Voxel-based Analysis of Cholinesterase Inhibitor Response in Lewy Body Disorders: A Prospective Observational Study

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

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2018

Abstract

Lewy body disorders (LBD) have visual hallucinations, fluctuations in attention and alertness, and parkinsonism as common features. While cholinesterase inhibitors (ChEIs) are an effective treatment for LBD, our understanding of the effects of these drugs in the brain in relation to response is limited. The objective is to investigate functional neuroimaging correlates of response to ChEIs. Standardized neuropsychiatric and neuropsychological batteries and brain perfusion SPECT scans were completed at baseline and after six-months following initiation of ChEIs. This study investigated longitudinal relationships between changes in battery test scores and perfusion using statistical parametric mapping. Treatment with ChEIs resulted in significantly increased occipital perfusion. Significant improvements in working memory, visuospatial performance, and executive functions were observed. Visual hallucinations were significantly reduced, which correlated with increased occipital perfusion. This study identified occipital perfusion as an important correlate of response to ChEIs in LBD, which could be further developed as a response biomarker.
Acknowledgements

I am fortunate enough to have many wonderful people to thank and acknowledge for their guidance, encouragement and support over the past few years. First and foremost, I would like to thank my supervisor and mentor, Dr. Mario Masellis. I began working for Dr. Masellis as a summer student, and I can confidently say he is a large reason for my decision to choose this field of research. His outstanding mentorship and support throughout this Master’s degree and beyond is greatly appreciated, and I thank him for the opportunity to engage in this interesting and important work. I would next like to thank my advisory committee, Dr. Sandra Black, Dr. Nathan Herrmann, and Dr. Brad MacIntosh, for their insightful comments, suggestions, guidance and critical review of this work. I am a better researcher for as a result of your help, which is very much appreciated.

To my past and present colleagues that I have met and with whom I have worked throughout the years – thank you for your friendship, help and for making my days here so enjoyable.

I would not be the person I am today without the love and support of my amazing family and friends. To my parents, Donna and Aldo; my nonne, Natalie and Dora; and Stefano, Anthony and Mike – thank you for your constant and unwavering support, love, and encouragement. There aren’t enough words to express my gratitude to you all, but I thank you for joining me in this journey.

Finally, I would like to thank the incredible men and women who volunteered their time to participate in this research during such a challenging period of their lives.
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List of Abbreviations

α-syn α-synuclein

β-CIT 2-β-carbomethoxy-3-β-(4-iodophenyl)-tropane

$^{18}$F-dopa $^{18}$F-6-fluorodopa

$^{99m}$Tc-ECD Technetium-99m ethyl cysteinate dimer

AChE Acetylcholinesterase

AD Alzheimer’s disease

ADL Activities of Daily Living

ANCOVA Analysis of covariance

ANOVA Analysis of variance

AOO Age of onset

APOE Apolipoprotein E

BAs Brodmann Areas

BNT Boston naming test

CGC-plus Clinical global change-plus

CGIC Clinician’s global impression of change

CT Computerized Tomography

CVLT California Verbal Learning Test

CYP Cytochrom P450

D2 Dopamine D2 receptor

DAD Disability Assessment for Dementia

DAT Dopamine transporter

DLB Dementia with Lewy Bodies

DNA Deoxyribonucleic acid
DRS Dementia Rating Scale
DSM-IV Diagnostic and Statistical Manual - IV
EEG Electroencephalography
FAS F-, A-, S-word phonemic fluency
FDG Fluorodeoxyglucose
FDR False discovery rate
fMRI Functional Magnetic Resonance Imaging
FWE Family-Wise Error
FWHM Full width at half maximum
GLM General linear model
HMPAO Hexamethylpropyleneamine Oxime
iADL Instrumental Activities of Daily Living
L-dopa Levodopa
MDRS Mattis Dementia Rating Scale
MMSE Mini Mental Status Examination
MNI Montreal Neurological Institute
MRI Magnetic Resonance Imaging
n Sample size
nAChRs Nicotinic acetylcholine receptors
nbM Nucleus basalis of Meynert
NFTs Neurofibrillary tangles
NPI Neuropsychiatric Inventory
p Probability value
PD Parkinson’s disease
PET Positron Emission Tomography
rCBF Regional cerebral blood flow
ROI Regions of interest
SD Standard deviation
SNCA Alpha-synuclein
SPECT Single-Photon Emission Computed Tomography
SPM Statistical Parametric Mapping
SPM12 Statistical Parametric Mapping version 12
SPSS Statistical Package for the Social Sciences
TMT-A Trail Making Test A
TMT-B Trail Making Test B
WAIS-III Wechsler Adult Intelligence Scale
WCST Wisconsin Card Sort Test
WMS-R Wechsler Memory Scale-Revised
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Chapter 1
Literature Review

1.1 Lewy body spectrum disorders: historical perspective

Fritz Jakob Heinrich Lewy first described cellular inclusions that are characteristic of Parkinson’s disease (PD) in some brain regions outside the substantia nigra (dorsal motor nucleus of the vagus nerve, nucleus basalis of Meynert, and some thalamic nuclei) in 1912 (Lewy, 1912). These published results were based on his examination of the brains of 25 individuals, who had a diagnosis of PD. The inclusions he described were eosinophilic, and insoluble in alcohol, chloroform and benzene, indicating the presence of a large protein component. Two years later, Tretiakoff reported the presence of these same inclusions in the substantia nigra in PD, and named them after Lewy (1919). Further, Tretiakoff hypothesized a relationship between degeneration of the substantia nigra and clinical findings of rigidity, and tremor. However, in 1923, Lewy was only able to confirm Tretiakoff’s hypothesized relationship in 11 of 50 cases of PD (Lewy, 1923). Despite these conflicting observations, Tretiakoff’s observations that parkinsonism is due to degeneration of the substantia nigra were later confirmed in 1938 (Hassler, 1938). It is now well-established that there is degeneration of neurons within the ventrolateral pars compacta in PD (Goedert, Spillantini, Del Tredici, & Braak, 2012). Greenfield and Bosanque determined that, in PD, Lewy bodies were always present in brainstem nuclei; whereas neurofibrillary tangles (NFTs) are present instead in post-encephalitic parkinsonism (Greenfield & Bosanque, 1953). In 1960, Bethlem and Den Haltog Jager reported on the distribution of Lewy bodies both in the central and autonomic nervous systems (Bethlem & Den Hartog Jager, 1960).
The relationship between cortical Lewy bodies and dementia was described by Okazaki and colleagues in 1961 (Okazaki, Lipkin, & Aronson, 1961). Throughout the seventies, Kosaka and colleagues studied autopsied cases with Lewy body pathology, and proposed the term “diffuse Lewy body disease”, a new disease entity, based on 20 cases (Kosaka, Yoshimura, Ikeda, & Budka, 1984). In the years that followed, the new disease entity was confirmed by two other groups (D. W. Dickson et al., 1987; Hensen et al., 1990).

In rare families segregating PD and DLB, the diseases were linked to three missense mutations in SNCA, which is the gene that codes for α-synuclein (α-syn) protein (Krüger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004). In 1997, Spillantini found Lewy bodies and Lewy neurites in PD to be immunoreactive for α-syn, establishing the relevance of α-syn aggregation for cases of Lewy body PD (Spillantini et al., 1997). This was one of the earliest works that identified PD, DLB and multiple systems atrophy (MSA) as part of a new group of neurodegenerative diseases characterized by α-syn inclusions (i.e., synucleinopathies).

Prior to the First International Workshop of the Consortium of Dementia with Lewy Bodies (DLB), where the term DLB was recommended (I G McKeith et al., 1996), there were many terms used to describe dementia in the context of Lewy bodies: diffuse Lewy body disease, Lewy body dementia, senile dementia of the Lewy body type and dementia associated with cortical Lewy bodies (McKeith et al., 2004).

DLB is now understood to occur along a spectrum of Lewy body diseases, which include PD, PD with dementia (PDD) and DLB (I McKeith, 2007; Ian G. McKeith et al., 2017). The clinical criteria for diagnosis of probable and possible DLB and PDD were last updated in 2017, and 2015, respectively (Ian G. McKeith et al., 2017; Postuma et al., 2015). While an arbitrary ‘one-
year rule’ continues to be used to differentiate DLB from PDD based on the temporal progression of the disease, according to the consensus criteria for DLB in 2017 (Ian G. McKeith et al.), the Movement Disorder Society (MDS) criteria for PD, published in 2015 (Postuma et al.), made this distinction less clear. The one-year rule states that if cognitive decline is reported as an issue/concern at the first assessment, or within one year of parkinsonism symptoms onset, then the diagnosis is DLB. Alternatively, if parkinsonism presents at least one year prior to cognitive decline, then the diagnosis is PDD (Ian G. McKeith et al., 2017). However, in the MDS criteria for PD, contrary to the one-year rule, dementia is not considered as an exclusion criteria for PD, “regardless of when it occurs in relation to parkinsonism onset” (Postuma et al., 2015).

The same group published an introductory manuscript discussing why they disagreed with the status quo (Berg et al., 2014). The reasons that they give are the similar nature of DLB and PDD with regard to dementia presentation, neuropsychological findings, non-motor profile, imaging, prodromal stage, genetics, and pathology. They then illustrate counterarguments to consider retaining the current distinction: although there are overlapping patterns, imaging differences have been found (Lippa et al., 2007), parkinsonism can present differently in DLB compared to idiopathic PD (Goldman, Goetz, Brandabur, Sanfilippo, & Stebbins, 2008), and the course disease for PDD and DLB can be very different. With that being said, this task force suggested the omission of the one-year rule for the separation of DLB and PDD, with dementia no longer an exclusion criteria for PD (Berg et al., 2014). While this was the case for the MDS criteria for PD (Postuma et al., 2015), it should be noted that the DLB consensus criteria still included the one-year rule in their criteria two years later (Ian G. McKeith et al., 2017).

The core clinical features of DLB are fluctuating cognition (attention and alertness), recurrent visual hallucinations, REM sleep behaviour disorder (RBD) and parkinsonism (bradykinesia, rest
tremor, or rigidity). Supportive clinical features of the disease are: severe sensitivity to antipsychotic drugs, postural instability, repeated falls, syncope or other transient episodes of unresponsiveness, severe autonomic dysfunction, hypersomnia, hyposmia, hallucinations in other modalities, delusions, apathy, anxiety and depression. Indicative biomarkers of DLB are reduced dopamine transporter uptake in basal ganglia on single photon emission computed tomography (SPECT) or positron emission tomography (PET), abnormal $^{123}$Iodine-MIGB ($^{123}$I-MIGB) myocardial scintigraphy, and polysomnographic (PSG) confirmation of rapid eye movement (REM) sleep without atonia. Supportive biomarkers are relative preservation of the medial temporal lobe on computed tomography (CT) or magnetic resonance imaging (MRI) scans, generalized low uptake on SPECT perfusion or PET metabolism scans with reduced occipital activity with or without the cingulate island sign on fludeoxyglucose-PET (FDG-PET) imaging, and prominent posterior slow-wave activity on electroencephalogram (EEG) with periodic fluctuations in the pre-alpha/theta range (Ian G. McKeith et al., 2017). As of the 2007 clinical criteria for PDD, the core features are a diagnosis of PD and a dementia syndrome with insidious onset and slow progression defined as “impairment in more than one cognitive domain, representing a decline from premorbid level, and deficits severe enough to impair daily life, independent of the impairment ascribable to motor or autonomic symptoms” (Emre et al., 2007). The associated cognitive features are impaired attention, executive and visuospatial functions, and memory, but relatively preserved language functions. Behavioural features include apathy, changes in personality and mood (depression and anxiety), hallucinations, delusions and excessive daytime sleepiness.
1.2 Epidemiology of Lewy body dementias

Currently, DLB is the second most common form of neurodegenerative dementia in older people, representing 10-15% of cases (McKeith et al., 2004). Population studies have come up with varying results. The prevalence of DLB in two population studies of people 65 and older ranged from 0.1% (de Silva, Gunatilake, & Smith, 2003) to 2.0% (Stevens et al., 2002). Using the same clinical criteria, two studies also investigated the prevalence of DLB in dementia cases and found that it ranged from 2.8% (Yamada, Hattori, Miura, Tanabe, & Yamori, 2001) to 30.5% (Stevens et al., 2002). In studies of populations over 70 years of age, the prevalence of DLB was found to range from 0.1% (Yamada et al., 2001) to 5% (Rahkonen et al., 2003) of the overall population, and 0.03% (Yamada et al., 2001) to 21.9% (Rahkonen et al., 2003) among participants with dementia. Since each of these was a community-based study, the disparity of results may lie in the geographical populations that were observed. The studies were conducted in Japan (Yamada et al., 2001), England (Stevens et al., 2002), Sri Lanka (de Silva et al., 2003), and Finland (Rahkonen et al., 2003). Furthermore, Rahkonen and colleagues investigated an older population that was 75 years or older, whereas the other three studies investigate a population that was 65 years or older.

Prior to any diagnostic criteria being published for either DLB or PDD, Cummings reviewed 27 studies, involving a total of 4336 participants with PD, and found that the prevalence of dementia in PD was 40%, although DLB participants were not excluded from the group (Jeffrey L Cummings, 1988). In a more recent systematic review of PD and PDD, PDD accounted for 24 to 31% of PD participants. Of the demented population, 3% to 4% is accounted for by PDD. The prevalence of PDD, they concluded, is between 0.2% to 0.5% of the general population aged 65 years or older (Aarsland, Zaccai, & Brayne, 2005). In other epidemiological studies, the
prevalence of dementia in PD was 29% in a Brazilian PD population (Fernandes, Socal, Francisco, Schuh, & Rieder, 2015), 22% in the Rotterdam Study (de Lau et al., 2005), and 21% in Chinese PD patients (Zhang, Wang, Li, Wen, & Xu, 2014).

The incidence of DLB has been investigated in two studies. The Cache County Study (Cache County, UT) estimated that the DLB incidence rate in a population over 65 years of age was 0.1%. This corresponded to an incidence rate of 3.2% in cases with dementia (Miech et al., 2002). Another study was conducted in Olmsted County, MN, between 1991 and 2005. The authors determined that the incidence of DLB was 3.5 per 100,000 person-years, that it increased with age, and that it was higher in men than women. They also investigated the incidence of PDD and found that the incidence was 2.5 per 100,000 person-years overall, that it also increased with age, but that it was similar in men and women (2.3 and 2.7, respectively). Combined, the incidence of DLB and PDD was 5.9 per 100,000 person-years overall (Rodolfo Savica, Grossardt, Bower, Ahlskog, & Rocca, 2013). Based on the rapid growth of the aging segment of the population, the incidence rate of DLB is predicted to increase by 131% by 2050 in the USA (Bach, Ziegler, Deuschl, Dodel, & Doblhammer-Reiter, 2011).

1.3 Neuropathological characteristics of Lewy body spectrum disorders

Lewy body spectrum disorders, including PD, PDD, and DLB, are characterized by Lewy-related pathology (LRP), which includes Lewy bodies (LBs) and Lewy neurites (LNs) (Dennis W Dickson et al., 2009; Emre et al., 2007; Ian G. McKeith et al., 2017). While DLB was originally described as a clinicopathologic entity, the only neuropathological requirement in the first consensus criteria was the presence of LBs anywhere in the brain (I G McKeith et al., 1996),
which led to as many as 60% of AD cases meeting the pathological criteria for DLB. Almost none of these patients would have clinical presentation of the DLB syndrome. The inclusion of these AD cases that met this broad pathological confirmation of DLB resulted in low sensitivity of the clinical criteria for DLB (I G McKeith et al., 2005). Updated criteria account for both LRP as well as AD-type pathology in explaining the probability that pathological findings explain the clinical DLB presentation (Ian G. McKeith et al., 2017). The consensus is that DLB is related to severity of LRP and inversely related to severity of AD-type pathology. A detailed description of LRP in LBD is presented in this section, and pathological criteria for the diagnosis of DLB and PDD will be presented in section 1.5.

1.3.1 Major component of Lewy pathology: α-synuclein

The protein α-syn was first discovered in 1988, when it was described as a presynaptic neuron-specific protein (Maroteaux, Campanelli, & Scheller, 1988). A major advance in our understanding of LRP was made when α-syn was discovered as a major component of LBs and LNs (Spillantini et al., 1997). LBs and LNs are also associated with intermediate filaments, chaperone proteins and elements of the ubiquitin-proteasome system, features which are not specific to LBs and LNs, as they are found in other inclusions in neurons (I G McKeith et al., 2005). Although its exact role is still not well understood, α-syn is thought to play a regulatory role in maintaining cellular homeostasis through exo- and endocytosis (Lautenschläger, Kaminski, & Kaminski Schierle, 2017). In songbirds, α-syn is upregulated when birds are learning new songs, suggesting a likely involvement in neural plasticity (George, Jin, Woods, & Clayton, 1995). Wild-type α-syn protein has an alpha-helical structure. The primary initiation for transformation from wild-type to abnormal α-syn is the formation of a β-sheet protein, which allows the formation of fibrillar aggregates (Cole & Murphy, 2002). The neuritic dystrophy
hypothesis describes the pathologic process that leads to Lewy-related neurodegeneration in
LBD. In a normal neuron, the production of \( \alpha \)-syn occurs in the soma, and the protein is then
transported down the axon to the nerve terminal, where it acts. In the diseased neuron, abnormal
formation of \( \alpha \)-syn into \( \beta \)-sheets leads to the formation of LNs within the axon. The aggregation
continues to grow through both the addition of more abnormal \( \alpha \)-syn, as well as the sequestration
of other proteins and cellular elements. This buildup blocks axonal transport, leading to an
accumulation of cellular elements that normally undergo anterograde transport. The cell soma
becomes saturated with \( \alpha \)-syn, which predisposes the neuron to the formation of somatic LBs.
Ultimately, the blockade of axonal transport prevents the functionality of the distal axon, leading
to axonal and neuronal degeneration (Duda, 2004).

1.3.2 Identification of Lewy related pathology

Although hematoxylin and eosin (H&E) histologic staining is sufficient for staining and
detection of brainstem type LBs, it is not satisfactory for detection of cortical LBs and cannot
detect LNs. Additionally, ubiquitin immunohistochemistry can only be used in cases with
minimal AD pathology, as ubiquitin is also present in NFTs (IG McKeith et al., 2005). The 2005
consensus criteria first recommended, and the current 2017 criteria continues to recommend, the
use of immunohistochemical staining for \( \alpha \)-syn for the detection of LBs and LNs (IG McKeith
et al., 2005; Ian G. McKeith et al., 2017). Figure 1 shows substantia nigra from patients with PD
and cingulate cortex from patients with DLB immunostained for \( \alpha \)-syn. The LBs and LNs are
stained for both \( \alpha \)-syn (brown) and ubiquitin (blue), but none appear to be blue only, because all
ubiquitin-immunoreactive structures are also \( \alpha \)-syn-immunoreactive (Spillantini, Crowther,
1.3.3 Distribution and staging of Lewy related pathology

Three studies (Braak et al., 2003; Braak & Del Tredici, 2008; Kosaka, Tsuchiya, & Yoshimura, 1988) have found that similar stereotypic progression of LRP occurs in both DLB and PD. The staging proposed by Braak and colleagues for PD roughly corresponds with the criteria proposed by Kosaka and colleagues (1988). According to Braak et al. (2003), PD stages 1-2 correspond with the presence of LRP in the vagal dorsal motor nucleus, lower raphe nuclei, and locus coeruleus, which is in line with the definition of brainstem LBD, proposed by Kosaka et al (1988). Stages 3-4 of Braak PD correspond to the following regions showing LRP: the substantia nigra pars compacta, amygdala, nucleus basalis of Meynert (nbM), intralaminar thalamic nuclei, and hippocampal CA2 sector, which are the potentially affected areas in transitional LBD, as proposed by Kosaka et al (1988). Finally, Braak PD stages 5-6 describes presence of LRP in the

cingulate, temporal, frontal and parietal cortices, which corresponds with the diffuse LBD stage, proposed by Kosaka et al. (Braak & Del Tredici, 2008; Kosaka et al., 1988). Furthermore, Braak and colleagues found that, regardless of the anatomical region affected in the brain, it was the accumulation of LNs that occurred first, always preceding the accumulation of LBs. This indicates that the accumulation of LNs might predispose neurons to the formation and accumulation of LBs (Braak et al., 2003). This staging method has been the subject of debate (Burke, Dauer, & Vonsattel, 2008; Jellinger, 2009; Kalaitzakis, Graeber, Gentleman, & Pearce, 2008). In those studies, however, there was considerable variability in the severity of LRP in different regions of the nervous system, which could account for the considerable clinical variability of LBD (Halliday, Holton, Revesz, & Dickson, 2011).

1.4 Neurotransmitter abnormalities in Lewy body dementias

Some of the clinical symptoms of DLB and PDD are related to neurotransmitter abnormalities, as the Lewy related neuronal lesions ultimately leads to neuronal dysfunction in other parts of the brain than where LRP first presents (Lippa, Smith, & Perry, 1999). These neurotransmitter abnormalities, and their relationship to symptomatology and LRP, are presented in this section.

1.4.1 Cholinergic deficit

The nbM in the basal forebrain (BF) supplies the cerebral cortex with cholinergic input (Figure 2) (M-Marsel Mesulam & Geula, 1988; M.-Marsel Mesulam, Mufson, Levey, & Wainer, 1983). Lippa and colleagues predicted, correctly, that because of the extra-cortical origin of the widely distributed cortical cholinergic input, choline acetyltransferase (ChAT) levels (a measure of cholinergic activity) would correlate more closely with neuronal loss in the nbM than with LRP in cortical regions where ChAT levels are reduced. They found that a greater loss of neurons in
the nbM correlated with cortical ChAT levels in DLB patients (Lippa et al., 1999). Furthermore, they found a negative correlation (Pearson r = -0.81) between the cortical ChAT levels and a combined factor of both LBs and neuronal loss in the nbM.

**Figure 2.** Cholinergic pathways in the brain. Cholinergic neurons from the basal forebrain (BF), including the nucleus basalis of Meynert, the diagonal band of Broca, the substantia innominata and the medial septal nucleus, project to the neocortex (frontal, parietal, and occipital cortices) and the mesial temporal limbic system (amygdala and hippocampus). Cholinergic neurons also project from the pontine cholinergic system toward the thalamus, basal ganglia and brainstem. Reprinted from The Lancet, Vol. 16, Richard L Doty, Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? Pages 478-88, Copyright 2017, with permission from Elsevier.

Since cholinergic input from the nbM is required for diverse cognitive functions, degeneration of the nbM contributes to the decline in cognitive performance in DLB (Duda, 2004). In an analysis of postmortem neurochemical data, Perry et al. found that neocortical cholinergic deficits are more marked in LBD than in AD, even when controlling for dementia severity (Elaine K Perry et
al., 1994). Furthermore, they concluded that neocortical cholinergic deficits occur in LBD, rather than archicortical deficit (which occurs in AD). An investigation of 182 AD, 49 LB variant of AD, 11 PD, 6 pure DLB participants, and 16 normal controls found marked losses in midfrontal ChAT activity in participants with LBs, independent of any concurrent AD-type pathology. The authors defined participants with LB variant of AD as those who met the criteria for AD, had LB in the brainstem, archicortex, and neocortex, and presented with dementia before any extrapyramidal signs occurred (Tiraboschi et al., 2000). This is true despite the fact that there is generally less brain atrophy in DLB relative to AD (Barber, Ballard, McKeith, Gholkar, & O’Brien, 2000; J T O’Brien et al., 2001).

1.4.2 Cholinergic receptor abnormalities in Lewy body dementias

The five muscarinic receptors (M1-M5) are G-protein-coupled receptors involved in signal transduction in many cells, including neurons (Figure 2). In the cerebral cortex, the most common muscarinic receptor subtype on post-synaptic neurons is the M1 receptor. While M1 receptors were spared or up-regulated in the temporal cortex in DLB relative to AD (C. Ballard et al., 2000; Shiozaki et al., 1999), M1 receptors were reduced in the hippocampus in both DLB and AD (Shiozaki, Iseki, Hino, & Kosaka, 2001).

Nicotinic receptors are also present at the synapse (Figure 3), and their stimulation on pre-synaptic neurons modulates acetylcholine (ACh) release, as well as the release of other neurotransmitters involved in cognition and behaviour (Alkondon, Pereira, Eisenberg, & Albuquerque, 2000). In a comparison of high-affinity nicotinic binding in PD, AD and DLB, Perry and colleagues found that binding is reduced in the substantia nigra in both PD and DLB. While neuronal loss accounts for much of the nicotinic receptor loss in PD (70% neuronal loss and 70% reduction in binding), participants with DLB had a 70% reduction in nicotinic receptor
binding, but only 40% neuronal loss (E. K. Perry et al., 1995). From these findings, it can be inferred that loss of cholinergic function precedes loss of neurons in the substantia nigra in DLB. In AD, loss of high-affinity nicotinic receptors in the temporal cortex corresponded with reductions in ChAT and acetylcholinesterase (AChE), which is the enzyme that breaks down ACh. In contrast to the situation in the substantia nigra, despite a greater reduction of ChAT in DLB compared to AD, the relative loss of temporal nicotinic receptors in DLB was smaller than in AD (E. K. Perry et al., 1995). Compared to AD participants and control subjects, low-affinity nicotinic receptors are reduced in DLB participants (Wonnacott, 1997).

1.4.3 Dopaminergic Deficiencies in dementia with Lewy bodies

In both PD and DLB, there is neuronal loss within the substantia nigra. In PD, this loss is more asymmetric than in DLB, with a relatively less affected medial substantia nigra in PD (Ransmayr et al., 2001). One study found a significantly larger dopaminergic loss in PDD compared to PD in the ventral striatum, right caudate nucleus, and anterior cingulate area, using $^{18}$F-dihydroxyphenylalanine ($^{18}$F-dopa) uptake (K. Ito et al., 2002). Colloby and colleagues showed, using $^{123}$I-FP-CIT SPECT (FP-CIT SPECT) as a measure of dopamine transporter activity, that there is significant progressive dopaminergic loss in PD, DLB and PDD, and that there was no significant difference between the three diagnoses (2005). Moreover, dementia severity and motor impairment were both predictors of percentage decline, implying a role of dopaminergic loss in cognitive and motor decline. Also, using FP-CIT-SPECT, dopamine transporter binding was shown to be significantly reduced in the caudate, anterior and posterior putamen in PDD, PD and DLB (John T O’Brien, Colloby, Fenwick, & Williams, 2004). PDD cases showed the largest reduction in binding, while PD and DLB cases showed similar reductions, and all three had greater reductions than that seen in AD.
1.5 Diagnostic criteria for Lewy body spectrum disorders

Clinical and pathologic criteria have been published for the diagnosis of DLB. These criteria were put together by the International Consortium on DLB first in 1996, and have been updated three times since, based on current research in the field. The most up to date version, which is presented in this section, was published in 2017. A similar task force was put together by the Movement Disorders Society to develop consensus criteria for the diagnosis of PDD. These criteria were based on clinical data only, and published in 2007. The recommendations presented in these criteria are presented in this section.

1.5.1 Consensus diagnostic criteria for dementia with Lewy bodies

The first consensus criteria for the diagnosis of DLB were published in 1996 (I G McKeith et al., 1996). Prior to this, any attempt to relate clinical symptoms to autopsy confirmed DLB were retrospective case note reviews (R. H. Perry, Irving, Blessed, Fairbairn, & Perry, 1990). These reviews resulted in early operationalized criteria to differentiate DLB from AD, which included fluctuating cognitive impairment with episodic delirium, prominent psychiatric symptoms (especially visual hallucinations), extrapyramidal features (parkinsonism), which are either spontaneous or part of an abnormal sensitivity to neuroleptic medication (I G McKeith et al., 1996). Due to many factors, including the need for caution with neuroleptic medication, a high prevalence of in hospital diagnosed cases, and the proportion of DLB individuals who are responders to ChEI therapy, it was determined that there was a need for criteria allowing for an ante mortem clinical diagnosis of DLB. Based on a 2-day workshop, during which researchers and clinicians presented data and formulated consensus criteria, the first report of the consortium on DLB International Workshop was published.
In 1999, the report of the second dementia with Lewy body international workshop was published (I G McKeith, Perry, & Perry, 1999). The primary objectives of this workshop were to compare the experiences of each clinical site in their own clinical application of the initial criteria and determine areas, if any, where modifications were necessary. Three critical areas were open for debate at this meeting. The first was the applicability of the consensus criteria, regarding reliability, specificity, and sensitivity, in different referral systems and clinical practices. The next was to identify whether current existing criteria for AD and PD needed to be modified to account for the emerging concept of DLB. Finally, another purpose of the meeting was to determine whether a registry of DLB cases, relating to clinical diagnosis and treatment trials (cholinergic and otherwise), would better facilitate DLB research.

In two published studies (Holmes, Cairns, Lantos, & Mann, 1999; Mega et al., 1996) and five of six unpublished studies presented during the workshop, a high specificity of the consensus criteria was confirmed, indicating that the criteria were appropriate to confirm a diagnosis of DLB. However, the criteria were of limited value in screening for DLB, due to their low sensitivity, and under-diagnosis remained a problem at that time. Visual hallucinations and extrapyramidal symptoms (EPS) were assessed with adequate inter-rater reliability, but inter-rater reliability was low for the characterization of cognitive fluctuations (time course, persistence and magnitude).

The participants at the workshop agreed that the criteria had not been used long enough to justify modifications to the core features. There were, however, two additional areas of clinical symptomatology that were not considered in the first guidelines, but were brought up at the second workshop, which were RBD and depressive symptoms (I G McKeith et al., 1999).
The consortium met once more, and published its report from the third meeting with updated guidelines for diagnosis and management of DLB (I G McKeith et al., 2005). In this report, the criteria were modified to increase sensitivity, which remained low. Progressive disabling mental impairment remained a mandatory requirement for a DLB diagnosis, and no major modifications were made to the three core clinical features, though improved methods for clinical assessment of these features were recommended. The feature with the lowest inter-rater reliability remains fluctuations in cognition and attention. As such, the consortium recommended that at least one of the available formal measures be used to assess fluctuations, and be administered by a trained staff person when the diagnostic criteria are being applied.

To increase sensitivity, three suggestive features were included in the consensus criteria: RBD, severe neuroleptic sensitivity, and low dopamine transporter uptake in basal ganglia on SPECT or PET scans. If one or more suggestive features are present, in addition to one or more core feature, the diagnosis is probable DLB. If, however, there is the presence of one or more suggestive features in the absence of a core feature, then a diagnosis of possible DLB can be made. Further, supportive features, some of which were included in the 1996 criteria, are described as features which are commonly seen in DLB, but are not specific enough, diagnostically, to be considered either core or suggestive features. The exclusion criteria were also modified to include that a diagnosis of DLB should not be made if parkinsonism only occurs in a stage of severe dementia.

In an attempt to differentiate DLB from PDD, the criteria included the arbitrary one-year rule, suggested in 1996 (I G McKeith et al., 1996), which separates the two diseases based solely on the temporal order in which symptoms appear. If, for example, motor symptoms present first, and are present for at least one year prior to the presentation of cognitive symptoms, then the
diagnosis is PDD. If however, the cognitive symptoms present first, or within one year of motor symptoms, then the diagnosis is DLB (Emre, 2003). This one-year rule has since been up for debate, as the MDS criteria for PD have omitted it (Postuma et al., 2015), however, it was still included in the updated versions of the DLB criteria to be discussed in the next sections (Ian G. McKeith et al., 2017). Other than age of onset, temporal course and responsiveness to levodopa there are no major differences between PDD and DLB in terms of the cognitive profile, attentional performance, neuropsychiatric features, sleep disorders, autonomic dysfunction, type and severity of parkinsonism, neuroleptic sensitivity, and responsiveness to ChEIs. Although there has been some debate as to whether PDD and DLB are different in more ways than just their temporal course, no convincing evidence has been presented of any significant pathological, neurochemical or imaging differences between the two (Aarsland, Ballard, & Halliday, 2004; Lippa et al., 2007). Guidelines and diagnostic criteria for PDD are presented in section 1.4.2.

The first report in 1996 only required, neuropathologically, that there be a presence of LBs somewhere in the brain of a patient with a clinical history of dementia. While this criterion is wide-ranging, newer, more sensitive methods for the detection of LBs have illustrated that many cases will meet this criterion pathologically, which do not meet DLB criteria clinically. Thus, this pathological criterion had low sensitivity. The third report made new recommendations in assessing whether pathological findings are associated with a DLB clinical syndrome, which account for both Lewy-related and AD-type pathology. They are based on the observation that a DLB clinical syndrome is directly related to LRP, but inversely related to AD-type pathology (I G McKeith et al., 2005; Merdes et al., 2003).

The fourth and most recent consensus report from the DLB consortium was published in January 2017 (Ian G. McKeith et al., 2017). The revisions made in this report aimed to distinguish
between clinical features and diagnostic biomarkers. They also provided guidance on optimal methods that might aid in the establishment and interpretation of these features and markers.

The features of the criteria were preserved; however, clinical features are now divided into either core or supportive features, and suggestive features have been reassigned throughout the new criteria (as this category no longer exists). Furthermore, candidate biomarkers are divided into indicative or supportive categories. The decision for the new groupings is based on the quality evidence that had been gathered in the decade since the previous criteria, as well as on the specificity that each feature or marker provides for diagnosis (Ian G. McKeith et al., 2017).

In addition to the three previously described core clinical features (I G McKeith et al., 2005), RBD has been included in the 2017 criteria as a core clinical feature (Ian G. McKeith et al., 2017). It was included because it occurs in 76% of autopsy confirmed DLB cases, compared to only 4% in non-DLB cases (Ferman et al., 2011). New to the list of supportive features are hypersomnia (Ferman et al., 2004a) and hyposmia (S. S. Williams et al., 2009).

Biomarkers were included in the new 2017 criteria due to the usefulness of many markers as indirect methods of DLB diagnosis (Ian G. McKeith et al., 2017). Included as indicative biomarkers are reduced uptake of dopamine transporter in the basal ganglia on SPECT or PET scans, low uptake on $^{123}$I-MIBG myocardial scintigraphy, and PSG confirmed REM sleep without atonia. Supportive biomarkers are relative preservation of medial temporal lobe structures on CT/MRI scans, low global uptake on SPECT/PET perfusion/metabolism scans with occipital hypoperfusion/hypometabolism plus or minus the cingulate island sign on FDG-PET imaging, and prominent posterior slow-wave activity on EEG. Diagnoses of probable and
possible DLB were revised to include biomarkers, though probable DLB should not be diagnosed based on biomarkers alone.

The *one-year rule* continues to be recommended for the distinction of DLB and PDD according to the consensus criteria for DLB (Ian G. McKeith et al., 2017), but not the MDS criteria for PD (Postuma et al., 2015). While the most appropriate term based on clinical features and temporal progression should be used, general terms such as Lewy body disease may also be helpful in general clinical practice.

1.5.2 Clinical diagnostic criteria for the diagnosis of probable and possible Parkinson’s disease with dementia

The lack of operationalized criteria to diagnose dementia in PD prompted the MDS to put together a task force to define clinical diagnostic criteria for PDD (Emre et al., 2007). This task force agreed that PDD and DLB share pathological and clinical features, and suggest that the two might be two clinical entities on a spectrum of Lewy body disease. They maintain the recommendation of the Third Report of the DLB Consortium (I G McKeith et al., 2005) to use of the *one-year rule* to temporally differentiate the two. The clinical features of PDD and criteria for diagnosis of probable and possible PDD are presented in Table 1 and Table 2, respectively. Generally, the clinical features of PDD include cognitive impairments in attention, executive and visuospatial functions and memory, and a preservation of language. The cognitive profile that predominates is dysexecutive, with hallucinations, apathy, and mood changes being the common behavioural features. Postural instability-gait dysfunction (PIGD) is the motor phenotype with which dementia is most associated. A core feature for the diagnosis of PDD is the diagnosis of PD according to the Queen Square Brain Bank criteria, which is a feature that is not included in the diagnosis of DLB (Ian G. McKeith et al., 2017).
While these criteria specifically aimed at defining the clinical diagnostic criteria for PDD, an MDS task force for the redefinition and update of PD released the MDS-PD Clinical Diagnostic Criteria for PD, which does not include dementia as an exclusion criteria for PD (Postuma et al., 2015). They state in their criteria that “for those patients with dementia who already carry a diagnosis of dementia with Lewy bodies (according to consensus criteria (I G McKeith et al., 2005)), the diagnosis can optionally be qualified as ‘PD (dementia with Lewy bodies subtype)’” in an attempt to clarify how to use their criteria if there is dementia present.
1. **Core features**
   a. Diagnosis of Parkinson’s disease according to Queen Square Brain Bank criteria
   b. A dementia syndrome with insidious onset and slow progression, developing within the context of established Parkinson’s disease and diagnosed by history, clinical, and mental examination, defined as:
      i. Impairment in more than one cognitive domain
      ii. Representing a decline from premorbid level
      iii. Deficits severe enough to impair daily life (social, occupational, or personal care), independent of the impairment ascribable to motor or autonomic symptoms
2. **Associated clinical features**
   a. Cognitive features:
      i. Attention: Impaired. Impairment in spontaneous and focused attention, poor performance in attentional tasks; performance may fluctuate during the day and from day to day
      ii. Executive functions: Impaired. Impairment in tasks requiring initiation, planning, concept formation, rule finding, set shifting or set maintenance; impaired mental speed (bradyphrenia)
      iii. Visuospatial functions: Impaired. Impairment in tasks requiring visual-spatial orientation, perception, or construction
      iv. Memory: Impaired. Impairment in free recall of recent events or in tasks requiring learning new material, memory usually improves with cueing, recognition is usually better than free recall
      v. Language: Core functions largely preserved. Word finding difficulties and impaired comprehension of complex sentences may be present
   b. Behavioral features:
      i. Apathy: decreased spontaneity; loss of motivation, interest, and effortful behavior
      ii. Changes in personality and mood including depressive features and anxiety
      iii. Hallucinations: mostly visual, usually complex, formed visions of people, animals or objects
      iv. Delusions: usually paranoid, such as infidelity, or phantom boarder (unwelcome guests living in the home) delusions
      v. Excessive daytime sleepiness
3. **Features which do not exclude PD-D, but make the diagnosis uncertain**
   a. Co-existence of any other abnormality which may by itself cause cognitive impairment, but judged not to be the cause of dementia, e.g. presence of relevant vascular disease in imaging
   b. Time interval between the development of motor and cognitive symptoms not known
4. **Features suggesting other conditions or diseases as cause of mental impairment, which, when present make it impossible to reliably diagnose PD-D**
   a. Cognitive and behavioral symptoms appearing solely in the context of other conditions such as: Acute confusion due to
      i. Systemic diseases or abnormalities
      ii. Drug intoxication
   b. Major Depression according to DSM IV
   c. Features compatible with “Probable Vascular dementia” criteria according to NINDS-AIREN (dementia in the context of cerebrovascular disease as indicated by focal signs in neurological exam such as hemiparesis, sensory deficits, and evidence of relevant cerebrovascular disease by brain imaging AND a relationship between the two as indicated by the presence of one or more of the following: onset of dementia within 3 months after a recognized stroke, abrupt deterioration in cognitive functions, and fluctuating, stepwise progression of cognitive deficits)

Probable PDD
1. Core features: Both must be present
2. Associated clinical features:
   a. Typical profile of cognitive deficits including impairment in at least two of the four core
cognitive domains (impaired attention which may fluctuate, impaired executive functions,
impairment in visuospatial functions, and impaired free recall memory which usually
improves with cueing)
   b. The presence of at least one behavioral symptom (apathy, depressed or anxious mood,
hallucinations, delusions, excessive daytime sleepiness) supports the diagnosis of Probable
PD-D, lack of behavioral symptoms, however, does not exclude the diagnosis
3. None of the group III features present
4. None of the group IV features present

Possible PDD
1. Core features: Both must be present
2. Associated clinical features:
   a. Atypical profile of cognitive impairment in one or more domains, such as prominent or
receptive-type (fluent) aphasia, or pure storage-failure type amnesia (memory does not
improve with cueing or in recognition tasks) with preserved attention
   b. Behavioral symptoms may or may not be present
   OR
3. One or more of the group III features present
4. None of the group IV features present

Table 2. Criteria for the diagnosis of probable and possible PDD. Reproduced with permission. Emre, M.,
diagnostic criteria for dementia associated with Parkinson’s disease. Movement Disorders, 22(12), 1689–
1707. Copyright © 2007 Movement Disorder Society

1.5.3 Pathologic diagnostic criteria for Lewy body dementias

The pathologic diagnostic criteria presented in the Third Report of the Consortium of DLB take
into account both the LRP as well as AD-type pathology in assessing the probability that the
neuropathological findings explain the clinical symptoms of DLB (IG McKeith et al., 2005). In
the third report, the assessment included AD-type pathology, categorized by NIA-Reagan
criteria, which uses the CERAD method for assessing neuritic plaques and a staging method for
neurofibrillary degeneration that is comparable to Braak stage (Montine et al., 2012), as well as
LRP characterized as diffuse neocortical, limbic/transitional, or brainstem-predominant. Based
on both the AD-type pathology and the LRP, a likelihood that pathologic findings are associated
with a DLB clinical syndrome can be assessed and graded from low to high. In the fourth report
of the consortium of DLB, they recommended the continued use of the recommendations from the third report, with the addition of two more categories of LRP: amygdala-predominant and olfactory bulb only (Ian G. McKeith et al., 2017).

A semi quantitative grading of lesion density is recommended (I G McKeith et al., 2005). The consortium recommends LB scoring as the following: 0 score if there are no LBs, 1 score if there are sparse LBs or LNs (mild), 2 score if there are more than one LB in a low power field and sparse LNs (moderate), 3 score if there are four or more LBs and scattered LNs in a low power field (severe), and 4 score if there are numerous LBs and numerous LNs (very severe). Potential cases of DLB should follow the National Institute of Aging-Alzheimer’s Association (NIA-AA) guidelines to assess the stage of AD-type pathology (Montine et al., 2012).

1.6 Clinical presentation of Lewy body dementias

This section will describe, in more detail, the clinical features that are included in the consensus criteria for the diagnosis of DLB and PDD. Although DLB and PDD are clinically similar entities, the diagnostic criteria are structured differently. For this reason, the clinical features of DLB and PDD are presented separately in this section.

1.6.1 Clinical and imaging features of dementia with Lewy bodies

The only essential feature for a diagnosis of DLB is dementia, which is defined by the DLB consortium as “progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational functions, or with usual daily activities” (Ian G. McKeith et al., 2017). They further specify that deficits in attention, executive functions and visuospatial ability may occur early in the disease process, and memory impairment does not necessarily occur early on,
but is often evident as DLB progresses. Tests such as the Mini Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) are useful in characterizing the global cognitive deficits in DLB, however, neuropsychological assessments that are more specific to DLB may be more useful in the differential diagnosis. These include Stroop task, trails making task, and phonemic fluency as measures of divided attention, and tasks of figure copy, visual assembly, spatial matching, and perceptual discrimination as measures of spatial and perceptual difficulties. While no DLB-specific assessment batteries have been established, Galvin published a composite risk score that can be used to improve the clinical detection of LBD (Galvin, 2015).

There are four core clinical features of DLB: fluctuating cognition with variations in attention and alertness, recurrent visual hallucinations, RBD, and parkinsonism. There are additional supportive clinical features of DLB: severe sensitivity to antipsychotic agents, postural instability, repeated falls, syncope, severe autonomic dysfunction, hypersomnia, hyposmia, hallucinations in other modalities, systematized delusions, apathy, anxiety and depression (Ian G. McKeith et al., 2017).

Fluctuation in cognitive performance is a characteristic of DLB, described as early as 1992 (the disease was called senile dementia of the Lewy body type, at the time of publication), and has since been included as a core clinical feature in all consensus criteria (I G McKeith et al., 1996, 2005, 1999; I G McKeith, Perry, Fairbairn, Jabeen, & Perry, 1992; Ian G. McKeith et al., 2017). In early stages of DLB, cognitive deficits may present intermittently with periods of normal or near-normal function (I G McKeith et al., 1996). Fluctuations are described as delirium-like, presenting as changes in cognition, attention and arousal, which occur spontaneously (Ian G. McKeith et al., 2017). They include episodes of behavioural inconsistency, incoherent speech, variable attention and/or altered consciousness. The third report of the consortium acknowledged
that much of the variability in the diagnostic accuracy of DLB was due to the difficulty in assessing fluctuating cognition (I G McKeith et al., 2005). There are cognitive fluctuation scales, including those completed both by a clinician and a non-clinician, which have been used in assessing fluctuations in the past. The Clinician Assessment of Fluctuation is designed for use by an experienced clinician, and involves the questioning of a reliable informant about the fluctuating confusion and impaired consciousness (during the past month before the assessment is conducted) (M. P. Walker et al., 2000). This scale was used in an investigation of the validity of the 1996 Consensus criteria (I G McKeith et al., 1996), where its sensitivity was 83% and its specificity was 91% (I G McKeith et al., 2000). The One Day Fluctuation Assessment Scale can be used by a non-clinician, and includes seven items of confused behaviour: falls, fluctuation, drowsiness, attention, disorganized thinking, altered level of consciousness, and communication (M. P. Walker et al., 2000). In a comparison of these two scales, Walker and colleagues found that scores from both scales correlated significantly with each other and with neuropsychological and electrophysiological measures of fluctuation, suggesting that they are both useful scales. In 2014, Lee and colleagues designed the Dementia Cognitive Fluctuation Scale that includes four items—marked differences in daytime functioning, daytime somnolence, daytime drowsiness, and altered levels of consciousness during the day—that provide questions for reliably identifying fluctuating cognition and differentiating LBD from other causes of dementia, such as AD and vascular dementia (Lee et al., 2014). The most updated guidelines recommend the use of at least one measure of fluctuation should be used when applying the DLB criteria (Ian G. McKeith et al., 2017). Further, they emphasize that early presentation of cognitive fluctuation is a better predictor for DLB, as fluctuations also occur in later stages of other dementias.
Visual hallucinations, described as recurrent, complex, and often well-formed occur in up to 80% of patients with DLB (Ian G. McKeith et al., 2017). They typically feature people, children or animals. Hallucinations in other modalities, including auditory, have been described, but are less common in DLB (I G McKeith et al., 1996). One useful tool for the assessment of frequency and severity of visual hallucinations is the Neuropsychiatric Inventory (NPI), which is an informant-based assessment (I G McKeith et al., 2005). Compared to PDD and DLB participants without visual hallucinations, those with visual hallucinations had significantly worse visual perception (Mosimann et al., 2004). Individuals with DLB are often able to report their experiences with visual hallucinations, as are caregivers (Ian G. McKeith et al., 2017), however, caregivers often underreport visual hallucinations (I G McKeith et al., 2005). Occipital hypoperfusion is associated with LBD (S. J. Colloby et al., 2002; Lobotesis et al., 2001), and hypometabolism in LBD occurs in primary and secondary visual cortices (Higuchi et al., 2000). Moreover, it was demonstrated that there are significant changes to synaptic proteins and ChAT in both primary and secondary visual cortices in DLB (Mukaetova-Ladinska et al., 2013). Imamura et al. demonstrated that DLB individuals with a history of visual hallucinations had a relative hypoperfusion compared to those without hallucinations in the occipital lobe, particularly in the primary visual cortex (1999). Individuals with visual hallucinations in DLB had significantly decreased perfusion on brain SPECT in the left anterior cingulate cortex, left orbitofrontal cortex and left cuneus compared to the DLB group without visual hallucinations (Heitz et al., 2015).

Another study demonstrated, using FDG-PET, that occipital hypometabolism often seen in DLB is associated with increased frequency and severity of visual hallucinations (Firbank, Llloyd, & O’Brien, 2016). Furthermore, a greater deficit in ACh is associated with visual hallucinations (E. Perry & Perry, 1995), and one study showed that the presence of visual hallucinations may
predict an improvement following cholinesterase inhibitor (ChEI) therapy (Ian G McKeith, Wesnes, Perry, & Ferrara, 2004).

Parkinsonism in PD, as defined by the most updated MDS-PD criteria, is bradykinesia, combined with rest tremor, rigidity, or both, and these features must not be attributable to confounding factors (Postuma et al., 2015). The parkinsonism in DLB often falls short of this, so just one cardinal feature of parkinsonism is required for the diagnosis of DLB (Ian G. McKeith et al., 2017). The criteria also require that the features of parkinsonism must be spontaneous, and not due to the antidopaminergic medications or stroke. These features occur eventually in 85% of DLB individuals. In both PDD and DLB, postural instability, gait, and speech disturbance are more common than in PD without dementia, while a tremor dominant phenotype is less common (Burn et al., 2003). Levodopa (L-dopa) is well tolerated in DLB, however, responsiveness to L-dopa is less than in PD and there is a tendency for dopaminergic drugs to exacerbate neuropsychiatric features (Lucetti et al., 2010; Molloy, McKeith, O’Brien, & Burn, 2005).

RBD, which is a parasomnia, is characterized by complex and vigorous behaviours during attempts to enact dreams, and increased phasic and/or tonic electromyographic activity during REM sleep (Schenck, Bundlie, Patterson, & Mahowald, 1987). A diagnosis of RBD is more likely if the bed partner has sustained injuries from the movement and if the theme of dreams involves attacking or chasing (Ferman et al., 2011). While RBD was previously included as a supportive feature (I G McKeith et al., 2005), the most updated consensus criteria include this as a core clinical feature because it occurs in 76% of DLB individuals compared to just 4% of individuals without DLB (Ferman et al., 2011). RBD often presents years before the other clinical symptoms of DLB and may become less severe over time (Ian G. McKeith et al., 2017). The consortium recommends the use of a scale that questions either the individual or their bed
partner such as the Mayo Sleep Questionnaire for the diagnosis of RBD, as well as the use of PSG.

Supportive clinical features are commonly present in DLB. Further, the consensus criteria stipulate that “although lacking specificity, such symptoms may indicate DLB in a patient with dementia, particularly when they persist over time or if several occur in combination” (Ian G. McKeith et al., 2017). Since the publication of the 2005 consensus criteria, hypersomnia and hyposmia have been added as supportive clinical features (Ian G. McKeith et al., 2017). Hypersomnia usually presents as excessive daytime sleepiness (Ferman et al., 2004) and hyposmia tends to present earlier in DLB than in AD (S. S. Williams et al., 2009). Due to the increased awareness of severe antipsychotic sensitivity in DLB, prescription of D2 receptor blocking antipsychotics has decreased, leading the consortium to list antipsychotic sensitivity as a supportive feature (Ian G. McKeith et al., 2017). Autonomic impairment, including constipation, orthostatic hypotension, and urinary incontinence, are common in DLB (Horimoto et al., 2003). Transient episodes of unresponsiveness may be an extreme presentation of cognitive fluctuation, and may be difficult to differentiate from true syncope (Ian G. McKeith et al., 2017).

1.6.2 Clinical features of Parkinson’s disease with dementia

A similar detailed description was also published for PDD (Emre et al., 2007). In addition to the requirement for a diagnosis of PD, the second core feature of PDD, according to these criteria, is dementia, which is defined as “impairment in more than one cognitive domain, representing a decline from premorbid level, and deficits severe enough to impair daily life, independent of the impairment ascribable to motor or autonomic symptoms”. As dementia is no longer an exclusion criteria for PD according to the current MDS-PD criteria, presentation of dementia in
conjunction with parkinsonism could also be qualified as PD (dementia with Lewy bodies type) (Postuma et al., 2015). Associated clinical features, according to the 2007 criteria published by Emre et al., include both cognitive and behavioural features. Cognitive impairments are observed in attention, executive and visuospatial function, and memory, but language functions are largely preserved. Attentional fluctuations are also found in PDD (C. G. Ballard et al., 2002). Impairments were comparable in individuals with DLB and PDD, but less so than impairments seen in DLB without parkinsonism. There is significant overlap between the clinical profiles in DLB and PDD (Lippa et al., 2007). The role of neuropsychological assessment was not considered to have been conclusive in the differential diagnosis of DLB and PDD (Emre et al., 2007).

The behavioural and neuropsychiatric symptoms that occur most commonly in PDD are hallucinations and delusions, mood disturbance and apathy (Emre et al., 2007). Delusions and hallucinations occur in 29% and 54% of PDD patients, respectively (Aarsland et al., 2004). They are more common in DLB (57% and 76%), but less common in PD without dementia (7% and 14%). Overall, individuals with PDD trend toward having less severe or frequent psychiatric symptoms than DLB, however, this could be the result of less severe dementia in these individuals (Emre et al., 2007).

The postural-instability-gait-disturbance (PIGD) phenotype of motor symptoms is more common in PDD than the tremor-dominant phenotype (Burn et al., 2003). RBD and excessive daytime sleepiness are also common in PDD, and may be useful in differentiating between PDD and AD (Sinforniani et al., 2006). Severe neuroleptic sensitivity was reported in 40% of PDD individuals exposed to neuroleptic drugs (Aarsland, Perry, et al., 2005).
1.7 Biomarkers of Lewy body dementias

There are currently no direct biomarkers of LRP, however, there are many useful indirect methods that could help improve the detection and diagnosis of DLB and PDD. Biomarkers have been included in the current consensus criteria for the diagnosis of DLB (Ian G. McKeith et al., 2017). While there was no definition set in these criteria for what constitutes a biomarker, in 2017 the FDA-NIH Biomarker Working Group defined a diagnostic biomarker as “a biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease.” (FDA-NIH Biomarker Working Group, 2017). Though not included in the diagnostic criteria for PDD (Emre et al., 2007), studies of biomarkers in PDD are also presented in this section.

1.7.1 Single photon emission computed tomography in Lewy body dementias

Dopamine transporter (DAT) is a sodium coupled transmembrane protein that mediates reuptake of dopamine from the synaptic cleft into presynaptic nigrostriatal nerve terminals (Beaulieu & Gainetdinov, 2011). $^{123}$I-FP-CIT SPECT is the most commonly used agent in the measure of DAT density because, compared to other available ligands, it has faster kinetics, higher selectivity, and can be performed in the presence of L-dopa treatment (Saeed et al., 2017). There is significantly decreased binding in striatum, including the caudate, and anterior and posterior putamen, on SPECT in DLB, PD, and PDD compared to AD and to normal controls (John T. O’Brien et al., 2004; Z. Walker et al., 2002). Abnormal $^{123}$I-FP-CIT scans detected probable DLB with a sensitivity of 78% and a specificity of 90% in participants with probable or possible DLB, or with a non-DLB dementia (Ian McKeith et al., 2007). For this reason, abnormal binding...
on $^{123}$I-FP-CIT SPECT scans is now included as an indicative biomarker for the diagnosis of DLB (Ian G. McKeith et al., 2017).

Using $^{99m}$Tc-hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) SPECT, it was shown that, while there is similarly temporoparietal hypoperfusion in both AD and DLB, occipital hypoperfusion is the only measure that separated DLB and AD (Lobotesis et al., 2001). Using $^{99m}$Tc-HMPAO SPECT, this group correctly classified DLB with a sensitivity of 65% and a specificity of 87%. Another study of diagnostic accuracy of $^{99m}$Tc-HMPAO SPECT found that it had a sensitivity of 71% and specificity of 70% (John T O’Brien et al., 2014). Though it may be helpful for diagnosis, the sensitivity and specificity are not high enough for it to be characterized as an indicative biomarker, and, instead, perfusion SPECT is a supportive biomarker (Ian G. McKeith et al., 2017).

1.7.2 Positron emission tomography in Lewy body dementias

Similar to SPECT imaging, PET imaging can be used to measure the activity of DAT, for example, using the ligand $^{18}$F-FP-CIT. In an investigation of DAT binding in parkinsonian syndromes, Jin et al. showed that striatal DAT binding on PET scans was also abnormal in DLB and PD groups (Jin et al., 2013). PET imaging of DAT is included with SPECT imaging as an indicative biomarker for DLB (Ian G. McKeith et al., 2017).

Cerebral glucose uptake is measured using $^{18}$F-FDG PET and is meant to approximate metabolism. In DLB compared to AD, there is more prominent occipital hypometabolism, but with a preservation of hippocampal metabolism (Mosconi et al., 2008). This method was successful at discriminating DLB from AD with a sensitivity of over 90% and a specificity of 71%. In a regional analysis, the most sensitive area for detecting DLB using $^{18}$F-FDG PET
imaging was hypometabolism in the lateral occipital area (88%), but the cingulate island sign had the highest specificity at 100% (Lim et al., 2009). The cingulate island sign refers to a relative preservation of posterior cingulate metabolism. The cingulate island sign is also a predictor of lower Braak NFT stage in DLB, and is not associated with amyloid-β load (Graff-Radford et al., 2014). Reduced occipital metabolism on PET scans and the cingulate island sign on FDG-PET imaging are included as a supportive biomarker for DLB (Ian G. McKeith et al., 2017). No study to date has been completed that investigates the presence of the cingulate island sign in PDD.

1.7.3 Metaiodobenzylguanidine myocardial scintigraphy
Metaiodobenzylguanidine (MIBG), when radiolabeled with iodine-123 (\(^{123}\)I-MIBG), can be used for cardiac scintigraphy to assess postganglionic presynaptic cardiac sympathetic nerve endings, in a non-invasive way (Orimo, Yogo, Nakamura, Suzuki, & Watanabe, 2016). Impairments in the heart-to mediastinum (H/M) ratio of count densities were independent of duration and severity of parkinsonian and autonomic symptoms (Yoshita, Taki, & Yamada, 2001). \(^{123}\)I-MIBG H/M ratios are reduced in LBD compared to AD (Hanyu et al., 2006; Taki, Yoshita, Yamada, & Tonami, 2004) and normal controls (Hanyu et al., 2006; Yoshita et al., 2001). This method positively identified DLB and PDD with a sensitivity of 95% and a specificity of 87% in one study (Hanyu et al., 2006) and discriminated between DLB and AD with a sensitivity and specificity of 100% (Yoshita et al., 2006). Low uptake on \(^{123}\)I-MIBG myocardial scintigraphy is an indicative biomarker of DLB (Ian G. McKeith et al., 2017).
1.7.4 Polysomnographic confirmation of rapid eye movement sleep without atonia

Polysomnographic (PSG) confirmation of REM sleep without atonia is a highly specific predictor of LRP. Among individuals with dementia and a history of RBD, those who had a PSG study that showed REM sleep without atonia, had dementia due to a synucleinopathy 90% of the time (Boeve, Silber, & Ferman, 2004). Due to its high specificity, PSG confirmation of REM sleep without atonia is sufficient for a probable DLB diagnosis, even in the absence of any other core feature or biomarker (Ian G. McKeith et al., 2017).

1.7.5 Electroencephalography in Lewy body dementias

The use of electroencephalography (EEG) has also been investigated as a biomarker of LBD. Group differences were observed between AD and DLB on EEGs in posterior derivations, and dominant frequency variability also differed between the two groups (Bonanni et al., 2008). Less than half of PDD individuals exhibited the same abnormal EEG results as those with DLB. Moreover, in PDD and DLB, but not in AD, the degree to which residual alpha bands appeared correlated with the presence and severity of cognitive function. This study also completed a two-year follow-up, where EEG abnormalities from posterior leads were seen in all DLB individuals, and 75% of PDD individuals. Together, the pre-alpha-dominant frequency, either stable or mixed with alpha, theta, or delta activities in pseudoperiodic patterns can distinguish DLB from AD in over 90% of cases (Ian G. McKeith et al., 2017). It is therefore included as a supportive biomarker for the diagnosis of DLB.
1.8 Treatment and management of Lewy body dementias

Once a diagnosis of probable or possible DLB or PDD has been made based on the clinical features, and the indirect biomarkers of these diseases, the clinician will begin to treat a patient’s symptoms. A correct diagnosis is important for the treatment of LBD because of the severe sensitivity that these individuals have to antipsychotic agents (Ian G. McKeith et al., 2017). Cholinesterase inhibitors (ChEIs) are used in the treatment of cognitive and neuropsychiatric features of LBD, and will be discussed in detail in this section.

1.8.1 Cholinergic neurotransmission

ACh is synthesized from choline and acetyl coenzyme A. The ACh molecules are then packaged into vesicles. When the neuron is depolarized, these vesicles fuse with the plasma membrane and release the ACh into the synaptic cleft, where it can bind to cholinergic receptors on the postsynaptic neuron, activating downstream cell signaling pathways through ion influx or activation of secondary messengers. Under normal conditions, the enzyme acetylcholinesterase (AChE) catalyzes the hydrolysis of ACh, preventing it from further binding to its receptors. Butyrylcholinesterase (BChE) is another enzyme that catalyzes the hydrolysis of esters of choline, which includes ACh, in addition to butyrylcholine and succinylcholine (Silver, 1974). The use of a ChEI prevents this hydrolysis, and ACh accumulates in the synapse. This leads to an increase in stimulation of cholinergic receptors on the postsynaptic neuron, and an increase in the downstream effects mediated by the binding of ACh to its receptors (Pope, Karanth, & Liu, 2005).
**Figure 3.** Mechanism of Action of Cholinesterase Inhibitors. Under normal conditions, vesicles containing acetylcholine (ACh) fuse with the plasma membrane following depolarization of the presynaptic neuron, and ACh is released into the synaptic cleft (1). ACh binds to nicotinic (N receptor) and muscarinic receptors (M receptor) on the postsynaptic neuron, resulting in cellular responses within the postsynaptic neuron (2). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) hydrolyze ACh (3) into choline and acetic acid, which can then be returned to the presynaptic nerve (4). Cholinesterase inhibitors prevent this hydrolysis, allowing ACh to build up in the synapse. Rivastigmine can inhibit AChE and BChE, and donepezil can inhibit AChE only (5). Galantamine additionally allosterically modulates nicotinic ACh receptors (N-receptor) (6).

### 1.8.2 Pharmacology of cholinesterase inhibitors

In response to cholinergic deficits seen in LBD (Tiraboschi et al., 2000), cholinesterase inhibitors have been used to treat these diseases. ChEIs are the current recommended treatment for cognitive and neuropsychiatric symptoms of DLB (Ian G. McKeith et al., 2017). There are three ChEIs that are commonly used: rivastigmine, donepezil and galantamine. The common mechanism of all ChEIs is to stop degradation of ACh, thus increasing its availability in the
synapse, such that there is an overall increase in the activation of cholinergic receptors (Pope et al., 2005).

Pharmacokinetics describe how a drug is absorbed from the site of administration, distributed in various tissues, metabolized, and finally excreted from the body (ADME framework) (Greenblatt, Harmatz, von Moltke, & Shader, 1995). Donepezil and galantamine are both metabolized by cytochrome P450 2D6 (CYP2D6) and CYP3A4 enzymes in the liver, and undergo extensive first pass metabolism (Jann, Shirley, & Small, 2002). The CYP2D6 metabolite of donepezil is as pharmacologically active as the parent compound, and the CYP2D6 metabolite of galantamine is three times more potent than the parent compound (Lam, Hollingdrake, Kennedy, Black, & Masellis, 2009). Unlike galantamine and donepezil, rivastigmine is not metabolized by oxidative CYP enzymes in the liver. Instead it is metabolized by cholinesterases, and is rapidly eliminated by the kidneys (Jann et al., 2002).

Pharmacodynamics describe how an organism is affected by drug concentrations (Greenblatt et al., 1995). Rivastigmine reversibly binds to and inhibits both AChE and BChE, and blocks their function at their active site (Farlow & Hake, 1998). Donepezil is a reversible competitive inhibitor that binds to AChE with a high selectivity (Sugimoto, 2001). Galantamine has a dual purpose: in addition to the competitive inhibition of AChE, it is also an allosteric modulator of nicotinic ACh receptors (nAChRs) (Albuquerque, Santos, Alkondon, Pereira, & Maelicke, 2001). Under normal conditions, ACh stimulates nicotinic and muscarinic receptors both centrally and peripherally. Inhibition by ChEIs increases the levels of ACh available in the synapse, thereby indirectly increasing ACh neurotransmission at its receptors (Lam et al., 2009). Galantamine also allosterically modulates nAChRs, further increasing cholinergic neurotransmission (Albuquerque et al., 2001). Cognitive enhancing effects of those drugs are the
result of central inhibition, while dose-dependent side effects are linked to peripheral inhibition (Lam et al., 2009).

1.8.3 Cholinesterase inhibitors in the treatment of Lewy body dementias

Many studies, including many randomized controlled trials (RCTs), have been done to investigate the efficacy of these drugs, and three meta-analyses have recently been conducted to assess the pooled results (Matsunaga, Kishi, Yasue, & Iwata, 2015; Stinton et al., 2015; Wang et al., 2015). The three meta-analyses that have been conducted on these RCTs of ChEI therapy in LBD were limited to measures of general cognition and some neuropsychiatric data because these were the only commonalities between many of the RCTs. In their pooled analyses, these meta-analyses found significant improvements, compared to placebo, on MMSE and MoCA (Matsunaga et al., 2015), or MMSE and CGIC (Stinton et al., 2015; Wang et al., 2015). Since the meta-analyses largely analyzed global measures, the main large studies that have been completed will be discussed in this section. Emre et al. conducted a study investigating the efficacy of rivastigmine in the treatment of PDD (n=410) (2004). They found significant improvements in PDD participants compared to placebo on their primary efficacy variables, which were ADAS-cog score (p<0.001) and CGIC score (p=0.007), as well as all their secondary efficacy variables (ADL, NPI-10, MMSE, Cognitive Drug Research power of attention test, Verbal Fluency test, Clock Drawing score). Another large-scale study was conducted in 2012 by Dubois and colleagues, investigating efficacy of donepezil at treating PDD (n=550). While they did not find a significant change on their first primary efficacy variable, which was ADAS-cog, they found a significant improvement on Clinician’s Interview-Based Impression of Change plus caregiver input for the donepezil 10mg group compared to placebo (p=0.04). There were also significant
improvements for donepezil therapy compared to placebo for their secondary measures (MMSE, Delis-Kaplan Executive Function System, and Brief Test of Attention) (Dubois et al., 2012).

McKeith and colleagues conducted the first study investigating the efficacy of rivastigmine in the treatment of DLB in 2000 (n=120). In this study, the NPI-4 subscore, comprising of NPI subscores identified as the main Lewy-body cluster (delusions, hallucinations, apathy and depression), was first used (I McKeith et al., 2000). They found significant increases on the NPI-4 in last observation carried forward and the observed cases analyses, but not for their intent to treat analysis for rivastigmine treatment compared to placebo. They also found significant increases for rivastigmine compared to placebo for all analyses using NPI-10. Their other secondary variables were not found to be significantly different following rivastigmine therapy compared to placebo (MMSE, clinical global change-plus (CGC-plus)). A study was conducted in 2012 by Mori and colleagues, which investigated the efficacy of donepezil for the treatment of DLB (n=140). They found significant improvements following donepezil therapy compared to placebo on a number of tests, including MMSE, Wechsler Memory Scale-Revised (WMS-R) attention/concentration, category fluency, letter fluency, Wechsler Adult Intelligence Scale (WAIS-III), NPI-10, NPI-2, NPI-4, and Zarit Caregiver Burden Interview (E. Mori, Ikeda, & Kosaka, 2012). A more recent publication by the same group similarly investigated donepezil efficacy for the treatment of DLB (Ikeda, Mori, Matsuo, Nakagawa, & Kosaka, 2015). They found a significant increase on MMSE in the 10mg donepezil group compared to placebo, but found no significant improvements on NPI-2 (hallucinations and cognitive fluctuation subscores) compared to placebo.
1.8.4. Investigations of cerebral perfusion with cholinesterase inhibitor therapy

Three studies have investigated changes in perfusion on brain SPECT following treatment with ChEIs in LBD in an attempt to determine the neural correlates of cognitive and neuropsychiatric improvement, but the results have been heterogeneous (Ceravolo et al., 2006; T. Mori, Ikeda, Fukuhara, Nestor, & Tanabe, 2006; John T. O’Brien, Firbank, Mosimann, Burn, & McKeith, 2005).

The first study investigating the relationship between brain perfusion changes on SPECT and ChEI therapy was published in 2005 (John T. O’Brien et al., 2005). The authors investigated a combined group of 29 DLB and PDD participants at a baseline time point, and a follow-up time point one year later. These participants were not all drug naïve at baseline, as eight participants were already taking a ChEI. Those who began the drug during the study did so within the first month, but at follow-up, there remained two participants still not on ChEI therapy. They found a significant improvement in the NPI hallucination subscore over the one year, but no significant improvement in mean score on the Cambridge Cognitive Examination (CAMCOG) or MMSE. From baseline to follow-up, over one year, there was no significant change in perfusion either globally or through a region of interest (ROI) approach. However, in comparing changes in cognitive or neuropsychiatric tests with changes in perfusion, they found a significant relationship between decrease in hallucination subscore and increase in perfusion in the midline parietal area (posterior cingulate, precuneus, and superior parts of cuneus). They also found significant associations between increased fluctuations of consciousness and increased perfusion in the thalamus, and between increased fluctuations and decreased occipital perfusion. They found no association between changes in perfusion and beginning ChEI therapy. Their results
included both participants who were on the drug at baseline, and those who were not, and were therefore not a direct analysis of changes in relation to drug therapy.

In a study of 19 PDD participants treated with donepezil or rivastigmine over six months, there was a significant increase in perfusion within the anterior bilateral cingulate, subgyral and bilateral superior, middle and inferior frontal gyri (Ceravolo et al., 2006). Separately, they observed a significant cognitive improvement, assessed using the Alzheimer’s Disease Assessment Scale-cognitive (ADAS-cog) total score. The total MMSE score showed a small but non-significant improvement, and the MMSE attention subscore significantly improved. Finally, there was no significant change in motor performance, assessed using the UPDRS-motor subscale. They therefore concluded that there was an association between cognitive improvement, specifically on attention, and the functional changes that were seen on perfusion imaging, although no direct analysis was conducted between the two.

Finally, Mori and colleagues conducted a similar study to that conducted by Ceravolo et al. (2006), but only with DLB participants (n=20) who had a history of visual hallucinations (T. Mori et al., 2006). The participants were treated with donepezil for three months. After three months, they saw a significant improvement on both MMSE and NPI total score, with the most significant NPI subscore being hallucinations. This significant improvement in a shorter period of time might be related to their inclusion of only participants who have a history of visual hallucinations, as they have been shown to respond better to ChEI therapy (Ian G McKeith et al., 2004). Separately, Mori and colleagues also found a significant increase in perfusion in occipital regions (2006). They concluded that occipital cholinergic deficits, occipital hypoperfusion and visual hallucinations might be related. They speculated that the increase in occipital perfusion is associated with improvement of visual hallucinations, although no direct association was made.
To date, no study investigating the direct relationship between changes in brain perfusion following the treatment of drug naïve individuals with ChEI and the cognitive or neuropsychiatric changes has been reported.
2.1 Synopsis and Overall Research Aims

Since LBD is the second most common form of neurodegenerative dementia, there has been, in the past two decades, a growing body of research into this disease. Early studies were interested in the clinical and pathological features, and developing and validating consensus criteria for diagnosing this disease. Clinicopathologic correlation studies sought to determine how pathological features translated into clinical symptomology. As it became clear that there was a large role of cholinergic deficits in cognitive and neuropsychiatric symptoms of the disease, ChEIs were evaluated for their efficacy in treating LBD. The treatment studies of PDD and DLB were not consistent in their results. Some studies showed positive primary outcomes and some studies showed differences in cognitive and/or behavioural outcomes, but other studies were unable to do so (see section 1.8). There is clearly heterogeneity of response, as displayed by RCTs.

Imaging studies aimed to determine biomarkers to better recognize the disease in clinical and research settings, and to potentially explain cognitive and behavioural presentations of LBD. Few studies, however, have tested brain-behaviour correlations as they relate to the clinical improvement seen when LBD individuals are treated with ChEIs. Using functional neuroimaging to outline these brain-behaviour correlations remains an important aspect in understanding this disease, as it may localize some of the observed clinical improvements following the treatment with ChEIs. The few studies that have investigated both functional neuroimaging and cognitive
and/or behavioural symptoms produced conflicting results. This could be the result of differences in methodology. First, different subjects were used in these three studies. The participants ranged from PDD and DLB participants already taking ChEI, to PDD participants only, to DLB participants with a history of visual hallucinations. While each of these participant groups was selected for a particular reason in these studies, they are not representative of LBD as it would present in a clinical setting. Second, there was only one study that directly tested the association between cognitive and behavioural improvements and functional neuroimaging changes in response to ChEI treatment. As the participants in this study were not ChEI naïve at baseline, their results cannot be definitively attributed to ChEI therapy. Finally, the sample sizes in these studies have been quite small, with the largest study group being 29 individuals. Consequently, similar studies with a larger sample size are warranted.

To summarize, while it has been established that ChEIs are an effective treatment of cognitive and neuropsychiatric symptoms of LBD, our understanding of the direct effects of these drugs in the brain is limited. With the heterogeneity of results in ChEI response studies, and limited evidence of brain-behaviour relationships of this response, a predictive response biomarker like SPECT might be useful for the purpose of a “personalized medicine” approach to treatment and management of this disease. Therefore, the overall research aim of this thesis is to investigate the functional neuroimaging correlates of response to ChEIs on brain SPECT in LBD.
2.2 Specific research aims and corresponding hypotheses

The specific research aims and the corresponding hypotheses are as follows:

A) Research aim 1: To assess the clinical effects of ChEI therapy on cognitive and behavioural symptoms of LBD in ChEI naïve individuals with DLB and PDD.
Hypothesis 1: ChEI naïve individuals with LBD will show cognitive and behavioural improvements following ChEI therapy.
This is based on previous RCTs and meta-analyses (discussed in detail in Chapter 1) that show ChEIs are effective at treating clinical symptoms, or preventing significant decline in LBD.

B) Research aim 2: Identify regions in the brain where changes in brain perfusion on SPECT occur in response to ChEI therapy.
Hypothesis 2: Blood flow changes, identified using brain SPECT, will occur in participants after six months of ChEI therapy. Increased blood flow is hypothesized to occur in the occipital lobe.
Although no consensus was reached as to where perfusion increases occur following treatment with ChEIs, all three studies that investigated a longitudinal change in perfusion with ChEI therapy found an increase in perfusion in at least one cortical region of the brain. Since one of these studies found an increase in occipital perfusion (though there was not a direct association made in that study) (T. Mori et al., 2006), and since it is well-known that LBD participants present with occipital hypoperfusion, it is hypothesized that these changes will be observed in the occipital region.

C) Research aim 3: To identify brain SPECT perfusion correlates of clinical response to six months of ChEI therapy.
Hypothesis 3: Improvements in clinical symptoms in response to ChEI therapy will directly correlate with regional changes in perfusion on brain SPECT.

Although two studies did not directly investigate how clinical changes correlate with functional imaging changes, and the study that directly analyzed this correlation was not on ChEI naïve individuals, it is plausible that improvements clinically would have neural correlates, and the aim is to show a direct relationship through this research thesis.
Chapter 3
Methods

3.1 Subjects

Unrelated participants with clinical diagnoses of PDD (Emre et al., 2007) or DLB (I G McKeith et al., 2005) were recruited (ClinicalTrials.gov Identifier: NCT01944436). Participants in the PDD group were taking a stable parkinsonian medication, have a diagnosis of clinically definite PD more than one year prior to onset of any cognitive deficit with at least two of the following symptoms: asymmetric resting tremor, rigidity or bradykinesia, and definite motor response to dopaminergic agents. Participants in the DLB group had a diagnosis of clinically probable or possible DLB with at least one of the core clinical features. Demographic (age, sex, level of education) and clinical (age of onset, and duration of disease at investigation) data were ascertained. Inclusion criteria for patients with DLB or PDD included: age > 50 years; English-speaking; mild-moderate or severe dementia (Mini-Mental State Exam [MMSE] > 9); contact on at least four of seven days/week with a responsible caregiver; education ≥ grade 8; and Hoehn & Yahr motor stage ≤ 4. Exclusion criteria were: age < 50, contact on less than four of seven days/week with a responsible caregiver, Hoehn & Yahr stage > 4, medication changes over the preceding month including changes to quetiapine or clozapine (if used), use of anticholinergic medications, active psychiatric disorder not attributable to LBD, history of prior stroke, brain tumor, hospitalization for head injury, active central nervous system illness, prior deep brain stimulation, enrolment in another study using neuropsychological testing in the past 12 months, and lack of consent from either the subject or an identified close contact.
3.2 Neuropsychological, neuropsychiatric and functional assessments

Each participant underwent a comprehensive and standardized neuropsychological battery, as well as assessments of neuropsychiatric and functional ability at a baseline time point and a follow-up time point, six months later. These assessments were conducted by the clinical staff in the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre. The assessments used are described in this section.

General cognition was assessed using Folstein’s MMSE (Folstein, Folstein, & McHugh, 1975), Mattis Dementia Rating Scale (DRS) (Mattis, 1970), and Clock Drawing Test (Rouleau, Salmon, Butters, Kennedy, & McGuire, 1992). These tests give an indication of the overall level of cognitive impairment in LBD. To assess language function and naming, the Boston Naming Test (BNT) and semantic fluency test were used. BNT is used to measure confrontational word retrieval, by asking the individual to name each picture they are shown (B. W. Williams, Mack, & Henderson, 1989). In the semantic fluency test, individuals are asked to name as many words in a semantic category, such as animals, as they can in a set period of time (Gladsjo et al., 1999). Memory was assessed using the California Verbal Learning test, where an individual is asked to remember as many words from a list as they can, in any order (Delis, Kramer, & Ober, 1987). Attention and working memory were assessed using the Forward and Backward Digit Span tests from the Wechsler Memory Scale-Revised (WMS-R), in which participants are asked to remember a set of numbers, in sequence, either forward or in reverse (Wechsler, 1987). A number of tests were used to assess executive function: phonemic (F- A- and S- word) fluency, in which individuals are asked to list as many words as they can that begin with a specified letter.
(Lezak, 1983); Trail Making Test A and B, where an individual is asked to connect a set of dots, as fast as they can, in order (Lezak, 1983); and the Wisconsin Card Sorting Test (WCST), which is a test of set shifting abilities (Heaton, 1981). Finally, visuospatial ability was assessed using the Rey-Osterrieth Complex Figure test, where individuals must reproduce a complex drawing (Lezak, 1983); and the Benton Judgment of Line Orientation, which assesses an individual’s ability to match line angle and orientation in space (Lezak, 1983).

Behavioural symptoms were assessed using the Neuropsychiatric Inventory (NPI), which evaluates dementia related behavioural symptoms using 12 subscores: depression, hallucinations, anxiety, apathy, night-time behaviour, appetite and eating behaviour, agitation/aggression, delusions, aberrant motor behaviour, irritability, disinhibition, and euphoria (J L Cummings, 1997). The NPI is scored out of a total of 144. Each of the 12 subscores is scored out of 12, with each score representing the frequency multiplied by the severity of each neuropsychiatric symptom. Higher scores indicate higher frequency and severity of symptoms, and a score of zero indicates that the symptom is not present. The NPI was scored as both a total score, encompassing all 12 subscores, as well as a DLB related, abbreviated score, which included the four subscores that were more commonly associated with DLB: delusions, hallucinations, apathy and depression (McKeith 2000).

Motor symptoms were evaluated using the Unified Parkinson’s Disease Rating Scale (UPDRS) (Fahn, Elton, & Committee, 1987). The Disability Assessment for Dementia (DAD) was used to assess basic self-care and instrumental activities of daily living (Gélinas, Gauthier, McIntyre, & Gauthier, 1999)
3.3 Recruitment and informed consent

Participants for this study were recruited from memory and movement disorders clinics. Subjects, who were potential candidates for the study, underwent screening in a clinical setting, to ensure that they met all the inclusion and exclusion criteria (Section 3.1). If they were eligible to participate in the study, an informed consent form for patients and caregivers was given, reviewed, and completed. This form included a request for the individual’s participation, the purpose and procedures of the study, any risks and benefits associated with their participation, and a description of confidentiality of their information. Once they read through these sections, a voluntary consent form was provided by both the participant and their substitute decision maker (Appendix B).

3.4 Study Design

This was a prospective and longitudinal observational study of rivastigmine efficacy in LBD. All participants were ChEI naïve at baseline. Following baseline imaging and testing, ChEI therapy was initiated, and participants were treated for six months with rivastigmine, which was selected because of level I evidence for benefits in LBD and because it is the current standard of care, as rivastigmine is approved for clinical use in Canada. If participants had intolerance to this drug, they were switched to another ChEI, most commonly, donepezil. Standard doses and titration schedules were employed. Rivastigmine was administered at 1.5 mg bid for 1 month, 3 mg bid for 1 month, 4.5 mg bid for one month and up to maximum of 6 mg bid or to the maximally tolerated oral dose. Donepezil was administered at 5 mg od for one month then 10 mg od, up to the maximum tolerated dose. Six participants were on a rivastigmine patch (three participants at 5 mg TD od and three at 10 mg TD od). Figure 1 shows the study timeline.
A standardized and comprehensive neuropsychological battery (Section 3.2) was administered at baseline and at follow up after approximately six months of ChEI therapy. The Unified Parkinson’s Disease Rating Scale (UPDRS) (Fahn et al., 1987) was also administered at these time points to assess motor function. Standardized $^{99}$mTc-ECD SPECT scans were performed at baseline and were also repeated after six months of ChEI therapy. SPECT was chosen as the method of functional neuroimaging given that it is more widely available and cost effective than FDG-PET, and is clinically available in Ontario for brain disease, which will increase compliance and reduce likelihood of artifacts due to motion (S. Colloby & O’Brien, 2004). Note: During the study, $^{99}$mTc-ECD became unavailable, and $^{99}$mTc-HMPAO was used in some patients. Tracer type was used as a covariate to correct for this in the analyses. Six participants discontinued the drug early. Since this number is too small for a statistically meaningful control group, they were included in the analysis, which was done on an intention to treat basis.

**Figure 1.** Individual subject study participation timeline.
3.5 Single-photon emission computed tomography data acquisition

Participants were imaged using a triple-headed rotating gamma camera (Picker 3000XP; Phillips Medical Systems Inc., Cleveland, Ohio) equipped with an ultra-high-resolution, low-energy, fan-beam collimator, approximately 15 to 30 minutes following injection of tracer. Patients were injected in a quiet environment with their eyes open during the injection phase (Misch et al., 2014). One hundred and twenty views were acquired on a 128 x 128-pixel image in a continuous acquisition mode. The total imaging time was roughly 19 minutes. Images were iteratively reconstructed. Gamma ray attenuation was corrected for using the Chang method (Chang, 1978), and the attenuation correction also accounted for scatter.

3.6 Image processing

Image data were transferred to a Windows-based computer and converted to ANALYZE format. Data were analyzed using MATLAB R2016b (The MathWorks. Inc.), and Statistical Parametric Mapping version 12 (SPM12, Wellcome Department of Imaging Neuroscience, University College London, London, UK). Baseline scans for each participant were iteratively registered to an MNI-derived SPECT template using linear registration, as well as a combination of affine and elastic registration. A group average of these spatially transformed scans was calculated, and was used as a group template for further spatial registration. Each participant’s repeat scan was linearly registered to the participant’s baseline scan, using an SPM12 linear registration. All transformations were mathematically combined into a single tri-linear interpolation into 2 x 2 x 2 mm³ MNI space, to reduce interpolation artifacts. Each image was divided by mean cerebellar perfusion to normalize scan intensity and smoothed using a 12mm Gaussian kernel. Proportional
scaling was used to account for inter-participant differences in image intensity (S. J. Colloby et al., 2002). Images were masked for whole brain to eliminate extracranial voxels from the analysis.

3.7 Statistical calculations

Statistical analysis of demographic, clinical and neuropsychological variables was performed using the Statistical Package for the Social Sciences (SPSS), version 23. Imaging analysis was completed using Statistical Parametric Mapping (SPM) software, version 12.

3.7.1 Demographic, clinical and neuropsychological measures

Normally-distributed demographic information was compared using a Student’s t-test or else equivalent non-parametric tests were used. Normality of demographic and neuropsychological data was determined through visual examination of Q-Q probability plots. Categorical data were evaluated with Chi-squared test. For the comparison of neuropsychological test scores, age-matched, normalized Z-scores were calculated (Appendix D). The mean Z-score was 0, with a standard deviation (SD) of 1. Positive Z-scores indicate that an individual is above the average for a population their age, based on normative data (Appendix D), while a negative Z-score indicates that they are below average, relative to age-matched controls. Regression analyses were completed to determine whether there was a significant impact of four covariates on longitudinal changes on neuropsychological and neuropsychiatric tests: time between visits, disease duration, baseline MMSE, and years of education. Where there was a significant effect on the longitudinal changes, the covariate was included in the analysis, and a repeated measures ANCOVA was used. If no covariate significantly affected the longitudinal change, a paired T-test was used. Equivalent non-parametric tests were used for non-normally distributed results. Since the first
part of this study, which investigated clinical response over time, was performed to establish the
typical response profile in our sample as compared to that shown in other studies, we did not
correct for multiple testing, as this was done to assess whether our sample was similar to other
LBD groups.

3.7.2 Statistical parametric mapping: voxel-wise single photon emission computed tomography analysis

Voxel-wise changes in perfusion following six months of ChEI therapy were assessed using
repeated measures ANCOVA implemented on SPM12. Statistical parametric maps were
thresholded at 100 voxels. As all three previous studies found either a direct or indirect
relationship between change in perfusion and improvement in visual hallucinations, both visual
hallucinations and visuospatial ability, which are well-established as being impaired in LBD,
were the focus of the longitudinal SPECT correlations. Relationships between changes in
perfusion and neuropsychological and neuropsychiatric changes were assessed using repeated
measures ANCOVA in SPM12, where change in perfusion was the dependent variable and
independent variables included baseline and follow-up scores for neuropsychological scores and
NPI test scores. This method adds power to the analysis, as both baseline and follow-up
perfusion images and scores were included in the model, rather than comparing calculated
difference images against calculated difference scores. Due to the observational nature of the
study, ensuring a constant time period of six months in between assessments was not always
possible. Therefore, time between visits was included as a covariate in all primary longitudinal
perfusion analyses. Both a peak-level and cluster-level of significance were calculated using
SPM. The peak level is “the chance (p) of finding (under the null hypothesis) a peak with this or
a greater height (T- or Z-statistic), corrected/uncorrected for search volume” and the cluster level
is “the chance (p) of finding a cluster with this or greater size (ke), corrected/uncorrected for
search volume” (Ashburner et al., 2016). Statistical parametric maps were initially thresholded at p<0.05, corrected for Family Wise Error Rate (FWE) and False Discovery Rate (FDR). A FWE is a false positive anywhere in the statistical parametric maps, and the FWE rate is the proportion of statistical parametric maps that contain a false positive. Correcting for FWE at the level of p=0.05, means that 1 in every 20 statistical parametric maps that are created contains one or more false positives (Ashburner et al., 2016). The FDR is the proportion of false positives (null hypothesis incorrectly rejected) among those tests (where multiple tests were performed) for which the null hypothesis was rejected (Genovese, Lazar, & Nichols, 2002). If the threshold for FDR or FWE correction was not met, statistical parametric maps were thresholded at an exploratory and uncorrected p<0.001. Corresponding coordinates for maximal peaks were converted to Talairach space using the Yale Non-linear MNI to Talairach Converter (http://www.bioimagesuite.org/Mni2Tal/index.html). Sensitivity analyses were also conducted, using tracer type and age of onset as additional covariates.
Chapter 4
Results

4.1 Participant discontinuation

Fifty-seven participants were recruited for this study, and completed the baseline assessment. After six months of active therapy, 10 participants did not return for follow-up (Figure 1). This includes four participants who discontinued the drug due to adverse events, including nausea, gastrointestinal (GI) symptoms, syncope, autonomic symptoms, orthostatic hypotension and dizziness; one participant who did not take the prescribed medication throughout the six months of active therapy phase; one participant who had a catatonic episode; and four participants were lost to follow-up. Forty-seven participants completed at least a follow-up MMSE. Three participants refused further testing. Of the 44 participants who did return for follow-up assessments, four participants did not complete a follow-up SPECT scan. Forty participants completed both baseline and follow-up SPECT scans and at least part of the neuropsychological and neuropsychiatric batteries, and were included in the primary SPECT analysis. Six participants discontinued the drug, but returned to complete follow-up assessments. The reasons for the discontinuation included increases in temperature, blood pressure, tremor and drooling, nausea, diarrhea, difficulty swallowing, coughing, sleepiness, lethargy, refusal to eat, akathisia, RBD, weight loss, anxiety, and lack of compliance with medication directions (Figure 1). Thirty-four participants remained on the drug for the full six months of active therapy and underwent both SPECT studies; they were included in a post-hoc sensitivity analysis for participants who completed both baseline and follow-up assessments and were on drug for the full duration of the study.
Figure 1. Flow chart of participant drop-out.
4.2 Demographic and clinical data

There were significantly more men in the PDD group compared to the DLB group (p<0.05), which is consistent with prior literature (R. Savica, Boeve, & Logroscino, 2016). As a result of the PDD diagnostic criteria used (Emre et al., 2007), the age of parkinsonism onset was significantly lower in the PDD group compared to the DLB group, where parkinsonism onset was later and in most cases contemporary with dementia onset. At baseline, the PDD group had a significantly higher UPDRS total motor score, compared to the DLB group, but there was no significant difference in cognitive ability, as measured by the MMSE at baseline (Table 1). There were no other significant demographic differences between the PDD and DLB group, and as such, they were combined into one LBD group for the purpose of the analysis relating to ChEI response.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>DLB (n=25)</th>
<th>PDD (n=32)</th>
<th>All (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex*</td>
<td>13 M 12 F</td>
<td>27 M 5 F</td>
<td>40 M 5 F</td>
</tr>
<tr>
<td>Handedness</td>
<td>24 R 1 L</td>
<td>31 R 1 L</td>
<td>55 R 2 L</td>
</tr>
<tr>
<td>Years of Education (mean ± SD years)</td>
<td>14.56 ± 4.0</td>
<td>15.31 ± 3.6</td>
<td>14.98 ± 3.8</td>
</tr>
<tr>
<td>Age of Dementia Onset (mean ± SD years)</td>
<td>70.62 ± 7.6</td>
<td>70.57 ± 9.4</td>
<td>70.59 ± 8.5</td>
</tr>
<tr>
<td>Age of Parkinsonism Onset (mean ± SD years)*</td>
<td>71.40 ± 6.9</td>
<td>65.74 ± 9.2</td>
<td>68.16 ± 8.7</td>
</tr>
<tr>
<td>Duration of Dementia at Investigation (mean ± SD years)</td>
<td>3.70 ± 1.9</td>
<td>2.94 ± 2.9</td>
<td>3.30 ± 2.5</td>
</tr>
<tr>
<td>Baseline MMSE</td>
<td>23.80 ± 4.5</td>
<td>24.22 ± 3.5</td>
<td>24.04 ± 3.9</td>
</tr>
<tr>
<td>Baseline UPDRS motor score*</td>
<td>25.71 ± 7.6</td>
<td>30.63 ± 9.0</td>
<td>28.52 ± 8.7</td>
</tr>
</tbody>
</table>

*Significantly different, p<0.05

Table 1. Demographic features of participants with DLB and PDD. The number of patients (n) tested is listed next to the individual measures. Missing values are due to the patients being lost to follow-up. F = Female; M = Male; DLB = Dementia with Lewy bodies; PDD = Parkinson’s disease with dementia; n = sample size; SD = Standard deviation.
4.3 Longitudinal Neuropsychological and Neuropsychiatric Battery Changes

Results from the neuropsychological and neuropsychiatric batteries are shown in Table 2. The neuropsychological battery scores are presented as age matched Z scores. Missing values are a result of participants being lost to follow-up, refusing to complete the task, or being unable to do so due to progression of their dementia. There was no significant change in measures of general cognition following six months of ChEI therapy (p<0.05). Following treatment with ChEIs, there were significant improvements on working memory, executive, language, memory and visuospatial tasks: Forward Digit Span (Figure 2), corrected for baseline MMSE (p=0.006, n=42, effect size=0.175); Boston Naming, corrected for visit latency (p=0.006, n=43, effect size=0.169); CVLT long delay free recall (p=0.044, n=38, effect size=0.108); WCST – Category score (p=0.027, n=31, effect size=0.153); and Benton Judgment of Line Orientation (Figure 3, p=0.043, n=33, effect size=0.122). There was no significant decline on any test score during the course of this study. Additionally, there was no significant increase in UPDRS total motor score, indicating that there was no motor worsening over the course of ChEI therapy.
<table>
<thead>
<tr>
<th></th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Motor ability</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPDRS motor score</td>
<td>28.18 ± 8.0</td>
<td>28.49 ± 9.6</td>
</tr>
<tr>
<td><strong>General cognition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMSE [n=47]</td>
<td>-2.14 ± 2.2</td>
<td>-2.10 ± 2.6</td>
</tr>
<tr>
<td>DRS Total Score [n=42]</td>
<td>-1.35 ± 1.1</td>
<td>-1.40 ± 1.0</td>
</tr>
<tr>
<td>Clock Drawing Test</td>
<td>-1.16 ± 1.9</td>
<td>-1.00 ± 1.6</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston Naming* [n=43]</td>
<td>-0.18 ± 1.8</td>
<td>0.079 ± 1.9</td>
</tr>
<tr>
<td>Semantic Fluency [n=35]</td>
<td>-1.83 ± 0.8</td>
<td>-1.79 ± 0.9</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVLT Long Delay Free Recall* [n=38]</td>
<td>-1.82 ± 1.0</td>
<td>-1.76 ± 1.3</td>
</tr>
<tr>
<td>CVLT Long Delay Cued Recall [n=40]</td>
<td>-1.85 ± 1.2</td>
<td>-1.69 ± 1.2</td>
</tr>
<tr>
<td><strong>Attention &amp; working memory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span – forward* [n=42]</td>
<td>2.04 ± 2.8</td>
<td>2.34 ± 2.4</td>
</tr>
<tr>
<td>Digit Span – backward [n=39]</td>
<td>0.822 ± 2.5</td>
<td>1.40 ± 2.4</td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WCST – categories* [n=31]</td>
<td>-1.81 ± 0.6</td>
<td>-1.60 ± 0.7</td>
</tr>
<tr>
<td>WCST – perseverative errors [n=30]</td>
<td>0.87 ± 0.6</td>
<td>0.30 ± 1.3</td>
</tr>
<tr>
<td>Phonemic (FAS) fluency [n=38]</td>
<td>-1.12 ± 1.0</td>
<td>-0.94 ± 1.2</td>
</tr>
<tr>
<td>Trails Making Test A [n=42]</td>
<td>-1.89 ± 0.9</td>
<td>-1.62 ± 0.7</td>
</tr>
<tr>
<td>Trails Making Test B [n=38]</td>
<td>-2.14 ± 0.6</td>
<td>-1.93 ± 0.9</td>
</tr>
<tr>
<td><strong>Visuospatial ability</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rey – Copy [n=39]</td>
<td>-4.14 ± 3.8</td>
<td>-4.01 ± 3.5</td>
</tr>
<tr>
<td>BJLOT /30* [n=33]</td>
<td>-0.52 ± 1.0</td>
<td>-0.16 ± 1.1</td>
</tr>
</tbody>
</table>

*Significantly different, p<0.05

**Table 2.** Mean Z-scores on neuropsychological battery (± SD)

The number of patients (n) tested is listed next to the individual measures. Missing values are either due to the patients being lost to follow-up, or due to the inability of participant or caregiver to complete the test.

MMSE = Mini Mental State Examination; DRS = Dementia Rating Scale; CVLT = California Verbal Learning Test; WCST = Wisconsin Card Sorting Test; Rey = Rey Osterrieth Complex Figure Test; BJLOT = Benton Judgment of Line Orientation; n = sample size; SD = Standard deviation.

a Model corrected for visit latency.
b Model corrected for duration of disease.
c Model corrected for baseline MMSE score.
Figure 2. Mean Z-scores on Forward Digit Span (n=42) at baseline and follow up (± SD). Missing values are either due to the patients being lost to follow-up, or due to the inability of participant to complete the test.

Figure 3. Mean Z-scores on Judgment of Line Orientation Test (n=33) at baseline and follow up (± SD). Missing values are either due to the patients being lost to follow-up, or due to the inability of participant to complete the test.
Based on the known effect of ChEIs on visual hallucinations, this was one of our primary outcomes of interest (I McKeith et al., 2000; T. Mori et al., 2006; John T. O’Brien et al., 2005). The mean hallucination subscore of the NPI (Figure 4) was significantly lower following six months of ChEI therapy (p=0.01, n=41, effect size=0.287). This subscore measures the severity and frequency of hallucinations, including visual hallucinations, which is one of the core clinical features of LBD (Ian G. McKeith et al., 2017). There was additionally a significant decrease in aberrant motor behaviour (p=0.011, n=41, effect size=0.284) and a significant increase in night-time activity (p=0.044, n=41, effect size=0.222). Although there was a trend for a small improvement on the total mean neuropsychiatric inventory score, this was not statistically significant. The change in NPI-4 following ChEI therapy was not significant (p=0.304).

**Figure 4.** Mean Neuropsychiatric Inventory-Hallucination subscores (n=41) at baseline and follow up (± SD). Missing values are either due to the patients being lost to follow-up, or due to the inability of participant to complete the test.
In terms of neuropsychiatric symptoms, 95% (49/52) of LBD participants presented with at least one symptom at baseline. Furthermore, 90% (46/52) of participants presented with one or more of the four symptoms that are most common in DLB, as represented by a non-zero value on the NPI-4 composite score. The frequencies for the 12 subscores, at baseline, in order of declining frequency, were as follows: depression, 63% (33/52); hallucinations, 58% (30/52); anxiety, 52% (27/52); apathy, 50% (26/52); night-time behaviour, 44% (23/52); appetite and eating behaviour, 35% (18/52); agitation/aggression 35% (18/52); delusions, 31% (16/52); aberrant motor behaviour, 29% (15/52); irritability, 21% (11/52); disinhibition, 10% (5/52); euphoria, 0%.

At follow-up, 93% (40/43) of participants remaining in the study had at least one neuropsychiatric symptom. At least one of the four symptoms that are most common in DLB, as measured by the NPI-4 composite score, was present in 88% (38/43) of remaining participants. The frequencies for the 12 subscores, at follow-up, in order of declining frequency, were as follows: depression, 67% (19/43); anxiety, 51% (22/43); apathy, 51% (22/43); night-time behaviour, 51% (22/43); hallucinations, 49% (21/43); irritability, 35% (15/43); appetite and eating behaviour, 33% (14/43); agitation/aggression 33% (14/43); delusions, 23% (10/43); disinhibition, 16% (7/43); aberrant motor behaviour 14% (6/43); euphoria, 9% (4/43).

With respect to functional changes assessed using the DAD, there was no significant change over the course of the six months of ChEI therapy. This was true for both activities of daily living, as well as for instrumental activities of daily living.
## Table 3. Mean scores (± SD) on behavioural and functional measures at baseline and follow-up time points.

The number of patients (n) tested is listed next to the individual measures. Missing values are either due to the patients being lost to follow-up, or due to the inability of caregiver to complete the test. DAD = Disability Assessment for Dementia; ADL = Activities of daily living; iADL = Instrumental activities of daily living; NPI = Neuropsychiatric Inventory; DLB = Dementia with Lewy bodies. The NPI-4 DLB composite score includes delusions, hallucinations, apathy and depression subscores, which are frequently seen in DLB (I McKeith et al., 2000).

<table>
<thead>
<tr>
<th>Neuropsychiatric Symptoms</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPI total /144 [n=41]</td>
<td>17.22 ± 18.1</td>
<td>16.44 ± 16.1</td>
</tr>
<tr>
<td>NPI-4 DLB composite score /48 ~</td>
<td>7.73 ± 8.2</td>
<td>6.6 ± 7.2</td>
</tr>
<tr>
<td>NPI hallucinations /12 *</td>
<td>2.46 ± 3.3</td>
<td>1.37 ± 2.2</td>
</tr>
<tr>
<td>NPI appetite and eating behaviour /12</td>
<td>2.29 ± 3.5</td>
<td>1.78 ± 3.0</td>
</tr>
<tr>
<td>NPI apathy /12</td>
<td>2.05 ± 2.9</td>
<td>2.46 ± 3.1</td>
</tr>
<tr>
<td>NPI night-time behaviour /12 *</td>
<td>1.98 ± 3.0</td>
<td>3.00 ± 3.3</td>
</tr>
<tr>
<td>NPI depression /12</td>
<td>1.68 ± 2.0</td>
<td>1.85 ± 2.0</td>
</tr>
<tr>
<td>NPI anxiety /12</td>
<td>1.54 ± 2.1</td>
<td>1.61 ± 2.3</td>
</tr>
<tr>
<td>NPI delusions /12</td>
<td>1.49 ± 3.0</td>
<td>0.95 ± 2.3</td>
</tr>
<tr>
<td>NPI aberrant motor behaviour /12 *</td>
<td>1.24 ± 2.6</td>
<td>0.46 ± 1.5</td>
</tr>
<tr>
<td>NPI agitation/aggression /12</td>
<td>1.20 ± 2.4</td>
<td>1.05 ± 2.0</td>
</tr>
<tr>
<td>NPI irritability/lability /12</td>
<td>0.90 ± 2.0</td>
<td>1.17 ± 2.2</td>
</tr>
<tr>
<td>NPI disinhibition /12</td>
<td>0.37 ± 1.4</td>
<td>0.41 ± 1.5</td>
</tr>
<tr>
<td>NPI euphoria /12</td>
<td>0.00 ± 0.0</td>
<td>0.17 ± 0.7</td>
</tr>
<tr>
<td>NPI caregiver distress /12</td>
<td>11.50 ± 10.2</td>
<td>12.00 ± 11.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activities of daily living</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DAD (%) [n=41]</td>
<td>71.41 ± 23.0</td>
<td>69.42 ± 23.4</td>
</tr>
<tr>
<td>DAD ADL (%)</td>
<td>85.20 ± 18.5</td>
<td>81.35 ± 22.0</td>
</tr>
<tr>
<td>DAD iADL (%)</td>
<td>62.02 ± 28.4</td>
<td>60.56 ± 28.9</td>
</tr>
</tbody>
</table>

*Significantly different, p<0.05.
4.4 Longitudinal Perfusion Changes

Figure 5 shows average perfusion distribution on brain SPECT for those participants who completed both baseline and follow-up scans (n=40). In the whole LBD group (n=40), there was a significant increase in perfusion in occipital regions, following 6 months of ChEI therapy (p=0.035, corrected for Family Wise Error [FWE]). Figure 6 and Table 4 show the voxels where perfusion significantly increased compared to baseline. The peak voxels that showed significantly increased perfusion were in the right and left visual association areas (Brodmann’s area 18). These results were controlled for visit latency.

<table>
<thead>
<tr>
<th>Anatomical locus (Brodmann area)</th>
<th>Talairach coordinates</th>
<th>Number of Voxels</th>
<th>SPM t-score (P-value)</th>
<th>Cluster-level P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Visual Association Area (18)</td>
<td>18  -89  26</td>
<td>1775</td>
<td>4.54 (P&lt;0.001)</td>
<td>P=0.035*</td>
</tr>
<tr>
<td>Left Visual Association Area (18)</td>
<td>-18  -89  27</td>
<td></td>
<td>3.94 (P&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Left Visual Association Area (18)</td>
<td>-38  -87  13</td>
<td></td>
<td>3.81 (P&lt;0.001)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Areas of increased perfusion on brain SPECT following ChEI treatment, controlled for visit latency.
*Corrected for Family-Wise Error. SPM = Statistical parametric mapping; SPECT = single-photon emission computed tomography; ChEI = Cholinesterase inhibitor.
Figure 5. Average perfusion on brain SPECT (n=40) at baseline (A) and follow-up (B). White arrows indicate occipital areas of interest.
Figure 6. Statistical Parametric Map depicting regions of increased perfusion on brain SPECT following cholinesterase inhibitor therapy, controlled for visit latency. Clusters presented contain at least 100 voxels. SPECT = single-photon emission computed tomography
Sensitivity analyses were conducted to include covariates that may additionally affect the model. Of the 40 participants who completed both baseline and follow-up brain SPECT scans, 24 had brain SPECT scans using ECD as the tracer. Two participants had baseline brain SPECT scans with ECD tracer, and follow-up brain SPECT scans with HMPAO tracer. Brain SPECT scans were completed at both time points with HMPAO tracer for 14 participants. For this reason, tracer type was one of the covariates that were included in the sensitivity analysis.

When the model was corrected for tracer type, there remained a significant cluster with peak voxels located in the right and left visual association areas (Brodmann’s area 18, p<0.001, uncorrected), but power was less due to inclusion of the covariate. With tracer as a covariate, perfusion was also significantly increased in the right inferior parietal lobe (Brodmann’s area 7, p<0.001, uncorrected).

A second sensitivity analysis was conducted that removed the two participants for whom there was a change in tracer type. Again, the results remained largely the same, with peak voxels in the right and left visual association areas (<0.001, uncorrected), and local maximums in Brodmann’s area 7, bilaterally, which is the inferior parietal lobe (p<0.001, uncorrected).

A separate model with age of onset as a covariate showed a similar increase in perfusion in the right and left visual association areas (p<0.001, uncorrected); this model also identified increased perfusion in the left inferior occipital gyrus (Brodmann’s area 19, p<0.001, uncorrected).
4.5 Longitudinal correlations between cognitive and behavioural scores and perfusion

There was a significant association between increased perfusion in the occipital regions and decreased frequency and severity of visual hallucinations following treatment (p<0.001, uncorrected). Peak voxels for this relationship, shown in Figure 7 and Table 5, were also in the right and left visual association areas (Brodmann’s area 18) and the right and left inferior occipital gyrus (Brodmann’s area 19).

There was also a smaller cluster wherein there was a significant association between improved Rey-Osterreith Complex Figure test score and increased perfusion (p<0.001, uncorrected). The peak voxel for this correlation, shown in Figure 8 and Table 5, is located in the right inferior occipital gyrus (Brodmann’s area 19).

<table>
<thead>
<tr>
<th>Anatomical locus (Brodmann area)</th>
<th>Talairach coordinates</th>
<th>Number of Voxels</th>
<th>SPM t-score (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPI – hallucinations subscore</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right visual association area (18)</td>
<td>18</td>
<td>-89</td>
<td>26</td>
</tr>
<tr>
<td>Right inferior occipital gyrus (19)</td>
<td>24</td>
<td>-85</td>
<td>34</td>
</tr>
<tr>
<td>Left visual association area (18)</td>
<td>-14</td>
<td>-85</td>
<td>27</td>
</tr>
<tr>
<td>Rey-Osterrieth Complex Figure test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right inferior occipital gyrus (19)</td>
<td>22</td>
<td>-87</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 5. Areas where improvements in visual hallucinations and visuospatial ability correlate with increased perfusion on brain SPECT following ChEI treatment; model controlled for visit latency. SPM = Statistical parametric mapping; SPECT = Single photon emission computed tomography; ChEI = Cholinesterase inhibitor. NPI = neuropsychiatric inventory.
Figure 7. Statistical Parametric Map depicting regions where increased perfusion on brain SPECT following cholinesterase inhibitor therapy correlate with improvements in visual hallucinations, controlling for visit latency. Clusters presented contain at least 100 voxels. SPECT = single-photon emission computed tomography
Figure 8. Statistical Parametric Map depicting regions where increased perfusion on brain SPECT following cholinesterase inhibitor therapy correlate with improvements in visuospatial ability, assessed using the Rey Osterreith Complex Figure test and controlled for visit latency. Clusters presented contain at least 50 voxels. SPECT = single-photon emission computed tomography.

The same sensitivity analyses were conducted to include tracer type and age of onset as covariates. The perfusion results as they relate to visual hallucinations remained the same when correction for age of onset was added to the model. When the model for the association of visual hallucinations and perfusion was instead corrected for tracer type, the peak voxels for the relationship between increased perfusion following ChEI therapy and decreased frequency and severity of visual hallucinations were in the right and left visual association areas (Brodmann’s area 18) and in the right inferior occipital gyrus (Brodmann’s area 19), but not the left inferior occipital gyrus (p<0.001, uncorrected).

When age of onset was added to the model of significant associations between increased perfusion following six months of ChEI treatment and improvement on Rey-Osterreith score,
there remained significantly increased perfusion in the right inferior occipital gyrus (Brodmann’s area 19). There was additionally an increase in perfusion in the right visual association area using this model (Brodmann’s area 18, p<0.001). Using tracer type as a covariate instead, there was a significant association between increased perfusion following six months of ChEI therapy and improvement on Rey-Osterreith test in the left and right inferior occipital gyrus (Brodmann’s area 19, p<0.001), as well as the right visual association area (Brodmann’s area 18, p<0.001). These clusters were smaller than those in the original model: right hemisphere cluster $k_E=48$, left hemisphere cluster $k_E=26$.

The results presented in this section, describing significant associations between longitudinal improvements in cognitive and behavioural measures and increased perfusion did not survive correction for multiple testing using false discovery rate (FDR) or FWE methods.

4.6 Post-hoc sensitivity analysis

Six participants discontinued the drug before the six month active treatment period was complete (Figure 1), but completed the follow-up assessment, nonetheless. These participants were included in the previously described analysis using intent to treat analytical methods. To further investigate whether the effects that were seen were due to the effect of the drug, a post-hoc sensitivity analysis was completed using only those who remained on the drug for the duration of the investigation. Table 6 is an outline of the areas in which perfusion increased in those participants who remained on cholinesterase inhibitors (n=34) for the full six months of treatment. Though the clusters were smaller than the analysis with a larger sample size, the
results remained largely unchanged, with peak voxels of increased perfusion in the right and left visual association areas.

<table>
<thead>
<tr>
<th>Anatomical locus</th>
<th>Talairach coordinates</th>
<th>Number of Voxels</th>
<th>SPM t-score (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right visual association area (18)</td>
<td>18  -89  26</td>
<td>298</td>
<td>4.19 (P&lt;0.001)</td>
</tr>
<tr>
<td>Left visual association area (18)</td>
<td>-38  -85  11</td>
<td>99</td>
<td>3.77 (P&lt;0.001)</td>
</tr>
</tbody>
</table>

Table 6. Areas of increased perfusion on brain SPECT following ChEI treatment, for those participants who remained on drug for 6 month duration.
*Corrected for Family-Wise Error. SPM = Statistical parametric mapping; SPECT = single-photon emission computed tomography; ChEI = Cholinesterase inhibitor.
5.0 Summary and General Discussion

This thesis examined the changes in cognitive, behavioural and functional features of individuals who met criteria for PDD or DLB (Emre et al., 2007; Ian G. McKeith et al., 2017) after they were treated with ChEIs for approximately six months. Further, neural correlates of these changes in clinical measures were assessed using a voxel-wise approach to data analysis of SPECT perfusion images. This thesis provides insight into both LBD as a disease, and response to ChEI from a brain-behaviour correlative perspective, and confirms findings and associations deduced from previous studies. Our findings will be discussed in this chapter in the context of prior literature. Strengths, limitations, and future directions will also be discussed.

5.1 Neuropsychological, neuropsychiatric and functional responses to cholinesterase inhibitor therapy in Lewy body disease

The first specific research aim of this thesis was to assess the clinical effects of ChEI therapy on cognitive, behavioural, and functional symptoms of LBD in ChEI naïve individuals. We hypothesized that ChEI naïve individuals would show improvement following ChEI therapy. Though we did not find a significant improvement on tests of general cognition (MMSE, DRS, Clock drawing test), there was no significant decline in cognitive ability, as measured by these tests. Changes on MMSE scores in placebo groups from RCTs over 24 weeks ranged from declining by 0.2 points, to improving by 0.1 points (Dubois et al., 2012; Emre et al., 2004; I
McKeith et al., 2000). Over a 12 week duration, changes on MMSE scores ranged from declining by 0.4 points to improving by 0.6 points in placebo groups in RCTs with LBD (Ikeda et al., 2015; E. Mori et al., 2012). With that being said, we were able to detect domain-specific cognitive improvements. Specifically, these improvements were shown on tests of attention and working memory, as well as executive and visuospatial ability, which are the domains that are most likely to be impaired in the LBD cognitive profile (Emre et al., 2007; Ian G. McKeith et al., 2017). These improvements were measured using the Digit Span test (backward), WCST category subscore, and Benton Judgment of Line Orientation test (BJLOT), respectively (p<0.05). These findings were consistent with our hypothesis that ChEI naïve individuals would show cognitive improvements following six months of ChEI therapy. Furthermore, there was no significant decline on measures of cognitive function, as measured by any domain specific cognitive test. Previous studies have found similar results with respect to domain specific neuropsychological results. Significant improvement in attention following rivastigmine therapy in PDD was shown using CDR power of attention tests (Emre et al., 2004). Donepezil similarly improved attention compared to placebo in PDD, as measured by the BTA (Dubois et al., 2012). Attention was also shown to improve through donepezil therapy in DLB, using the WMS-R attention/concentration test (E. Mori et al., 2012), and through rivastigmine therapy in DLB on the three attentional tests from the computerized cognitive assessment (I McKeith et al., 2000). Executive function was significantly improved following donepezil and rivastigmine therapy in PDD, which was shown using D-KEFS Verbal Fluency Test (Dubois et al., 2012; Emre et al., 2004). Executive function was also shown to improve following donepezil therapy in DLB compared to placebo, using the Verbal Fluency Test (E. Mori et al., 2012). Visuospatial ability was only measured in one of the larger studies investigating ChEI efficacy in LBD (E. Mori et
al., 2012). Although they did not find this to improve following donepezil therapy, the authors speculate that this was due to a ceiling effect.

The NPI was used to assess changes in neuropsychiatric and behavioural symptoms, over the course of six months of ChEI therapy. Our results found no significant change in NPI total score or NPI-4 DLB composite score (I McKeith et al., 2000). Individually, the subscores that significantly improved were hallucinations and aberrant motor behaviour. There was no significant worsening in most symptom domains, including appetite and eating behaviour, apathy, depression, anxiety, delusions, agitation and aggression, irritability and lability, disinhibition and euphoria. There was a significant increase in night-time behaviour. This could be due to a worsening of RBD, as a result of acting out dreams. Three previous studies, which were included in meta-analyses investigating ChEI efficacy in LBD, specifically investigated behavioural improvements following ChEI therapy in DLB and PDD using the NPI (Emre et al., 2004; I McKeith et al., 2000; E. Mori et al., 2012). In a study of donepezil in DLB (n=140), individuals on ChEI therapy, compared to placebo, improved significantly on NPI-4, but not NPI-10 (10 item version of NPI, which does not include night time activity or appetite and eating change) (E. Mori et al., 2012). Individually, they also saw some improvement in delusions, hallucinations, and cognitive fluctuation in those individuals on therapy, whereas there was deterioration in those symptoms on placebo. They did not report individual significance levels for each NPI subscore. Two studies investigated change in NPI with the treatment of rivastigmine, which was the first line of therapy in our study (Emre et al., 2004; I McKeith et al., 2000). McKeith et al. investigated the drug in individuals with DLB (n=120), and found a significant improvement on NPI-4 in treated participants compared to placebo. There was also a significant improvement, compared to placebo, on the NPI-10, for observed cases and last
observation carried forward cases, but not in intent to treat cases. The domains where they saw improvement were apathy, indifference, anxiety, delusions, hallucinations, and aberrant motor behaviour. There was a decline in depression, agitation, irritability, night time behaviour, and appetite and eating change. A second study, investigating PDD individuals (n=410), found that NPI-10 significantly improved with treatment compared to placebo (Emre et al., 2004). Given that our sample size was much smaller than these studies and we were detecting within subject changes, rather than comparing against placebo, there was less power to detect potentially small changes. However, visual hallucinations had the highest score at baseline, indicating that the combined frequency and severity were the highest, and thus there was more power to detect an improvement in this subscore (mean change was -1.09). Our results are consistent with previous studies insofar as there was an overall trend toward decrease in neuropsychiatric symptoms, though most of these decreases were non-significant. McKeith et al. included in their study a 3-week discontinuation period, during which all improvements re-emerged rapidly (2000), suggesting that, while this treatment is not disease modifying, it is able to treat the symptomology, including one of the core clinical features.

As mentioned, since this part of the thesis was largely to demonstrate a typical response profile shown in these studies, we did not correct for multiple comparisons. Furthermore, the cognitive tests used are highly correlated with each other, which was a further reason not to correct for multiple testing. Our primary outcome of longitudinal perfusion was corrected for multiple testing (FWE-corrected).

All of the larger trials presented in this section investigated either PDD or DLB; however, our cohort included both, allowing a representative sample of LBD. While our sample did not include placebo groups like the larger studies that have been discussed (Dubois et al., 2012;
Emre et al., 2004; I McKeith et al., 2000; E. Mori et al., 2012), we found similar results to these studies with regard to domain specific neuropsychological and neuropsychiatric responses to ChEIs. This validates our use of this sample as a representative sample of ChEI response in both PDD and DLB and the use of this sample for our hypotheses relating to functional neuroimaging.

5.2 Longitudinal functional imaging changes following cholinesterase inhibitor therapy

The second specific research aim was to identify regions in the brain where changes in perfusion on SPECT occur in response to ChEI therapy. We found a significant increase in perfusion in the right and left visual association areas (BA 18) following six months of ChEI therapy. This comparison survived correction for multiple comparisons (FWE). In the context of longitudinal imaging studies in this disease group, the sample size for this comparison (n=40) was larger than those completed in the past, which had between 19 and 29 participants (Ceravolo et al., 2006; T. Mori et al., 2006; John T. O’Brien et al., 2005). As previously described, these studies produced discordant results, and found increased perfusion in different areas of the brain. We hypothesized, that, since it is well known that there is occipital hypoperfusion on brain SPECT in participants with LBD (Lobotesis et al., 2001; John T O’Brien et al., 2014), this would be the area most likely to show increased perfusion following ChEI therapy, and our results were consistent with this hypothesis in an unbiased, voxel-based analysis.

It has been suggested that, in addition to cholinergic projections from the nbM modulating neuronal activity in cortical regions of the brain, intracerebral cholinergic nerve fibers are also vasodilators, and have a role in increasing CBF to cortical regions to prepare for increased neuronal activity (Sato & Sato, 1992). By this logic, Fong et al. suggested that the occipital
hypoperfusion often seen in LBD could be the result of both decreased neuronal activity due to decreased cholinergic input, as well as vascular effects, from the nbM (2011). Furthermore, occipital gray matter has been shown not to change, structurally, on MRI in DLB individuals (Middelkoop et al., 2001). Our results, showing increased occipital perfusion following treatment with ChEIs, suggest the possibility that an increase in cholinergic activity, through ChEI therapy, results in some reversal of the occipital hypoperfusion normally seen in DLB. This is in line with Fong and colleagues, and suggests that occipital hypoperfusion is likely due to decreased cholinergic input, which may be at least partially reversed through the use of ChEIs. In the post-hoc sensitivity analysis, which included only those who remained on active ChEI therapy for the full duration of the study, there remained significantly increased perfusion in the right and left visual association areas, similar to the results of the intent to treat analysis. This suggests that the increased perfusion in these areas was a result of drug therapy, rather than that of another confounding factor. The clusters that resulted from the post-hoc analysis were much smaller than that which included all 40 participants, which could be the result of decreased statistical power, due to a smaller sample size. More details regarding the cognitive and behavioural implications of these findings will be described in the next section.

5.3 Longitudinal correlations between cognitive and behavioural scores and perfusion following cholinesterase inhibitor therapy

The final specific research aim was to identify brain SPECT perfusion correlates of clinical response to ChEI therapy. Consistent with our hypothesis, we saw direct correlations between cognitive and behavioural changes and perfusion changes. The correlations between improvement in visual hallucinations and visuospatial ability with increased occipital perfusion
highlight direct brain-behaviour correlations following ChEI therapy that have not yet been shown in this disease group. One explanation of this novel finding is that visual hallucinations and visuospatial deficit observed in LBD may be attributable to occipital hypoperfusion, and increased perfusion in this region could be indicative of the cognitive and behavioural benefits that have been shown following ChEI therapy. Since this result was uncorrected for multiple comparisons, this could be is a false positive result, however, given that this correlation is in the same region as that in which increased perfusion was observed, it is less likely to be so.

Mori et al. found both elementary and higher-order visuoperceptual dysfunction in participants who had DLB compared to AD, and concluded that this reflects dysfunction of the visual cortices. Furthermore, they suggest that this plays a role in the development of behavioural and cognitive symptoms of DLB that are related to vision, such as visual hallucinations (Mori 2000). There have been studies conducted in DLB participants that compare imaging patterns in individuals with hallucinations to those without. Imamura and colleagues compared DLB participants with hallucinations, DLB participants without visual hallucinations and AD participants using FDG PET imaging (Imamura et al., 1999). Patients had a history of hallucinations, but were not hallucinating during the PET study. They found a relatively preserved metabolism in the right temporoparietal association areas and hypometabolism in the primary visual cortex in the DLB group with visual hallucinations, and concluded that these metabolic patterns might be associated with the presence of visual hallucinations. In a study using $^{18}$F-FDG PET imaging, decreased uptake was found in downstream visual association areas, rather than the primary visual cortex, including the right occipito-temporal junction and the right middle frontal gyrus in DLB participants with visual hallucinations compared to those without (Perneczky et al., 2008). The occipital hypometabolism often described in DLB,
especially compared to AD, was also described as being localized to the visual association cortex (Higuchi et al., 2000). In addition to involvement of posterior primary visual and visual associative areas, other causes of visual hallucinations have been postulated. The Perception and Attention Deficit model theorizes that there is a combination of top-down and bottom-up processes that involve impaired attentional binding and perceptual processes, respectively, that result in the activation of incorrect perceptual proto-objects (Collerton, Perry, & McKeith, 2005). A study that supports this theory, showed significantly lower perfusion on brain SPECT in participants with visual hallucinations, compared to those without, in the left anterior cingulate cortex, left orbitofrontal cortex and the left cuneus (Heitz et al., 2015). In addition to a comparison of two separate groups of DLB participants with and without hallucinations, they also correlated perfusion with hallucination severity. In doing so, they found a significant correlation between hallucination severity and decreased perfusion in the anterior cingulate cortex, left orbitofrontal cortex, right parahippocampal gyrus, right inferior temporal cortex and left cuneus. They concluded, in line with the Perception and Attention Deficit model, that there is involvement of both top-down (anterior) and bottom-up (posterior) processes in visual hallucinations. The heterogeneity of the body of research attempting to determine neural correlates of visual hallucinations could be explained by the large variability between individuals within this disease, as well as the differing methodology and imaging modalities used across these studies.

Similarly, attempts to investigate neural correlates in the context of cholinesterase inhibitors has led to heterogeneous results, likely due to differences in methodology and large variability in this disease group. Of the three studies investigating perfusion changes during ChEI therapy, one found anterior perfusion increases (Ceravolo et al., 2006), while two others found posterior
perfusion increases (T. Mori et al., 2006; John T. O’Brien et al., 2005). The study by Ceravolo and colleagues, which found frontal and bilateral cingulate perfusion increases, investigated PDD participants only, and did not present any information on visual hallucinations, except to say that hallucinations did not worsen with treatment (2006). Therefore, the increase in anterior perfusion cannot be linked to visual hallucinations in these participants based on those results. A second study, investigating both PDD and DLB participants directly compared perfusion with visual hallucinations, although not in participants who were ChEI naïve at baseline (John T. O’Brien et al., 2005). They found that a decreasing hallucination score was associated with increased perfusion in the posterior cingulate. They concluded that their results were in line with the model that visual hallucinations are both visual and attentive dysfunction (Collerton et al., 2005). They suggested that improvement in attention to visual stimuli may be caused by improved integration of primary and associative visual areas, as a result of increased cholinergic input due to ChEI therapy. This is in line with past studies that have connected visual hallucinations to visual associative areas, as well as one that found involvement of the primary visual cortex. Since many of these participants were already on ChEI therapy at baseline, and the study took place over one year, increased parietal perfusion could also be a longer-term effect of cholinergic therapy. The third study investigating visual hallucinations in relation to ChEI therapy, investigated only participants with a history of visual hallucinations (T. Mori et al., 2006). Though their analysis did not involve a direct correlation between the severity of hallucinations and perfusion, they concluded that increased occipital perfusion and improvement of visual hallucinations were likely related, as all of their participants had a history of this symptom. They found increased perfusion, following ChEI therapy in the right and left inferior occipital lobe. Their peak coordinates correspond with the right and left visual association areas.
(BA 18), which is similar to our results. They concluded that occipital cholinergic deficits, occipital hypoperfusion, and visual hallucinations may be related. The results of this thesis show that there is a direct correlation between improved visual hallucinations and improved visuospatial ability and increased occipital perfusion, which strengthens Mori and colleagues’ proposed relationship between the two. While our results do not necessarily rule out the findings that have shown improvements to perfusion in anterior parts of the brain, they do show improved posterior perfusion following ChEI therapy. Moreover, they draw a direct relationship between these posterior increases in perfusion with visual deficits, including visuospatial ability and visual hallucinations.

5.4 Clinical impact and significance

The significance of this study is that it is the first to directly analyze the relationship between change in perfusion on brain SPECT scans and modifications in cognitive and behavioural symptoms following ChEI therapy in LBD individuals, who were ChEI naïve at baseline, in the largest sample published to date. The potential clinical impact of this study is that it sets the stage for the development of a functional neuroimaging response biomarker to ChEI therapy. This could aid clinicians in objectively monitoring a LBD patient’s response to ChEI, instead of only relying upon subjective clinical observation. Occipital hypoperfusion is characteristic of LBD, and is included as a supportive biomarker for the diagnosis of DLB (Ian G. McKeith et al., 2017). Our results show that increased perfusion in the occipital lobe should be investigated as a response biomarker for the efficacy of ChEI therapy in LBD, with particular emphasis on improving visuospatial ability and decreasing frequency and severity of visual hallucinations. Although meta-analyses have shown that the overall effect of ChEI therapy for LBD is an
improvement in cognitive and behavioural symptoms, there is still considerable variability among individual response. Future development of increased occipital perfusion following ChEI therapy as a response biomarker would fill a gap in the research, which is that there are no validated imaging response biomarkers to ChEI therapy in LBD. Furthermore, those LBD patients with occipital hypoperfusion at baseline could indicate a potentially more favourable response to ChEIs, which is characteristic that should also be investigated further. This region could also be monitored throughout the process, and if no increase in perfusion in this area is observed, this may indicate non-responders early in the treatment process, which will allow a clinician to switch over to an alternative therapy. This trend toward “personalized medicine” could reduce both the potential for adverse effects in an individual who is not showing clinical benefit of taking the drug, and as well, improve cost effectiveness by preventing continued therapy that is not successful in that individual.

5.5 Comment on neuroimaging methods
The approach used for neuroimaging analysis in this thesis (Ceravolo et al., 2006; S. J. Colloby et al., 2002; John T. O’Brien et al., 2005) was an unbiased voxel-wise analysis of change in perfusion on brain SPECT using SPM. Statistical parametric maps are image processes, where the values of voxels are distributed, under the null hypothesis, according Student’s T or F distribution (Friston, Ashburner, Kiebel, Nichols, & Penny, 2007). The values measured at each voxel, in the case of SPECT relate to intensity, which is an indication of relative perfusion. Statistical analysis is performed on a voxel-by-voxel basis using both the general linear model and Gaussian random field theory to make inferences over the whole volume of the brain.
Voxels with changes in intensities which cluster together identify areas of changed relative perfusion from the first time point to the second, within the group.

As described in Chapter 1, the two main functional neuroimaging techniques that are used to characterize cerebral metabolism and perfusion in LBD as compared to controls and other disease groups are PET and SPECT, respectively. A brief description of these two imaging modalities will now be provided in order to compare and contrast their strengths and weaknesses as they relate to longitudinal imaging in LBD. The focus will be on FDG-PET and either HMPAO or ECD SPECT.

Both PET and SPECT use radioactive nuclides, which can be used in tracer studies to investigate brain function *in vivo* (S. Colloby & O’Brien, 2004). The most commonly used PET tracer is FDG, which is a marker of cerebral glucose metabolism (Bailey, Townsend, Valk, & Maisey, 2005). FDG is radioactively labeled with an $^{18}$F isotope of fluorine, which is synthesized in a cyclotron through the acceleration of protons into the nuclei of oxygen atoms. As a result of this process, the extra proton in the nucleus of fluorine renders the isotope unstable, with a relatively short half-life of 110 minutes. The isotope is then incorporated into a deoxyglucose molecule to form FDG, which is administered intravenously and regionally distributed to the brain.

Following a delay to allow appropriate uptake of the isotope, the individual is placed in a PET scanner. As the isotope undergoes positron emission decay, the emitted positron travels a short distance, and then collides with an electron. This interaction results in two gamma photons, which travel in opposite directions, and are simultaneously detected by two opposing gamma detectors that encircle the individual’s head. Throughout the whole scanning process, many detections of this nature are made along one plane, from which a three-dimensional image of the brain can be derived.
Brain SPECT is another method of functional neuroimaging that is frequently used in LBD and other dementias. The differences between SPECT and PET underlie the advantages and limitations associated with the use of SPECT instead of PET. The most common tracers in LBD for a study of regional cerebral perfusion are HMPAO and ECD, both labeled with $^{99m}$Tc, as they are taken up proportionally to cerebral blood flow (Matsuda, Yagishita, Tsuji, Hisada, & Matsuda, 1995). SPECT imaging employs the use of gamma cameras, comprised of collimators, detection crystals and photomultiplier tubes to detect the gamma radiation that is emitted by the SPECT tracers trapped in the brain (Masdeu, Ph, & Arbizu, 2008). The collimators reject photons that are outside a very small angular range, such that the angle of incidence can be determined, and the origin of the gamma ray can be localized (Rahmim & Zaidi, 2008). The gamma camera in a SPECT tracer rotates around the individual’s head, acquiring two-dimensional images, which can be reconstructed to achieve a three-dimensional view of the brain. The limitation of detecting just one gamma photon for SPECT is that the spatial resolution that results from this imaging technique is lower than that seen in PET. The main advantage, however, for SPECT over PET is that the radioisotopes used for SPECT have a relatively long half-life, lasting between a few hours to a few days (S. Colloby & O’Brien, 2004). As a result, they are relatively cheap and easy to produce, making SPECT imaging a cheaper and more readily available method of imaging in a hospital setting. For this reason, and based on availability of SPECT imaging in the clinical setting, SPECT was chosen as the tracer for this study, because the results are meant to be translated into regular clinical practice.

Studies have been conducted in the past investigating differences in perfusion between ECD and HMPAO tracers in normal and AD patients (Inoue et al., 2003; H. Ito et al., 2006; Koulibaly et al., 2003; Koyama et al., 1997). In a study of healthy adults, higher radioactivity was found in
the midbrain, right caudate, bilateral putamen, bilateral thalamus, and left superior temporal gyrus in HMPAO compared to ECD. Radioactivity was found to be higher in ECD compared to HMPAO in the left lingual gyrus (Koyama et al., 1997). Differences between HMPAO and ECD were shown in another study of healthy adults, although significance levels were reported as compared to PET as opposed to within SPECT tracers (H. Ito et al., 2006). Differences with age were investigated in a study of healthy adults, with two groups: ECD and HMPAO. They found an increase in perfusion with age in the right anterior frontal lobe in the HMPAO group, and a decrease in perfusion with age in the bilateral retrosplenial cortex in the ECD group (Inoue et al., 2003). This was not a longitudinal study, and age was included as a covariate. Finally, a study investigated how the tracers perform in AD found significantly higher uptake ratio values in ECD compared to HMPAO in the right and left precentral gyri (BA 4), right cuneus, left superior and middle temporal gyri (BA 21), and in HMPAO compared to ECD in the right globus pallidus, right and left hippocampus, left mesencephalon and left caudate nucleus (Koulibaly et al., 2003). Due to circumstances beyond our control, the ECD tracer became unavailable during the study, and HMPAO was used instead. Since this study is looking at the longitudinal differences in tracer uptake within subjects, rather than comparing uptake between subjects, the effect of this variability due to tracer differences will have less of an impact. Further, the areas that have been described as variable between the two tracers are different from those in which we found significant changes, longitudinally. In our sensitivity analyses, tracer type was included as a covariate, and the results remained largely the same, though it no longer survived correction for multiple comparisons. In addition, a further sensitivity analysis was performed, with the two participants removed for whom the tracer was different at baseline and follow-up. Again, the results were largely the same, but did not survive correction for multiple comparisons.
5.6 Limitations

The limitations associated with this study are common in this field of research and also related to the nature of the phenotype of LBD. The first limitation is the challenge of performing a study of this nature without a placebo control group. Early studies that investigated the efficacy of ChEIs at treating LBD compared a treatment group to a control group of LBD participants who were not receiving treatment (i.e., placebo). Although it would be informative to compare perfusion changes over six months in LBD participants who were receiving ChEI therapy with those who were not, having such a control group would not be possible. Rivastigmine, the primary ChEI used in this study is approved by Health Canada as the standard of care for effective use in the treatment of PDD, as a result of positive results from these earlier studies. Donepezil is also approved in Canada. For this reason, it would be unethical to withhold an effective therapy from LBD individuals for six months.

Another limitation is the potential bias in the model due to the fact that those participants who remained in the study may have been those who benefitted from the drug. Those who dropped out of the study could be those who could not tolerate the drug due to side effects, or those who continued to decline. The six month duration of the study was designed based on the duration of some of the larger ChEI studies that have been conducted, which found a beneficial effect of ChEIs in LBD (Dubois et al., 2012; Emre et al., 2004). Unfortunately, during that time, there were some drop-outs. The dropout rate in this group was taken into consideration in the number of participants that were recruited at baseline (n=57). At follow-up, 40 participants completed both baseline and follow-up SPECT scans, which is larger than that of past studies that investigated ChEIs and perfusion (n=19-29) (Ceravolo et al., 2006; T. Mori et al., 2006; John T.
O’Brien et al., 2005). Drop out in this disease group is not uncommon, for a number of different reasons, including those that were observed in our sample: diarrhea, nausea, difficulty swallowing, coughing, sleepiness, lethargy, akathisia, RBD, weight loss, and anxiety, as well as increases in temperature, BP, tremor, and drooling (Chapter 4). This limitation should continue to be factored into the recruitment process in future studies of this disease group, to ensure the final analysis includes enough participants. Although our sample size would be considered large compared to other studies (Ceravolo et al., 2006; T. Mori et al., 2006; John T. O’Brien et al., 2005), it had a low power to detect differences, which was particularly apparent in the SPM analysis, where correlations were not able to survive correction for multiple testing.

Furthermore, due to the clinical and observational nature of this study, an exact time difference between the first and last appointments was not always exactly six months. This could have an effect on the perfusion changes that were observed. For this reason, the time between visits for each participant was included as a covariate in the primary longitudinal perfusion analyses.

Since participants completed the same cognitive battery at both baseline and follow-up, and in the absence of a placebo control, there is potential for practice effects. Six months’ time between visits would help mitigate some of this effect.

A final point that should be mentioned is that participants were not hallucinating while they were injected with the SPECT tracer. For this reason, our results that are related to hallucinations are indicative of a trait rather than state. The NPI probes caregivers for the presence of psychiatric symptoms in the recent past. Therefore, the correlation of visual hallucinations with perfusion is indicative of a change in the trait of visual hallucinations. Since they are so variable, it would be difficult to time the scan to correspond with visual hallucinations. With that being said, although
the NPI provides subjective information related to the observations of the caregiver, the longitudinal nature of this study reduces some of the within subject variability that may arise from such a test.

5.7 Future Directions

The results of this thesis illustrate the neural correlates of cholinesterase inhibitor efficacy in LBD. We have shown that ChEIs are effective at reducing some of the characteristic symptoms of LBD, including, but not limited to, visual hallucinations. Furthermore, the longitudinal effect of ChEI therapy on cerebral perfusion was shown to be a relative increase in occipital perfusion, which is well-established as a region of hypoperfusion (supportive diagnostic biomarker) in LBD. There was also a direct correlation between increased occipital perfusion and improvements in visuospatial ability and visual hallucinations. Our results warrant replication in a larger sample size to increase the power of the analysis. Although our sample was large compared to other functional neuroimaging studies of ChEIs in this disease group, a replication in an even larger sample will increase the power, such that correlation analysis can confirm our novel results, and survive correction for multiple comparisons.

Though these results fill a gap in the body of research on this topic that has been done to date, there are more gaps yet to be filled. While the overall effect of ChEI is one of cognitive and behavioural benefits (Matsunaga et al., 2015; Stinton et al., 2015; Wang et al., 2015), response is still quite variable from individual to individual, ranging from no response, to slowed rate of cognitive decline, to improvement. Moreover, adverse effects are also common, and limit the use of ChEI in certain individuals, which was evidenced by the participants who were unable to
complete our study due to adverse events. The answer to the variability in both response and adverse effects may, in part, be explained by genetic differences. Genetic variation, or polymorphisms, can determine how an individual handles a drug (pharmacokinetics) or the way the drug interacts with targets in the body of the individual (pharmacodynamics). Including pharmacogenetic analysis in a replication study of these results would add a novel line of investigation, and, in combination with imaging biomarkers, would help define response to these drugs in an objective way.
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Appendix A
Consent Form

L. C. CAMPBELL COGNITIVE NEUROLOGY
RESEARCH UNIT

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Co-Investigators: Dr. Sandra Black, Sunnybrook Health Sciences Centre; Dr Nathan Herrmann, Sunnybrook Health Sciences Centre; Dr. Bradley MacIntosh, Sunnybrook Health Sciences Centre.

INFORMED CONSENT FORM FOR PATIENTS AND SUBSTITUTE DECISION MAKERS

PARKINSON’S RELATED DEMENTIA: A PHARMACOGENOMIC STUDY OF CHOLINESTERASE INHIBITORS INVESTIGATING COGNITIVE BENEFIT, MOTOR WORSENING, AND BRAIN SPECT.

1. REQUEST FOR PARTICIPATION

We are asking for your participation in this research project. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take as much time as you need to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. We appreciate you taking the time to read, inquire about, and understand the following information. Your primary care physician will be notified if you decide to participate in the study.

2. PURPOSE

Dementia with Lewy Bodies and Parkinson’s Disease with Dementia are neurodegenerative conditions which cause significant disability, and reduced quality of life in the elderly. The mainstay of treating the symptoms is through the use of rivastigmine or other cholinesterase inhibitors (e.g. donepezil or galantamine). These are drugs used to alter levels of the brain chemical, acetylcholine. This chemical is deficient in these disorders. Not all people respond the same way to this medication; that is, some people have an excellent response, while others have a poor response. The purpose of this research is to investigate this variability from person-to-person in the response to cholinesterase inhibitors. By using advanced brain imaging techniques,
changes in specific imaging measures over time and how they correlate with memory and other cognitive functions in affected patients will be examined. These changes over time in response to cholinesterase inhibitors will then be correlated with variation in genes that are thought to be involved in the action of the drug.

3. PROCEDURES

In the first part of the assessment, participants will be asked to complete a number of cognitive tests which will involve solving problems designed to investigate memory and other mental processes. The tests include activities such as answering questions, learning simple material and sorting cards. The testing procedure will take approximately three to four hours to complete (including breaks). These tests are often administered for clinical investigation of cognitive impairment. In addition, some computerized reaction time tasks and questionnaires on mood state and activities of daily living may be administered. Participants will then be asked to undergo brain scanning at Sunnybrook using Single Photon Emission Computerized Tomography (SPECT). SPECT uses brain blood flow to measure brain function. It requires the injection of a small amount of radioactive tracer through a vein in the arm. A radioactive tracer is a chemical that is radioactively labeled and is taken up by the brain briefly to assess blood flow and then is eliminated from the body. SPECT scans are usually obtained in the initial clinical work up and the results are reported as part of the participant’s clinical record. These scans take about 45 to 60 minutes to complete.

The second part of the study begins with the initiation of treatment with a cholinesterase inhibitor. These drugs are NOT experimental and are the standard of care used to treat Lewy body diseases. The dose of this drug will be increased slowly according to standard protocols and will be adjusted based on your tolerability and response. After 12 weeks of treatment, we will contact you to find out how you are tolerating the drug. After 24 weeks of treatment, you will be asked to return to Sunnybrook to repeat the cognitive tests and SPECT scans that were done in the first part of the study.

Advances in genetics are also making it possible to look at variation in genes that may predict response to cholinesterase inhibitors. Genes provide the instructions for processes in the body and for characteristics such as eye colour. Everyone's genes differ to some degree. In other words, everyone has a unique genetic blueprint or genetic variation and this is called polymorphism. In order to look at genetic predictors of cholinesterase inhibitor response, a small blood sample will be taken from patients to determine genetic variation or polymorphism. Because these genetic relationships are currently under investigation, the results of these tests will not be made available under research practices.

3.1 Duration of the research project

The research program is currently funded by the Parkinson’s Society of Canada through to September 2013.

3.2 Scope of the research project
The research project is centered at Sunnybrook Health Sciences Centre. The genetic studies are performed at the Centre for Addiction and Mental Health. Our partner institutions include: Baycrest Centre for Geriatric Care, University Health Network, North York General Hospital, Parkwood Hospital, the University of Toronto, and the University of Western Ontario.

### 3.3 Storage and Safekeeping of DNA Samples

Your name will NOT be included with your samples. Your samples will be linked to your information by number, and this information will be available only to Dr. Masellis and his research team. Therefore, no one else will be able to associate you with the results of any tests performed on your sample.

The samples will be stored anonymously (that is, using a code number only) for up to 25 years. Only members of the research team will have access to the DNA samples and information pertinent to the DNA samples. The research team will never divulge any information concerning the genetic sampling to anyone without your prior written consent. After 25 years, all the samples will be destroyed.

### 4. RISKS

In regards to the cognitive testing, there is no anticipated risk of physical discomfort associated with these tests. SPECT scanning requires an injection with a small amount of a radioactive tracer; there is negligible blood lost through this procedure and it may cause minor discomfort and a small amount of subsequent bruising. No adverse effects have been reported with the SPECT tracer, which is not a dye, since its introduction for clinical purposes in Canada in 1987. The risk associated with exposure to this amount of radiation is approximately equivalent to the long-term risk to your health of being exposed to air pollution by living in Boston or New York for one year. SPECT scanning also involves lying still for approximately twenty minutes. In regards to the blood sample for genetic testing, there may be some pain, bruising or swelling around the area of the blood draw, and fainting can rarely occur when blood is drawn.

### 5. BENEFITS

The results of this research may not benefit individual participants but may help in the understanding of the mechanism through which cholinesterase inhibitors help to improve the clinical symptoms of Lewy body diseases. The results may also help in determining better methods to assess for response to these drugs.

### 6. CONFIDENTIALITY

#### 6.1 Safety/security of the data

All of the personal information obtained in this study will remain confidential. Samples will not be used for commercial purposes. Your confidentiality will be respected, and no information that discloses your identity will be released or published without your consent. The results of this
study may be published or communicated in other ways, but it will be impossible to identify you. This information will be coded and kept under lock and key at Sunnybrook Health Sciences Centre under the responsibility of Dr Mario Masellis.

The results of the research will not appear in your medical record.

6.2 Third-party Access to Results

For the purposes of ensuring the proper management of the research, it is possible that a member of the Research Ethics Board at Sunnybrook may consult your research data in order to verify the research procedures and/or data; however, this will be done in such a way as to maintain your confidentiality, and will be done only to the extent permitted by the applicable laws and regulations. By signing this consent form, you are authorising this access. Your rights under any applicable data protection laws are not affected.

7. COMMUNICATION OF RESULTS

You can communicate with the research team to obtain information on the general progress or the results of the research project. If you desire, project updates can be provided annually and at the end of the project. However, we will not communicate any individual results to you.

8. FREEDOM OF PARTICIPATION AND PERIOD OF REFLECTION

You are free to withdraw from the study at any time. You may take the time necessary to reflect on your decision and discuss your participation in the project with persons close to you before giving us your answer.

If you withdraw, your DNA sample will be retraced and destroyed. Also if you withdraw, your care will not be affected and we will continue to actively follow you for your ongoing medical issues.

9. COMPENSATION FOR EXPENSES INCURRED

You will be reimbursed (up to $30) for out-of-pocket expenses (e.g., parking or lunch vouchers).

10. CONTACTS

If you have any questions you can contact Dr. Masellis, the Principal Investigator of this study, at 416-480-6100 Ext. 1620 or the Research Ethics Board at Sunnybrook Health Sciences Centre at 416-480-4276.

11. VOLUNTARY CONSENT
I acknowledge that the research procedures described above have been explained to me and that any questions I have asked have been answered to my satisfaction. The possible risks and discomforts have been explained to me. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that any information obtained during the study will be confidential. No identifying particulars will be made available to anyone other than the investigators, except by my authority. I understand that participation in this study is voluntary and I may withdraw from the study at any time without affecting my health care in any way. I have been fully informed of the details of this research and I hereby give approval to participate in the study. A copy of this consent form has been given to you to keep for your records and reference.

_________________________________
Name of Participant (please print)

_________________________________
Signature of Participant Date

_________________________________
Signature of Substitute Decision Maker (if required) Date

_________________________________
Name of Witness

_________________________________
Signature of Witness Date

_________________________________
Signature of Dr. Mario Masellis Date
# Appendix B

## Single-photon emission computed tomography Protocol

**Table B1.** SPECT acquisition and processing parameters

<table>
<thead>
<tr>
<th>SPECT Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected Dose</td>
<td>740 (20 mCi) $^{99m}$Tc-ECD</td>
</tr>
<tr>
<td>Delay prior to imaging</td>
<td>15 to 30 minutes</td>
</tr>
<tr>
<td>Patient position</td>
<td>Supine</td>
</tr>
<tr>
<td>Number of views</td>
<td>120</td>
</tr>
<tr>
<td>Acquisition mode</td>
<td>Continuous</td>
</tr>
<tr>
<td>Radius of Rotation</td>
<td>$&lt;13.5$ cm</td>
</tr>
<tr>
<td>Total acquisition time</td>
<td>18.7 minutes</td>
</tr>
<tr>
<td>Collimators</td>
<td>Low-energy, ultra high resolution, fan beam</td>
</tr>
<tr>
<td>Acquisition matric size</td>
<td>128 x 128</td>
</tr>
<tr>
<td>Attenuation correction</td>
<td>$\mu=0.12$ cm$^{-1}$, Chang 1st order</td>
</tr>
</tbody>
</table>
# Appendix C

## Normative Data References

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>

MMSE = Mini Mental State Examination; DRS = Dementia Rating Scale; CVLT = California Verbal Learning Test; WCST = Wisconsin Card Sorting Test; Rey = Rey Osterrieth Complex Figure Test; BJLOT = Benton Judgment of Line Orientation;
Appendix D
Statement of Contributions

**Jordana Compagnone** (Author) prepared this thesis in its entirety. The author contributed to the data entry and quality control processes, and the maintenance of the database in which the information was stored. The author participated in the development of the objectives and data analysis plans for the study presented in this thesis. The author learned to use Statistical Parametric Mapping (SPM) software, which was used in the imaging analysis. The author completed the reconstruction of the scans, to correct for attenuation, and convert the images for analytical use, using the original workbench associated with the scanner in the Nuclear Medicine department at Sunnybrook Health Sciences Centre. With the help and guidance of Dr. Mutsaerts, the author further completed the preprocessing of the scans necessary for analysis. Finally, the author completed the analysis of all neuropsychological, neuropsychiatric and imaging data using Statistical Package for Social Sciences (SPSS) software and SPM. The study was designed and conceived by Dr. Masellis and clinical evaluations were performed by him and his research team. This work was funded by a New Investigator Award from the Parkinson Canada as well as an Early Researcher Award from the Ministry of Research, Innovation, and Science (Ontario) awarded to Dr. Mario Masellis.

**Dr. Mario Masellis** (Thesis Supervisor and Program Advisory Committee Member): provided mentorship; principal investigator for this study; conceived of the objectives and design for the study; recruited participants and carried out the study protocol; provided oversight for the preparation and final draft of the thesis; critically reviewed and provided input on the thesis; provided laboratory resources.

**Dr. Sandra Black** (Thesis Co-supervisor and Program Advisory Committee Member): provided mentorship; critically reviewed and provided input on the study presented in this thesis.

**Dr. Bradley MacIntosh** (Program Advisory Committee Member): provided mentorship; critically reviewed and provided input on the study presented in this thesis.

**Dr. Nathan Herrmann** (Program Advisory Committee Member): provided mentorship; critically reviewed and provided input on the study presented in this thesis.

**Dr. Henk Mutsaerts** aided in my training on the Statistical Parametric Mapping software that was used to analyze the brain imaging data, helped develop scripts to speed up my analytical processes and helped with any issues that came up during the analysis.

**Julian Li** performed data entry, quality control and preliminary analysis on the partial data that was present early on in the study.

**Drs. Morris Freedman, Galit Kleiner, Joyce Lee, James Kennedy, Robert Chen, David Tang-Wai and Anthony Lang** participated in the recruitment process for the study presented in this thesis.
Copyright Acknowledgements

Chapter 1:


Figure 2: The Lancet, Vol. 16, Richard L Doty, Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? Pages 478-88, Copyright 2017, with permission from Elsevier.
