform in terms of location, just different in terms of extent. In PD, the severity of disease progression at a single point in time can be most accurately estimated by dihydrotetrabenazine (DTBZ) PET (Au et al., 2005), which is less subject to regulatory and compensatory changes than $^{18}$F-dopa PET. Specifically, subjects can be stratified, at a specific point in time, as follows:

<table>
<thead>
<tr>
<th></th>
<th>Few clinical symptoms (by UPDRS motor score)</th>
<th>Many clinical symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild disease (by DTBZ PET)</td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Severe disease</td>
<td>Group C</td>
<td>Group D</td>
</tr>
</tbody>
</table>

TABLE 1.1: Stratification of PD subjects

By contrasting the fMRI activity in Group A with Group B, it would be feasible to interpret which aspects of activity are compensatory. Group A presumably shows better compensation than Group B since symptoms are fewer despite the same disease progression. Similarly, Group C shows better compensation than Group D.

In the second approach, subjects perform tasks of progressive difficulty as part of an fMRI study and network(s) unique to a disease group are probed to see if increased activity correlates with task performance at increasing levels of difficulty (and is therefore presumed compensatory). For example, Grady and colleagues performed a H$_2^{15}$O PET study in which mildly demented AD patients and healthy elderly controls performed semantic and episodic memory tasks (Grady et
al., 2003). They determined that a network, unique to the AD patients and including bilateral prefrontal and temporo-parietal cortices, correlated with the ability of the patients to perform the tasks accurately, suggesting a compensatory mechanism.

Although method 1 is attractive, the number of subjects required to have undergone a PET scan in the past 12 months in order to achieve sufficient power in the comparison shown in table 1.1 unfortunately prevented this method from being feasible for the current thesis work. Instead, the following work has focused on using tasks with several difficulty levels to infer compensation by showing increased recruitment with increasing task difficulty. This method relies on the assumption that disease-related changes are static across variations in the difficulty of a task, whereas compensatory mechanisms, which are by definition recruited to improve performance, are expected to show a degree of correlation to the difficulty of a task.

1.10. SUMMARY

There are several lines of evidence for compensatory mechanisms in PD that exist at a systems level, involving not just the basal ganglia but several downstream cortical regions. However more work is needed that incorporates methodologies designed to assist in disentangling compensation from disease-related effects. The growing emphasis on networks in neuroimaging reflects the increasing acknowledgment of the importance of distributed representation (a single function is subserved by a combination of spatially separate regions) and neural context (neural activity at a given region appears to be highly dependent on the neural context in which it occurs). The following body of work will study individuals with early-stage PD both off and on medication, applying regional and network approaches to investigate possible compensatory activation.


CHAPTER 2: LEVODOPA-SENSITIVE, DYNAMIC CHANGES IN EFFECTIVE CONNECTIVITY DURING SIMULTANEOUS MOVEMENTS IN PARKINSON’S DISEASE

2.1. PREAMBLE

Since the proposed approach for differentiating disease-related and compensatory mechanisms requires tasks that can be performed with more than one level of difficulty, we researched tasks which are of particular difficulty in PD. These include, but are not limited to, simultaneous bimanual movements, complex sequential movements, and internally generated movements. For our first study in this body of work, we selected to investigate simultaneous, bimanual task performance using fMRI. A continuous, visually-guided right-handed tracking task was chosen as the unimanual task (easy level of task difficulty), since externally guided task performance is relatively unimpaired in PD. This was compared to performance of a simultaneous task (hard level of difficulty), where a simple button press task was added in the left hand. Continuous tasks were chosen in order to saturate the hemodynamic response (Berns et al., 1999). Eight PD subjects off and on L-dopa medication and 10 age-matched healthy control subjects performed both simultaneous and unimanual motor tasks in the fMRI scanner. Changes in effective connectivity between Regions of Interest (ROIs) during simultaneous and unimanual task performance were determined with Structural Equation Modelling (SEM), and changes in the temporal dynamics of task performance were determined with Multivariate Autoregressive modelling (MAR).

2.2. INTRODUCTION

One of the characteristic features of Parkinson’s disease (PD), a neurodegenerative condition with greatest impact on the motor system, is difficulty in performing tasks which require either sequential or simultaneous movements (Johnson et al., 1998), even when each task can be performed individually without difficulty (Benecke et al., 1986; Benecke et al., 1987; O’Shea et al., 2002). Compared to healthy controls, PD subjects show slower movement times and longer pauses between movements (Horstink et al., 1990; Lazarus & Stelmach, 1992; Shimizu et al., 1987) unlike patients with other movement disorders such as depressive motor retardation and neuroleptic induced parkinsonism (Fleminger, 1992). These deficits are task dependent, as PD subjects are able to perform a symmetrical bimanual task out of phase, but are more likely to perform the movement in-phase instead in the presence of external timing cues (Johnson et al., 1998).

The mechanisms underlying the difficulty PD subjects have in performing simultaneous tasks are poorly understood. Normally, successful performance of a simultaneous motor task also depends on the extent to which the movements have become practiced or automatic. In neurologically intact individuals, evidence that a task has become automatic is suggested by the ability to perform a secondary task with little or no interference (Passingham, 1996). During performance of an automatic sequence, when the basal ganglia are typically less active (Wu et al., 2004), PD subjects demonstrate relatively increased activity in the cerebellum, premotor area, parietal cortex, precuneus, and prefrontal cortex, suggesting that they must recruit additional neural resources to achieve automaticity of movement (Wu and Hallett, 2005). If practiced unimanual movements already require more resources in PD subjects, it follows that they may be more prone to performance errors when asked to complete a secondary or simultaneous task.
Previous neuroimaging studies of dual-task performance have attempted to compare activity during simultaneous bimanual tasks to the sum of activity from each unimanual task, although results have been conflicting (Herath et al., 2001; Ehrsson et al., 2000; De Weerd et al., 2003). Here we suggest that simultaneous tasks may not necessarily recruit separate loci of “co-ordination” regions, but rather that the network of activity binding individual regions together may be altered during dual task performance. Specifically, effective connectivity between regions usually recruited by the unimanual tasks may be modified due to increased task demands and co-ordination requirements. Additionally, when individual task networks have overlapping areas of activity, this may result in the potential for interference when both tasks must be performed together. This is supported by previous electrophysiology studies (Serrien et al., 2004) that have shown changes in cortico-cortical coherence during simultaneous tasks: the pattern of functional coupling between cortical regions during the bimanual task was greater than the sum of the individual unimanual networks. Because PD patients can successfully complete individual movements and are specifically impaired when they try to perform two movements together, their impairment most likely reflects an inability to effectively bind widespread cortical and subcortical areas necessary for dual-task performance. This inability may be a direct result of the impaired output from the basal ganglia, or a downstream effect of the hypoactivity or hyperactivity which is seen in several other brain regions in PD. We therefore hypothesise that the simultaneous-task network in PD subjects off-medication will show changes in effective connectivity compared to age-matched, healthy controls. Because L-dopa treatment leads to behavioural improvements and has been previously shown to normalise regional activity in PD, we additionally predict that any alterations in connectivity will be at least partly normalised following treatment with levodopa. We do not expect that the regions involved in the task-related network will show any changes in amplitude of BOLD signal when
comparing bimanual to unimanual task performance, since we predict that changes are predominantly related to connection strengths as opposed to amplitude.

Effective connectivity from fMRI data can be assessed using structural equation modelling (SEM; Buchel and Friston, 1997; McIntosh et al., 1994) which detects the network of regions which are actively connected during task performance. To observe any subtle differences in connectivity between PD subjects and in healthy controls, we designed a continuous trial lasting sufficient time to saturate the fMRI BOLD hemodynamic response (Berns et al., 1999). This right-handed task required continuous sinusoidal force production, which was performed with or without the addition of a secondary task with the left hand. The secondary task was a discrete button-press task designed to require only a brief motor output but to require continuous attention. The period between the cues for the button press was sufficiently long that the cue remained unpredictable, and required sustained attention. By comparing connectivity between PD and control subjects during the simultaneous compared to unimanual tasks, we aimed to determine whether there are changes in connectivity in PD during bimanual movement.

SEM is able to describe the underlying connectivity implied by the functional data. However, this approach does not take account of temporal information (Harrison et al., 2003). Since interactions within the brain do not happen instantaneously or independently of one another, one variable may have a historical influence on another. To model the inter-regional dependencies within the fMRI data in terms of this historical influence, we used Multivariate Autoregressive (MAR) modelling (Harrison et al., 2003). MAR detects significant inputs to a given ROI by comparing activity of all other ROIs in the previous time point to current activity of the ROI in question (Harrison et al., 2003). This was used to demonstrate the temporal dynamics of brain activity during simultaneous movement. We predicted that PD subjects would show changes in
temporal patterns of activity during simultaneous tasks, and that these temporal dynamics would be approximately normalised by levodopa.

2.3. MATERIALS AND METHODS

2.3.1. Subjects

Ten volunteers with clinically diagnosed PD participated in the study (7 men, 3 women, mean age 64 ± 8 years, all right-handed). Data from 2 PD subjects were subsequently removed from analysis due to problems with scanner and data reconstruction. All patients had mild to moderately severe PD (Hoehn and Yahr stage 1-3) (Hoehn & Yahr, 1967). Exclusion criteria included atypical parkinsonism, presence of other neurological or psychiatric conditions, and use of antidepressants, hypnotics, or dopamine blocking agents. All patients were taking levodopa (mean daily dose 675 ± 190 mg). Other medications included dopamine agonists, trihexyphenidyl, and amantadine. We recruited ten healthy, age-matched control subjects without active neurological disorders (9 females, 1 male, mean age 58 ± 11 years, all right-handed).

All patients stopped their antiparkinson medications overnight for a minimum of 12 hours before the study. The mean Unified Parkinson’s Disease Rating Scale (UPDRS) motor score during this “off-levodopa” state was 28 ± 9. There were no significant correlations between UPDRS motor scores and age. After completing the experiment in an off-medication state, PD subjects were given their usual morning dose of levodopa (mean 132 ± 29 mg Sinemet IR). They then repeated the same tasks post-medication following an interval of approximately 1 hour to allow levodopa to reach peak dose. Control subjects completed the tasks only once.
The study was approved by the appropriate Ethics Boards and all subjects gave written informed consent prior to participating. Ethics approval certificates for this study can be found in Appendix I.

2.3.2. Experimental Design

Subjects held a custom-built, in-house designed, rubber squeeze bulb in their right hand and were instructed to squeeze the bulb using an isometric hand grip and to keep their hand position constant throughout the study. Each subject had their maximum voluntary contraction (MVC) measured at the start of the experiment and all subsequent movements were scaled to this.

Using the squeeze bulb, subjects were required to control the width of a horizontal bar on the computer screen in order to keep the two ends of the bar within two thick white lines which formed a vertically scrolling, undulating tunnel or pathway (Figure 2.1). Applying greater pressure to the bulb increased the width of the bar, whilst releasing pressure from the bulb decreased the width of the bar. The undulating pathway was designed so that subjects had to modulate their pressure between 5 and 15% MVC, and a deviation of more than 2% from the optimal pressure resulted in touching the sides of the tunnel. If subjects touched either side of the white lines, visual “sparks” appeared where the bar was touching. To appear smooth, but not easily predictable, the pathway was a linear combination of two equal-amplitude sinusoids with periods of 10 and 18 seconds.

At regular intervals the bar changed colour from black to red. In some trials, subjects were asked to respond to the colour change by pressing a button with their left index finger. Each subject performed two 5-minute long trials. During one trial, subject used only the squeeze-bulb in the right hand. In another trial they were required to do both movements simultaneously, squeezing
FIGURE 2.1. The simultaneous bimanual task involved squeezing a pressure-responsive rubber bulb to control the width of the horizontal black bar such that the ends remained on the white, vertically scrolling pathway. At intervals the bar changed colour requiring a button-press response with the left hand.

the bulb with their right hand and pressing the button with their left hand. The tunnel was identical for all trials, and the order of the trials was pseudo-randomised across subjects.

Custom Matlab software (Mathworks) and the Psychtoolbox (Brainard et al., 1997; Pelli et al., 1997) was used to design and present stimuli, and to collect behavioral data from the response devices. Data from the squeeze-bulb device was sampled at 100Hz and plotted against the target force output. Error was calculated as the amount of time output force deviated from target force. An ANOVA was used to compare error rates, using the independent variables of group (control, PD off-medication, PD on-medication), task (bimanual, unimanual), subject, and task section (first half vs second half).

2.3.3. Data Acquisition

Subjects lay in the MR scanner with a response device placed in each hand. The right hand response device was an in-house built, pressure-sensitive rubber squeeze bulb connected to a pressure transducer outside the scanner room, and the left hand response device was a button
box with 5 buttons ergonomically placed below each finger (Lumitouch; Photon Control, Burnaby, Canada).

Functional MRI was conducted on a 3 Tesla scanner (Philips Achieva 3.0T; Philips Medical Systems, Netherlands) equipped with a head-coil. We collected echo-planar (EPI) T2*-weighted images with blood oxygenation level-dependent (BOLD) contrast. Scanning parameters were: repetition time 2000 ms, echo time 37, flip angle 90°, field of view (FOV) 240mm, matrix size = 128 x 128, in plane voxel size 1.9 x 1.9 mm. Each functional run lasted 5 minutes. Thirty-six axial slices of 3mm thickness were collected in each volume, with a gap thickness of 1mm. We selected slices to cover the dorsal surface of the brain and include the cerebellum ventrally. A high resolution, 3-dimensional T1-weighted image was acquired of the whole brain to facilitate anatomical localization of activation for each subject.

Head motion was minimized by a foam pillow placed around the subjects head within the head-coil. Subjects also used ear plugs to minimize the noise of the scanner. The subjects viewed visual stimuli on a screen through a mirror built into the head coil. The stimuli were programmed and run using Matlab (Mathworks, MA). Due to technical difficulties with the scanner and stimulus presentation equipment in two of the PD subjects, these data sets had to be removed from the analysis.

For PD patients, off-medication data were acquired after overnight withdrawal (minimum of 12 hours since last dose of levodopa), and on-medication approximately one hour after taking their usual morning dose of levodopa.
2.3.4. Data Analysis

Functional images were first pre-processed using Brain Voyager software (Brain Innovation B.V. Maastricht, The Netherlands) for 3D motion correction and slice scan time correction. Data were then exported as Analyze format. No temporal or spatial smoothing was performed on the data. The data were then further motion corrected with MCICA, a computationally expensive but highly accurate method for motion correction (Liao et al., 2004, 2005).

Structural scans were also converted to Analyze format using MRIcro. MRIcro’s Brain Extraction Tool was used to strip the skull from the structural scans and from the first volume of each functional run to facilitate more accurate co-registration of the structural and functional data. The high-resolution skull-stripped structural scan was aligned to the skull-stripped functional volume using custom scripts written for the Amira software (Amira 3D Visualization and Volume Modeling). ROIs were manually drawn on the transformed structural image using anatomical landmarks and guided by a brain atlas (Talairach & Tournoux, 1988). We drew 9 ROIs in each hemisphere, namely the caudate, putamen, globus pallidus, thalamus, cerebellum, primary motor cortex, supplementary motor area, anterior cingulate cortex, and pre-frontal cortex. Boundaries of each ROI were selected in accordance with standard Brodmann areas. A sample segmented slice is shown in Figure 2.2.

FIGURE 2.2. Example of manual ROI segmentation shown on an axial slice.
Functional data were resliced to produce isotropic voxels. The labels on the segmented anatomical scans were resliced at the fMRI resolution. The raw time courses of the voxels within each ROI were then extracted.

To examine the relative amplitude of the BOLD response in relation to task for each subject group we employed a sensitive method, local linear discriminant analysis (LLDA; McKeown et al., 2007), which accounts for the greater intersubject, within-group variability typical of Parkinson's disease (Li et al., 2008). In order to investigate the combinations of brain regions that were recruited by the experimental tasks we used Structural Equation Modelling (Buchel & Friston, 1997; McIntosh et al., 1994) to calculate the effective connectivity between regions during each task condition and for each subject group. To calculate the temporal dynamics, we used Multivariate Autoregressive Modelling (MAR: Harrison et al., 2003).

A full description of the procedures for calculating SEM and MAR networks can be found in Appendix II. A description of the procedure for LLDA has been published elsewhere (McKeown et al., 2007). While it would also be of interest to calculate the second-order MAR, that is, to predict current fMRI signals from not just the prior time point, but the previous two time points, this would require estimation of twice the number of parameters. Given the number of time points that we have per trial (150), we did not think that this would provide robust results for the second-order MAR. Future improvements in algorithms may make second-order MAR results feasible.
2.4. RESULTS

2.4.1. Behavioural Data

Error rates for each task within each subject group were calculated as the mean difference between the actual and target bar width. Error rates (arbitrary units) were: Control subjects 486.72 (simultaneous), 483.54 (unimanual); PD subjects Off medication 1455 (simultaneous) 1018 (unimanual); PD subjects On medication 683.92 (simultaneous) 565.2 (unimanual). Results suggest that PD subjects off medication performed worse than control subjects and PD subjects on medication, and that the performance difference between simultaneous and unimanual tasks was greater for PD subjects off medication. However it should also be noted that the error rates for PD subjects off medication are influenced by the effect of tremor on the squeeze bulb output therefore may be artificially inflated. ANOVA results demonstrated that there were no significant differences in the error rates between each subject group (F(2,37)=3.09, p>0.94), or between the bimanual and unimanual task (F(1,37)=0.09, p>0.23).

Figure 2.3 shows the squeeze-bulb force output for each subject against the target pathway. Individual forces were scaled to be comparable between subjects. The data demonstrates that all subjects performed the task accurately.

All subjects were given practice on the task before data collection to remove the possibility of learning or practice effects during task performance. To confirm that performance did not improve due to practice or deteriorate due to fatigue during the duration of the trial, we compared error rates for the first half and second half of each trial. There were no significant differences in task performance between first and second halves (F(1,37)=0.69, p>0.59).
FIGURE 2.3. Behavioural data for control subjects and PD subjects off and on medication. Red line represents target output. Each subjects output is displayed as a separate blue line, overlaid on target.
2.4.2. fMRI Amplitude Changes

Figure 2.4 demonstrates ROIs where the amplitude of the BOLD signal significantly differed between simultaneous and unimanual tasks for at least one subject group (control, PD off-medication, PD on-medication). We demonstrated that the PD subjects off-medication had greater activity of bilateral putamen during the unimanual compared to simultaneous task, and PD subjects on medication showed an increase in activity of the left thalamus and right M1 during simultaneous task performance. No other ROIs demonstrated significant differences in BOLD amplitude between tasks.

FIGURE 2.4. Relative activity in simultaneous (positive axis) vs unimanual squeeze only (negative axis) tasks.
2.4.3. fMRI Connectivity Changes

**Control Subjects**

In healthy control subjects (Figures 2.5A & 2.5B), simultaneous movements induced alterations in both effective connectivity and temporal dynamics in comparison to a unimanual task. Simultaneous movements were associated with increased connectivity between the left SMA and right prefrontal cortex (PFC) and thalamus, and between the left PFC and the right putamen, left globus pallidus, and left cerebellum. Decreased connectivity was seen during simultaneous movements between the right PFC and left cerebellum and globus pallidus, between left SMA and PFC, and between right primary motor cortex (M1) and putamen. Interhemispheric connectivity between bilateral M1, SMA, and cerebellum also decreased during the simultaneous task.

MAR modelling demonstrated that there was increased input during simultaneous movement from left anterior cingulate cortex (ACC) to right putamen and left cerebellum, from right SMA to right cerebellum, and from left to right PFC. Decreased input was seen from left globus pallidus to left M1, and from left thalamus to right cerebellum.

**PD Subjects Off Medication**

In PD subjects off-medication, there were several differences in the simultaneous network compared to control subjects (Figure 2.6A). Like control subjects, PD subjects showed an increase in connectivity from left SMA to right PFC and from left PFC to left cerebellum. They also showed decreased interhemispheric connectivity between bilateral M1, SMA, and cerebellum. In addition, they showed increased connectivity from left M1 to right PFC, left SMA, and left cerebellum, and from right caudate to left thalamus and left SMA. They showed
additional decreases in connectivity from left SMA to left thalamus, from left ACC to right caudate and PFC, and between right SMA and PFC.

Figure 2.6B demonstrates the alterations in temporal dynamics seen in PD subjects during simultaneous performance. These changes all involve either input or output from the basal ganglia. Specifically, input from left ACC to right caudate and putamen is increased in PD, as is input from left cerebellum to left caudate. Input from left SMA to left thalamus, and right cerebellum to left caudate is decreased. Output from left putamen to left PFC and from right caudate to right ACC is also decreased in PD subjects.

**Effects of Levodopa**

Figures 2.7A and B show that following a single dose of levodopa, effective connectivity patterns and temporal dynamics were largely normalised. In terms of effective connectivity, the PD subjects showed a decrease in connectivity from left PFC to right SMA, between left putamen and thalamus, and between bilateral cerebellum (Figure 2.7A). They also had decreased temporal input from right cerebellum to right thalamus and left caudate, and from left globus pallidus to left M1 (Figure 2.7B).
FIGURE 2.5A: Effective connectivity in control subjects performing simultaneous compared to unimanual tasks. Connections that are stronger during simultaneous tasks are shown in black, connections that are weaker during simultaneous movement are grey. For abbreviations refer to pg xii.
FIGURE 2.5B: Temporal dynamics in control subjects performing simultaneous compared to unimanual tasks. Connections that are stronger during simultaneous tasks are shown in black, connections that are weaker during simultaneous movement are grey.
FIGURE 2.6A: Differences of effective connectivity in PD subjects off medication performing simultaneous compared to unimanual tasks, in comparison to controls. For the individual task, between group comparison, connections that are stronger in PD compared to controls are shown in black, and connections that are weaker are shown in grey. For the comparison between tasks (lower figure) connections that are stronger during simultaneous tasks are shown in black, connections that are weaker during simultaneous movement are grey.
FIGURE 2.6B: Differences of temporal dynamics in PD subjects off medication performing simultaneous compared to unimanual tasks, in comparison to controls. For the individual task, between group comparison, connections that are stronger in PD compared to controls are shown in black, and connections that are weaker are shown in grey. For the comparison between tasks (lower figure) connections that are stronger during simultaneous tasks are shown in black, connections that are weaker during simultaneous movement are grey.
FIGURE 2.7A: Differences of effective connectivity in PD subjects on medication performing simultaneous compared to unimanual tasks, in comparison to controls. For the individual task, between group comparison, connections that are stronger in PD compared to controls are shown in black, and connections that are weaker are shown in grey. For the comparison between tasks (lower figure) connections that are stronger during simultaneous tasks are shown in black, connections that are weaker during simultaneous movement are grey.
FIGURE 2.7B: Differences of effective connectivity (2.8A) and temporal dynamics (2.8B) in PD subjects on medication performing simultaneous compared to unimanual tasks, in comparison to controls. For the individual task, between group comparison, connections that are stronger in PD compared to controls are shown in black, and connections that are weaker are shown in grey. For the comparison between tasks (lower figure) connections that are stronger during simultaneous tasks are shown in black, connections that are weaker during simultaneous movement are grey.
2.5. DISCUSSION

Our results confirm that changes in connectivity between brain regions, as opposed to activation of discrete loci, are important for the performance of simultaneous tasks. Even when using individually-drawn ROIs to avoid mis-registration errors caused by the usual practice of warping individual subjects' brain images to a common template (Nieto-Castanon et al., 2003), and using a discriminant method robust to intersubject variability (McKeown et al., 2007), we found only four ROIs had significantly different amplitude of BOLD activity between tasks (Figure 2.4). In contrast, both control subjects and PD subjects demonstrated several changes in connectivity (as measured by SEM and MAR) during performance of simultaneous tasks (Figures 2.5 & 2.6). These changes were not due to differences in task performance or error detection, as error rates did not significantly differ between subjects, tasks or the part of the task performed.

When control subjects were asked to perform bimanual simultaneous tasks compared to a unimanual task, they increased their inter-hemispheric connectivity between the attentional system and the left and right motor control loops (Figure 2.5A). Changes were seen in the pre-frontal cortex, which has an important role in performance monitoring (Owen et al., 1996), and in switching between tasks during simultaneous or sequential movements (Dreher and Grafman, 2003). The right pre-frontal cortex, which would be associated with the left-handed button press, showed increased connectivity to the left SMA, associated with the squeezing task. This is consistent with prior studies demonstrating a role for the SMA in timing and spatial co-ordination of bimanual tasks (Lang et al., 1990; Uhl et al., 1996). Conversely, the left pre-frontal cortex increased connectivity with the left cerebellum, which has important roles in visual guidance and timing (Stein and Glickstein, 1992). Interestingly, we did not see an increase in connectivity within the right hemisphere motor loop when we add a left-handed task. There are...
two possible explanations for this. Firstly, control subjects may have already recruited this network for the right-handed squeeze task. This possibility is consistent with prior studies that have demonstrated ipsilateral recruitment for complex unimanual motor tasks (Verstynen et al., 2005). Addition of a secondary task may therefore require an adjustment in connectivity between the left and right hemisphere, but not within the motor loops themselves. This is supported by the finding that only 3 out of the 13 significant changes we detected in connectivity were intrahemispheric. An alternative explanation is that the left-handed task, which required only a single button press every 30 seconds, did not produce sufficiently large changes in BOLD signal across the entire task to demonstrate altered connectivity within the associated motor network. However, the fact that we still observed changes in cortical connections indicates that the secondary task did require continuous changes in attention and movement planning.

PD subjects off-medication demonstrated a distinctly different pattern when they performed simultaneous movements relative to unimanual movements (Figure 2.6A), suggesting that automaticity may not have been achieved by the PD subjects. We observed enhanced connectivity from the left thalamus to the right caudate in PD subjects, which in turn had increased connectivity to the left SMA and decreased connections to the left M1 and ACC. The right caudate has been shown to play specific roles in timing and movement planning (Jech et al., 2005; Dagher et al., 2001). Both the caudate and SMA have been shown to be active during skill acquisition, particularly for motor sequences (Miyachi et al., 1997; Puttemans et al., 2005). After learning and consolidation, basal ganglia activity decreases and secondary tasks can be performed with minimal interference, when movements are considered to be automatic (Wu & Hallett, 2005).
The altered connectivity patterns demonstrated by PD subjects during bimanual performance may explain the mechanism of this commonly clinically-observed deficit. Performance of a secondary task alongside an automatic (over-learned) movement requires that two movement plans be operated independently through different systems. During the simultaneous task, there was a decrease in interhemispheric connectivity in the SMA, cerebellum, and primary motor cortex which may reflect modulation of normally observed low-frequency inter-hemispheric correlations between homologous regions in the resting state (Biswal et al., 1995; Cordes et al., 2000; Lowe et al., 2000; Xiong et al., 1999). If PD subjects are not able to execute two movement plans at the same time, it follows that a simultaneous movement would have to be executed by a more complex, combined plan. In contrast to controls, who were able to adjust inter-hemispheric connectivity to combine performance of the two tasks, PD subjects were unable to make such combination and must therefore perform the two tasks using a single motor plan.

Many of the brain regions observed with modulated connectivity during simultaneous performance have been previously demonstrated to be altered in PD. The cerebellum, important for visual guidance and timing (Stein & Glickstein, 1992), is extensively recruited by PD subjects who rely excessively on visual cues (Cooke et al., 1978; Jahanshahi et al., 1995). Likewise, the altered connectivity in M1 and cerebellum is consistent with patterns of cortical hypo- and hyper-activation demonstrated in previous studies (Jenkins et al., 1992; Playford et al., 1992; Rascol et al., 1992; Rascol et al., 1997; Sabatini et al., 2000; Samuel et al., 1997), and may reflect compensatory mechanisms. From the MAR results, we detected increased input to the striatum comes from the cerebellum ipsilateral to the button press, which occurred at regular intervals and required ongoing monitoring for the response cue, so this increase in connectivity is consistent with prior studies describing a role for the striatum in guiding responses to external
stimuli (Bailey & Mair, 2006), and for the cerebellum in visual guidance of movement (Stein & Glickstein, 1992).

Our MAR results further demonstrate that the temporal dynamics of the fMRI BOLD signal are also changed in PD subjects performing simultaneous tasks. The ACC contralateral to the arm performing the continuous squeeze task demonstrated an increase connectivity to the opposite striatum at the next fMRI TR interval, possibly reflecting the reduced automaticity of PD subjects. The decrease in connectivity from the right cerebellum to the left caudate during performance of simultaneous movements may reflect a reallocation of resources as monitoring for the button-press cue became more important than the visual feedback for controlling the squeeze task. Bimanual tasks require not only simultaneous monitoring of external cues and two separate streams of performance feedback from the two hands, but also require interactions between the two hands to facilitate proper task co-ordination, particularly in asymmetric tasks. Failure to adjust temporal inputs, along with failure to recruit the normal network as demonstrated by the SEM modelling, may help to explain their impaired performance on more complex simultaneous tasks.

Consistent with clinical experience demonstrating improvements to many PD symptoms after L-dopa medication, we observed that PD subjects on medication had connectivity patterns more similar to that of normal controls, but differences were still evident (Figures 2.7 A and B). There were fewer significant connections in PD subjects on medication than that of normal controls. This may in part be due to the increased intersubject variability evident in fMRI data of PD subjects (Li et al., 2008). An alternate explanation is that a system-level mechanism of L-dopa may be to attenuate excessive beta-band synchronization that permeates widespread areas in the cortex and basal ganglia (Brown, 2006).
The finding that levodopa does not completely normalise connectivity in either SEM or MAR models is consistent with prior studies showing that L-dopa may improve some cognitive symptoms of PD while having a detrimental effect on other cognitive symptoms (Cools et al., 2001). This is also consistent with what is observed clinically, where symptoms such as bradykinesia typically show improvement but other symptoms, such as postural instability, may not (Jankovic, 2008). Additionally, we suggest that some changes in connectivity may occur as a compensatory or adaptive response, and that medication may be unable to rapidly reverse these adaptive processes.

All of our PD subjects were at an early or moderate stage of PD. It would be of interest to determine whether there is any correlation between the severity of disease, as measured by the UPDRS motor score, and the extent of abnormal connectivity in these individuals. However, because we are using a large number of ROIs, we do not have sufficient statistical power to calculate changes in connectivity on an individual subject-by-subject basis. Rather, we pooled our data across all subjects within a group before determining changes in connectivity. As well as increasing statistical power, pooling the data reduces the likelihood of identifying changes in connectivity which were related to BOLD activity unrelated to task performance, since it is unlikely that brain activity unrelated to the task would be common across all subjects.

The use of two methods of connectivity analysis was able to determine that connectivity changes take place on several temporal scales, with the SEM analysis demonstrating covariance of regions, and the MAR demonstrating ROIs that exert an influence over other regions at a later TR interval, and are therefore more likely to represent truly causal influences. The identification of additional temporal information using the MAR technique compared to SEM analysis is important given that the low sampling frequency in fMRI studies often precludes discussion of
temporal aspects. We have shown here that some temporal information can be obtained even at a TR of 2 seconds.

A key clinical implication from this study is that simultaneous movement deficits in PD are due to an impaired ability to adjust connectivity in the normal fashion, rather than a specific regional impairment. This has important consequences for PD subjects while off medication, who should be careful to avoid doing secondary tasks whenever possible. For example, the risk of falling while taking a step back and opening a cupboard may be avoided by taking care to focus on one task at a time.

These results also have wider reaching implications for the study of nervous system disorders in general by demonstrating that foci of neurodegeneration can change not only regional activity in downstream areas, but also the connectivity between two or more other regions that may be located far from the area of damage caused by the disease. Connectivity changes may be more subtle than gross changes in the level of BOLD activity and may thus prove useful for explaining functional impairments that are not fully explained by changes in amplitude of activity alone.
2.6. REFERENCES


CHAPTER 3: φ, β BUT NOT α-BAND EEG CONNECTIVITY HAS IMPLICATIONS FOR DUAL TASK PERFORMANCE IN PARKINSON’S DISEASE²

3.1. PREAMBLE

In the previous chapter, we demonstrated significant changes in effective connectivity during performance of a simultaneous, bimanual task; a task which is typically impaired for individuals with PD. By using two different methods of connectivity analysis, SEM and MAR, we showed that different patterns of connectivity are detected when considering connections within the same time point (using SEM) compared to connections where information is fed from one time point to the next (using MAR). Because we showed significant connectivity changes in PD using the MAR approach, even at the limited temporal resolution of fMRI, we were interested to see what we could learn about connectivity during the same task performed while subjects underwent EEG recording from the cortex. This method has good temporal resolution and allows investigation of cortico-cortical synchronization, which can be subdivided into specific frequency bands as discussed in section 1.9. We recorded the EEG in 10 PD subjects off and on L-dopa medication performing the same simultaneous and unimanual tracking tasks used in Chapter 2. To deal with the inherent non-stationarity of the EEG during motor tasks, we segmented the data into task-related sections based on transient synchronization between independent components of the data, before assessing the mutual information (MI) between each EEG channel pair, a measure of the synchronization between two channels.

² A version of this chapter has been accepted for publication: Palmer SJ, Lee PW-H, Wang ZJ, Au W-L & McKeown MJ (in press) φ, β but not α-Band EEG Connectivity has implications for Dual Task Performance in Parkinson’s Disease. Parkinsonism and Related Disorders.
Clinical observations in PD suggest that patients have difficulty coordinating control of their upper limbs in tasks which require sequential (Benecke et al., 1987) or simultaneous movements (Benecke et al., 1986). A common interpretation of this phenomenon is that PD subjects have difficulty in simultaneously executing separate motor programs, although the exact pathological mechanisms responsible for such difficulty are unclear. A potential mechanism is abnormal synchronisation of activity between regions normally responsible for planning and sequencing of movements (Scnitzler and Gross, 2005).

Electrophysiological studies have demonstrated that the Parkinsonian state is characterized by pathological oscillations within both basal ganglia-cortical loops and cortico-cortical loops, and have shown synchronised basal ganglia activity is linearly related to activity of the cortex (Levy et al., 2000; Brown et al., 2001; Cassidy and Brown, 2001; Cassidy et al., 2002; Priori et al., 2002; Williams et al., 2002; Silberstein et al., 2005). EEG recordings in the resting state have shown that PD subjects demonstrate an increase in theta (5-8 Hz) and low alpha (8-10Hz) power and a decrease in power of beta and gamma frequency bands compared to controls (Stoffers et al., 2007). These resting-state changes are only slightly modulated by L-dopa (Stoffers et al., 2007). Changes in synchronisation between cortical regions are also seen in the resting state, with prominent increases in the alpha range connectivity apparent in early, untreated PD, and increases in theta and beta bands appearing later in the disease (Stoffers et al., 2008).

During movement, the basal ganglia may release task-related cortical areas from normal resting-state $\alpha$ activity (Brown and Marsden, 1998), which is manifest as a pre-movement desynchronisation of cortical EEG activity in the $\alpha$ band over the recruited regions. In PD, this
desynchronisation is delayed (Wang et al., 1999). Wang and colleagues demonstrated that L-dopa treatment improved the pre-movement desynchronisation of cortical activity in PD patients, and the extent of this improvement correlated with a reduction in bradykinesia (slowness of movement) during performance of a flex or squeeze movement with one hand (Wang et al., 1999). The location of this correlation shifted when both movements were performed simultaneously, indicating that simultaneous movements may require synchronisation between alternative or additional regions.

Because abnormalities in PD have been shown to be more apparent during performance of a dynamic motor task, typical approaches that assume stationarity of the EEG (such as coherence) may be problematic. Therefore we utilised a recently developed technique that segments the EEG into quasi-stationary, task-related segments based upon the temporal dynamics of the cross-spectrogram of the Independent components (ICs) (Lee et al., 2008). To determine the connectivity between EEG channels, we applied mutual information to calculate both linear and non-linear statistical dependencies. Using this approach, we have previously demonstrated that PD subjects are unable to independently recruit different areas of the brain while performing simultaneous movements, and instead recruited disparate clusters of synchronous activity (Lee et al., 2008).

Here, we investigate synchronisation between distributed regions during simultaneous movements by measuring mutual information between pairs of EEG channels. We investigated the same PD subjects both off and on L-dopa medication in order to determine whether connectivity changes during simultaneous movements are modulated by dopamine, since prior studies have suggested that task-dependent changes in the functional coupling of EEG signals in
PD are dopamine-dependant, with fewer changes during performance of a motor tracking task
during an off-medication state compared to a medicated state (Cassidy and Brown, 2001).

3.3. MATERIALS AND METHODS

3.3.1. Subjects

This study was approved by the University of British Columbia Ethics Board and all subjects
gave written, informed consent prior to participating. Ethics Approval certificates for this study
can be found in Appendix III. Seven volunteers with clinically diagnosed PD participated in the
study (5 men, 2 women, mean age 63.7 ± 7.1 years, 6 right-handed, 1 left-handed). All patients
had mild to moderately severe PD (Hoehn and Yahr stage 1-3) (Hoehn and Yahr, 1967) with
mean symptom duration of 7.1 ± 2.8 years. All patients were taking L-dopa (mean daily dose
728 ± 228 mg), with an average morning dose of 222 ± 106 mg. Other medications included
dopamine agonists, trihexyphenidyl, and amantadine. We recruited six healthy, age-matched
control subjects without active neurological disorders (1 man, 5 women, mean age 60.5 ± 11.3
years, 6 right-handed, 1 left-handed). Exclusion criteria included atypical parkinsonism,
presence of other neurological or psychiatric conditions, and use of antidepressants, hypnotics,
or dopamine blocking agents.

All PD subjects withdrew from L-dopa medication overnight for a minimum of 12 hours before
the EEG study. Those subjects who were also taking dopamine agonists or anticholinergics
stopped this medication for a minimum of 18 hours prior to the study. The mean Unified
Parkinson’s Disease Rating Scale (UPDRS) motor score during this off-medication state was 28
± 7. After completing the experiment in an off-medication state, PD subjects were given their
usual morning dose of L-dopa (mean 168 ± 80 mg Sinemet IR). They then repeated the same
tasks post-medication following an interval of approximately 1 hour to allow L-dopa to reach peak dose.

3.3.2. Experimental Design

Subjects sat facing a computer screen 80 cm away, with stimuli subtending a visual angle of 7.15 degrees. In their right hand they held a custom-built, in-house designed, rubber squeeze bulb that they were instructed to squeeze using an isometric handgrip, keeping their hand position constant throughout the study. Each subject had their maximum voluntary contraction (MVC) measured at the beginning of the study and subsequent movements were scaled to this.

The experimental task has been described in detail in Chapter 2.3.2. Briefly, subjects performed a tracking task with their right hand requiring sinusoidal force output, with or without the addition of a cued button-press task in the left hand. Refer to Figure 2.1. for an illustration of the task.

3.3.3. Data Acquisition

The EEG was recorded using a standard electrocap (Electro-cap International, Inc). The International 10-20 System of electrode placement was used, with 19 scalp and 2 auricular tin electrodes. A reference electrode was placed at the tip of the nose. Two additional electrodes were used to record eye movements, with one positioned at the upper outer canthus of the right eye, the other at the lower outer canthus of the left eye. The EEG was recorded, amplified, digitized (Ceegraph 6.71, Gamma II Netlink, Biologic System Corps), and sampled online at 512Hz. Offline, the data were re-referenced to the average reference, desampled at 128Hz, and bandpass filtered at 0.55-55Hz using a 4th order Butterworth filter prior to data analysis.
3.3.4. Data Analysis

Behavioural data from the squeeze bulb was used to calculate the error rates during tracking tasks in both simultaneous and unimanual conditions. Mean error rates across both task conditions were compared between each subject group using one-way ANOVA.

Artifactual components, for example eye blinks, cardiac signals, and muscle contamination, were removed using Independent Component Analysis, described in detail elsewhere (Jung et al., 2000). We segmented the EEG into task-related segments in order to minimize task-unrelated activity and additionally address the non-stationarity of the EEG data (Kaplan et al., 2005). Segmentation based upon behavioural data alone may be misleading since final motor output depends not only on cortical activity (as measured by the EEG) but also on subcortical and brainstem circuitry.

After artifact removal using this method, task-related EEG segments were determined by examining autocorrelations of the cross-spectrogram of the ICs over three physiologically relevant frequency bands; 5-8 Hz (theta), 8-12 Hz (alpha) and 12-30 Hz (beta). The cross-spectrogram of every pair of ICs was examined using a short-term time shifting window (3 seconds shifted by 0.5 seconds). Task-related sections were obtained by selecting segments with a peak at 10 or 18-20 seconds (the periods used for the simultaneous task). After appropriate segmentation, the task-related sections were concatenated and used to derive a mutual information network. The reader is referred to Wang et al (2009) for a full description of the segmentation and mutual information methods.
The pair-wise information (MI) of two random variables $X$ and $Y$ is defined as

$$I(X, Y) = \sum_{x,y} P_{XY}(x,y) \log_2 \frac{P_{XY}(x,y)}{P_X(x)P_Y(y)},$$

where $P_{XY}(x,y)$ denotes the joint probability distribution function (pdf) of $X$ and $Y$, and $P_X(x)$ and $P_Y(y)$ are the marginal pdfs of $X$ and $Y$ respectively. Mutual information (MI) measures the mutual dependence or information gained about one signal from another, or in other words, the amount of information about $X$ that $Y$ contains (Cover et al., 2006). The higher the MI between two signals, the more information they contain about each other and hence the more likely that the two signals are biologically related.

For this study, segmented EEG data was separated into 4 second epochs for MI computation to increase sample size and to make the distribution more Gaussian.

To compare mutual information measures when multiple factors of variability are present, for each frequency band, a two-way ANOVA with factors ‘task’ (unimanual, simultaneous; repeated measures) and ‘group’ (controls, PD off-medication, PD on-medication; independent measures) was first conducted to yield a preliminary set of interested connections. Further, based on the preliminary assessment from the two-way ANOVA, to investigate the effects of task, MI networks for the unimanual and simultaneous tasks were compared using one-way ANOVA, and connections where the MI significantly differed between tasks were displayed graphically. To investigate the effects of medication, the MI networks for PD subjects off and on medication were compared using one-way ANOVA and connections where MI significantly differed between medication conditions were displayed graphically.
3.4. RESULTS

3.4.1. Behavioural Data

Error rates were calculated as the mean difference between the actual and target bar width; Error rates (arbitrary units) were: Control subjects 1410.3 (simultaneous), 844.39 (unimanual); PD subjects Off medication  692.23 (simultaneous), 653.85 (unimanual); PD subjects On medication  1210.0 (simultaneous), 837.97 (unimanual). There was no significant difference in the tracking error across the normal, PD subjects off medication, and PD subjects on medication groups (F(17,3) = 0.567, p = 0.58).

3.4.2. Comparison between Simultaneous and Individual Movements

Figure 3.1 demonstrates connections in which MI significantly differed between the unimanual and simultaneous tasks in each of the three frequency bands (theta: 5-8Hz; alpha: 8-12Hz; beta:12-30Hz). Healthy control subjects (Figure 3.1, upper row) demonstrated few changes in MI when a simultaneous task was performed compared to a unimanual task. Decreased connectivity within parietal and occipital regions was seen in the left hemisphere in the alpha band, and bilaterally in the beta band during simultaneous tasks.

In contrast, PD subjects off medication (Figure 3.1, middle row) showed several changes in connectivity when performing a simultaneous task compared to the unimanual one, with widespread increases in theta-band connectivity during simultaneous performance, and beta-band increases in connectivity between right hemisphere occipital and temporal areas but decreases in the left hemisphere and midline frontal and central areas. Few changes were observed in the alpha band.
Following L-dopa medication (Figure 3.1, lower row), similar to the normal case, connectivity was similar between simultaneous and unimanual tasks in theta and alpha bands, although some differences remained in the beta band that were not seen in control subjects.

FIGURE 3.1: Headplots showing significant changes (p<0.05) in synchronisation for simultaneous task performance compared to unimanual task performance within theta, alpha, and beta bands. Solid lines indicate increases in mutual information, dotted lines indicate decreases in mutual information during simultaneous task performance.
3.4.3. Comparison between PD Subjects and Controls

Figure 3.2 shows changes in PD subjects compared to healthy controls. During unimanual tracking, PD subjects demonstrated increased theta- and alpha-band connectivity between frontal and central EEG channels, and a decrease in connectivity between parietal and occipital channels compared to controls (Figure 3.2, upper left). In the beta band, PD subjects showed frontal and occipital decreases in MI and central and parietal increases (Figure 3.2, upper right). Increases in theta and alpha activity were more pronounced in PD subjects performing a simultaneous task (Figure 3.2, upper left).

After L-dopa medication, PD subjects performing unimanual tracking showed fewer increases in frontal connectivity in the theta range, suggesting some normalisation of activity within this frequency range (Figure 3.2, lower left). Normalisation in theta connectivity was more pronounced when performing the simultaneous task, during which there were only two frontal connections showing increased MI compared to controls. In the alpha band, normalisation occurred predominantly over the right hemisphere in connections between central and frontal areas (Figure 3.2, lower center).

In the beta band, levodopa medication partially normalised connectivity in both unimanual and simultaneous tasks. Remaining differences between controls and PD subjects on-medication were seen mostly in the left hemisphere over temporal and central/parietal motor areas (Figure 3.2, lower right).

In contrast, there were minimal changes seen in the alpha band after L-dopa medications with contrasts between PD subjects off medication vs controls being similar to PD subjects on medication vs controls (Figure 3.2, lower center).
FIGURE 3.2: Headplots indicating significant (p<0.05) changes in synchronisation for PD subjects compared to controls. Solid lines indicate increased mutual information for PD subjects, dotted lines indicate decreased mutual information.
Extensive prior research has demonstrated changes in resting state synchronization of the EEG in PD, with PD subjects showing a global increase in theta, alpha, and beta band activity across much of the cortex (Stoffers et al., 2007, 2008a). These changes have been shown to occur at different points in the disease progression, with alpha changes occurring early in the disease, followed by theta and beta changes at a later stage (Stoffers et al., 2008a). Resting-state studies have shown conflicting evidence of the effect of L-dopa treatment. Silberstein and colleagues (2005) showed that levodopa resulted in decreased coherence between 10-35 Hz in correlation with improved motor function. In contrast, Stoffers and colleagues showed an increase in 4-30Hz connectivity with levodopa treatment (Stoffers et al., 2008b), with a negative correlation between increase in local beta synchronisation and symptom improvement. However, in subjects who improved the most following L-dopa, local beta band synchronisation actually decreased. Taken together these studies show that resting-state EEG may differ over the course of PD, and that dopamine has complex effects on synchronisation which may vary substantially between individuals.

Although the PD symptom of tremor is apparent during the resting state, other symptoms such as bradykinesia become apparent during movement, thus task-related changes are also important for distinguishing between control subjects and PD. However, due to the non-stationary nature of the EEG, prior studies that have examined synchronisation over a prolonged movement period may have included data points which were contaminated by resting-state connectivity. By using a recently developed segmentation method, we were able to ensure that such potentially contaminated segments of the EEG were discarded from analysis. Accordingly, we demonstrated task-related and L-dopa dependent altered patterns of connectivity in PD subjects.
that involved frequency-specific, regional changes. Unlike prior studies which have identified global increases in synchronisation, we found both increases and decreases in connectivity with specific topographic distributions.

**Changes during Simultaneous Movement**

Both PD subjects and normal controls demonstrated altered pattern of synchronisation in the beta band during simultaneous task performance. Concurrent up- and down-regulation of beta activity has been suggested to play a role in suppressing and facilitating competing responses (van Wijk et al., 2009), which would be particularly important in a bimanual task. In contrast to healthy control subjects, bimanual task performance in PD subjects off medication was characterised by an increase in right hemisphere connectivity and a decreased connectivity in the left hemisphere. We suggest that in control subjects, a simultaneous task can be performed by execution of two motor plans in parallel without competition for the increased recruitment of additional resources. However, in PD subjects each motor plan requires additional, compensatory resources which then compete during simultaneous movement.

**Effects of L-dopa**

The task-related changes in activity seen in this study were modulated by L-dopa, suggesting dopamine is able to at least partially normalise connectivity changes related to simultaneous movement. PD subjects on-medication showed little change in connectivity patterns between the simultaneous and unimanual tasks, similar to what was seen in control subjects. Between-group comparisons showed that L-dopa was able to partially normalise task-related changes in theta and beta bands, although little effect was seen on the alpha range connectivity, in contrast to a prior motor study in which L-dopa had a similar effect across all frequency bands (Cassidy and Brown, 2001). Due to the EEG segmentation methodology applied, the alpha band changes seen
in the PD subjects in this study were still task-related changes, but these changes were not specific to simultaneous versus unimanual performance and presumably related to a common aspect of the motor task being performed.

**Frequency-specific Effects of L-dopa**

The finding that connectivity changes between cortical areas are not completely normalized following treatment with L-dopa may suggest that dopamine is not responsible for all of the changes in cortical synchronisation seen in PD, particularly those changes seen in the alpha range. Interestingly, our finding that theta and beta changes appear to be subserved by a different mechanism than alpha-band changes in connectivity is supported by a prior study in which where theta and beta changes, but not alpha changes, correlated with the severity of motor symptoms as measured by the UPDRS (Stoffers et al., 2008a). However, increased alpha-range connectivity at rest was seen at an earlier stage than theta and beta changes, and alpha-band changes correlated with disease duration, suggesting that dopamine-sensitive changes in connectivity may not be the first to appear.

A recently proposed staging system for PD neuropathology by Braak and colleagues (2004) suggests that one of the earliest pathological stages in PD is degeneration of brainstem nuclei that give rise to thalamocortical projections, including noradrenergic projections from the locus coeruleus and serotonergic neurons in the dorsal raphe nuclei, as well as dopaminergic neurons. Degeneration in these projections may thus contribute to non-dopaminergic changes in cortical oscillatory activity. In addition, it has been shown that oscillations in the alpha band recorded from motor areas may arise partly from somatosensory cortex (Salmelin & Hari, 1994), while those in the beta band arise from primary motor cortex (Baker and Baker, 2003), suggesting that motor task-related changes in PD may arise from several sources, not all of which may be
dopamine dependent. While not possible to directly conclude from surface EEG whether the synchronisation arises from a cortico-cortical coupling or from a common subcortical influence, we suggest that early changes in PD arise from the effects of subcortical degeneration, while later changes may include downstream alterations in cortico-cortical connections.

**Conclusions**

Understanding the changes in cortical synchronisation in PD is important given that basal ganglia dysfunction exerts its damaging effects on movement via motor projections that pass through the cortex (Silberstein et al., 2005). EEG changes in PD may therefore be indicated initially by alterations in resting alpha-band activity, possibly on the basis of early locus coeruleus and/or dorsal raphe nuclei involvement. Later, task-related dopaminergic-sensitive theta and beta changes may represent a marker for greater recruitment required to accomplish an individual task. It is this greater recruitment for individual tasks that leads to an inability to perform simultaneous tasks without interference, resulting in performance breakdown when simultaneous tasks are required. The alterations in theta and beta connectivity may be a quantitative marker of the conjecture that PD subjects have difficulty in simultaneously executing separate motor programs.


4.1. PREAMBLE

In Chapters 2 and 3, we demonstrated significant changes in effective connectivity during performance of a simultaneous task. While this task is typically more difficult for PD subjects than healthy controls, the comparison of a bimanual versus unimanual task allowed for only two levels of difficulty, in which the comparison for PD subjects was between an impaired versus a relatively unimpaired task. Additionally, there was likely little difference in difficulty between tasks for the healthy control subjects. This methodology made inferences regarding compensation difficult. To provide stronger evidence that altered activation in PD is compensatory, we required a task that could be performed at 3 or more levels of difficulty, and that would increase in difficulty for both the healthy controls and PD subjects. Since the use of a visually guided tracking task had proved successful in identifying changes in PD, we chose to develop this tracking task to incorporate increasing movement speeds, in comparison to a static force output. This could be performed with 3 movement speeds, and would become increasingly harder for both subject groups as target speed increased. Healthy subjects have been shown to draw on neuronal reserves with increasing task difficulty, resulting in increased activation amplitude. We hypothesized that PD subjects may be able to utilize this normal reserve as a form of compensation, in addition to recruitment of novel areas.

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Parkinson’s Disease (PD) symptoms develop only after 50-60% of dopaminergic cells in the substantia nigra degenerate (Fearnley and Lees, 1991; Hornykiewicz and Kish, 1987). The ability of the brain to perform without functional impairment until damage reaches a critical threshold has been termed *neuronal reserve* (Mortimer et al., 1981; Satz, 1993). Implicit within this definition is that neuronal reserve is a passive process incorporating redundancy. However, an active form of reserve may also be important for overall manifestations of disease (Stern, 2002). In Alzheimer’s disease, an *active cognitive reserve* has been postulated, whereby the normal task-related networks are recruited to a greater extent than normal to maintain performance (Stern et al., 2004). Here, we expand this concept into the motor domain and investigate whether PD subjects demonstrate *active motor reserve*, recruiting the normal motor network to a greater extent when performing tasks of increasing difficulty. We distinguish this form of compensation from *novel area recruitment (NAR)* whereby new areas and/or networks are recruited.

In the healthy brain, active and passive reserves provide additional resources when task difficulty increases (Grady et al., 1996; Grasby et al., 1994). Demonstration of *active motor reserve* in PD therefore requires demonstrating increased recruitment of a network that monotonically increases with task difficulty in normal subjects. Since a typical clinical characteristic of PD is slowness of movement or bradykinesia, a motor task of increasing speeds will likely represent progressive difficulty for the motor system. Unlike disease-related activation changes that are constant and a direct result of the disease, we assume that compensatory, NAR changes will: (1) be regions activated by the patient population but not normal subjects and (2) scale with task difficulty. Detection of NAR suggests that patients are increasingly recruiting novel regions to maintain near-normal performance (Grady et al., 1996).
Because treatment with L-dopa medication may improve movement speed in PD and hence may influence NAR, we propose investigating the same PD subjects both off and on medication.

Detection of increased activation in a brain region via the indirect measure of fMRI is not straightforward, as the traditional assumption that only amplitude, as opposed to spatial extent of activity, is modulated by task difficulty may be over-simplistic (Thickbroom et al., 1998; Ng et al., 2008). For example, Buhmann and colleagues (2003) showed the primary motor cortex contralateral to the affected hand in PD has fewer active voxels than the unaffected cortex, despite finding that the average BOLD signal had no significant difference in peak height.

To assess active motor reserve in PD, we investigated how PD subjects recruited regions of interest (ROIs) that normally scale with movement speed in control subjects. To investigate NAR, we determined whether PD subjects recruited new regions that also monotonically increased with movement speed. In addition, we investigated the spatial distribution of activation within those ROIs. *By identifying activity that changes with task difficulty, we aimed to show that active motor reserve and NAR in both amplitude and spatial domains are part of the compensatory process in PD.*

4.3. MATERIALS AND METHODS

4.3.1. Subjects

The study was approved by the University of British Columbia Ethics Board, and all subjects gave written informed consent prior to participating. Ethics certificates can be found in Appendix I. We recruited ten volunteers with clinically diagnosed, mild to moderate (Hoehn and
Yahr stage 2-3) (Hoehn & Yahr, 1967) PD (4 men, 6 women, mean age 66 ± 8 years, 8 right-handed, 2 left-handed, mean symptom duration 5.8 ± 3 years). Ten healthy, age-matched subjects without active neurological disorders participated as control subjects (3 men, 7 women, mean age 57.4 ± 14 years, 9 right-handed, 1 left-handed). Exclusion criteria included atypical Parkinsonism, presence of other neurological or psychiatric conditions and use of antidepressants, hypnotics, or dopamine blocking agents. All PD subjects were taking L-dopa with an average daily dose of 685 ± 231 mg, although some subjects also took other anti-parkinson’s medications including ropinirole, bromocriptine, and domperidone.

PD subjects stopped their L-dopa medications overnight for a minimum of 12 hours before the study. Dopamine agonists were withheld for a minimum of 18 hours. The mean Unified Parkinson’s Disease Rating Scale (UPDRS) motor score during this “off-L-dopa” state was 26±8. PD subjects first completed the study in the off-L-dopa state. They were then given the equivalent to their usual morning dose of L-dopa/carbidopa in immediate release form (mean 132 ± 29 mg of L-dopa). After a 1 hour interval to allow L-dopa to reach peak dose they then repeated the same tasks in the on-L-dopa state.

4.3.2. Experimental Design

To investigate amplitude and spatial distribution of activity among cortical and subcortical brain regions in PD subjects and age-matched controls, we performed an fMRI, ROI-based pre/post medication study of sinusoidal force production at 3 frequencies (0.25, 0.5, and 0.75 Hz). Frequencies were chosen based upon prior tracking tasks, and pilot data confirmed that this range of frequencies could be accomplished by PD subjects off-medication. Prior studies that
have used a greater range of frequencies have typically employed a simpler task that did not offer much resistance, such as button pressing. Using our squeeze-bulb system, faster frequencies of force production were difficult or not possible in both control and PD subjects. We compared rhythmic force output to static contractions as opposed to rest to ensure that changes in activity were due to movement frequency and not force output per se. We note that other prior studies, such as a comparison of manual tracking to eye tracking only have also used a non-rest comparison condition (Turner et al., 1998). Since the examined frequencies in this study are too fast to be directly measured by the hemodynamic response, we utilized a block design experiment, restricting the block durations to 20-s to avoid problems with reduced amplitude of movement secondary to bradykinesia.

4.3.3. Experimental Procedures

Subjects viewed a computer screen via a projection-mirror system while laying in the magnetic resonance scanner. In their right hand, they held an in-house designed MR-compatible rubber squeeze bulb, which was connected to a pressure transducer outside the scanner room. They lay with their forearm resting down in a stable position, and were instructed to keep the same hand grip position throughout the task and to squeeze the bulb using an isometric grip. Subjects had their maximum voluntary contraction (MVC) measured at the start of the experiment and subsequent target movement for all tasks was scaled to vary between 5-15% of MVC.

Subjects performed a visually guided tracking task in which they were required to control the width of an “inflatable ring” (shown as a black horizontal bar on the screen) using the squeeze bulb to keep the ring within a scrolling pathway without scraping the sides (Figure 4.1). The width of the bar could be increased by applying greater pressure to the bulb, and releasing pressure from the bulb decreased the width of the bar. The target force output varied
sinusoidally from 5-15% MVC. To avoid scraping the sides of the tunnel, the required pressure had to be within 1% of the target force. Subjects had to monitor their own performance carefully and no additional visual feedback or error reporting was given when subjects went outside the white target lines. We used a block design for the pathway, with sinusoidal sections in three different frequencies (0.25, 0.5 and 0.75 Hz) presented pseudo-randomly, and straight parts in between where the target force needs to be constant at 10% MVC. Task blocks lasted 19.85 seconds (exactly 10 TR intervals), alternating between sinusoidal and constant force for a total of 4 minutes. All subjects performed the 4-minute task once only with the right hand.

FIGURE 4.1: Experimental task required subjects to control the width of a horizontal bar using the squeeze bulb to keep ends within the vertical scrolling pathway

PD subjects performed the task once after an overnight withdrawal (minimum of 12 hours since their last dose of L-dopa, minimum of 18 hours since the last dose of dopamine agonists) of their anti-Parkinson drugs and again one hour after admission of L-dopa. All subjects practiced the task at each frequency before the first scanning session until errors stabilized and they were familiar with the task requirements.
Stimuli were designed and presented using custom Matlab software (Mathworks) and the Psychtoolbox (Brainard, 1997; Pelli, 1997). Matlab was also used to collect behavioral data from the response device.

4.3.4. Data Acquisition

Functional MRI was acquired on a Philips Achieva 3.0 T scanner (Philips, Best, the Netherlands) equipped with a head-coil. Subjects viewed the task on a mirror attached to the head-coil. Echo-planar (EPI) T2*-weighted images with blood oxygenation level-dependent (BOLD) contrast were collected using the following scanning parameters: repetition time 1985 ms, echo time 37 ms, flip angle 90°, field of view (FOV) 240.00 mm, matrix size = 128 x 128, pixel size 1.9 x 1.9 mm. We collected 36 axial slices with 3mm thickness and a gap thickness of 1 mm. The FOV was positioned to include the dorsal surface of the brain and include the cerebellum ventrally. Each functional run lasted 4 minutes. To facilitate anatomical localization for each subject, a high resolution, 3-dimensional T1-weighted image consisting of 170 axial slices was acquired of the whole brain. A foam pillow was placed around the subject’s head within the coil to minimize head motion and to help block out auditory noise from the scanner. Subjects also used ear plugs to minimize the noise of the scanner.

4.3.5. fMRI Data Pre-processing and Analysis

Pre-processing of functional MRI data was completed using Brain Voyager trilinear interpolation for 3D motion correction and Sinc interpolation for slice time correction. No temporal or spatial smoothing was performed on the data. The data were then further motion corrected with MCICA correction (Liao et al, 2004, 2005), implemented in Matlab. MRCro’s
Brain Extraction Tool (Rorden and Brett, 2000) was used to remove the skull from the anatomical and first functional images to facilitate more accurate alignment of the functional and anatomical scans. Co-registration of anatomical and functional images was performed using custom scripts in Amira software (Mercury Computer Systems, San Diego, USA). After co-registration, eighteen regions of interest (ROIs) were manually drawn on each unwarped, aligned structural scan.

The following ROIs were drawn separately in each hemisphere, based upon anatomical landmarks and guided by a neurological atlas (Talairach & Tournoux, 1988): primary motor cortex (M1) (Brodman Area 4), supplementary motor cortex (SMA) (Brodman Area 6), prefrontal cortex (PFC) (Brodman Area 9 and 10), caudate (CAU), putamen (PUT), globus pallidus (GLP), thalamus (THA), cerebellum (CER) and anterior cingulate cortex (ACC) (Brodman Area 28 and 32). The ROI labels outlined on the high-resolution anatomical scan were resliced to isotropic voxels at the fMRI resolution, and the raw time courses of the functional data from voxels within each ROI were extracted.

Due to the sluggish hemodynamic response inherent to the BOLD fMRI signal, which prevents direct measurement of the task-related frequencies, we used a block design for analysis. A hybrid Independent Component Analysis (ICA) / General Linear Model scheme was used to contrast each of the 3-frequency blocks with the static force blocks (McKeown et al., 2003) to create statistical parametric maps (SPMs).

To measure amplitude changes, we first restricted our analysis to voxels that had a t-value > 1.96. We then calculated the mean t-statistic for each ROI and compared the mean t-statistic between each of the 3 frequencies and between each of the 3 subjects groups (Controls, PD Off medication, PD On medication).
To determine the networks of brain regions active at the different frequencies, the SPMs were further analyzed. Feature vectors, equal in length to the number of ROIs, from each SPM were obtained by repetitively sampling t-statistics from each ROI as previously published (McKeown & Hanlon, 2004). This process was repeated for all S subjects in the group, and the $P$-variate vectors ($P = 18$, the number of ROIs) were assembled into a $P \times M$ matrix, $X$, where $M$ is the number of bootstrap samples ($M$=3000). A different $X$ matrix was calculated for each frequency-based SPM. This whole process was repeated for the PD subjects to provide another $P$ by $S \cdot M$ matrix, both off- and on-medication.

To identify the linear combination of ROIs where activity scaled with increasing movement speed we used a robust multiple regression analysis approach. The above matrices were used in a multivariate linear regression model using the equation,

$$ Y = X_{\text{reg}} \cdot \beta + \varepsilon $$

(1),

where $Y = [f_1, f_2, f_3]^T$ represents the three levels of frequency of squeezing, $X_{\text{reg}} = [X_1, X_2, X_3]^T$ represents the matrices of feature vectors described above at the different frequencies, $\beta$ is the vector of regression weights, which is the linear combination of ROIs that linearly scales with frequency and $\varepsilon$ are the residuals. The regression coefficients $\beta$ were estimated using iteratively re-weighted least squares with the bisquare weighting function, as implemented in Matlab. Once the $\beta$ coefficients had been determined for normal subjects, we calculated the projection of the PD SPMs on the normal network, $\beta$, determined above.

$$ Y_{\text{pred, normal}} = X_{\text{reg,normal}} \cdot \beta_{\text{normal}} $$

$$ Y_{\text{pred, PD}} = X_{\text{reg,PD}} \cdot \beta_{\text{normal}} $$
We then plotted the relative activity across this network at each movement frequency. This was plotted separately for each subject group to determine whether PD subjects show relatively increased or decreased recruitment of the normal network during the task. Networks specific to PD subjects on and off medication, where activity scaled linearly with movement speed, were calculated using the same regression technique as for the control subjects.

To characterize the spatial distribution of ROI activation, we employed 3D moment invariants as previously described (Ng et al., 2008). In brief, this method permits one to measure the spatial variance of activation statistics within each ROI, and determine if this spatial characteristic systematically varies across groups and tasks. The reader is referred to (Ng et al., 2008) for a full theoretical and practical discussion of this approach.

4.4. RESULTS

4.4.1. Behavioural Data

The power spectra of the squeeze data for all movement blocks from all subjects are shown in Figure 4.2, demonstrating that both PD subjects and control subjects could perform the task successfully with output at 0.25, 0.5 and 0.75 Hz in slow, medium and fast blocks respectively.

Error was calculated as the mean difference between the actual and target bar width, scaled as a percentage of the target width. Error rates across all frequencies were 24% for controls, 31% for PD subjects off medication, and 25% for PD subjects on medication. An ANOVA on the error rates with subject group as a factor indicated that error rates did not significantly differ between
any of the groups ($F(2,166)=1.56, p>0.05$). In PD subjects, tremor did not cause any prominent changes in task performance.

**Power spectra of behavioural squeeze data**

![Power spectra of behavioural squeeze data](image)

**FIGURE 4.2:** Power spectra of behavioural squeeze data for control subjects and PD subjects on and off-medication during each of the 3 target task frequencies.

### 4.4.2. Mean t-statistics

Table A.1 (Appendix IV) shows the results of a 2-way mixed ANOVA comparing the mean t-statistic in each ROI between frequencies and between subject groups. There was a significant main effect of group (Normal, PD off, PD on) in bilateral primary motor cortex, and a significant main effect of frequency in the left primary motor cortex, bilateral SMA, and bilateral cerebellum. There were no significant interactions between group and frequency. Note that independent ANOVAs were calculated for each ROI, rather than including ROI as another factor, since the latter approach would only tell us that there was a main effect of ROI, whereas we were interested in the effect of group and frequency within each given ROI.
Comparisons between control subjects and PD subjects at each of the task frequencies revealed no significant differences in the mean t-statistic in any of the 18 ROIs. Comparisons between task frequencies for a single subject group revealed significant differences between the medium and fast frequency for control subjects within the right PFC and ACC, and for PD subjects off-medication in the right cerebellum, left SMA, and right ACC. For PD subjects on-medication, there were significant differences in mean t-statistics in the left putamen between slow and medium frequencies (Table A.1; Appendix IV).

4.4.3. Multiple Linear Regression

We used multiple linear regression to determine the combination of ROIs in which activity linearly increased with increasing movement speed in healthy control subjects. We found that, in control subjects, increasing frequency is associated with increased activity in a widespread network incorporating bilateral putamen, contralateral globus pallidus, bilateral thalamus, bilateral SMA, bilateral cerebellum, ipsilateral M1 and bilateral PFC, and decreasing activity of bilateral caudate and contralateral ACC (Figure 4.3).

FIGURE 4.3: Network of regions in control subjects in which activation monotonically increased (positive axis) or decreased (negative axis) with increasing movement speed. Red bars indicate ROIs where this was statistically significant.
Using this network, control subjects successfully recruited additional resources at each frequency (Figure 4.4, left upper). PD subjects off-medication recruited the normal network to a higher extent even at the slowest frequency (Figure 4.4, upper center). The increased activity of the normal network was partially normalised by L-dopa (Figure 4.4, right upper). At all frequencies the relative activity of the normal network was decreased by levodopa in the majority of PD subjects (Figure 4.4, lower).

The network that scaled with movement frequency in PD subjects off medication (Figure 4.5 upper) demonstrated more prominent involvement of bilateral cerebellum and bilateral M1, and to a lesser extent, bilateral PFC. Scaling of bilateral SMA and thalamus, and right putamen, were decreased in PD subjects off medication compared to controls. Activity of ipsilateral putamen, caudate, and globus pallidus decreased in PD subjects as movement speed increased, unlike control subjects where only the bilateral caudate was negatively associated with movement speed.
Following L-dopa medication (Figure 4.5, lower), PD subjects again demonstrated prominent involvement of cerebellum and M1, but activity in the basal ganglia and thalamus was largely normalized, with right putamen and bilateral thalamus increasing with movement frequency while right caudate decreased.

**Relative Activity in Normal Network for Movement Speed**

![Graphs showing relative activity in normal network and PD subjects off and on medication at different task frequencies.](image)

**FIGURE 4.4:** (Upper) Relative activity in the normal network at each of the task frequencies in each subject group. (Lower) Relative activity in the normal network subject in PD subjects off and on medication at each task frequency. Note that although the absolute scale is arbitrary, the scaling is the same across all graphs and can therefore be directly compared.
4.4.4. 3D Moment Invariant Results

Spatial variance of fMRI activation maps within each ROI for each frequency and subject group was compared using a 2-way mixed ANOVA (Table A.2: Appendix IV). This demonstrated a significant main effect of group in bilateral SMA, cerebellum and M1. Bilateral M1 showed significant changes between controls and PD subjects off-medication at the medium and fast
frequencies, and left cerebellum and left SMA demonstrated significantly different spatial variance between controls and PD subjects on medication only at the fastest frequency.

Frequency had a significant main effect of spatial variance in thalami, cerebella, and SMA cortices bilaterally, and in right PFC at p<0.01. There was an additional main effect of frequency in right M1 at p<0.05. At p<0.01 these effects were not apparent in the comparison of frequency within individual subject groups, with the exception of bilateral thalamus in control subjects between slow and fast frequencies. However, at the less restrictive threshold of p<0.05, control subjects demonstrated a change within bilateral cerebellum, right SMA and right PFC when comparing slow and fast frequencies, PD subjects off-medication showed a change in left cerebellum and right PFC, and PD subjects on medication showed change in right thalamus, right M1, right cerebellum and right SMA along with left caudate.

In order to determine the effect of frequency on spatial variance, we plotted the spatial variance in primary motor cortex, cerebellum and supplementary motor area as a function of frequency (Figure 4.6). In bilateral M1, spatial variance was increased in medium and fast frequencies and appeared to show a stronger linear relationship to frequency similar to the findings from multiple linear regression. In the bilateral cerebellar hemispheres, spatial variance was increased by frequency in both groups but appeared to be increased more in PD subjects even at the slowest frequency. In contrast, in the SMA, spatial variance was reduced and the linear relationship to speed was weaker in PD compared to controls.
FIGURE 4.6: Spatial variance of activation in M1, SMA and cerebellum (CER) for each frequency, within representative control and PD subjects.
4.5. DISCUSSION

Our results suggest that PD subjects demonstrate *active motor reserve*, whereby they recruit normal networks to a greater extent at lower speeds, as well as demonstrate *novel activation recruitment* (NAR) in both amplitude and spatial domains. The task that the subjects performed was an externally visually-guided task, where PD subjects demonstrate relatively normal motor performance compared to tasks where movement is self-initiated (Jahanshahi et al., 1995).

As expected, in normal subjects movement frequency modulated the mean fMRI t-statistic within the contralateral primary motor cortex, bilateral SMA, and bilateral cerebellum. These regions are consistent with a cerebello-thalamo-cortical motor pathway known to be active during tracking tasks (Miall et al., 2001). The right prefrontal cortex and anterior cingulate cortex were the only regions in healthy controls to show significant changes in mean t-statistic between the medium and fast frequencies, and there were no significant changes between slow and medium frequencies using this technique. Because we utilised a non-rest, static force comparison condition in order to detect regions specific to movement speed, we expected that observed changes in BOLD signal would be relatively small and that techniques which consider ROIs in isolation may not be sensitive enough. In support of this, multivariate linear regression was able to identify several additional regions where combined activity changed with increasing movement speed in normal controls. The most prominent of these regions were the thalamus and putamen bilaterally, consistent with prior studies showing that activity in the basal ganglia is related to increasing movement amplitude and rate (Taniwaki et al., 2003).

In the bilateral caudate, the BOLD signal had an inverse relationship to movement speed in control subjects. It has been shown that bilateral caudate activation occurs when feedback about task performance is given (Elliott et al., 1998), and that caudate activation is increased and
sustained following reward but dips sharply after punishment (Delgado et al., 2000). Although no performance feedback was given in the current task, self-monitoring was likely, and it is therefore feasible that as the task became more difficult with increasing movement speed, subjects perceived that their performance worsened resulting in decreased activation of the caudate.

By using multiple linear regression to identify regions in control subjects where activity scales linearly with movement speed, we identified a network that was subsequently probed in PD subjects in order to determine how the normal network was utilized in PD subjects performing the same task. We found that PD subjects had almost maximally recruited this network at the slowest frequency (Figure 4.4), suggesting that PD subjects can still recruit the normal network, but must do so to a greater extent, utilizing motor reserve to retain an optimal level of task performance. This allowed them to perform the slower task relatively “normally”, but when speed was further increased, PD subjects could no longer increase recruitment of this network. This is consistent with prior studies that have demonstrated that the preferred movement speed in subjects performing motor tasks is slower in PD (Phillips et al., 1994).

When PD subjects off medication had already maximally recruited the normal network, they switched to a different, compensatory network, demonstrating NAR. Off-medication, PD subjects showed less recruitment of the basal ganglia and thalamus in the network that increased with movement speed, and instead showed monotonically increasing activity in bilateral M1 and cerebellum, together with bilateral PFC, compared to controls (Figure 4.5, upper), possibly representing compensatory overactivation of these regions to counteract dysfunctional striato-frontal motor circuits (Sabatini et al., 2000; Samuel et al., 1997). Using a quantitative measure of spatial variance, we also demonstrated that significant changes in the spatial distribution of
fMRI activity occurred in PD subjects in cerebellum and M1, and that the increase in spatial variance showed a monotonic relationship to movement speed, suggesting that compensatory hyperactivity of these regions occurs at both amplitude and spatial scales (Tables 4.1 and 4.2, Appendix IV).

Interestingly, the ipsilateral putamen and globus pallidus showed a decrease in activity as movement speed increased in PD subjects off-medication. Because these regions are part of the normal speed-related network identified in control subjects, and because this network is almost maximised in the PD subjects at the lowest speed, we suggest that this decrease reflects the switch from a more normal network at slower speeds to the NAR recruitment at higher speeds, which requires a shift from basal ganglia-thalamo-cortical loops to cerebello-thalamo-cortical loops.

The increased recruitment of normal networks and recruitment of novel areas shown here is consistent with research into cognitive deficits in PD. For example, during a working memory task, PD subjects showed increased activation of prefrontal and parietal areas compared with controls, which correlated with task performance, and a novel correlation of posterior cingulate activity with task performance (Marié et al., 2007). Similarly, increases in the spatial extent of activation have been shown in the cognitive domain in PD, for example, Monchi and colleagues (2004) showed that the prefrontal cortex activation had increased amplitude and spatial extent (by voxel counts) during a set-shifting task. The results of this study thus support the idea that reserve and NAR are common mechanisms across both motor and cognitive domains.

The idea that there may be a “cost” of compensation has previously been discussed in the context of subjects recovering from stroke (Mulder et al., 2002). In the current study, the near-maximal recruitment of the normal network at slower frequencies by PD subjects off-medication
implies that increased effort is required to maintain adequate behavioural output. This increased effort of moving at a more normal rate requires activation of active motor reserve and reflects a cost of compensation (Stern, 2002). NAR at higher speeds by PD subjects can be considered as an even further cost of compensation.

After L-dopa medication, recruitment of the normal network in PD subjects was reduced, suggesting that PD subjects no longer needed to utilize motor reserve to the same extent (Figure 4.4, upper right and lower). As the graphs in Figures 4.3 and 4.5 represent the contribution of each ROI to the linear scaling of movement frequency, we demonstrated that frequency-dependent activity in the basal ganglia and thalamus was relatively normalized in PD subjects following medication (Figure 4.5, lower). However, PD subjects still showed prominent frequency-dependent activity of the cerebellum and M1 even after medication, suggesting that L-dopa can modulate disease-specific activity, but may be unable to quickly adjust compensatory NAR in other regions.

An alternative explanation for the decreased recruitment of the normal network in PD subjects on medication is that dopaminergic agents are able to improve the efficiency of processing within task-related networks by increasing the signal-to-noise ratio (Mattay et al., 2000). Similar findings have been shown in a cognitive task in PD, where blood flow in the frontal cortex was increased relative to controls in the off-medication condition, but decreased relative to controls following L-dopa (Cools et al., 2002).

Following L-dopa, the modulation of spatial variance with increasing frequency was partially normalized predominantly in the ipsilateral cortex. Spatial variance can also be influenced by motor learning, which should be considered here as PD subjects performed the task a second time during the on medication session. However, we chose a simple sinusoidal task that would
not require extensive learning. Furthermore, prior studies have shown that practice results in an increased spatial extent (Karni et al., 1995) rather than a decrease, and PD subjects did not show an improvement in task performance during the on medication session compared to the off medication session, so it is unlikely that our results can be explained as a result of practice effects. The demonstration that spatial variance in PD subjects was modulated by the difficulty of the task adds support for this phenomenon occurring as a compensatory mechanism.

Another potential mechanism for compensation is altered connectivity between regions as has been shown in PD (Palmer et al., 2009). The multivariate linear regression approach we employed here investigated co-activation between regions. This will not capture the direction of information flow between regions, which requires more specialized models (Li et al., 2008) that are beyond the scope of the current paper.

Interestingly, research into other neurodegenerative conditions has also identified increases in recruitment of both normal and novel networks, suggesting that compensation is a general mechanism important for understanding of neurodegenerative disorders (Palop et al., 2006). In Alzheimer’s Disease, Grady and colleagues (2003) showed that patients recruited a novel dorsolateral prefrontal and posterior cortex network during a semantic and episodic memory task, and activity of this network correlated with better performance on both tasks. This increased recruitment was similar to that seen in healthy older adults compared to younger adults in a previous study (Grady et al., 2002). Similarly, Reddy and colleagues (2000) have shown that patients with MS have a fivefold increase in the amplitude of ipsilateral sensorimotor cortex activity compared to controls. Thus it appears that utilisation of reserve and NAR are common mechanisms of compensation across several disorders. Better understanding of these
mechanisms may lead us to treatments that will enhance compensation, providing greater symptomatic relief from the effects of degeneration.


CHAPTER 5: JOINT AMPLITUDE AND CONNECTIVITY
COMPENSATORY MECHANISMS IN PARKINSON’S DISEASE

5.1. PREAMBLE

The previous chapter demonstrated convincing evidence that CTC regions are recruited as a compensatory strategy in PD, with increases in both the amplitude and spatial extent of the BOLD signal. Next, we considered the relationship between amplitude and functional connectivity during the same task, in PD subjects off medication. To extend the prior amplitude findings using mean t-statistics, amplitude in this chapter was investigated using a novel technique, local linear discriminant analysis, which maximizes group differences while minimizing intersubject variability within a group. This approach identifies the combination of regions in which activation amplitude maximally discriminates between subject groups, or between task conditions. We predicted that compensatory cerebellar involvement in PD would induce changes in the networks of ROIs, as well as the amplitude within individual ROIs. In addition, because of growing interest in the fMRI literature regarding the resting state, or “default-mode” of brain activation, we chose to add two additional ROIs (precuneus and posterior cingulate) to our analysis to investigate whether changes occurred within default networks in PD, in addition to the changes in the STC and CTC networks that we could predict based upon our prior studies.

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5.2. INTRODUCTION

PD is a common movement disorder, affecting 1% of the population over the age of 65. Although the main site of neurodegeneration in PD is a small subcortical region in the basal ganglia, several neuroimaging studies have shown widespread downstream changes in the amplitude of neuronal activity within both subcortical and cortical structures (Jahanshahi et al., 1995; Playford et al., 1992; Rascol et al., 1997; Sabatini et al., 2000; Samuel et al., 1997; Thobois et al., 2000; Turner et al., 2003). More recent studies have shown changes in patterns of functional and effective connectivity in PD during rest (Wu et al., 2009), motor tasks (Helmich et al., 2009; Palmer et al., 2009b), and cognitive tasks (Rowe et al., 2002), suggesting that normal functional networks are disrupted by the basal ganglia degeneration in PD, and that alternative networks may be activated as a compensatory strategy.

Prior neuroimaging studies, which have focused on the amplitude of activation in PD, have produced conflicting results. For example, supplementary motor area (SMA) activity is reduced in tasks that require attentional or selection processes (Rowe et al., 2002), but appears normal in tasks that are externally cued and highly repetitive (Turner et al., 2003). Primary motor cortex (M1) activation has been shown to be normal during joystick movements in a freely chosen direction cued at a regular pace (Jenkins et al., 1992; Playford et al., 1992), but “hyperactive” (i.e., excessively recruited compared to normal controls) during a freely chosen joystick movement cued with random inter-trial intervals (Haslinger et al., 2001). Similarly, M1 was hyperactive in a study by Sabatini and colleagues in which subjects performed a learned finger tapping sequence (Sabatini et al., 2000), but hypoactive during self-chosen regularly paced finger taps in another study (Buhmann et al., 2003).
Some of these discrepancies may be explained by differences in the tasks used, which range from simple finger tapping tasks, to force production tasks, tracking tasks, and performance of complex learned sequences. Another explanation proposed for hyperactivation seen in PD is that subjects may have difficulty in correctly selecting motor programs that correspond to the type of task they are performing (Thobois et al., 2004), and/or be unable to inhibit inappropriate motor circuits (Boecker et al., 1999). Alternatively, increased activity may be a form of compensatory neuronal plasticity for movement in the presence of dysfunctional basal ganglia (Rowe et al., 2002).

A further reason why there may be discrepancies between previous studies is for technical reasons, namely the common practice of warping brain images to a common atlas template in order to facilitate anatomical localisation and group inferences. Standard normalisation techniques do not fully accommodate wide inter-subject variability, and even after registration with advanced techniques there can be considerable inter-subject discrepancy between the location and shape of regions defined anatomically. Nieto-Castanon and colleagues (2003) showed that the percentage voxel overlap between homologous regions defined on individual anatomical scans and then warped to a common template was less than 40% for all regions considered, and worsened with increasing group size, reaching less than 5% overlap once the group had 9 subjects. The use of template ROIs to anatomically label a normalised brain may thus result in significant error in estimation of mean activation, particularly for small subcortical regions such as the basal ganglia of particular importance in PD (Ng et al. 2009; Chen et al. 2009). Segmenting ROIs in the subject’s native co-ordinate space, and utilizing analysis methods that do not require a common-space representation, will avoid these mis-registration errors.
Since regions of the brain, rarely, if ever, act in isolation, an increase or decrease in regional activity likely affects the overall pattern of activation within a functional network. However, the relationship between changes in amplitude of activity within a region, and changes in connection strengths between regions is unclear. For example, Morgan and Price found that continuous performance of a finger tapping sequence did not significantly alter functional connectivity between motor regions compared to rest (Morgan and Price, 2004). However, other studies have shown that functional connectivity between motor regions is increased in tasks requiring bimanual co-ordination compared to the same tasks performed separately with each hand (Rissman et al., 2004; Sun et al., 2004). Studies which measure both activation amplitude and functional connectivity are important to provide further understanding of the relationship between regional and network activity, and how this may be impaired in disorders such as PD.

Here, we examine changes in the amplitude and connectivity of neural activity measured by functional MRI while subjects performed a visually guided tracking task, requiring sinusoidal force production at three different speeds. Tasks with several difficulty levels such as this are helpful for differentiating compensatory from non-compensatory disease changes (Grady et al., 2003), as changes that are a direct consequence of the disease process are likely to be static across task conditions, whereas compensatory mechanisms that are recruited to improve performance may vary according to task demands. We have previously shown that this tracking task resulted in compensatory recruitment of the cerebellum and M1 in PD subjects while off medication, which was only partially normalised by levodopa (Palmer et al., 2009a). In the current study, we applied a recent analysis method, local linear discriminant analysis (LLDA) (McKeown et al., 2007), to determine the combinations of regions in which activation amplitude maximally discriminated between the fast and slow movement speeds, while accounting for enhanced inter-subject variability expected in disease states. We additionally applied LLDA to
determine the combination of regions, across all task frequencies, which maximally discriminated between subject groups. Further, to investigate changes in functional connectivity, we applied a newly developed approach, which examines the statistical dependence between pairs of ROIs, conditional upon all others, while controlling the false discovery rate for detecting significant edges (Li and Wang, 2009). For both approaches we a priori specified our regions-of-interest based upon prior studies, including both striato-thalamo-cortical (STC) and cerebello-thalamo-cortical (CTC) motor loops, and resting-state or ‘default-mode’ regions. We predicted that there would be joint changes in the amplitude of motor-related regions in PD compared to controls, consistent with prior studies. We further predicted that these regional activation changes would result in changes in patterns of connectivity.

5.3. MATERIALS AND METHODS

5.3.1. Subjects

All subjects gave written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the University of British Columbia Research Ethics Board. Ethics approval certificates can be found in Appendix I.

We recruited 10 individuals with clinically diagnosed PD, and 10 age-matched healthy controls. Description of subjects can be found in Chapter 4.3.1. PD subjects stopped their L-dopa medications overnight for a minimum of 12 hours before the study. If also taking dopamine agonists, these were withheld for a minimum of 18 hours. The mean Unified Parkinson’s Disease Rating Scale (UPDRS) motor score off medication was 26±8.
5.3.2. Experimental Design

The experimental design has been previously described (please refer to Chapter 4.3.3. and figure 4.1.). Briefly, subjects performed a visually guided tracking task at 3 different sinusoidal frequencies, which required squeezing a rubber bulb between 5 and 15% of maximum voluntary contraction (MVC), interspersed with periods of static force at 10% MVC. Frequencies of 0.25, 0.5 and 0.75Hz were chosen based upon prior tracking studies.

Since the examined frequencies in this study are too fast to be directly measured by the hemodynamic response, we utilized a block design experiment, restricting the block durations to 20 seconds (10 repetition time intervals) to avoid problems with reduced amplitude of movement secondary to bradykinesia.

5.3.3. Experimental Procedures and Data Acquisition

The experimental procedures and parameters for data acquisition are described in section 4.3.3 and 4.3.4.

5.3.4. fMRI Data Pre-processing and Analysis

Pre-processing was conducted as described previously in Chapter 4.3.5.

The following ROIs were drawn separately in each hemisphere, based upon anatomical landmarks and guided by a neurological atlas (Talairach and Tournoux, 1988): primary motor cortex (M1) (Brodman Area 4), supplementary motor cortex (SMA) (Brodman Area 6), prefrontal cortex (PFC) (Brodman Area 9 and 10), caudate (CAU), putamen (PUT), globus pallidus (GLP), thalamus (THA), cerebellum (CER) and anterior cingulate cortex (ACC) (Brodman Area 28 and 32). Additionally, the right and left posterior cingulate cortex and precuneus were outlined using Freesurfer software (http://surfer.nmr.mgh.harvard.edu/).
The ROI labels outlined on the high-resolution anatomical scan were resliced to isotropic voxels at the fMRI resolution, and the raw time courses of the functional data from voxels within each ROI were extracted.

### 5.3.5. Amplitude Analysis - Local Linear Discriminant Analysis (LLDA)

Due to the sluggish hemodynamic response inherent to the BOLD fMRI signal, which prevents direct measurement of the task-related frequencies, we used a block design for analysis. A hybrid Independent Component Analysis (ICA) / General Linear Model scheme was used to contrast each of the 3-frequency blocks with the static force blocks (McKeown, 2000) to create statistical parametric maps (SPMs). Subsequent analysis was restricted to voxels that had a t-value > 1.96 in at least one of the task frequencies.

The LLDA method is fully described elsewhere (McKeown et al., 2007). In brief, the method involves repetitively sampling t-statistics from each ROI to create feature vectors, equal in length to the number of ROIs, to create a matrix. Two matrices are compared, these two can either be across tasks (e.g. comparing activation t-statistics at fast (0.75 Hz) vs static squeezing to and slow (0.25 Hz) vs static squeezing) or two groups (e.g. PD subjects vs controls for one or more conditions). The matrix comparisons are done by performing a linear discriminant analysis locally on each possible pairs of subjects containing one subject from each group and/or condition. The ultimate result is a weighting of each ROI which maximally discriminates between groups, whilst taking into account intersubject variability. Bootstrapping (n=1000) is used to assess the significance of each weighing by permuting the group labels of subjects. To determine the effects of movement frequency on activation amplitude, we applied LLDA to the SPMs for a single task frequency and determined the linear combination of ROIs that maximally discriminated between activation at the fast (0.75 Hz) and slow (0.25 Hz) frequencies for both
PD and control groups. In order to compare amplitude changes that were robust to task frequency but consistently different across groups, we performed another LLDA analysis by pooling the t-statistics from each of the 3 frequencies and determined the combination of ROIs that discriminated between PD and controls.

5.3.6. Connectivity Analysis - PCfdr

The connectivity network was computed with the PCfdr algorithm (Li et al., 2008). This method is designed to deal with the situation of a large number of ROIs but relatively few time points, typical for fMRI experiments. It takes the mean time course from each ROI (after linear detrending) and determines the conditional (in)dependence of each pair of ROIs conditional on all other ROIs. The algorithm is able to asymptotically curb the false discovery rate (FDR) (Benjamini and Yekutieli, 2001) of inferred connections under a user-specified level. In other words, the expected ratio of spurious connections to all the connections learnt with the algorithm is controlled under a predefined threshold (Li et al., 2008). The method estimates a p-value with hypothesis tests for the existence of each possible connection, and applies an FDR-control procedure to the p-values collectively to adjust the effect of simultaneously testing multiple connections. We set the FDR threshold at 5% in this study. Connectivity networks were calculated for each subject group and each task frequency independently.

5.4. RESULTS

5.4.1. Frequency-dependent Changes in Activation Amplitude

Figure 5.1 shows the combination of regions which maximally discriminated between fast and slow frequencies, for each subject group. In control subjects, bilateral thalamus, cerebellum,
SMA, and right PFC showed increased amplitude in fast versus slow frequency movement. Contralateral (left) precuneus showed a decrease in activation in fast versus slow movement.

In PD subjects, the thalamus was no longer influenced by movement speed. Instead, left caudate showed a significant increase in fast versus slow movement, together with bilateral cerebellum, and right M1. Right posterior cingulate also showed increased amplitude in fast relative to slow frequency movement.

**FIGURE 5.1.** Relative activity in the combination of regions which maximally discriminates between fast (positive axis) and slow (negative axis) frequencies.
5.4.2. Changes in the Amplitude of Activation between PD Subjects and Controls

The LLDA method demonstrated that PD subjects showed several regions with increased activation (relative to static force production) compared to control subjects (Figure 5.2). These were left cerebellum, bilateral M1, right SMA, PFC and ACC, left globus pallidus, and bilateral precuneus and posterior cingulate. Two regions showed increased activation in control subjects relative to PD, namely the left caudate and right globus pallidus.

![Control Subjects versus PD Subjects Off-Medication](image)

**FIGURE 5.2:** Relative Activation Amplitude in Control (positive axis) versus PD subjects (negative axis), with weighting showing combination of ROIs which maximally discriminates between subject groups, units arbitrary

5.4.3. Effect of Frequency on Functional Connectivity

Using the PCfdr approach, we determined the connectivity patterns for each task frequency, for each subject group. Within each subject group, task frequency did not alter the network recruited by the task, that is, all connections shown in the subsequent figures were common to all three task frequencies.

**Normal patterns of connectivity**

Figure 5.3 shows the connections that occurred in control subjects and indicates whether or not these connections were also found in PD subjects. Only a small number of connections found in
control subjects were also present in the PD group, namely from the left anterior cingulate to left prefrontal cortex, left SMA to M1, left globus pallidus to putamen, left PCC to precuneus, right ACC to PCC and right putamen to left cerebellum.

The majority of connections found in control subjects were absent in PD subjects. These included connections within the basal ganglia (between right caudate and right thalamus, and from right globus pallidus to right thalamus), connections from thalamus to cortex (left thalamus to right SMA and cerebellum) or cortex to thalamus (right PFC to left thalamus, right M1 to right thalamus), between cortex and other basal ganglia regions directly (right M1 to right globus pallidus, right caudate to left PFC, left posterior cingulate to left putamen, left SMA to left caudate) and between cortical regions (left M1 to right precuneus, left precuneus to left SMA, left ACC to left PCC, left ACC to left PFC, right SMA to right M1, right PFC to right ACC, and right PCC to right SMA).

**Connectivity changes unique to PD**

While figure 5.3 showed the normal connections missing in PD subjects, figure 5.4 demonstrates the unique pattern of connectivity in PD subjects. This novel recruitment included intrahemispheric connections from left cerebellum to left M1, left PFC to left SMA, right ACC to right PFC, right PFC to right SMA, right cerebellum to right M1, right putamen to right globus pallidus, and right globus pallidus to right SMA. Interhemispheric connectivity was recruited from left ACC to right caudate, left SMA to right M1 and right cerebellum, left caudate to right putamen and thalamus, right caudate to left thalamus, right putamen to left globus pallidus and left thalamus, and right PCC to left putamen.
Normal networks in PD

FIGURE 5.3: Connectivity patterns in control subjects performing the tracking task. Black lines show connections also present in PD, grey lines show connections missing in PD. Regional amplitude changes are indicated by the symbols as described.
FIGURE 5.4: Connectivity unique to PD subjects (off medication) performing the tracking task. Regional amplitude changes are indicated by symbols as described.
Summary of changes in amplitude and functional connectivity

Table 5.1 shows a summary of the direction of change in amplitude and connectivity in PD subjects compared to age-matched controls, within three functional loops. The STC loop ROIs included connections from the striatum to thalamus, to motor cortex, while the CTC loop involves connections from cerebellum to thalamus to motor cortex. The default-mode ROIs were the precuneus and posterior cingulate.

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TABLE 5.1 Changes in amplitude and connectivity in PD subjects versus controls.

Within the STC loop, PD subjects showed decreased amplitude of task-related BOLD activation in the left hemisphere, together with decreased functional connectivity bilaterally, although inter-hemispheric connectivity between basal ganglia regions showed a strong increase. In the CTC loop, PD subjects showed bilateral increases in both amplitude and connectivity. In the resting state or default mode regions, bilateral activation amplitude increased, but functional connectivity decreased.

5.5. DISCUSSION

5.5.1. Changes in Basal Ganglia Recruitment and STC Loops

As expected, given the site of neurodegeneration in PD, activation amplitude within the left hemisphere STC loop was reduced in PD subjects off medication compared to controls while
performing a right handed task. In terms of functional connectivity, we demonstrated that few connections were common to control subjects and PD subjects, suggesting that functional connectivity is significantly altered by PD. Normal connections between ipsilateral basal ganglia regions and from ipsilateral cortical regions to the basal ganglia were missing in PD subjects, suggesting that not only is output from the basal ganglia disrupted in PD, but that normal patterns of input to the basal ganglia are functionally impaired as well.

Novel connections were recruited in PD subjects, with a dramatic increase in the number of inter-hemispheric connections between basal ganglia regions. This bilateral recruitment of basal ganglia structures may be a compensatory change to ameliorate impaired basal ganglia function by recruiting the homologous region in the opposite hemisphere. Recruitment of ipsilateral networks with increasing task demands has been previously shown in healthy subjects (Verstynen et al., 2005). It is reasonable that this recruitment would require functional connections between the two hemispheres in order to pool resources. However, functional coupling between homologous regions has also been observed at rest (e.g. Macaluso et al., 2007), thus the increased inter-hemispheric connectivity seen here may alternatively reflect a loss of function of STC loops, which remain in a default state of connectivity.

5.5.2. Compensatory Recruitment of CTC Pathway

PD subjects demonstrated increased recruitment of several areas, in line with our previous reports of increased activation amplitude in this task (Palmer et al., 2009a), and with prior studies (Sabatini et al., 2000; Haslinger et al., 2001). These areas included regions previously suggested to be compensatory in PD, including the cerebellum and bilateral primary motor cortex. Functional connectivity analysis demonstrated a novel functional network during motor task performance in PD subjects, which included prominent connectivity between many of the
purportedly compensatory regions, most predominantly within bilateral CTC pathways. Together, these results add further support for a compensatory role of these regions.

5.5.3. Changes in the Default-mode Network in PD

Overactivation of bilateral precuneus and posterior cingulate was found in PD subjects. These regions are part of the ‘default mode network’, that is typically active during the resting state. At rest, motor networks may remain functionally connected in an ‘equilibrium’ state and be ready for action, being recruited when needed for voluntary actions (Wu et al., 2009). Thus, in PD, a change in the balance of resting state versus motor networks may impair successful task-related recruitment. An inability to rapidly perturb networks from a resting state may contribute to slowness of movement initiation. Disruption of the default mode of activity has also been shown to be abnormal in other neurological conditions, for example in Alzheimer’s Disease (Greicius et al., 2004), and therefore may also be a common mechanism to other neurodegenerative diseases.

Connectivity to and from the default mode regions was reduced in PD. Thus, unlike STC and CTC loops where amplitude and connectivity changes in PD were seen in parallel, amplitude and connectivity changes in default mode regions were in opposite directions. We suggest that a reduction in connectivity between default mode regions may disrupt a normal resting pattern of inhibition resulting in an increase in the regional amplitude.

5.5.4. Comparison of Amplitude and Connectivity Analysis

When considering the effects of task frequency, several regions demonstrated changes in amplitude, but there was no change in the functional connectivity within a subject group as task frequency increased. Combined degenerative and compensatory changes in PD presumably lead
to a novel pattern of anatomical and functional connectivity related to voluntary movement that would remain fixed across different movement conditions, even when task conditions result in changes in the amplitude of activation within one or more of the regions involved. The finding that neither PD nor control subjects showed frequency related changes in connectivity supports the idea that underlying functional networks are fixed, whereas amplitude can vary rapidly with increasing task demands.

A between-groups comparison of the results obtained from amplitude and connectivity analyses demonstrates several regions with differing functional connectivity between PD and control subjects, without showing any significant changes in amplitude. This further highlights the important of jointly considering both amplitude and connectivity changes, and implies that studies which choose regions-of-interest for connectivity analysis based on functional regions from task activation maps may miss important changes in connectivity.

Our results add further support to the notion that a non-trivial relationship exists between amplitude and connectivity in brain disorders. A recent fMRI study of children with high-functioning autism performing a motor task demonstrated increased activation only in the SMA, but fairly diffuse decreased connectivity across the entire motor network (Mostofsky et al., 2009). Similarly, a study of patients with major depression revealed increased activation of dorsolateral prefrontal cortex and increased connectivity between this region and the cerebellum, but also decreased connectivity between inferior parietal, superior prefrontal and frontopolar regions (Vasic et al., 2009).

One reason for the complex relationship between amplitude and connectivity changes is that activation amplitude, as measured by the BOLD signal, includes influence from both excitatory and inhibitory connections. The balance of these two connections may influence the relationship
between two separate regions. It is currently unclear how excitatory and inhibitory activation can be disentangled from current functional MRI data. However, since a specific region may receive excitatory input from one region and inhibitory input from another region, connectivity patterns derived from statistical methods may help to provide information regarding the likely basis of observed increases in signal amplitude. In addition, there are also negative fluctuations in the BOLD signal, or ‘deactivations’, and again it is not clear how these should be interpreted, though they may reflect the release of a region from a resting or default state to be recruited by the task, or to allow resources to be allocated to other areas. Studying functional connectivity alongside amplitude changes may help to determine whether a particular ROI becomes functionally disconnected from the resting state network during task performance, and possibly becomes connected to an alternative network related to the experimental task.

5.5.5. Conclusions

In conclusion, the findings of amplitude increases within cerebello-thalamo-cortical regions in PD adds further support to a large body of literature suggesting that this recruitment is compensatory, and we extend this knowledge further by demonstrating functional connectivity changes that recruit these pathways for a motor task. We additionally show that there are inter-hemispheric connectivity changes between basal ganglia structures that may also be compensatory. The findings that network changes were constant across task frequencies (unlike amplitude), and that several regions showed connectivity but not amplitude changes in PD versus controls, suggest that functional connectivity is a particularly suitable method for detecting compensatory changes in recruitment of widespread regions. Further, we add support for the suggestion that default-mode regions are also altered by PD, which may impair rapid recruitment of appropriate motor networks from rest.
5.6. REFERENCES


Chen J, Palmer SJ, Khan AR, McKeown MJ, Beg MF (2009) Freesurfer-Initialized Large Deformation Diffeomorphic Metric Mapping with application to Parkinson’s Disease. SPIE Medical Imaging; 2009; Orlando, Fla..


CHAPTER 6: LEVODOPA OVER-RESTORES AMPLITUDE BUT FAILS TO RESTORE COMPENSATORY CEREBELLO-THALAMO-CORTICAL LOOP CONNECTIVITY IN PD\textsuperscript{5}

6.1. PREAMBLE

In Chapter 5, we extended our evidence of compensatory CTC loop recruitment in PD to include changes in functional connectivity. In addition, we showed changes in connectivity of the STC loop consistent with the neurodegeneration seen in PD, as well as changes in the amplitude and connectivity of the default-mode networks. Although the expected effects of L-dopa on compensation are unclear, it is known that L-dopa reliably improves some of the motor symptoms of PD, and therefore we were interested to see the effects of L-dopa on the functional connectivity changes observed within STC, CTC and default loops. In the following chapter we therefore studied the same PD subjects performing the same tracking task after L-dopa administration. We utilized the same analysis techniques as in the prior chapter to compare the changes in amplitude and connectivity pre and post medication.

\textsuperscript{5} A version of this chapter will be submitted for publication: Palmer SJ, Li J, Wang ZJ, & McKeown MJ (2009) L-dopa over-restores amplitude but fails to restore compensatory cerebellar connectivity in PD.
6.2. INTRODUCTION

Neurodegeneration of the nigrostriatal pathway in PD induces widespread downstream changes in both activation amplitude and functional connectivity as measured by fMRI, including changes resulting directly from the disease process, and compensatory changes that attempt to maintain function. L-dopa, the gold standard for treatment of PD, results in significant clinical improvements; however the effects of L-dopa on both disease and compensatory changes in PD are still incompletely understood.

It is clear that L-dopa has complex and at times conflicting effects in PD, possibly related to the inverse-U shaped relationship between dopamine levels and performance (Williams & Goldman-Rakic, 1995). L-dopa has been shown to normalize both hypo- and hyper-activations of the same ROIs (see for example, Haslinger et al., 2001 versus Buhmann et al., 2003). While L-dopa generally improves overall motor function in PD, it may have complex motor effects (Au et al., 2009) and possibly worsen the cognitive symptoms associated with PD (Cools, 2006). A recent study found that L-dopa restores the amplitude of motor-cortico-striatal loops but has no effect on cognitive cortico-striatal regions (Jubault et al., 2009). Another study demonstrated that, in healthy controls, L-dopa increases functional connectivity in motor pathways originating in the putamen, but had mixed effects on connections originating in the caudate (Kelly et al., 2009). We have previously shown that L-dopa was able to partially normalise effective connectivity in PD subjects performing a bimanual task (Palmer et al., 2009a).

Interpreting the effects of L-dopa is further complicated by the fact that prior studies have frequently failed to measure both amplitude and connectivity, yet these may be both be affected by L-dopa, as well as the spatial extent of activation (Palmer et al., 2009a-b; Ng et al., 2009).
We have recently applied both amplitude and functional connectivity analysis to healthy controls and PD subjects (off medication) performing a tracking task at 3 frequencies (Palmer et al., under review). We found decreases in amplitude of activation and connectivity in ROIs within striato-thalamo-cortical (STC) loops, and parallel increases in cerebello-thalamo-cortical (CTC) loops. In contrast, regions associated with the so-called ‘default mode’, or resting state, had dissociation between activation and connectivity, showing increased amplitude but decreased connectivity in PD. While amplitude changes were task-dependent, and influenced by the speed of movement performed, connectivity changes were fixed across task frequencies.

Here, we investigate the effect of L-dopa on amplitude and connectivity during a manual tracking task consisting of sinusoidal squeezing at three different frequencies. We a priori specify ROIs based upon prior studies, including ROIs contributing to striato-thalamo-cortical (STC) and cerebello-thalamo-cortical (CTC) motor loops, and resting-state or ‘default-mode’ regions.

We predict that L-dopa will at least partially normalise changes within the STC loop. The expected effect of L-dopa on compensation is less clear. Successful normalisation of dopaminergic transmission may negate the requirement for compensatory activation. Alternatively, L-dopa may fail to fully normalize activation patterns in PD, resulting in continuing need for compensation. Additionally, compensatory changes in functional networks that involved synaptic plasticity may remain apparent even after administration of L-dopa.

6.3. MATERIALS AND METHODS

The subjects, experimental paradigm, and analysis methodology for this Chapter are identical to those described in Chapter 5.3. For the on-medication results, the same PD subjects were
scanned approximately one hour after receiving their usual morning dose of L-dopa. All subjects were scanned on the same morning in which they completed the off-medication task. For the purposes of this study, we did not consider the effect of task frequency on activation amplitude, only the differences between subject groups. We did confirm that task frequency had no effect on connectivity, as in the previous chapter.

6.4. RESULTS

Changes in the amplitude of activity within ROIs and connectivity changes between ROIs were examined between PD subjects off and on medication, and between PD subjects on medication and controls. Within subject groups, task frequency did not alter the network recruited, thus subsequent reports of connectivity are common to all frequency conditions.

6.4.1. Effect of L-dopa on Activation Amplitude

Comparing the activation amplitude within PD subjects pre/post L-dopa medication across all frequencies (Figure 6.1A) demonstrated that the medication decreased the activation amplitude in bilateral thalamus, cerebellum, M1, SMA, precuneus, left putamen, and right prefrontal cortex and posterior cingulate (PCC).

Following L-dopa medication, the majority of ROIs showed decreased relative activation in PD subjects relative to control subjects (Figure 6.1B), including bilateral putamen, caudate, thalamus, M1, cerebellum, and SMA, left PFC, and right globus pallidus (GLP). Only two regions, left PCC and left precuneus, showed increased activation in PD subjects after L-dopa compared to controls.
FIGURE 6.1: (A) Relative activation between PD subjects off and on medication (upper figure), positive axis represents greater activation in the off medication group; (B) Relative activation between controls and PD subjects on-medication (lower figure), positive axis represents greater activation in the control group.
6.4.2. Effect of L-dopa on Functional Connectivity

Figure 6.2 shows the effect of L-dopa on connectivity patterns in PD subjects. L-dopa normalised several of the connections unique to PD, including left PFC to left SMA, left SMA to right M1 and right cerebellum, right cerebellum to right M1, left caudate to right putamen and thalamus, right caudate to left thalamus, right putamen to left GLP and left thalamus, right GLP to right SMA, and right PCC to left putamen. These connections were abnormally present in PD subjects off medication, and this was corrected by L-dopa (normalisation). No normal connections were re-established by L-dopa, that is, if a normal connection was missing in PD subjects off-medication, it remained missing after L-dopa.

Some connections were not normalised by L-dopa, that is they were found in both PD groups but not in controls (Figure 6.3, grey arrows), specifically left cerebellum to left M1, left ACC to right caudate, right ACC to right PFC, right PFC to right SMA, and right putamen to right GPL. In addition, other connections were recruited only following L-dopa administration (Figure 6.3; black arrows), such that they were unique to the on-medication group, including connections from left caudate to left putamen, right caudate to right putamen and ACC, right ACC to left putamen; right M1 to right SMA and PCC, right PCC to left SMA, left PCC to left ACC and left M1, right precuneus to left putamen and PCC, left precuneus to right cerebellum, and right cerebellum to right thalamus and SMA.
FIGURE 6.2: Functional connectivity that is normalised by L-dopa. ROIs in which amplitude also decreased are shown with a red square.
FIGURE 6.3: Functional Connectivity in PD subjects on medication; connections found in PD off-medication that are not normalised by L-dopa are shown in grey, connections unique to the on-medication group are shown in black.
6.4.3. Summary of Changes in Amplitude and Functional Connectivity

Table 1 summarises the changes in amplitude and connectivity in PD subjects off and on medication, compared to controls, within the three loops specified *a priori*. The STC loop ROIs included connection from the striatum to thalamus, to motor cortex, while the CTC loop involves connections from cerebellum to thalamus to motor cortex. The default-mode ROIs were the precuneus and posterior cingulate.

<table>
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<th>STC</th>
<th>CTC</th>
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<td>PD Off</td>
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In summary, L-dopa resulted in a bilateral decrease in the amplitude of both STC and CTC loops, and an increase in left hemisphere default mode amplitude. Connectivity was relatively normalised within STC loops bilaterally, but increased in left hemisphere CTC loops. Default mode regions showed increased connectivity bilaterally.
6.5. DISCUSSION

6.5.1. Effects of L-dopa on Activation Amplitude

In PD subjects on medication, we demonstrated a reduced amplitude of activation within STC regions compared to both healthy controls and to the same PD subjects off-medication, suggesting L-dopa is unable to fully normalize defective STC circuits. In contrast, prior studies found that L-dopa largely restored the amplitude of activation within motor-striatal regions during a cognitive task (Jubault et al., 2009), and reduced expression of the PD-related-pattern of resting state regional metabolism (Feigin et al., 2001). It is possible that motor tasks retain the most impairment in PD even after L-dopa administration, as PD predominantly affects voluntary movement.

In addition to the inadequate restoration of STC amplitude with L-dopa, we found that L-dopa changed CTC circuits from being hyperactive to being hypoactive. This “overshooting” of activity in the CTC after L-dopa was unexpected, and may be due to the focusing effect of L-dopa (Ng et al., 2009), which may result in reduced mean amplitude across a given ROI.

In the default network, left hemisphere regions showed an increase in activation amplitude compared to control subjects. Prior studies have shown that activity and connectivity of the default-mode are reduced during task performance (McKiernan et al., 2003; Fransson, 2006), together with an increase in activation of task-associated regions. Dopamine depletion has been shown to reduce task-related suppression of the default mode in healthy subjects (Nagano-Saito et al., 2008), and we have previously shown a bilateral increase in the amplitude of default-mode regions in PD subjects off medication (Chapter 5). Here, we show that L-dopa fails to normalise this hyperactivity of default-mode regions in the contralateral hemisphere. Failure to
suppress the default-mode during task performance is associated with behavioural impairments (Polli et al., 2005, Weissman et al., 2006).

6.5.2. Effects of L-dopa on Functional Connectivity

The majority of the novel PD off-medication connections were normalised (i.e. removed) by L-dopa treatment, suggesting that restoring dopamine levels in the basal ganglia can at least partially restore functional connectivity in the basal ganglia and downstream systems. However, rather than returning PD subjects to a normal pattern of connectivity, L-dopa resulted in another, unique pattern of functional connectivity. There may be several reasons for this failure to completely normalise functional networks. First, some of the observed changes in network activity may be compensatory mechanisms that have developed over a prolonged time period utilising structural, plastic changes, and these may not be rapidly readjusted following L-dopa administration. Secondly, prolonged disruption of the normal network in the early stages of PD, before treatment has begun, may lead to a downregulation of normal pathways that can no longer be restored at a later date with pharmacological therapy. Indeed, Palop and colleagues (2006) describe how long-term changes in neurotransmission may result in network changes may create a ‘vicious cycle’ whereby downstream changes cause further changes in other neurotransmitter systems (Palop et al., 2006). Once individuals with PD reach a clinical stage and receive treatment it is feasible that network changes may not be easily reversible.

We found that L-dopa increased the functional connectivity both between motor regions and between so-called default or resting state regions, but it was only in the motor regions that we saw a decrease in the amplitude of activation. This suggests that there exist dopaminergic mechanisms in both sensorimotor and resting state functional networks, but these are differentially affected by L-dopa treatment. As previously mentioned, the focusing effect of L-
dopa may account for the reduction in mean amplitude within the motor network, so it is of interest that this effect appears confined to the motor loop and does not appear to affect default-mode regions of the brain in the same way.

Functional connectivity of the default-mode was increased in PD subjects following L-dopa in this study, in contrast to prior studies by Tinaz and colleagues (2008) in which functional connectivity did not differ between PD and control subjects, and Kelly and colleagues, in which L-dopa reduced connectivity in the default mode in healthy controls (Kelly et al., 2009). This discrepancy may be explained by the difference in task used, as these prior studies used a cognitive (semantic event sequencing) task and resting-state task respectively as opposed to the predominantly motor task here.

A limitation of the connectivity analysis used in this study is that it does not calculate path coefficients, which would have provided additional information regarding the weighting of connections with the recruited networks, for example, the relative importance of STC versus CTC regions in PD. For the current task, there were insufficient time points to utilize other connectivity techniques such as Dynamic Bayesian Networks (Li et al., 2008) that would provide information on connection strengths, and future work is thus needed to ascertain the influence of task parameters on this aspect of connectivity.

6.5.3. Comparison of Amplitude and Connectivity Analysis

Functional connectivity was not altered by task frequency in PD subjects following L-dopa, consistent with our prior findings in controls and PD subjects off medication. It is feasible that combined degenerative and compensatory changes in PD would lead to a novel pattern of anatomical and functional connectivity related to voluntary movement that would remain fixed across different movement conditions, even when specific aspects of the movement may result
in changes in the amplitude of activation within one or more of the regions involved. The finding that neither PD nor control subjects showed frequency related changes in connectivity supports the idea that underlying functional networks are fixed, whereas amplitude may vary rapidly with increasing task demands.

A between-groups comparison of the results obtained from amplitude and connectivity analyses revealed that several regions demonstrated changes in functional connectivity between PD and control subjects, without showing any significant changes in amplitude. This further highlights the important of jointly considering both amplitude and connectivity changes. Additionally, this further suggests that studies which select regions-of-interest for connectivity analysis based on functional regions from task activation maps may miss important changes in connectivity from other brain areas.

Our results add further support that there is not a simple relationship between amplitude and connectivity whereby increased amplitude necessarily corresponds to increased connectivity and vice versa. This is in line with prior studies in many brain disorders, for example, a fMRI study of children with high-functioning autism performing a motor task demonstrated increased activation only in the SMA, but fairly diffuse decreased connectivity across the entire motor network (Mostofsky et al., 2009). Similarly, a study of patients with major depression revealed increased activation of dorsolateral prefrontal cortex and increased connectivity between this region and the cerebellum, but also decreased connectivity between inferior parietal, superior prefrontal and frontopolar regions (Vasic et al., 2009).

One reason for the complex relationship between amplitude and connectivity changes is that activation amplitude, as measured by the BOLD signal, includes influence from both excitatory and inhibitory connections. The balance of these two connections may influence the relationship...
between two separate regions. This may be of particular importance when considering the effects of L-dopa, as dopamine has opposing excitatory and inhibitory effects on D1 and D2 receptors respectively (West and Grace, 2002). It is currently unclear how excitatory and inhibitory activation can be disentangled from current functional MRI data. However, since a specific region may receive excitatory input from one region and inhibitory input from another region, connectivity patterns may help to provide information regarding the likely basis of observed increases in signal amplitude.

In addition, there are also negative fluctuations in the BOLD signal, or ‘deactivations’, and again it is not clear how these should be interpreted, though it is suggested that they may reflect the release of a region from a resting or default state to be recruited by the task, or to allow resources to be allocated to other areas. Studying functional connectivity alongside amplitude changes may help to determine whether a particular ROI becomes functionally disconnected from the resting state network during task performance, and possibly becomes connected to an alternative network related to the experimental task.

6.5.4. Conclusions

In conclusion, we have shown that although STC regions are hypoactive following L-dopa administration, connectivity patterns within the basal ganglia are largely normalised. In compensatory CTC regions, L-dopa over-normalises the activation amplitude such that these regions show bilateral hypoactivation in PD subjects on-medication, while connectivity of CTC loops is altered, but not normalised, by L-dopa. Default-mode regions in the contralateral hemisphere remain abnormally recruited during task performance, which may contribute to behavioural difficulties.
The findings that network changes were constant across task frequencies, and that several regions showed connectivity but not amplitude changes in PD versus controls, suggest that functional connectivity is a particularly suitable method for detecting movement-related changes in recruitment of widespread regions.
6.6. REFERENCES


CHAPTER 7: CEREBELLO-THALAMO-CORTICAL
COMPENSATION OCCURS IN TREMOR-DOMINANT, BUT NOT
AKINETIC-RIGID PD

7.1. PREAMBLE

Thus far we have provided several lines of evidence for a compensatory role of cerebellar
activation in Parkinson’s Disease, demonstrating that this compensation can involve amplitude,
spatial, and connectivity changes during performance of a motor task. In addition, we have
demonstrated that L-dopa incompletely normalised these compensatory changes.

However, increased cerebellar activation may come with a price. Indeed, Hoshi and colleagues
(2005), who demonstrated a direct pathway between cerebellum and the input area of the basal
ganglia, showing that the cerebellum is well placed to compensate for BG dysfunction, ended
their discussion with the question “When basal ganglia activity is abnormal, is cerebellar input
part of the problem or part of the solution?”. Although compensation is designed to maintain a
near-normal level of function, and thus by definition is beneficial, compensation that
successfully maintains the homeostasis of dopaminergic transmission in one pathway may
conceivably lead to detrimental changes in other interconnected pathways, and compensation
that recruits alternative networks to accomplish a specific task may at the same time impair
performance on tasks that this network normally subserves.

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6 A version of this chapter will be submitted for publication: Palmer SJ, Ty E, Li J, Wang ZJ, McKeown MJ (2009)
Cerebello-thalamo-cortical compensation occurs in tremor-dominant, but not akinetic-rigid PD.
Increasing evidence suggests that the cerebellum may actually play a critically important role in tremor generation in PD. PD is a heterogeneous disorder, and although tremor is listed as one of the key symptoms, not all individuals with PD will develop parkinsonian resting tremor. Together, these findings led us to speculate that the role of the cerebellum may differ between subjects with tremor-dominant versus those with akinetic-rigidity dominant PD. To date, no study has investigated differences between the subtypes of PD using functional MRI. To investigate this hypothesis, we thus recruited two groups of PD subjects, one for each subtype, and examined connectivity within STC and CTC loops whilst they performed a visually guided tracking task.

7.2. INTRODUCTION

Several studies have demonstrated increased activation of the cerebellum in Parkinson’s disease, with increasing evidence that this overactivation represents a compensatory mechanism (Wu and Hallett, 2005; Yu et al 2007; Palmer et al 2009a,b; Palmer et al, under review). Neuroimaging studies of the functional role of the cerebellum and basal ganglia in healthy individuals have demonstrated dissociable roles for striato-thalamo-cortical (STC) versus cerebello-thalamo-cortical (CTC) loops (Jueptner and Weiller, 1998; Taniwaki et al 2006), implying that compensatory recruitment of CTC pathways in PD may (i) occur only during tasks which normally recruit CTC pathways, or (ii) may occur abnormally in tasks that typically recruit STC pathways, which may provide both benefit and detriment.

Indeed, the cerebellum is increasingly being named as a likely locus of the pathological mechanism of tremor generation, with several lines of evidence supporting this role. First, the severity of tremor in PD does not correlate with the severity of dopamine loss in the striatum, or
with progression of clinical symptoms (Zaidel et al., 2009). Secondly, primate and rodent models of PD, produced by chemically lesioning the midbrain, do not typically produce a resting tremor. Early animal studies concluded that experimental rest tremor required damage to both nigro-striatal dopamine projections and cerebellar outflow to the red nucleus and thalamus (Marsden, 1984). Lastly, in human PD, pathological oscillations occur in the GPi and STN at tremor frequency or double tremor frequency; however, studies have failed to show coherence between these oscillations and simultaneous tremor recordings (Lemstra et al, 1999; Hurtado et al 2005). In contrast, high coherence has been found between tremor recordings and the firing of the cerebellar-receiving neurons in the ventralis-intermedius (Vim) of the thalamus (Lenz et al 1994), and surgical lesioning of the Vim successfully ameliorates parkinsonian and other tremors (Lenz et al 1995; Papavassilou et al 2008).

Interestingly, tremor does not present in all PD subjects. Two major PD subtypes have been described, namely those patients whose dominant symptom is tremor (PDt) and those whose dominant symptoms are akinesia and rigidity (PDar). Studies have suggested that the pathology of PDt differs to that of PDar, with more severe pathology of the retrorubral area in the PDt form (Paulus and Jellinger, 1991), and greater incidence of cortical Lewy bodies in the PDar form (Selikhova et al., 2009). Patients with the tremor dominant form of PD have a better prognosis and typically slower disease progression than PDar patients (Lewis et al., 2005). A compensatory role for cerebellar activity, which may also contribute to tremor generation, is thus consistent with these clinical findings.

Basal ganglia and cerebellar contributions to movement can been dissociated using self-initiated versus externally guided tasks respectively (Mink and Thatch, 1991; Stein and Glickstein, 1992; Jueptner et al, 1996a). The cerebellum is more reliant on sensory feedback, particularly visual
feedback, during motor performance, and as such plays a greater role in tasks that are externally cued, while the basal ganglia plays a more important role in tasks that are self-initiated. Perhaps because of this distinction, PD subjects show increased reliance on visual cues during voluntary movement (Glickstein and Stein, 1991; Cunnington et al., 1995). A study by Jahanshahi and colleagues (1995) showed that, compared to controls, PD subjects had increased activation in supplementary motor area (SMA), anterior cingulate, left putamen, left insular cortex, right DLPFC, and right parietal cortex during self-initiated finger tapping, but showed no difference to controls in a finger tapping task that was externally triggered. In contrast, Cerasa and colleagues (2006) showed that PD subjects had increased activation in cerebellum, putamen, SMA, thalamus, right inferior frontal gyrus and insula cortex during externally guided movement, and increased activity of the cerebello-thalamic pathway only during self-guided performance. Neither of these studies differentiated between PDt and PDar subtypes. Here, we compared the activity within STC and CTC loops in one group of PDt patients and one group of PDar patients, compared to healthy controls, while they performed a visually-guided tracking task requiring sinusoidal force production. Manual tracking of a visual pathway has been shown to recruit both STC and CTC pathways when compared to rest or eye movements alone (Jueptner and Weiller, 1998), and both STC and CTC regions have been shown to play a role in the visuomotor process during continuous force production tasks (Vaillancourt et al., 2003). We predicted that both PD groups would show reduced recruitment of STC pathways compared to controls, and that the PDt group would show a greater amount of compensatory cerebellar recruitment compared to PDar subjects.
7.3. MATERIALS AND METHODS

7.3.1. Subjects

The study was approved by the local Ethical Review Board, and all subjects provided written informed consent in accordance with the Declaration of Helsinki. We recruited 10 subjects with tremor dominant PD and 10 subjects with akinetic-rigid PD. Subtypes were determined based upon the ratio of scores obtained from a subset of the Unified Parkinson’s Disease Rating Scale motor section, as described by Schiess et al (2000).

Despite completion of a training session outside the scanner, 3 PDt and 2 PDar subjects were unable to successfully perform the self-guided task at the required frequency and their data were subsequently excluded. The remaining groups thus consisted of 7 PDt subjects (5 men, 2 women, 5 right-handed, 2 left-handed, mean age 67.1 years, mean symptom duration 65.3 months) and 8 PDar subjects (8 male, 8 right-handed, mean age 66.8 years, mean symptom duration 70 months). In addition, we recruited 10 age-matched healthy control subjects (mean age 58.6 years, 3 male, 8 right-handed). Exclusion criteria included atypical parkinsonism and presence of other neurological or psychiatric disorders, as well as use of anti-depressants, hypnotics, or dopamine blocking agents.

In order to be studied in an effectively “off medication” state, PD subjects withdrew from L-dopa for a minimum of 12 hours prior to the study, and withdrew all other parkinson’s medication for at least 18 hours, consistent with CAPSIT-PD guidelines (Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease).
7.3.2. Experimental Design

Stimuli were designed and presented using Matlab, which also was used to collect behavioural output from the response device. Subjects were asked to perform a tracking task at 1 Hz which required squeezing a pressure-responsive rubber bulb (in-house designed to be MR compatible) with their right hand to control the width of a horizontal bar on the screen, so that the width of the bar remained touching a vertically scrolling pathway that had a sinusoidal pattern. A block design was used such that the tracking task was performed for 3 blocks of 100 seconds each, interspersed with 100 second periods of rest, where a straight pathway with parallel lines appeared such that subjects had to completely rest their grip so that the bar would fit between the parallel pathway (Figure 7.1). All subjects practiced the task outside the scanner until errors had stabilised and they felt comfortable with the requirements.

![Figure 7.1: Illustration of experimental task.](image-url)
7.3.3. Experimental Protocol

Subjects lay in the MRI scanner viewing the computer screen via a projection-mirror system attached to the head coil. The squeeze bulb was held in the right hand using an isometric grip and attached to a pressure-transducer in the control room via a fluid-filled tube.

Subjects were instructed to maintain a consistent hand grip position throughout the task, and to squeeze the bulb using an isometric grip. Each subject had their maximum voluntary contraction (MVC) measured at the start of the experiment, and subsequent target movement was scaled accordingly so that target output was between 5 and 15% MVC.

Behavioural data from the squeeze bulb, i.e. the actual force output, was compared to the target force output to calculate the root mean squared error measurement for each subject (i.e. standard error). This performance measure was compared between subject groups to ensure that differences in task performance were not a confounding variable.

7.3.4. Data Acquisition

A Philips Achieva 3.0 T scanner (Philips, Best, the Netherlands) equipped with a head-coil was used to collect functional MRI data, consisting of echo-planar (EPI) T2*-weighted images with blood oxygenation level-dependent (BOLD) contrast (repetition time 1985 ms, echo time 37 ms, flip angle 90°, field of view (FOV) 240.00 mm, matrix size = 128 x 128, pixel size 1.9 x 1.9 mm). 36 axial slices were acquired, with 3mm thickness (1 mm gap thickness). The FOV was selected to include both the dorsal surface of the brain and the cerebellum ventrally. Additionally, a high-resolution (1x1x1 mm), whole brain 3-dimensional T1-weighted image was
acquired for each subject, consisting of 170 axial slices. Head motion was minimized using a ‘memory foam’ pillow placed around the subject’s head within the coil.

7.3.5. fMRI Data Pre-processing and Analysis

Pre-processing of functional data was conducted as previously described in Chapter 4.3.5. ROIs that constitute the STC and CTC loops were segmented based upon anatomical landmarks and guided by a neurological atlas (Talairach and Tournoux, 1988): including primary motor cortex (M1), supplementary motor cortex (SMA), putamen (PUT), globus pallidus (GLP), thalamus (THA), and cerebellum (CER). The ROI labels were subsequently resliced to isotropic voxels and desampled to the fMRI resolution, and the raw time courses of the functional data from voxels within each ROI were extracted.

7.3.6. Connectivity Analysis

A PCfdr algorithm was used to compute connectivity during performance of the tracking task, for each subject group independently (controls, PDt, and PDar). A description of this method can be found in Chapter 5.3.6.

7.4. RESULTS

Analysis of behavioural data showed that there was no significant difference in the standard error between any of the subject groups [RMS error for Normals 0.5067, PDar off med 0.5007, PDar on med 0.5163, PDt off med 0.4315, PDt on med 0.4824; ANOVA result F(4.23)=0.41 p>0.8021 ]; thus changes in connectivity in the following analysis are not attributable to changes in the accuracy of task performance.
Figure 7.2 shows the networks recruited during the manual tracking task, compared to rest, for each of the subject groups. In control subjects, connectivity was seen within the contralateral STC loop (left putamen ↔ left GPi ↔ left thalamus ↔ left SMA ↔ left putamen) and CTC loop (right cerebellum ↔ left thalamus ↔ left SMA). Additionally, some connectivity was seen within ipsilateral STC and CTC loops (right putamen ↔ right GPi and right SMA; left cerebellum ↔ right thalamus). The SMA and primary motor areas were connected in both hemispheres, and inter-hemispheric connectivity was seen between contralateral (left) M1 and ipsilateral SMA. Contralateral SMA was connected to ipsilateral GPi and thalamus. The two loops of interest were functionally connected to each other between left cerebellum and right putamen. Left cerebellum additionally connected to right SMA.

In tremor-dominant PD subjects, connectivity remained within the basal ganglia bilaterally, between putamen and globus pallidus, but output from the BG was lost contralaterally. Contralateral left M1 was connected to the ipsilateral putamen, which was also connected with right thalamus and right M1. Contralateral thalamus was connected to ipsilateral SMA. Left cerebellum remained connected to right SMA but lost connections to right thalamus and right putamen. Connections between SMA and M1 bilaterally and between right cerebellum and contralateral thalamus remained. The connection from left cerebellum to right putamen was lost, but became connected to left putamen.

In akinetic-rigid type PD subjects, like controls and PDt, connectivity remained between putamen and globus pallidus bilaterally, and between M1 and SMA bilaterally. Like PDt, the right putamen connected with right thalamus. Novel connections in this group were right SMA↔ right thalamus and left SMA ↔ bilateral putamen. Like controls, the CTC and STC loops were connected between left cerebellum and right putamen.
FIGURE 7.2: Functional Connectivity during visuomotor tracking in PDt and PDar Subjects off medication and Healthy control subjects.
7.5. DISCUSSION

In healthy control subjects, both the STC and CTC loops were recruited to perform the visually guided tracking task, consistent with prior studies of target-tracing (Jueptner and Weiller, 1998) and of tracking involving continuous (Vaillancourt et al., 2003) or variable (Floyer-Lea and Matthews, 2004) force production. In both tremor and akinetic-rigid subtypes of PD, normal output from the GPi to the thalamus contralateral to the movement being performed was absent. Instead, ipsilateral putamen and thalamus were directly connected. Anatomical tracing studies in primates have identified converging inputs from motor cortex and thalamus to the dorsal putamen (McFarland and Haber, 2002), suggesting that this region may play a role in integrating cortical information with motor output from the striatum. A recent meta-analysis of basal ganglia connectivity in humans revealed that both left and right putamen co-activated with bilateral thalamus (Postuma and Dagher, 2006), and this connectivity was localised to the rostral putamen consistent with a role in movement preparation (Schultz and Romo, 1992). In PD, increased recruitment of thalamo-striatal projections involved in the control and preparation of upcoming movement may thus reflect the increased difficulty that these individuals have in performing finely tuned movement.

In the PDar group, the CTC loop was no longer recruited. Studies in animal (rodent and primate) models of PD have shown reduced metabolic activity in both cerebellar and basal ganglia receiving areas of the thalamus (Rolland et al., 2007). In these models, resting tremor is either absent or present in only intermittent bursts together with action tremor, suggesting that reduced activity of both STC and CTC networks may be present in parkinsonian cases that present with only minimal tremor. In contrast, the PDt group showed recruitment of the cerebello-thalamo
pathway contralateral to movement, and, like control subjects, the left cerebellum was connected to the right SMA.

It should be noted that several prior studies have shown hyperactivity of the cerebellum in groups of PD subjects with predominantly akinetic-rigid symptoms (Elsinger et al., 2003; Rascol et al., 1997; Yu et al., 2007); however these studies did not investigate functional connectivity and therefore could not conclude that the CTC pathway was recruited. In the current study we demonstrated that PDar do not recruit normal connectivity between the cerebellum and thalamus, but the cerebellum remained connected to other regions and thus these pathways may have been overactive. Additionally, removal of inhibitory connections to the cerebellum would also result in regional hyperactivity.

In control subjects and in PDar subjects, the STC and CTC loops were functionally connected with each other between left cerebellum and right putamen. This connection appears to be important in PD, as Yu and colleagues (2007) demonstrated a negative correlation between activation in the putamen and the contralateral cerebellum in PD subjects without tremor. They suggested that compensatory recruitment of the cerebellum may be mediated via a direct cerebello-basal ganglia pathway, which has been demonstrated by anatomical studies in both rodents and primates (Ichinohe et al., 2000; Hoshi et al., 2005). This connection originates in the dentate nucleus of the cerebellum and communicates via disynaptic connections with the input regions of the striatum (caudate and putamen), supporting the idea that the cerebellum can directly modulate motor programs in the striatum on the basis of internal models and/or error signals (Doya, 2000). Additionally, neurochemical studies in animals have shown that stimulation of deep cerebellar nuclei can increase dopamine turnover in the contralateral striatum (Nieoullon et al., 1978).
Disruption of the interaction between cerebellum and basal ganglia may be an important pathological mechanism in disorders that affect either of these regions. In animal models of dystonia, which was traditionally thought to be a basal ganglia disorder, evidence suggests that expression of dystonic movements depends on influences from both BG and cerebellum (Neychev et al., 2008), and increased (compensatory) recruitment of the cerebellum has indeed been shown in human non-symptomatic carriers of the DYT1 gene mutation for dystonia (Carbon et al., 2008). In the current study, the PDt group did not show the connectivity between cerebellum and putamen that was observed in controls and in PDar subjects. This “disconnection” between STC and CTC loops may thus play an important role in tremor generation in PD. The purpose of such disconnection may be to allow the CTC loop to act more independently, allowing compensatory recruitment of the CTC pathway for tasks it may not normally subserve. We did observe a connection from left cerebellum to left putamen in the PDt group. The role of this intrahemispheric connection is not clear and has not been established in prior studies of the cerebello-striatal pathway. If loss of normal interhemispheric modulation of striatum by the contralateral cerebellum is responsible for tremor generation, then an abnormal intrahemispheric interaction between these regions may be an adaptive response, but this issue requires further investigation.

In conclusion, this is the first study that we know of to directly investigate the differences in activation patterns between tremor- and akinetic/rigidity-dominant PD subjects using fMRI. Taken together, our results strongly support the suggestion that compensatory recruitment of the cerebellum in PD is also linked to the generation of parkinsonian tremor. This may have implications for therapeutic approaches that aim to reduce the tremor in PD, since reduction of compensation may improve the symptoms of tremor but impair behavioural performance on
motor tasks. Future studies that investigate both pre-clinical and clinical PD subjects may contribute to our understanding of when compensatory changes in connectivity become also detrimental to normal motor function.


CHAPTER 8: GENERAL DISCUSSION

8.1. SUMMARY OF FINDINGS

The aim of this thesis was to provide convincing evidence of compensatory changes in individuals with early-stage, clinically diagnosed PD. To do this, we have utilized several analysis methods, considering the amplitude, connectivity, and spatial variation of the BOLD signal recorded using fMRI, in addition to temporal information at specific frequency bands in the EEG.

In Chapter 2, we showed that simultaneous task performance, which is typically impaired in PD (Benecke et al., 1986, 1987), was associated with alterations in the effective connectivity and in the temporal dynamics of communication between several subcortical and cortical regions. L-dopa was able to partially normalise relative change. This manuscript is among the first to demonstrate changes in connectivity during performance of a motor task in PD. In the next chapter, using EEG, we demonstrated that synchronization changes occurred in distinct frequency bands during this simultaneous task, and only some of these changes appeared to be dopamine dependent, highlighting the importance of a systems level approach that can identify changes influenced by all neurotransmitter systems.

In Chapter 4, we changed our experimental task to a tracking task with three target movement speeds, providing three levels of difficulty, in order to provide stronger inferences about the compensatory nature of the changes we observed. We applied an idea from cognitive research (Stern, 2002), that of active reserve, to the motor domain and considered whether PD subjects are able to recruit normal task-related networks to a greater extent as a form of compensation. Indeed, we demonstrated significant increases in the amplitude of activation within normal
networks in PD subjects, which likely assists them in attaining a similar level of performance as healthy controls. In addition, we provided strong evidence for compensatory recruitment of novel networks involving predominantly CTC pathways. This study extended prior evidence of compensatory cerebellar hyperactivity (Wu and Hallett, 2005; Yu et al 2007) by showing that this activation monotonically increased with increasing task difficulty. If purely a disease-related changes, we would expect that this cerebellar hyperactivation would be static across variations in the task itself.

We next investigated the joint changes in amplitude and functional connectivity during this multi-frequency tracking task. As expected given the site of neurodegeneration in PD, we saw a decrease in amplitude and connectivity within STC loops. This was accompanied by an increase in the amplitude and connectivity within CTC loops, providing further evidence that this loop is a primary locus of compensatory activity in PD. This study is the first to demonstrate compensatory changes in functional connectivity, as opposed to amplitude alone. Such distinction is important since demonstration that areas of hyperactivity form an interconnected network during performance of a motor task strongly suggests that these changes are functional in nature and not merely a downstream influence of nigrostriatal degeneration. Evidence of connectivity changes in PD also supports a growing body of evidence that network dysfunction is the key to many neurodegenerative diseases (Palop et al., 2006).

Building on the information gleaned from this tracking task, we went on to investigate the influence of L-dopa on these connectivity changes. L-dopa decreased the amplitude of both STC and CTC loops in PD subjects relative to controls, and reversed the majority of abnormal functional connections in PD. However, compensatory connections within the contralateral hemisphere remained even after L-dopa, despite over-correction of the hyperactivity observed
within the ROIs of this loop, suggesting that compensatory mechanisms result in long-lasting changes in anatomical networks that are not rapidly altered by pharmacological treatment.

Despite the large body of evidence we have collected that suggests a compensatory role for CTC loops in PD, other literature suggests that cerebellar activity in particular is associated with the generation of parkinsonian tremor (Lenz et al., 1995; Papavassilou et al., 2008; Zaidel et al., 2009). Prior fMRI studies in PD had never considered the differences between the two main clinical subtypes of PD, i.e. individuals with tremor, and individuals with predominantly akinetic-rigid symptoms. We designed a simple visually guided tracking task that we predicted would recruit both STC and CTC loops. We demonstrated, for the first time, that the networks recruited by PDt subjects differed dramatically from those recruited by PDar, strongly suggesting that compensatory CTC recruitment is limited to the PDt group, and that this compensation requires a disconnection between STC and CTC loops that may also play an important role in the mechanism of tremor generation.

Taken together this body of work has provided significant advances in the understanding of cerebellar compensation in PD and has extended the knowledge of PD obtained using fMRI. Some comment should be given regarding the technical aspects of this work. A major strength of this work was the use of novel or advanced fMRI analysis techniques, many of which have been developed through collaboration with investigators at UBC and SFU. Use of these techniques has allowed us to identify subtle changes in shape, amplitude, and connectivity which may not have been detected by the use of traditional techniques that largely rely on the mean t-statistics, derived from the percentage BOLD signal change. We have also demonstrated that the relationship between the variables of amplitude, connectivity, and spatial extent is not
uncomplicated, and it is important to therefore consider these changes in combination rather than isolation.

Our experimental tasks have been largely dependent on the in-house response device developed in collaboration with colleagues in the Electrical and Computer Engineering department at UBC. Traditional tasks have utilized paradigms that require no response device, such as finger tapping, or simple responses such as a joystick movement or button press. These response devices may be difficult to use for individuals with PD, particularly those with prominent tremor, and typically are used for discrete rather than continuous movements, even if those movement are assembled into complex sequences. Berns (2003) demonstrated the advantages of continuous paradigms for fMRI in providing saturation of the BOLD signal. With the use of our pressure-responsive device, we were able to utilize tracking tasks that required continuous modulation of force, maintaining continuous attention and requiring ongoing preparation and execution for a motor command throughout the entire task block. This was likely another factor that increased the sensitivity of our designs.

A further strength of this work is the use of manual segmentation of ROIs on an individual subject basis, removing the possibility of mis-registration to standard atlases, which may introduce error, particularly in small subcortical regions (Nieto-Castanon et al., 2003). This attention to accuracy of anatomical segmentation has allowed us to investigate spatial aspects of activation in subject’s native space, and the demonstration of variations in spatial extent attributable to task frequency, disease, and compensation in Chapter 4 highlights the advantages of this approach.
8.2. IMPLICATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Identification of compensatory mechanisms has several implications for Parkinson’s Disease. From a therapeutic prospective, it is critical to determine which changes in brain activity during the early clinical stages are a consequence of the disease (Palop et al., 2006), and which changes are a compensatory mechanism, since therapies should target only those changes that are an effect of the disease itself. In contrast, compensatory mechanisms represent another target of adjunctive therapies that could be developed to enhance or prolong the action of compensation in order to delay progression of the disease. Further work is needed to elucidate the microscopic and neurochemical nature of compensatory CTC recruitment in order to suggest possible mechanisms of such therapies. This will likely incorporate research from animal models as well as local field potential recordings from patients undergoing surgical intervention for PD.

Over the course of PD, it is suggested that compensatory mechanisms increase in the pre-clinical stages in parallel with increasing degeneration, but later may asymptote or even fail, such that they can no longer mask the symptoms of the disease and maintain normal performance. The severity of clinical symptoms represents the addition of disease related and compensatory changes. Here, we have shown that compensatory mechanisms do exist in the early clinical stages of PD. Identification of these mechanisms is important for longitudinal studies that assess the progression of PD using neuroimaging techniques, which will detect changes in both disease-related and compensatory activity over time. Future research is needed to ascertain how CTC recruitment changes with disease progression, and whether this correlates with the severity of specific symptoms.

Compensatory mechanisms that exist during the pre-clinical stage of PD may also be useful as a biomarker that can be used to determine those individuals who will go on to develop the disease.
Applications of this may be limited to individuals who are at increased risk, such as those with a known genetic mutation, since screening of the general population would be unrealistic in terms of both expense and time requirements. However, future investigation of the presence of compensatory recruitment in asymptomatic carriers of known PD-related gene mutations is warranted.

The implication that there is a pathological role for cerebellar compensation in the generation of tremor certainly requires further investigation and presents a working hypothesis for future studies. We have demonstrated the importance of studying the clinical subtypes of PD individually rather than attempting to make conclusions from groups with heterogenous symptoms. If a mechanistic link can be confirmed, then therapeutic strategies that aim to reduce tremor might also reduce beneficial compensation, and should thus be approached with caution. However, it would also be possible for therapies to reduce tremor indirectly by reducing the disease-related changes, and thereby negate the need for compensatory mechanisms and removing the detrimental side-effects of compensation. Although we have shown preliminary evidence that current pharmacological therapy does not completely reverse the compensatory changes in functional networks involving the cerebellum, future treatments may be more successful in completely reversing the effects of PD, including compensatory changes.

8.3. LIMITATIONS

The conclusions from this body of work were drawn from a population of individuals with early stage, mild to moderate idiopathic PD. The extent to which these findings generalize to individuals with pre-clinical or late stage PD remains to be determined. Investigation of pre-clinical PD is only possible in individuals who are identified as at risk (i.e. those with genetic
mutations that predispose them to PD), or in longitudinal studies that follow individuals over an extended period to see who will later go on to develop the disease. Since genetic forms of PD often take a different course to idiopathic PD, it is not clear whether results from such studies relate to all forms of Parkinsonism. Longitudinal studies may provide better information, but are time consuming and expensive to conduct. Study of individuals with later stage PD is possible, however the increasing severity of tremor and bradykinesia in these individuals presents difficulties with task performance, particularly with the motion sensitive technology of fMRI.

The applicability of results from a single type of motor task, in this case visuomotor tracking, to all voluntary movement also remains to be determined. Prior studies have suggested that activation is highly task dependent. However, the fact that cerebellar hyperactivation has been commonly found in PD across a wide range of tasks in prior research suggests that this form of compensation is a general mechanism that exists across various movement parameters.

The temporal resolution of fMRI is poor in comparison to the temporal scale of neuronal activation, and the BOLD signal an indirect measure of neuronal firing. Research in animal models that can record firing rates directly from implanted electrodes is useful for determining information regarding the synchronization between STC and CTC activation in PD, however the question of whether compensation involves the same network changes in animal models remains to be determined. EEG can be used to provide greater temporal resolution, and was used in this work to show dopamine-sensitive and non-dopamine-sensitive changes in connectivity between cortical regions, however cerebellar activity can only be measured indirectly in the EEG, and is difficult to disentangle from STC influences since these loops project to overlapping regions of the cortex.
8.5. CONCLUSIONS

In conclusion, this body of research has provided strong evidence that CTC loops are recruited as a compensatory strategy in PD. This alteration involves changes in the functional networks recruited by motor tasks which are not fully reversible by L-dopa, implying that plastic changes take place within normal functional networks responsible for voluntary movement. We have provided preliminary evidence that CTC compensation is recruited to a greater extent in individuals with tremor-dominant PD, and have suggested that this mechanism may involve a disconnection between the STC and CTC loops that enables the CTC pathway to be increased independently of the STC loop status, but at the cost of tremor generation. This work has implications for the treatment of PD as well as the study of PD progression, and may contribute to better understanding of the pathological mechanism of the key symptoms of PD.
8.6. REFERENCES


APPENDIX I: ETHICS APPROVAL FORMS FOR FMRI STUDIES

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

**ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL WITH AMENDMENTS TO THE STUDY**

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**INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:**

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**CO-INVESTIGATOR(S):**

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<td>Chong S. Lee</td>
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<td>Donald B. Calne</td>
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<td>A. Jon Stoessl</td>
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**SPONSORING AGENCIES:**

- Canadian Institutes of Health Research (CIHR) - "Making the connection: Methods to Infer Functional Connectivity in brain studies"
- Canadian Institutes of Health Research (CIHR) - "Making the connection: methods to infer functional connectivity in brain studies"
- Natural Sciences and Engineering Research Council of Canada (NSERC) - "Making the connection: Methods to Infer Functional Connectivity in brain studies"
- UBC Internal Grant - "Motor & Cognitive Functional Magnetic Resonance Imaging Studies in Parkinson's & Related Disorders"

**PROJECT TITLE:**

Making the connection: Methods to Infer Functional Connectivity in brain studies
The current UBC CREB approval for this study expires: May 4, 2010

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AMENDMENT APPROVAL DATE: May 4, 2009

CERTIFICATION:

**In respect of clinical trials:**

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

**Approval of the Clinical Research Ethics Board by one of:**

Dr. Peter Loewen, Chair
Dr. James McCormack, Associate Chair
Dr. John Russell, Associate Chair
Dr. Caron Strahlendorf, Associate Chair
Dr. Stephen Hoption Cann, Associate Chair
May 7, 2009

Dr. M.J. McKeown  
Department of Neurology  
Pacific Parkinson’s Research  
Rm M-36 – Purdy Pavilion  
Wesbrook Mall  
UBC Hospital

Vancouver Coastal Health Authority Research Study #V04-0091

RENEWAL CERTIFICATE OF APPROVAL

TITLE: Making the connection: Methods to Infer Functional Connectivity in brain studies

SPONSOR: Canadian Institutes of Health Research (CIHR); Natural Sciences and Engineering Research  
Council of Canada (NSERC); UBC Internal Grant – “Motor & Cognitive Functional  
Magnetic Resonance Imaging Studies in Parkinson’s & Related Disorders”

This is to inform you that your project has been renewed. Approval has been granted until May 4, 2010  
based on the following:

1. UBC Ethics Board Certificate of Renewal Approval – H04-70177
2. VCHA Request for Renewal reply.

Yours truly,

for:
Dr. Robert McMaster  
Interim Vice-President Research
APPENDIX II: TECHNICAL APPENDIX – SEM AND MAR

In order to calculate the connectivity patterns for both the MAR and SEM approaches, the data from each subject was first individually pre-processed. The raw fMRI data were first bandpass-filtered between 0.025 and 0.2 Hz using a 5th order Butterworth filter to capture activity at the frequency the subjects were squeezing the bulb. The filtered time courses from the voxels within a given ROI were then averaged and normalized to unit variance. This procedure was repeated for each ROI and each subject. This resulted in a \( p \times n \) matrix of de-noised fMRI data, \( Y \), where \( p \) is the number of ROIs, and \( n (= t \times s) \), is the total number of time points, \( s \) is the number of subjects, and \( t \) is the number of time points for a single subject (=150).

We estimated the connectivity matrices between ROIs as follows:

The first order multivariate autoregressive model is given by:

\[
y_t = A_1 y_{t-1} + e_t, \quad t = 2, \ldots, n
\]

where \( y_t \) is the \( t^{th} \) column of \( Y \), and \( e_t \) is assumed multivariate Gaussian ~ \( \mathcal{N}(0, \Sigma) \). We note that the elements of \( A_1 \) can be estimated in a regression framework by,

\[
(Y^r)^T_{2\rightarrow n} = Y^T_{1\rightarrow (n-1)} B^r + E^r \quad E^r \sim \mathcal{N}(0, \Sigma)
\]

where \( Y^r \) represents the \( r^{th} \) row of \( Y \), the subscripts represent the range of time points, and \( T \) denotes transpose. The regression coefficients \( B^r \) are estimates of the \( r^{th} \) row of \( A_1 \) in (1). The edge effects of concatenating different subjects together were ignored.

Similarly for the Structural Equation Model,
\[
(Y^s)_{2 \rightarrow n}^T = (Y^{-s})_{1 \rightarrow (n-1)}^T B^s + E^s \quad \quad E^s \sim N(0, \Sigma) \quad (3)
\]

\(Y^s\) represents the \(s^{th}\) row of \(Y\), \(Y^{-s}\) represents \(Y\) with the \(s^{th}\) row removed, the subscripts represent the range of time points, and \(T\) denotes transpose. The coefficients \(B^s\) are the (non-diagonal) estimates of the \(s^{th}\) row of the connectivity matrix \(A\) in:

\[
y_t = Ay_t + E_t \quad \text{with} \quad \text{diag}(A) = 0, \quad t = 1, \ldots, n, \quad \text{and} \quad E_t \sim N(0, \Sigma) \quad (4)
\]

The regression analyses for both the SEM and MAR connectivity matrices were implemented using an iteratively-weighted least squares and bisquare weighting algorithm implemented as the function "robustfit" in Matlab. The standard error in the coefficient estimates were used to determine the element-by-element significance resulting from subtracting the connectivity matrices from subjects performing both tasks to when they were only performing the squeezing task. For example, in Figure 2.8. we displayed the contrast (PD\{on med, simultaneous\} - PD\{on med, unimanual\}) - (Controls\{simultaneous\} - Controls\{unimanual\}).
**ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL WITH AMENDMENTS TO THE STUDY**

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**CO-INVESTIGATOR(S):**

| Joseph K.C. Tsui | A. Jon Stoessl |

**SPONSORING AGENCIES:**

- National Parkinson Foundation (US) - "Spatial & Temporal Aspects of Compensatory Mechanisms in PD Electroencephalographic Studies (EEG)"
- University of British Columbia - "Electroencephalographic Studies in Subjects with Parkinson's Disease and Related Disorders"

**PROJECT TITLE:**

Spatial & Temporal Aspects of Compensatory Mechanisms in PD Electroencephalographic Studies (EEG)

The current UBC CREB approval for this study expires: May 29, 2010

**AMENDMENT(S):**

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<td>Dr. Caron Strahlendorf, Associate Chair</td>
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<td>Dr. Stephen Hoption Cann, Associate Chair</td>
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May 29, 2009

Dr. M.J. McKeown
Department of Neurology
Pacific Parkinson’s Research
Rm M-36 – Purdy Pavilion
Wesbrook Mall
UBC Hospital

Vancouver Coastal Health Authority Research Study #V04-0242

RENEWAL CERTIFICATE OF APPROVAL

TITLE: Spatial & Temporal Aspects of Compensatory Mechanisms in PD Electroencephalographic Studies (EEG)

SPONSOR: National Parkinson Foundation (US) - "Spatial & Temporal Aspects of Compensatory Mechanisms in PD Electroencephalographic Studies (EEG)"; University of British Columbia "Electroencephalographic Studies in Subjects with Parkinson’s Disease and Related Disorders"

This is to inform you that your project has been renewed. Approval has been granted until May 29, 2010 based on the following:

1. UBC Ethics Board Certificate of Renewal Approval – H04-70291
2. VCHA Request for Renewal reply.

Yours truly,

for:
Dr. Robert McMaster
Interim Vice-President Research

A joint venture in research between the Vancouver Coastal Health Authority and The University of British Columbia.
Room 100 – 2647 Willow St., Vancouver, BC V5Z 3P1
Tel: 604-875-5641, Fax: 604-875-5684
www.vchri.ca
Table A.1: Comparison of BOLD signal amplitude between PD and Control subjects

**2-way Mixed ANOVA**
Significance level at p<0.01 shown in bold, p<0.05 shown in italics.

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Table A.2: Comparison of spatial variance of activation between frequencies and subject groups.

2-way mixed design
Significance level at p<0.01 shown in bold, p<0.05 shown in italics.

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