Investigating the relationship between cholinergic system integrity and Parkinson's disease symptoms using MRI and EEG

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Contents

Abstract

Cholinergic cells of the basal forebrain (cBF) and pedunculopontine nucleus (PPN) are implicated in Parkinson's disease (PD), but current understanding of their role in PD symptomology is limited. Neuropathological and recent *in vivo* imaging research implies that cBF and PPN degeneration is associated with PD cognitive and mobility impairments. There remains a need to identify and validate widely accessible markers of cholinergic system degeneration to better understand its contribution to these symptoms. The aim of this thesis was to investigate how structural changes in the cBF and PPN relate to cortical activity and cognitive and mobility performance in people with PD, people with mild cognitive impairment (MCI), and healthy age-matched controls.

T1 and diffusion-weighted images were used in combination with stereotactic maps of the cBF and PPN to extract volumetric and diffusivity metrics from these regions as *in vivo* surrogate markers of structural integrity. These structural measures were assessed for their relationship with resting-state EEG, and cognitive and functional mobility performance.

People with PD showed reduced cBF volumes compared to healthy controls, and elevated PPN diffusivity compared to people with MCI. Subregional cBF volumes correlated with EEG changes in the theta-alpha range in people with PD and people with MCI. Volume loss in the cBF was also shown to mediate the relationship between executive function and Timed Up and Go dual-task performance in people with PD. PPN diffusivity metrics demonstrated correlations with cognitive performance and EEG changes in the alpha range in people with PD, and in the beta-gamma range in people with MCI.

Cortical activity measured with EEG may hold physiological relevance for structural changes occurring in the cBF and PPN. Volumetric loss in the cBF may impair the attentional-executive control of mobility functions. Elevated PPN diffusivity may impair attentional performance during tasks that require sensorimotor integration.

Declaration

No material contained in the thesis has been used in any other submission for another academic award.

The following material has been published based on work contained in this thesis:

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List of figures

List of tables

Abbreviations

AChE – acetylcholinesterase
AD – Alzheimer's disease
aD – axial diffusivity
ANCOVA – analysis of covariance
cBF – cholinergic basal forebrain
Ch1-2 – cholinergic cell clusters of the medial septum and vertical limb of the diagonal band
Ch4 – cholinergic cell clusters of the nucleus basalis of Meynert
Ch4p – cholinergic cell clusters of the posterior nucleus basalis of Meynert
ChAT – choline acetyltransferase
CSF – cerebrospinal fluid
CVLT – California Verbal Learning Test
DARTEL – Diffeomorphic Anatomic Registration using Exponentiated Lie algebra
DBS – deep brain stimulation
DLB – dementia with Lewy bodies
DMS – dorsomedial striatum
DTI – diffusion tensor imaging
DWI – diffusion-weighted imaging
EEG – electroencephalography
FaD – free water-corrected axial diffusivity
FDR – false discovery rate
FFT – Fast Fourier transform
FmD – free water-corrected mean diffusivity
FOG – freezing of gait
FrD – free water-corrected radial diffusivity
FW – free water
IQR – interquartile range
LB – Lewy body
LBD – Lewy body disease
LDT – laterodorsal tegmental nucleus
MCI – mild cognitive impairment
Mini-BESTest – Mini-Balance Evaluation System Test

MLR – mesencephalic locomotor region

MNI – Montreal Neurological Institute

MoCA – Montreal Cognitive Assessment

mD – mean diffusivity

MRI – magnetic resonance imaging

nbM – nucleus basalis of Meynert

PD – Parkinson's disease

PDD – Parkinson's disease dementia

PET – positron emission tomography

PIGD – postural instability and gait difficulty

POA – power of attention

PPN – pedunculopontine nucleus

PSD – power spectral density

qEEG – quantitative electroencephalography

rD – radial diffusivity

ROCFT – Rey Osterreith Complex Figure Test

ROI – region of interest

RT – reaction time

SPECT – single-photon emission computerized tomography

TIV – total intracranial volume

TMT – Trail Making Test

TUG – Timed Up and GO

VAChT – vesicular acetylcholine transporter

VBM – voxel-based morphometry

WAIS – Wechsler Adult Scale of Intelligence

Chapter 1.**Introduction**

1.1 Parkinson's disease context

1.1.1 *Disease burden*

Parkinson's disease (PD) is a common and progressive neurodegenerative disorder. PD incidence has been reported as 38 and 61 per 100,000 in females and males (aged ≥ 40 years), respectively (Hirsch et al., 2016). These rates increase with age, rising sharply among those aged 70 and older. Socioeconomic factors such as increased longevity and an ageing population, declining smoking rates, and increased industrialisation have been proposed as key contributors to what appears to be a rapidly growing global disease burden (Dorsey et al., 2018; Feigin et al., 2019).

A hallmark of PD is a loss of dopaminergic neurons in the substantia nigra pars compacta. However, PD is a multisystem disorder, and neuronal loss occurs in many other brain regions including the locus coeruleus, nucleus basalis of Meynert, pedunculopontine nucleus, raphe nucleus, dorsal motor nucleus of the vagus, amygdala, and hypothalamus (Jellinger, 2012; Kalia and Lang, 2015).

Abnormal aggregation of α-synuclein into Lewy bodies represents another hallmark of PD (Jellinger, 2012), which is hypothesised to progress though the brain in six stages in a caudal-to-rostral gradient, corresponding with symptom progression (Braak et al., 2003). While this scheme provides a useful heuristic, it does not apply to all cases, as demonstrated by late-stage Lewy pathology with an absence of severe motor or cognitive symptoms (Parkkinen et al., 2008).

The symptomology of PD is heterogeneously expressed, with significant motor and cognitive features underpinned by extensive pathophysiology. The evolving complexity of PD therefore presents a considerable clinical challenge, including the need for accurate prognosis at the earliest disease stages, and in the management of symptoms as the disease progresses (Kalia and Lang, 2015).

1.1.2 *Clinical features*

Motor symptoms of PD include bradykinesia, rigidity, tremor, and postural and gait impairment. However, the heterogenous clinical presentation of PD (Foltynie et al., 2002), has motivated attempts to establish disease subtypes (Marras and Lang, 2013). Common clinical groupings include tremor dominant vs postural instability and gait difficulty (PIGD) dominant. The tremor dominant subtype has been associated with slower disease progression and less functional disability than PIGD (Foltynie et al., 2002; Post et al., 2007). Furthermore, PIGD is associated with accelerated cognitive decline and higher risk of dementia (Burn et al., 2006).

Non-motor symptoms are common even in early disease stages (Khoo et al., 2013), and include cognitive impairment, autonomic dysfunction, sleep disorder, depression, and anxiety. These symptoms have a major impact on daily functioning and health-related quality of life for people living with PD (Duncan et al., 2014; Lawson et al., 2016).

As PD progresses into later stages, treatment-resistant motor and non-motor symptoms become prominent. These include more severe posture and gait disturbances (including freezing of gait, FOG), falls, and PD dementia (PDD) (Hely et al., 2005; 2008). Importantly, and relevant to this thesis, mobility and cognitive impairments have been identified as two of the top ten research priorities for the management of PD, by people living with the disease (Deane et al., 2015). These symptoms form the focus of the following discussion.

1.2 Cognitive impairment in PD

First introduced in the context of Alzheimer's disease (AD) research, the construct of mild cognitive impairment (MCI) is defined as a transitional state between normal cognitive ageing and dementia (Petersen, 2004). MCI has since been adopted in PD research (PD-MCI) as a diagnostic entity proceeding PDD. Clinical consensus criteria has been developed by the Movement Disorder Society Task Force for the diagnosis of PDD (Emre et al., 2007) and PD-MCI (Litvan et al., 2011), outlined in Table 1.1.

Both criteria require an established PD diagnosis, and a subsequent decline in cognitive function one year or more after the onset of PD motor symptoms. Two possible levels of assessment are suggested based on the availability of formal tests to assess global and/or domain-specific cognition. Impairment is indicated by scores that fall between 1-2 standard deviations (SD) below controls or normative means. Level I can be assessed with limited expertise, with abbreviated, easily reproducible tests (i.e., global cognitive measures), whereas Level II requires a more comprehensive battery. For PDD classification, an individual should have deficits in at least two of the five core cognitive domains (attention, executive, visuospatial, language, or memory), considered severe enough to impair activities of daily activities. Those with PD-MCI are, by definition, functionally independent.

Table 1.1 Summary of clinical classification criteria proposed by the Movement Disorders Society

	Clinical diagnostic criteria
PD-MCI	
Core features	Established diagnosis of PD according to QSBB criteria. Cognitive decline (after PD diagnosis) reported by patient/carer/physician. Cognitive deficits on formal neuropsychological testing *or* a scale of global cognitive abilities. Cognitive deficits not sufficient to interfere with functional independence.
Exclusion criteria	PDD diagnosis. Other explanation for cognitive impairment (e.g., stroke, depression, delirium). Other PD-associated comorbid conditions (e.g., motor impairment, severe anxiety, psychosis) that may significantly influence cognitive testing.
PD-MCI Level I	Impairment on a scale of global cognitive abilities validated for use in PD (e.g., MoCA, SCOPA-COG, PD CRS, MDRS) *or* impairment on at least two tests, when a limited battery of neuropsychological tests is performed.
PD-MCI Level II	Neuropsychological testing that includes 2 tests within each of the 5 cognitive domains (attention/working memory, executive, language, memory, visuospatial). Impairment on ≥ 2 neuropsychological tests (either two impaired tests in one cognitive domain or two impaired tests in two different cognitive domains). Impairment on neuropsychological tests may be demonstrated by: - Performance approximately 1 to 2 SDs below appropriate norms or - Significant decline from estimated premorbid levels
PDD	
Core features	Diagnosis of PD according to QSBB criteria. Gradual cognitive decline after the diagnosis of PD. Impairment of at least 2 of 5 cognitive domains (attention, executive, visuospatial, memory and language). Cognitive deficits sufficient to interfere with functional independence.
Supporting features	Presence of at least one behavioural symptom (apathy, depressed or anxious mood, hallucinations, delusions, excessive daytime sleepiness). No presence of features suggesting other conditions that make it impossible to reliably diagnose PDD (i.e., time interval between motor and cognitive symptoms not known, vascular dementia, acute confusion, major depression (using DSM IV).

Summarised from (Emre et al., 2007; Litvan et al., 2011). Abbreviations: QSBB = Queens Square Brain Bank; MoCA = Montreal Cognitive Assessment; SCOPA-COG = Scales for Outcomes of Parkinson's disease Cognition; PD CRS = PD Cognitive Rating Scale; MDRS = Mattis Dementia Rating Scale; DSM = Diagnostic and Statistical Manual of Mental Disorders.

1.2.1 *Prevalence*

PDD affects approximately 30 of every 100,000 people in the general population, and accounts for around 4% of all dementia cases among those 65 and over (Aarsland et al., 2005). For people diagnosed with PD, the cumulative risk of developing PDD ranges from 78% after 8 years (Aarsland et al., 2003) to 83% at 20 years (Hely et al., 2008).

Cognitive impairment is not just a late-stage disease problem, however. Those newly diagnosed with PD are twice as likely to develop MCI than healthy elderly individuals (Aarsland et al., 2009). Even at the time of diagnosis (drug-naïve), up to 36% may present with cognitive impairment (Aarsland et al., 2003; Foltynie et al., 2004; Santangelo et al., 2015; Weintraub et al., 2015). Cross-sectional prevalence of PD-MCI ranges from 19%-55% (Caviness et al., 2007a; Aarsland and Kurz, 2010; Litvan et al., 2011) and longitudinal data in incident PD show that rates can increase up to 50% within 5 years (Broeders et al., 2013; Pedersen et al., 2013).

1.2.2 *Profiles*

Cognitive impairment in PD can be characterised by heterogeneity, with deficits manifesting in one or more cognitive domains, with varying severity, and at different stages of the disease (Goldman et al., 2014). Executive function (the ability to plan, organise, and initiate goal directed behaviour) is typically affected (Foltynie et al., 2004; Muslimović et al., 2005; Goldman et al., 2013). Such deficits are thought to reflect dopaminergic deficiencies in frontostriatal circuits, and can be detected with tests of planning, spatial working memory, and set shifting (Kehagia et al., 2010; 2013). However, even early in PD, impairments in other cognitive domains including attention, memory, and visuospatial function are also evident (Foltynie et al., 2004; Muslimović et al., 2005; Aarsland et al., 2009), and may be driven by non-dopaminergic changes (Kehagia et al., 2013).

Two large multi-centre analyses in PD-MCI agree that single domain impairment is more common than multiple domain impairment, however one reported greater prominence of memory impairment (Aarsland et al., 2010),

while the other indicated executive dysfunction (Kalbe et al., 2016). Such discrepancies not only highlight the heterogenous profile of PD-MCI, but may also reflect wider methodological inconsistencies related to, for example, differences in the number and selection of cognitive tests, and cut-off scores used to define PD-MCI. The extent of between-study variability was highlighted recently in a large multi-centre pooled analysis, which failed to recommend a test battery that would be sensitive to detect mild cognitive deficits in PD (Hoogland et al., 2018).

People with PDD share a similar cognitive profile to those with PD-MCI, albeit more severe and complex, with impairments affecting multiple domains (Emre, 2007). Abnormalities in attention and executive function are apparent from early in the course of PDD (Noe et al., 2004; Bronnick et al., 2007), and cognitive symptoms typically fluctuate (Tröster, 2008). PDD is also associated with profound visuoperceptual impairments (Mosimann et al., 2004).

1.2.3 *Progression*

Longitudinal studies of incident PD have provided key insight into the progression of cognitive decline. For example, the CamPaIGN study (n=142) reported findings from multiple time points over a 10-year follow-up period. At 3-5 years, 10% of the cohort converted to PDD, and 57% developed cognitive impairment (Williams-Gray et al., 2007). At 10-years, 46% had developed dementia. The most relevant baseline predictors of PDD were older age, motor impairment severity, posterior-cortical cognitive deficits (i.e., visuospatial abilities), and a particular tau protein genotype (MAPT). Furthermore, there was no association between frontostriatal executive dysfunction and later dementia (Williams-Gray et al., 2013). These findings suggest two distinct cognitive syndromes carrying a different prognosis for PDD risk, each with different underlying pathophysiology.

The likelihood of developing PDD may be increased among those with PD-MCI. For example, in a community-based PD cohort (n=147), one quarter developed PDD over a 5-year follow up period. MCI, and older age at baseline predicted later dementia, and baseline results of tests measuring episodic memory,

visuospatial function, semantic fluency, and mental flexibility differed between MCI converters and non-converters (Domellöf et al., 2015).

The progression from PD-MCI to PDD is not inevitable, however. In the Norweigan ParkWest study (n=182), among the 27% with MCI at baseline, 22% reverted to normal cognition at 1-year follow up (Pedersen et al., 2013). Similar findings have been reported in a drug-naïve cohort, in which reversal to normal cognition was related to young age at onset and high level of apathy at baseline (Santangelo et al., 2015). The relationship between early cognitive deficits and PDD prognosis is therefore nonlinear, and future studies are needed to characterise cognitive profiles that herald PDD.

Defining the pattern of MCI that is prodromal of PDD is an area of active research owing to its potentially predictive value, and the possibility that these deficits may respond better to treatments targeting distinct neuropathological substrates.

1.2.4 *Neural correlates*

A range of neuroimaging methods have been used to index the presence and degree of pathophysiological mechanisms underpinning PD cognitive dysfunction. Work related to neurotransmitter changes, proteinopathy, and neurodegeneration will be briefly outlined in the follow sections. There is also a growing interest in the use of electrophysiological methods – which will be discussed in more detail in section 1.10.

It should be noted that the neural correlates of PD discussed here have been selected for their relevance to this thesis and are therefore not proposed as an exhaustive list. Other pathologies have also been identified. For reviews see (Lin and Wu, 2015; Delgado-Alvarado et al., 2016; Kalia, 2018; Wolters et al., 2019).

1.2.4.1 *Neurotransmitter changes*

Several neurotransmitter systems have been implicated in PD cognitive impairment. Dopamine and acetylcholine are among the most widely studied and will be the focus of discussion here.

The ‘dual-syndrome’ hypothesis posits that early or mild cognitive impairment, particularly executive dysfunction, may be driven by frontostriatal dopamine losses. While progression to PDD may be driven by more posterior-temporal dysfunction (i.e., memory and visuospatial deficits), mediated by cholinergic losses (Kehagia et al., 2010; 2013). Similarly, the ‘compensatory hypothesis’ posits that frontostriatal dopaminergic losses may result in compensatory reliance on cortical cholinergic function (Bohnen et al., 2015; Kim et al., 2019a). Such that combined cholinergic and dopaminergic losses may predispose to PDD, while preserved cholinergic function may attenuate cognitive decline even in the context of dopaminergic loss.

The following section reviews evidence that informed the above hypotheses, highlighting observations of dopaminergic and cholinergic contributions to PD cognitive impairment.

1.2.4.1.1 Dopamine

Dopamine cell loss in early PD is most severe in the ventrolateral tier of the substantia nigra pars compacta, which projects primarily to the dorsal striatum (dorsal regions of the putamen and caudate). Projections from the dorsal striatum mainly innervate a select set of cortical regions including the motor and premotor cortex, supplementary motor area, and dorsolateral prefrontal cortex. Dopamine cells in the dorsal tier of the midbrain, including the ventral tegmental area, are less affected. These cells project to the ventral striatum (ventral parts of the putamen and caudate, and nucleus accumbens), which in turn connect with the amygdala, orbitofrontal cortex, anterior cingulate cortex, and inferotemporal cortex (Alexander, 1986; Kish et al., 1988; Roeper, 2013). The dopaminergic contribution to PD cognitive impairment depends on the severity of degeneration within these circuits, individual variation in baseline dopamine, and the cognitive function recruited by task demands, reviewed in (Ray and Strafella, 2012).

Executive impairments are common in early PD and implicate a range of abilities including planning, manipulating working memory, initiating and inhibiting actions, and adapting behaviour in response to changing environmental demands (Kehagia et al., 2010). Functional imaging in PD has

linked executive impairments to reduced activation within fronto-striatal loops connecting the dorsolateral and ventrolateral prefrontal cortices and the striatum (Lewis et al., 2003). Reduced presynaptic dopamine in the striatum has been correlated with impairments on tests probing executive functions (Rinne et al., 2000; Cheesman et al., 2005; Nobili et al., 2010; Polito et al., 2012). Furthermore, in drug naïve PD, reduced striatal dopamine binding has been associated with executive impairment, but not to memory or visuospatial impairments (Siepel et al., 2014).

Several lines of evidence suggest that dopamine can have variable effects on different components of executive tasks (Cools et al., 2001; Lewis et al., 2005; Cools, 2006). The relationship is complicated by the fact that striatal dopamine deficits are not uniform, as described above. For example, dopaminergic medications have been shown to normalise activity in dorsolateral prefrontal cortex during planning and working memory tasks, suggesting that functions relying on the more affected dorsal striatal circuits are ameliorated (Cools et al., 2002). Conversely, dopaminergic medication was shown to disrupt nucleus accumbens activity during a reversal learning task suggesting a detrimental 'over-dosing' of the less affected ventral striatal circuits (Cools et al., 2007).

Thus, dopaminergic medication appears to restore the more severely affected dorsal striatal circuit implicated in executive functions involving planning and working memory, but can impair (overdose) the less affected ventral striatal circuits implicated in learning and reward processing (Cools et al., 2001; Cools, 2006).

Although dopaminergic changes affect specific cognitive functions in PD, they do not appear to explain the development of PDD. For example, similar striatal dopaminergic deficits have been reported in PD with and without dementia (Hilker et al., 2005; Song et al., 2014). Furthermore, evidence from multitracer positron emission tomography (PET) studies has shown independent and interactive effects of cholinergic and dopaminergic degeneration across the spectrum of PD cognitive decline (Hilker et al., 2005; Klein et al., 2010; Bohnen et al., 2015).

1.2.4.1.2 Acetylcholine

Major central cholinergic projections originate in basal forebrain and brainstem nuclei. The forebrain nucleus basalis of Meynert (nbM) provides the principal cholinergic input to the entire cortex (Mesulam and Geula, 1988), while the brainstem pedunculopontine nucleus (PPN) connects with an array of structures including the thalamus, basal ganglia, cerebellum, spinal cord, and cortex (Mesulam et al., 1983b; Jenkinson et al., 2009).

Early neuropathological studies provided evidence for substantial loss of nbM and PPN neurons in PD (Candy et al., 1983; Hirsch et al., 1987). There is now strong evidence derived from *in vivo* imaging studies for the role of cortical cholinergic system changes in PD cognitive decline (Bohnen et al., 2018b; 2018a; Craig et al., 2020b).

Volumetric assessment of cholinergic basal forebrain nuclei (cBF) *in vivo* has been shown to predict risk of PD-MCI over a 5-year follow up period (Ray et al., 2018). This finding was later replicated, with additional diffusion tensor imaging (DTI) analyses showing that nbM mean diffusivity was a stronger predictor of future cognitive impairment in PD (Schulz et al., 2018). In a recent 10-year longitudinal study, current or future conversion to PDD was associated with lower nbM volumes, and extended atrophy (beyond nbM) in other cBF nuclei (Pereira et al., 2020). Thus, MRI based structural measures of cholinergic system show promise as early markers of PD cognitive impairment. These methods (including DTI) are discussed in more detail in Section 1.8 and 1.9.

Recognition of the contribution of cholinergic deficits to PD cognitive impairment has led to pharmacological investigations of acetylcholinesterase inhibitors, that act to sustain levels of circulating acetylcholine. A large study of Rivastigmine administration was shown to produce moderate but significant improvements in measures of global cognition, attention, and executive function in PDD (Emre et al., 2004). Similar cognitive improvements have been observed with Donepezil (Dubois et al., 2012). Interestingly, Donepezil administered at a prodromal stage of AD was shown to reduce annual cBF atrophy, suggesting that structural MRI may provide a useful marker of

cholinergic treatment response (Cavedo et al., 2017) however see (Teipel et al., 2016).

1.2.4.2 *Proteinopathy*

Clinicopathological correlations have revealed greater cortical and limbic Lewy body burden in PDD compared to PD without dementia (Kempster et al., 2010; Irwin et al., 2012). However, reports of PDD with few cortical Lewy bodies (Irwin et al., 2012), or extensive Lewy pathology without PDD (Colosimo et al., 2003; Parkkinen et al., 2008), implies that Lewy pathology may not be exclusive in the development of PDD. Concomitant AD pathology is common (Mattila et al., 2000; Sabbagh et al., 2009; Compta et al., 2011; Irwin et al., 2012) and has been associated with more rapid motor and cognitive decline (Irwin et al., 2017). A similar pattern of proteinopathy, albeit less advanced, has also been reported in a small study conducted in PD-MCI (Adler et al., 2010).

Multiple *in vivo* studies have assessed cerebral spinal fluid (CSF) markers of proteinopathy. A meta-analysis of α-synuclein studies revealed greater pathology in PD compared to controls and AD, but no differences between PD with or without dementia (Wang et al., 2015). Correlations between amyloid-β and cognitive tests have also been revealed in PD-MCI (Yarnall et al., 2014). Furthermore, amyloid-β has been shown to predict the progression to PDD (Alves et al., 2014). CSF amyloid-β may therefore be a promising biomarker candidate for cognitive decline in PD.

A meta-analysis of studies using molecular imaging and Pittsburgh compound B with PET demonstrated amyloid-β burden in dementia with Lewy bodies (DLB), PDD, and PD-MCI (Petrou et al., 2015). Baseline amyloid-β measured with PET has been linked to decline in executive function and conversion to PD-MCI or PDD (Gomperts et al., 2013). Furthermore, combined striatal and cortical β-amyloid has been associated with more severe cognitive impairment, independent of nigrostriatal dopaminergic and cortical cholinergic losses (Shah et al., 2016). These findings highlight the multisystem nature of PD cognitive impairment and suggest potential synergistic effects of PD and AD proteinopathy.

1.2.4.3 *Structural changes*

Widespread grey matter loss involving parietal, temporal, and occipital regions has been reported in PDD (Burton et al., 2004; Summerfield et al., 2005; Beyer et al., 2007a; Song et al., 2011; Weintraub et al., 2011; Hattori et al., 2012; Melzer et al., 2012; Hwang et al., 2013; Pagonabarraga et al., 2013; Zarei et al., 2013). Subcortical atrophy in the hippocampus (Junqué et al., 2005; Summerfield et al., 2005; Tam et al., 2005; Beyer et al., 2007b; 2007a; Weintraub et al., 2011; Melzer et al., 2012), thalamus, putamen, caudate nucleus (Burton et al., 2004), and amygdala (Junqué et al., 2005) has also been observed. Meta-analyses, including a total of 16 voxel-based morphometry (VBM) studies and 287 individuals with PDD, have consistently reported medial temporal lobe atrophy (Pan et al., 2013; Xu et al., 2016).

Compared to PD without cognitive impairment, PD-MCI have shown losses in temporal, parietal, and frontal cortices (Beyer et al., 2007a; Song et al., 2011; Melzer et al., 2012), in addition to the thalamus, nucleus accumbens (Mak et al., 2014), and hippocampus (Weintraub et al., 2011). These findings are not universal however, as revealed by non-significant changes in PD-MCI compared to healthy controls (Hattori et al., 2012; Yarnall et al., 2014) or PD without cognitive impairment (Dalaker et al., 2010; Mak et al., 2014).

Longitudinal data has illustrated the progression of cortical atrophy. In newly diagnosed PD, cortical atrophy was shown to extend from frontal regions to posterior temporal cortices over 18-months of follow-up (Mak et al., 2015). In a 2-year exploratory longitudinal study, people with PD-MCI who converted to PDD showed grey matter atrophy in prefrontal and insular cortices, caudate nuclei, and substantia innominata (Lee et al., 2014).

Diffusion tensor imaging (DTI) is sensitive to the magnitude (i.e., mean diffusivity, mD) and direction (i.e., fractional anisotropy, FA) of water molecule flow in brain tissue (Le Bihan et al., 2001), and is used as proxy for microstructural integrity. Degeneration of structural barriers, such as myelin and cell membranes in white matter, increases mean diffusivity and lowers fractional anisotropy.

White matter microstructure has been shown to deteriorate across cognitive stages in PD. For example, increases in mD and decreases in FA in major white matter tracts have been reported in PD-MCI and PDD (Melzer et al., 2013). In an incident PD cohort, those with executive impairments showed increased mD in frontal and parietal tracts, with preserved FA. This finding suggests that white matter changes in these regions occurs early, and may underpin PD cognitive dysfunction (Duncan et al., 2016). Furthermore, more rapid progression to PD-MCI has been associated with decreased FA and increased mD in frontal regions (Shin et al., 2016).

Studies analysing grey and white matter also suggest that white matter microstructural changes may precede grey matter atrophy (Agosta et al., 2014; Duncan et al., 2016), indicating that DTI may be more sensitive than volumetry for detecting the pathology that underpins cognitive impairment in PD. However, further work is needed to establish this temporal sequence. It must be noted, however, that many of the changes reported in the neuroimaging literature do not meet the sensitivity and specificity required for accurately distinguishing people with PD from controls, and as such the development of an imaging biomarker remains an unmet need in PD research.

1.3 Mobility impairment in PD

1.3.1 *Profile and progression*

Gait and postural impairments present early in PD, worsen as the disease progresses, and contribute to increased disability and falls risk, and reduced quality of life (Muslimović et al., 2008; Lord et al., 2016; Wilson et al., 2020a).

Early changes in PD include reductions in gait speed (small-steps), rhythmicity, arm swing, and postural control, and increases in gait variability and asymmetry (Baltadjieva et al., 2006; Galna et al., 2015; Mirelman et al., 2019; Wilson et al., 2020a). Changes in posture and range of motion further affect the magnitude of movements, and automaticity is further impaired with disease progression. Turning becomes slow and rigid, as more steps are required, and the normal turning sequence (i.e., eyes-head-trunk-feet) is replaced with simultaneous

rotation (Crenna et al., 2007; Mellone et al., 2016). In moderate-advanced stages, more complex and episodic symptoms emerge, such as festination and freezing of gait (sudden inability to initiate/maintain gait), which greatly increase the risk for falls (Okuma et al., 2018).

1.3.2 *Assessment of mobility*

Technology-enabled tools are now widely used in research settings to assess PD mobility (Mirelman et al., 2019). Devices such as optical 3D motion capture, wearable sensors, force-platforms, and pressure-sensitive walkways provide quantitative data that can be used to detect subtle deviations in gait and balance. Assessment of discrete gait characteristics has been informative for monitoring the progression of PD (Galna et al., 2015; Morris et al., 2017; Wilson et al., 2020a). Emerging evidence also suggests that these characteristics may be have different neuroanatomical and functional substrates (Rochester et al., 2017; Wilson et al., 2019; 2020b) and may therefore provide a target for early intervention. While these measures provide rich information, the need for specialist equipment and analysis techniques is currently a limiting factor in their application in clinics. Future work will therefore help to determine whether they can be translated into improvements in clinical decision making and patient outcomes.

In routine clinical settings, as well as many research studies, mobility assessment is performed with semi-quantitative performance scales including (but not limited to) the Mini-BESTest (Franchignoni et al., 2010) and the Timed Up and Go test (Podsiadlo and Richardson, 1991). These measures provide a gross indication of functional mobility and therefore do not offer the same degree of granularity as the quantitative methods outlined above. That said, they require little equipment, are quick and easy to administer, and show good reproducibility in people with PD (Huang et al., 2011; Leddy et al., 2011; Löfgren et al., 2014) thus making them well suited for use in clinics.

1.3.3 *Neural correlates*

Our understanding of the neuronal processes underlying actual walking has been limited by constraints of neuroimaging techniques. Methods that enable

whole brain imaging (i.e., cortical *and* subcortical) are not portable, largely restrict movement inside the scanner, and lack temporal resolution, while portable alternatives cannot image subcortical structures directly. Motor imagery has been combined with functional imaging techniques by way of assessing brain activity associated with movement. This approach takes advantage of the overlap in supraspinal activation during imagined and real locomotion (la Fougère et al., 2010). Studies using functional MRI and imagined gait, and molecular imaging following actual gait, have revealed key locomotor regions in humans (Figure 1.1), which include the frontal cortex, basal ganglia, brainstem tegmentum, and cerebellum (Jahn et al., 2008; la Fougère et al., 2010).

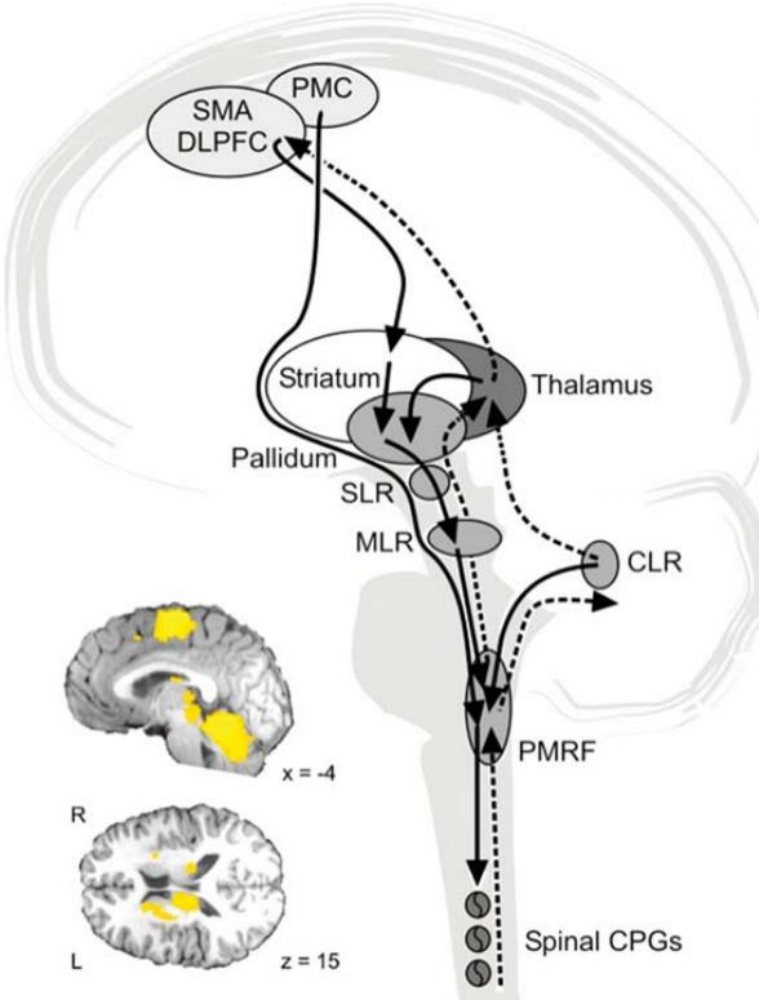


Figure 1.1 Schematic representation of the supraspinal locomotor network in humans

The presumed supraspinal locomotor network in humans is comprised of an indirect and a direct pathway. The direct pathway is recruited during steady-state unmodulated gait. This pathway goes from the primary motor cortex directly to the spinal cord, bypassing the basal ganglia and brainstem locomotor centres. A feedback loop runs from the spinal cord to the cerebellum, then to the cortex via the thalamus. During planning and modulation of gait in response to external demands, the indirect pathway is recruited. This pathway originates in the frontal cortex (prefrontal cortex, premotor/supplementary motor area, SMA) and proceeds, via the basal ganglia and (disinhibited) subthalamic and mesencephalic locomotor regions, to the spinal cord. Cerebellar signals also converge with descending mesencephalic locomotor signals. As with the direct pathway, ascending spinal cord signal reach the cortex via the cerebellum and thalamus, and cortical signals can be further modulated via thalamo-cortical-basal ganglia circuits (Jahn et al., 2008; la Fougère et al., 2010). The inset displays blood oxygen level-dependent signal increases during mental imagery of locomotion, from (Wutte et al., 2012). Figure taken from (Bohnen and Jahn, 2013).

1.3.3.1 *Structural changes*

VBM has been used to compare global grey matter changes between PD motor subtypes (PIGD vs. tremor-dominant). PIGD showed widespread atrophy including cortical motor areas (primary motor, premotor, SMA), cingulate cortex, inferior parietal cortex, hippocampus, dorsal striatum, amygdala, and cerebellum (Rosenberg-Katz et al., 2013; 2016).

Grey matter changes in PD with freezing of gait (compared to without freezing) have also been revealed with VBM. In particular, prominent atrophy has been observed in frontoparietal regions, thalamus, caudate nucleus, cerebellum, and mesencephalic locomotor region (Snijders et al., 2011; Kostić et al., 2012; Sunwoo et al., 2013; Herman et al., 2014; Jha et al., 2015).

DTI has also revealed changes in grey and white matter structures that are associated with posture and gait. For example, increased diffusivity within the global pallidus, substantia nigra, and PPN has been reported in PIGD (Nagae et al., 2016; Craig et al., 2020a). Furthermore, freezing of gait has been associated with reduced structural connectivity of white matter tracts between frontoparietal and temporo-occipital cortical connections in (Canu et al., 2015), and between PPN and a number of structures including the cerebellum, thalamus, and multiple regions of the frontal cortex (particularly in the right hemisphere) (Fling et al., 2013).

1.3.3.2 *Proteinopathy*

Cross-sectional PET imaging in PD without dementia has revealed an association between increased neocortical β-amyloid deposition and more severe PIGD symptoms, after controlling for striatal dopaminergic denervation, age, and cognitive impairment (Müller et al., 2013b). Longitudinal studies in newly diagnosed PD, have also linked CSF β-amyloid levels to declining gait characteristics (Rochester et al., 2017), and the development of freezing of gait (Kim et al., 2019c). Together these findings suggest that comorbid amyloid pathology may contribute to PD gait impairments.

1.3.3.3 *Changes in neural activation*

Functional MRI and imagined gait has revealed reduced activity in posterior parietal cortex, precuneus, and SMA as a function of severity of gait disturbances in PD, in addition to reduced activity in the mesencephalic locomotor region and cerebellum compared to controls (Crémers et al., 2012). In another study, people with PD showed activity reductions in basal ganglia (globus pallidus) during imagined walking, and increased activity in SMA during imagined turning compared to walking. The authors interpret this as a potential compensatory neural adaptation during more complex gait (Peterson et al., 2014). In that study, faster gait also correlated with greater activity in SMA, putamen, mesencephalic locomotor region and pallidum, in people with PD but not controls. Together, while not entirely consistent, these findings suggest that imagined gait in PD results in altered activation in several locomotor regions, and may vary depending on the complexity of the gait task.

Molecular imaging and treadmill walking in people with PD has revealed less activity in medial frontal cortex, precuneus, and left cerebellum, but increased activity in temporal cortex, insula, cingulate cortex and cerebellar locomotor region, compared to controls (Hanakawa et al., 1999b). Gait improvements have been reported in people with PD with the use of visual cues (lines on floor), which was accompanied by increased activation in the cerebellum and premotor cortex (Hanakawa et al., 1999a). In an exploratory meta-analysis of imaging studies, PD gait was consistently associated with lower activation in SMA and enhanced activity in cerebellar locomotor region (Gilat et al., 2019). The cerebellum receives sensory input and the cerebellar locomotor region is thought to regulate speed and timing of movements (Jahn et al., 2004). Thus, in PD, changes to basal ganglia output, resulting in reduced excitation of cortical motor regions, may lead to compensatory reliance on the cerebellum to modulate gait.

1.3.3.4 *Neurotransmitter changes*

1.3.3.4.1 Dopamine

Dopamine imaging (using PET) has shown that dopamine uptake in the putamen was decreased to a greater extent by walking in controls compared to people with PD – who showed greater uptake in the caudate and orbitofrontal cortex (Ouchi et al., 2001). The shift in activation from the nigrostriatal to mesocortical pathways during walking may represent a pathological mechanism in PD. Furthermore, reduced dopamine in the caudate was shown to incur risk of future PIGD in people PD diagnosed for 2-years or less (Craig et al., 2020a).

Dopaminergic medications remain the most common treatment for PD motor dysfunction but reported effects on gait and posture are variable. For example, speed, turning, and hypokinesia (i.e., small amplitude steps and reduced arm swing) show improvements with levodopa, while aspects of gait timing and postural sway show no improvement or are worsened (Curtze et al., 2015; Smulders et al., 2016). This evidence suggests that some features of gait are underpinned by non-dopaminergic pathology.

1.3.3.4.2 Acetylcholine

Accumulating evidence implicates cholinergic dysfunction in PD gait impairments (Yarnall et al., 2011; Bohnen and Jahn, 2013; Morris et al., 2019). The relationship between PD symptoms and cholinergic system changes is discussed in detail in following sections. A brief overview will be provided here.

As outlined previously, basal forebrain and brainstem cholinergic nuclei and their projections degenerate in PD. Molecular imaging has shown that comorbid cortical cholinergic dysfunction was a more robust marker of PD gait slowing than nigrostriatal dopaminergic alone (Bohnen et al., 2013). Short latency afferent inhibition (a transcranial magnetic stimulation technique that is thought to index cholinergic function (Di Lazzaro et al., 2000; 2002) has also revealed relationships between cortical cholinergic activity and gait speed and stride length (Rochester et al., 2012; Pelosin et al., 2016). Furthermore, a pharmacological investigation reported improvements in gait speed and

variability with cholinergic treatments (using AChE inhibitors to increase circulating levels of acetylcholine) (Henderson et al., 2016).

Greater PPN-thalamic cholinergic denervation has been associated with PD balance impairments, independent of motor and cognitive impairments (Müller et al., 2013a). Cortical *and* thalamic cholinergic losses have been reported in PD fallers (Bohnen et al., 2009). Furthermore, pharmacological studies have revealed a reduction in fall rates (Chung et al., 2010; Henderson et al., 2016) and improvements to balance (Henderson et al., 2016) although see (Chung et al., 2010), with acetylcholinesterase (AChE) inhibitors demonstrating potential benefits of cholinergic enhancement, particularly for alleviating fall risk.

1.4 The relationship between PD cognitive and mobility impairments

Studies assessing motor subtypes have shown that PIGD and attention deficits independently correlate with more rapid cognitive decline in PD without dementia (Taylor et al., 2008). Specific aspects of gait have also shown links with cognitive impairments. For example, gait timing and stability were associated with MCI in PD. In addition, visuospatial ability was strongly associated with development of PIGD and PD progression (Amboni et al., 2012). Using a comprehensive battery of gait and cognitive characteristics, pace, variability, and postural control predicted fluctuating attention and visual memory impairments in newly diagnosed PD within a 3-year follow up (Morris et al., 2017).

1.4.1 *Evidence from dual-task paradigms*

The interaction between gait and cognition has also been highlighted during dual-tasking (Kelly et al., 2012), for example, when completing gait with a secondary cognitive task (e.g., counting backwards). Studies in PD highlight a slower, asymmetric, and more variable gait under dual-task conditions (Yogev et al., 2005; Plotnik et al., 2011a; Rochester et al., 2014), which is particularly pronounced in those with FOG (Peterson et al., 2015). Deficits in attention and executive function have been shown to exacerbate these effects (Yogev et al.,

2005; Plotnik et al., 2011a). Increased fall risk in PD has been linked to a slower, more variable gait (Schaafsma et al., 2003; Ma et al., 2020), as well as impaired attention and executive functions (Allcock et al., 2009; Plotnik et al., 2011b). Similarly, converging evidence suggest that FOG is related to attention, executive, and visuospatial function (Kostić et al., 2012; Sunwoo et al., 2013; Jha et al., 2015).

1.4.2 *The role of cholinergic system changes*

The above findings imply that PD mobility impairments including PIGD, FOG, gait timing and variability characteristics, and falls – all of which respond poorly to levodopa, are closely related to deficits in attention, executive function, and visuospatial functions. As outlined in previous sections, cholinergic deficits have been shown to contribute to each of these symptoms, suggesting a shared underlying pathology (Yarnall et al., 2011).

Given the more pernicious disease course associated with PIGD, better understanding the early manifestation of this phenotype with regards to cholinergic changes represents an important area of research.

1.5 Central cholinergic system

1.5.1 *Overview of central cholinergic system projections*

There are three major sources of acetylcholine in the brain. These include cholinergic projection neurons with soma located in the basal forebrain, the brainstem, or interneurons contained within the striatum. The major cholinergic projection nuclei have been designated into six numbered cell groups (Ch1-6), each with projections to cortical and subcortical target regions (Mesulam et al., 1983b). According to this nomenclature, the major supply of cholinergic input to target regions is shown in Table 1.2. Together, Ch1-4 cell groups correspond to the cholinergic basal forebrain (cBF) complex, and Ch5-6 correspond to the cholinergic brainstem complex.

Table 1.2 Overview of central cholinergic projections

Cholinergic cell group	Corresponding nuclei	Target region
Ch1	medial septal nucleus	hippocampus
Ch2	vertical limb nucleus of the diagonal band of Broca	hippocampus
Ch3	horizontal limb nucleus of the diagonal band of Broca	olfactory bulb
Ch4	nucleus basalis of Meynert	neocortex and amygdala
Ch5	pedunculopontine nucleus	thalamus, basal ganglia
Ch6	laterodorsal tagmental complex	thalamus, basal ganglia, cerebellum, brainstem and spinal cord, cortex

Summarised from (Mesulam et al., 1983a; 1983b; Heckers et al., 1992; Jenkinson et al., 2009; Martinez-Gonzalez et al., 2011).

1.5.2 *Cholinergic basal forebrain organisation and connectivity*

The cholinergic basal forebrain (cBF) has been subdivided into four main cell groups corresponding to cholinergic neurons of the medial septum (Ch1), the vertical (Ch2) and horizontal (Ch3) limb of the diagonal band of Broca, and the nucleus basalis Meynert (nbM, Ch4) (Mesulam et al., 1983b; 1983a). Ch4 represents the largest cell cluster and has been further subdivided into six subsectors corresponding to anterior-medial (Ch4am) and anterior-lateral (Ch4al), intermediate (Ch4i), and posterior (Ch4p) based on their topographical innervation (Figure 1.2) – mapped with axonal tracing in primates (Mesulam et al., 1983a). In summary, anterior Ch4 innervates the limbic regions, posterior Ch4 projects to superior temporal and temporal polar regions, and intermediate Ch4 to the remaining cortical regions (Mesulam et al., 1983a; Liu et al., 2015).

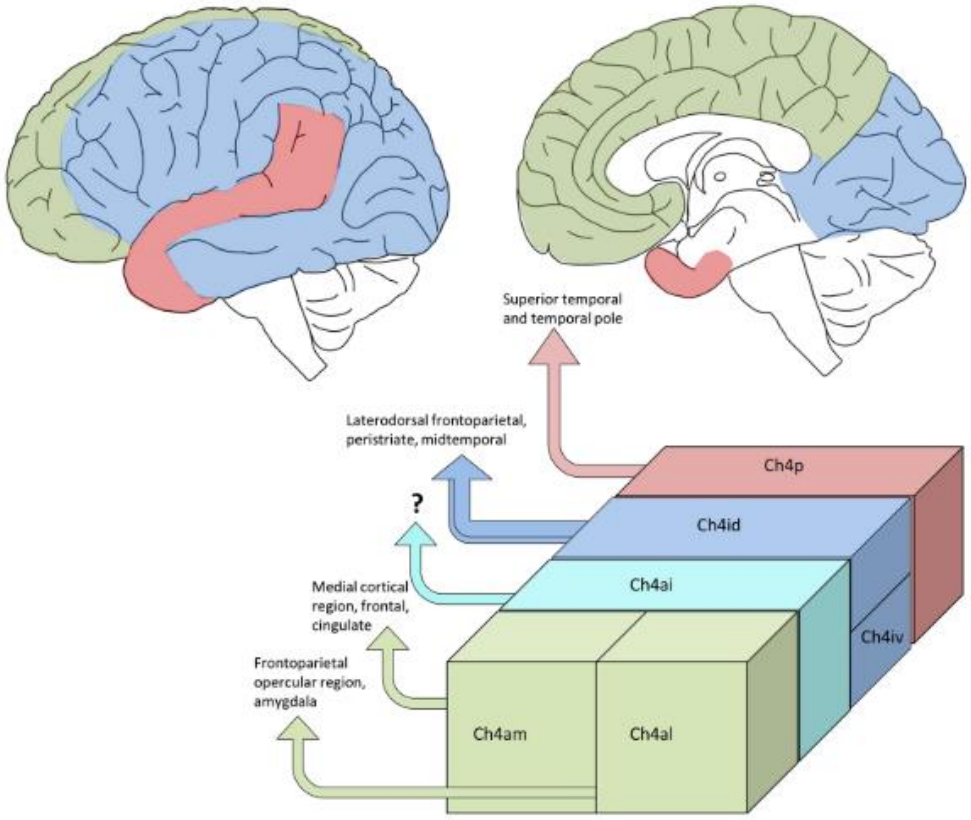


Figure 1.2 Topographical innervation of nucleus basalis of Meynert (Ch4)

Figure taken from (Liu et al., 2015).

Cholinergic cells in the basal forebrain are interspersed with non-cholinergic neurons including GABAergic and glutamatergic, each with different morphologies and projection patterns (Gritti et al., 2006). However, histochemical staining for AChE and choline acetyltransferase (ChAT) suggests that approximately 90% of all nbM neurons are cholinergic (Mesulam and Geula, 1988).

Primate and rodent studies have shown that Ch4 receives inputs from limbic cortical areas, amygdala, nucleus accumbens, hypothalamus, substantia nigra, ventral tegmental area, and several brainstem areas including the PPN, locus coeruleus and raphe nucleus (Zaborsky, 1991; Mesulam and Mufson, 1984). More recent studies using electron microscopy suggest that input to cholinergic basal forebrain neurons is diverse, but topologically specific (Záborszky et al.,

2018). For example, cholinergic neurons that project to motor cortex receive input from somatosensory cortices, while those that project to medial prefrontal cortex do not.

1.5.3 *Cholinergic basal forebrain functions*

The basal forebrain system has been implicated in a range of cognitive functions and may also contribute to posture and gait. Animal studies have informed much of our current understanding and will form the focus of discussion in the following sections.

1.5.3.1 *Attention and cue detection*

Increases in cortical acetylcholine have been observed upon presentation of stimuli designed to increase attention and arousal (Inglis and Fibiger, 1995). During sustained attention tasks, cortical acetylcholine release has been shown to increase with greater attentional demand (Himmelheber et al., 2000), and to correspond more to attentional demand than to task performance (Kozak et al., 2006). Such findings have provided the basis for the proposed involvement of cortical cholinergic transmission in attentional processes, with particular relevance to attentional effort (Sarter et al., 2006).

Cholinergic depletion studies further illustrate the role of acetylcholine in attentional processes. For example, selective cholinergic immunotoxin infusions into the nbM (resulting in loss of cortical input) was shown to elicit deficits in stimulus detection during a sustained attention task (McGaughy et al., 1996). Furthermore, performance deficits were larger during more effortful processing of multimodal cues (i.e., visual and/or auditory cues) compared to unimodal (Turchi and Sarter, 1997).

The prefrontal cortex is thought to play a key role in exerting top-down attentional control, and is also a significant target of cholinergic projections (Sarter et al., 2001). Lesions of cholinergic-prefrontal projections was shown to increase impulsive responses and sensitivity to distractor cues (Dalley et al., 2004; Newman and McGaughy, 2008). Furthermore, transient increases in prefrontal acetylcholine were observed in response to attention task-related

cues (Parikh et al., 2007). More recently, optogenetic stimulation was used to enhance these cue-related transient increases in acetylcholine, which improved cue detection. Furthermore, inhibition of basal forebrain cholinergic neurons also resulted in missed cues (Gritton et al., 2016). Together, these findings suggest that basal forebrain cholinergic projections to the prefrontal cortex play an important role in cue detection.

1.5.3.2 *Posture and gait*

The cBF has also been implicated in the attentional control of mobility functions. Rats with combined cortical cholinergic and striatal dopaminergic losses (modelling the lesions seen in PD) have been shown to exhibit gait and postural deficits and high fall rates during tasks designed to tax the attentional control of movement (Kucinski et al., 2013). Such tasks included walking across various dynamic runways (including rotating or inclined), also with additional distractions (e.g., walking through a doorway or retrieving food from a platform). Higher fall rates correlated with impaired performance on a test of sustained attention, as well as slower traversal speed, lower step frequency, slouched posture, and deficits in rebalancing after slips. Furthermore, partial striatal dopaminergic losses alone did not impair movement performance, thus it was proposed that the cBF may serve a compensatory attentional mechanism to prevent falls in the context of diminished striatal control of movement (Kucinski et al., 2013; Sarter et al., 2014).

Consistent with the above evidence for the role of cholinergic signalling in cue detection, cBF losses are thought to disrupt the cortical processing of movement cues that are instrumental for safe and effective gait (Sarter et al., 2014). Striatal circuitry would normally receive cue information via cortico-striatal projections, which would subsequently be used to select and update motor output. Thus, cBF losses may indirectly deprive the striatum of this information, resulting in impairments to attentional-motor integration, and an increased propensity for falls (Sarter et al., 2014).

1.5.3.3 *Modulation of cortex*

The cBF constitutes an important component of the neuromodulatory system controlling brain states. These neurons are more active during active waking, when gamma and theta EEG activity are maximal, and are inactive during slow-wave sleep (Lee et al., 2005). Furthermore, selective excitotoxin lesions or destruction of the nbM has been shown to increase slow wave EEG activity, indicative of drowsiness or sleep (Buzsaki et al., 1988; Riekkinen et al., 1990).

Attending to behaviourally relevant stimuli is thought to rely on increased activity of sensory neurons along with a reduction in the noise among intra-cortical neurons, such that the response of sensory neurons is enhanced (Cohen and Maunsell, 2009). Cholinergic signalling from the basal forebrain to sensory cortices has been shown to mediate this selective processing. For example, stimulation of cBF neurons in mice was shown to improve performance on a visual discrimination task, and to desynchronise cortical activity. Conversely, basal forebrain inactivation led to impaired visual responses and synchronised cortical activity (Pinto et al., 2013).

The above observations imply that cBF neurons are capable of modulating cortical dynamics, thereby contributing to cortical state regulation and enhancing sensory processing.

1.5.3.4 *Memory*

The ability to effectively encode relevant information is a defining aspect of memory. Acetylcholine is thought to play a key role in memory encoding processes. For example, selective immunotoxic cholinergic lesions of the medial temporal lobe was shown to impair encoding for subsequent visual recognition in primates (Turchi et al., 2005). Similar findings were reported for novel odours in rats (McGaughy et al., 2005). Furthermore, post-training stimulation of the nbM was shown to improve memory consolidation in animals with low initial learning ability (Montero-Pastor et al., 2001). These observations highlight the role of basal forebrain region in the storage of representations during learning.

Synaptic plasticity, thought to be the cellular substrate of learning and memory, has also been associated with the cBF (Drever et al., 2011). For example, pairing an auditory stimulus with nbM stimulation was shown to reorganise the auditory cortex (Kilgard, 1998). Furthermore, selective lesions of the cBF was shown to disrupt training-induced cortical reorganisation during motor skill learning (Conner et al., 2003). Plastic changes in the hippocampus have been associated with theta oscillations (Huerta and Lisman, 1995) – which are modulated by medial septal projections (Buzsaki, 2002) and tightly coupled with acetylcholine release (Zhang et al., 2010). Elevated hippocampal theta power has also been associated with improved learning (Griffin et al., 2004). Thus, cBF output could stimulate theta activity along with cortical plasticity to facilitate learning and memory processes.

The research described above provides just a small selection of findings to illustrate the potential contribution of cBF signalling to attention and memory processes. The specific mechanisms at the synapse, receptor, and circuit level paint a far more complex picture – a comprehensive discussion of which is beyond the scope of this thesis. For more in depth reviews, readers are directed to (Hasselmo and Sarter, 2011; Ballinger et al., 2016; Záborszky et al., 2018).

1.5.4 *Cholinergic brainstem organisation and connectivity*

The PPN and the laterodorsal tegmental nucleus (LDT) of the brainstem (classically defined by cholinergic cell groups Ch5 and Ch6, respectively (Mesulam et al., 1983b)) are made up of three main neuronal populations that are intermingled in different proportions; cholinergic, GABAergic, and glutamatergic (Wang and Morales, 2009). In the LDT, the density of all three neuronal populations is high and relatively constant, whereas in the PPN, a distinction is made between the rostral and caudal portions with respect to cellular composition. The most rostral part of the PPN (PPNr) contains a small number of cholinergic neurons and high concentration of GABAergic neurons, whereas the caudal PPN (PPNc) contains a larger density of cholinergic and glutamatergic neurons (Mena-Segovia, 2016).

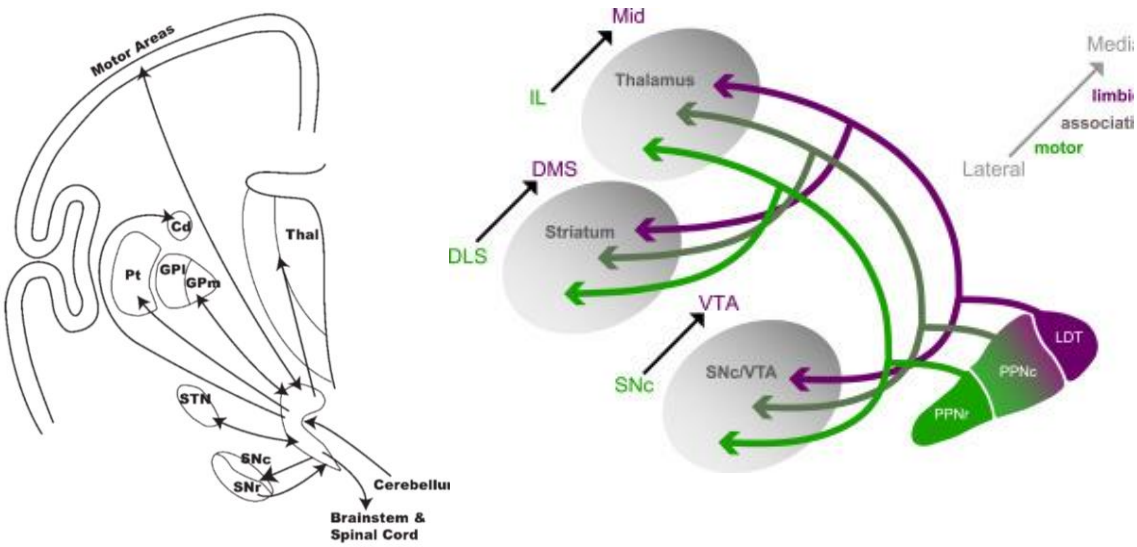


Figure 1.3 Topographical innervation of the pedunculopontine nucleus (PPN)

Overview of major efferent and afferent pathways of the PPN to the basal ganglia and other motor structures (left) – figure taken from (Jenkinson et al., 2009), and topographical organisation and innervation of cholinergic brainstem (right) – figure taken from (Mena-Segovia and Bolam, 2017). Abbreviations: Cd = caudate nucleus; GPi and GPm= globus pallidus internal, and medial, segments; DLS = dorsolateral striatum; DMS = dorsomedial striatum, IL = intralaminar thalamic nuclei; LDT = laterodorsal tegmental nucleus; Mid = midline thalamic nuclei; Pt = putamen; SNc = substantia nigra pars compacta; SNr = substantia nigra reticulata; VTA = ventral tegmental area.

Cholinergic brainstem nuclei give rise to long axons with up to six collaterals that innervate the dopaminergic midbrain, the striatum, and the thalamus (non-cholinergic give rise to two collaterals on average) (Mena-segovia et al., 2008). These ascending cholinergic projections maintain a clear topographical organisation (as shown in Figure 1.3 above). PPNr preferentially innervates motor-related circuits (substantia nigra pars compacta (SNc), dorsolateral striatum, and the intralaminar thalamus), the LDT targets limbic circuits (ventral tegmental area (VTA), dorsomedial striatum, and medial thalamus), and the PPNc provides innervation to both motor and limbic structures. Neurons in the PPN also innervate motor regions of the cortex, as well as the

lower brainstem, pons, medulla, and spinal cord (Jenkinson et al., 2009; Martinez-Gonzalez et al., 2011).

Neurons in the PPN receive afferents from functionally diverse areas of the brain including the cortex, thalamus, hypothalamus, pons, cerebellum, medulla, spinal cord, and basal ganglia. A complete review of specific cells that these afferents target is beyond the scope of this discussion, however two important neuronal systems – the basal ganglia and cortex, appear to target distinct regions of the PPN. GABAergic neurons from basal ganglia mainly innervate PPNr, while cortical and dorsal raphe neurons preferentially innervate PPNc (Martinez-Gonzalez et al., 2011).

1.5.5 *Cholinergic brainstem functions*

The cholinergic brainstem has been most notably studied for its role in movement, however, its potential role in cognitive processes in increasingly recognised. As with the cBF, animal studies have informed much of what we know about these functions and will therefore form the focus of discussion in the following section.

1.5.5.1 *Movement*

The PPN has traditionally been viewed as a locomotor centre, forming part of the mesencephalic locomotor region (MLR), together with the cuneiform nucleus (CnF) (Whelan, 1996). More recently however, the role of the PPN in locomotor control, and its function in the MLR, has been brought into question (Winn, 2008).

Complete, partial, and selective cholinergic PPN lesions have been shown to have little effect on spontaneous locomotion in rats (Inglis et al., 1994; Dellu et al., 2002; Gut and Winn, 2015). Electrical stimulation in decerebrate cats provided support for opposing roles of PPN neurons (Takakusaki et al., 2016). More specifically, cholinergic PPN stimulation reduced locomotion, whereas the dorsal PPN (containing high concentrations of glutamatergic neurons) induced locomotion. Cholinergic PPN neurons may therefore play a role in movement inhibition, while glutamatergic PPN neurons may induce movement.

Newer methods have allowed for more precise stimulation experiments *in vivo*. In mice, activation of glutamatergic MLR (but not cholinergic) was shown to initiate, maintain, and increase locomotion (Roseberry et al., 2016). When targeted separately, CnF stimulation increased running speed and initiated a change from walk to gallop. While PPN stimulation increased exploratory behaviour (as measured by head-dips in a hole-board), and locomotion gradually.

Inhibition of glutamatergic PPN neurons was shown to reduce locomotion and exploratory behaviour (Caggiano et al., 2018), while stimulation disrupted the locomotor cycle (Josset et al., 2018). Activating only PPN cholinergic neurons has shown mixed results. For example, it was not sufficient to initiate gait, but was shown to accelerate locomotion in mice that were already moving (Roseberry et al., 2016). In a subsequent study, it reset locomotor rhythm, and reduced locomotor speed (Josset et al., 2018).

One possible explanation for what appears to be discrepant findings between early and more recent stimulation studies was highlighted by (Gut and Winn, 2016; Mena-Segovia and Bolam, 2017). In earlier studies in decerebrate animals, descending control of the MLR was lost. Specifically, descending projections from basal ganglia that serve to inhibit PPN activity were severed. Removing this inhibitory control and stimulating PPN/CnF may then result in an increase in descending projections to lower brainstem motor systems, and increased motor output (Gut and Winn, 2016).

On a related note, ascending projections from the PPN (also severed in decerebrate animals) may also influence motor output. Specifically, locomotion was triggered in freely moving rats when cholinergic PPN axons in SNc (but not VTA) were stimulated (Xiao et al., 2016), and when cholinergic LDT axon in VTA (but not SNc) were stimulated (Dautan et al., 2016). These findings suggest that descending cholinergic brainstem projections may inhibit movement, while ascending projections may promote movement via function-specific pathways.

1.5.5.2 *Brain states and sensory processing*

Early electrical stimulation studies demonstrated that the transition to wakefulness was marked by EEG desynchronisation in the cortex, i.e., a shift from slow to fast oscillatory activity (Moruzzi and Magoun, 1949). Such effects were shown to arise from the projection of cholinergic brainstem nuclei to the cortex via the thalamus (Steriade et al., 1990). However, lesions of cholinergic PPN and LDT neurons had minimal effect on wakefulness and slow-wave sleep, therefore questioning their role in purely maintaining active states (Webster and Jones, 1988).

Electrophysiology in rats has demonstrated potentially different roles for distinct PPN neurons in behavioural states. For example, PPN neurons fire transiently during sensory stimulation (hind-paw pinch), while glutamatergic neuronal firing is prolonged, beyond the period of sensory stimulation (Petzold et al., 2015). Given that PPN and LDT neuronal activity has been shown to correlate with cortical EEG (Mena-segovia et al., 2008), cholinergic PPN neurons may be involved in transient cortical activation related to behavioural context (i.e., sensory information processing). While the maintenance of brain-state may be better explained by the dynamics of glutamatergic PPN neurons.

1.5.5.3 *Reward-prediction signalling and action selection*

A prominent feature of dopamine neurons is their reward-related activity. Their firing increases in response to unexpected, or better than predicted rewards, remains uninfluenced by events that are as good as predicted, and decreases in response to events that are worse than predicted (Schultz, 1998). Research suggests that the PPN (projecting to midbrain neurons) may influence dopamine-mediated behaviour. For example, distinct groups of PPN neurons were shown to carry reward information during a visually guided saccade task in primates (Okada et al., 2009). Specifically, one group fired persistently between target onset and reward delivery, the other fired quickly after the reward was delivered. These findings suggest that separate PPN neuronal populations may signal predicted and actual reward.

Furthermore, in mice performing an odour-cued spatial choice task (requiring them to orient left-right to receive a reward), PPN activity was related to direction selection, and an overlapping population of neurons fired in relation to movement direction and reward outcome. The authors highlight the potential role for the PPN in sensorimotor decision making (Thompson and Felsen, 2013). Together, the findings outlined above suggest that different populations of PPN neurons are capable of influencing goal-directed actions.

1.5.5.4 *Behavioural flexibility*

PPN cholinergic neurons have also been considered for their influence on behavioural flexibility (Mena-Segovia and Bolam, 2017; Gut and Mena-Segovia, 2019). As outlined above (section 1.5.4), PPN cholinergic neurons project to multiple divisions of the thalamus that in turn project to the striatum (Mena-segovia et al., 2008). Furthermore, it was recently was shown that PPN/LDT cholinergic neurons provide direct innervation to the striatum (Dautan et al., 2014).

The dorsomedial striatum (DMS) supports learning that requires a shift in strategies, i.e., reversal learning (Palencia and Ragozzino, 2004), which is supported by acetylcholine (Palencia and Ragozzino, 2006). Increases in DMS acetylcholine are blocked by thalamic inactivation (Brown et al., 2010) and removing thalamic input to cholinergic DMS interneurons impairs reversal learning (Bradfield et al., 2013). Thus, thalamostriatal pathways are important for goal-directed actions that requires updates to behaviour based on outcome. Lesions to the posterior PPN in rats have been shown to mimic these behavioural impairments i.e., resulting in a failure to update task response despite a change in task contingency (MacLaren et al., 2013). These findings highlight the role of the PPN in behavioural adaptation, particularly in tasks that rely on striatal-thalamic interactions.

Deficits in behavioural flexibility could also conceivably contribute to postural and gait deficits. In PPN-lesioned rodents, performance on a fixed speed rotarod test was unaffected, while performance was impaired when rats were challenged with adapting to the change in speed on an accelerating rotarod (Maclaren et al., 2014). Thus, PPN lesions impaired the ability to continually

update motor programs in response to changing task demands. In a more recent study, rats with damage to the PPN (produced by electrode implantation) displayed freezing of gait episodes only during contextual movements (i.e., when trained to run along a confined corridor), but not in their home cage (Gut and Winn, 2015). These results suggest that the contribution of the PPN to motor behaviour is contingent on environmental demands. A failure to update action-outcome associations may lead to impaired sensorimotor integration and inappropriate postural and gait adjustments, thus resulting in a greater propensity for falls.

1.6 Cholinergic system pathology

Cholinergic system changes that accompany neurodegenerative disease have been assessed in post-mortem brains and will be reviewed in the following section.

1.6.1 *Cortical cholinergic deficits in Alzheimer's disease*

Most of the early investigations of cholinergic degeneration were performed in people with AD (Bartus et al., 1982). Substantial cortical cholinergic denervation became a universal finding in advanced AD, particularly evident in the temporal lobes (Geula and Mesulam, 1996). Around the same time, substantial (-90%) cell loss in the nbM was reported in advanced AD (Whitehouse et al., 1982). In a review of early pathological studies, Lui and colleagues used nbM subdivisions (Mesulam and Geula, 1988) to identify a caudo-rostral gradient of neuronal loss, with more severe degeneration in the posterior nbM region (Liu et al., 2015).

While much of the pathological evidence for cortical cholinergic degeneration involved advanced AD cohorts, losses in early AD were also reported (Perry et al., 1981). Biopsy samples of the frontal lobe obtained within a year of symptom onset displayed up to 95% reductions in cholinergic activity (Bowen et al., 1982; 1983). Despite losses in cholinergic function, the number of Ch4 neurons was shown to be relatively preserved in MCI (Candy et al., 1983; Gilmor et al., 1999).

It was later shown that cholinergic system changes may be accompanied by compensatory mechanisms. For example, an upregulation of ChAT was reported in surviving cholinergic synapses in the hippocampus and prefrontal cortex in early-stage AD, thereby appearing as normal cholinergic function (Dekosky et al., 2002). Furthermore, AD-pathology and cholinergic axon abnormalities were observed in cBF neurons even in non-demented individuals (Mesulam et al., 2004; Geula et al., 2008). Together these findings suggest that morphological changes may occur early in the disease course, even with no apparent change in cortical cholinergic activity.

Ch3 (projecting to the olfactory bulb) neuronal loss was also reported in AD (Loy et al., 1990) which corresponds with findings of early and severe involvement of the olfactory bulb in the degenerative process in AD (Christen-Zaech et al., 2003). In contrast to the degeneration of Ch3 and Ch4 neurons, the hippocampal projecting Ch1-2 nuclei appeared to be less affected (Vogels et al., 1990; Mufson and Kordower, 1992; Fujishiro et al., 2006). Similarly, the cholinergic brainstem system remained intact (Jellinger, 1988; Davis et al., 1999). Findings from post-mortem studies therefore indicate a selective pattern of cholinergic loss in AD.

1.6.2 *Cortical cholinergic deficits in Parkinson's disease*

Early studies reported cortical cholinergic losses in the neocortex and hippocampus of people with PD (Ruberg et al., 1982; Dubois et al., 1983; Aubert et al., 1992; Lange et al., 1993). Deficits were more pronounced in the presence of dementia (Dubois et al., 1983), particularly in frontal regions (Ruberg et al., 1986). Temporal cholinergic losses were also shown to correlate with nbM neuronal loss and more severe cognitive impairment (Perry et al., 1985).

Quantitative morphological studies conducted around this time revealed significant depletion (up to 87%) of nbM cholinergic neurons in PD compared to controls (Arendt et al., 1983; Candy et al., 1983; Nakano and Hirano, 1984; Rogers et al., 1985), with more substantial losses observed in PDD (Whitehouse et al., 1983; Rogers et al., 1985). In direct comparisons, nbM neuronal loss was shown to be equivalent (Rogers et al., 1985), or more extensive in people with PD compared to AD (Arendt et al., 1983; Candy et al., 1983; Perry et al., 1985).

Early evidence identified a potential relationship between Lewy pathology and degeneration of the cBF. For example, people with AD with concomitant Lewy pathology were shown to have limited AD pathology in the nbM despite having greater nbM neuronal loss compared to pure AD (Cullen and Halliday, 1998).

A more recent study combined stereological analysis of the cBF with assessment of α-synuclein pathology and cholinergic activity in people with PD. Ch4 cell loss was moderate in PD, but severe in PDD compared to controls (Hall et al., 2014). In contrast, Ch1 and Ch2 neurons were largely spared. While Ch4 cell loss was variable, cortical cholinergic activity was reduced in both PD and PDD, suggesting significant dysfunction in cortical cholinergic pathways before frank neuronal degeneration. PDD was also differentiated from controls by reduced hippocampal cholinergic activity and increased α-synuclein pathology in the cBF and hippocampus. These findings suggest that Ch4 atrophy, hippocampal cholinergic denervation, and α-synuclein may play a synergistic role in the development of dementia in PD.

1.6.3 *Brainstem cholinergic deficits in Parkinson's disease*

Around half of cholinergic neurons in the PPN *pars compacta* have been shown to degenerate in people with PD (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989; Gai et al., 1991; Lee et al., 2000; Rinne et al., 2008), whereas these neurons were generally shown to be spared in people with AD (Jellinger, 1988; Davis et al., 1999). Clinical neuropathological data is relatively sparse, however, relationships between PPN *pars compacta* neuronal loss and the severity of PD symptoms have been reported (Zweig et al., 1989; Rinne et al., 2008).

A more recent post-mortem study retrospectively assessed UPDRS scores and showed that the number of AChE containing neurons in the PPN was significantly lower in PD patients with balance deficits and falls, compared to those without (Karachi et al., 2010). These early observations therefore provide evidence for the involvement of cholinergic brainstem degeneration in PD mobility impairments.

Post-mortem studies have provided valuable insight into cholinergic system pathology that accompanies neurodegenerative disease. However, by their

nature, these methods are often limited to end-stage disease cases when degenerative processes and clinical symptoms are fully established. This approach therefore provides limited information with regards to changes that occur earlier in the disease course. *In vivo* methods serve to overcome some of the limitations of post-mortem methods and allow for the assessment of brain–behaviour relationships in living individuals.

1.7 *In vivo* molecular imaging of the cholinergic system

1.7.1 *Molecular imaging markers*

Several radioactive ligands have been developed to target specific molecular components involved in cholinergic neurotransmission (Figure 1.4). Molecular imaging techniques such as PET and single-photon emission computed tomography (SPECT) enable *in vivo* assessment of these processes.

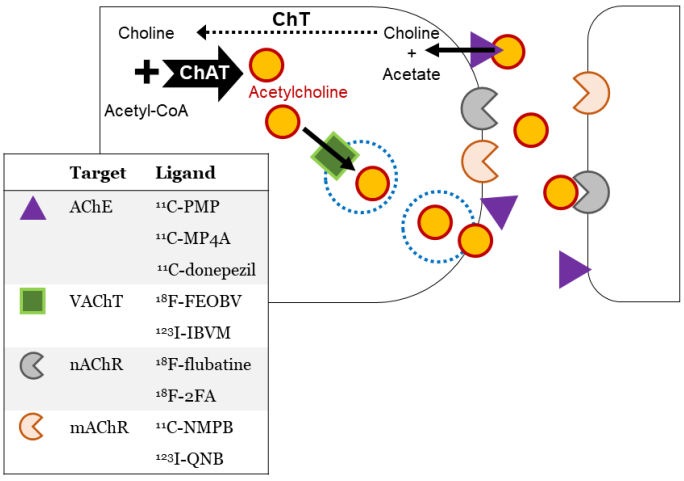


Figure 1.4 Schematic representation of cholinergic nerve terminal and synaptic cleft

Cholinergic imaging targets and example ligands displayed in table inset. Acetylcholine (ACh) is synthesised, via acetyltransferase (ChAT), and packaged into synaptic vesicles by the vesicular ACh transporter (vAChT), before fusing to the presynaptic membrane. Upon neuronal stimulation, ACh is released into the synaptic cleft where it binds to nicotinic (nAChRs) or muscarinic (mAChRs) ACh receptors. Within the synapse, ACh is inactivated and degraded into choline and acetate by acetylcholinesterase (AChE), which is bound to pre and postsynaptic membranes. Free choline can be recycled by the high affinity choline transporter (ChT) (Picciotto et al., 2012).

1.7.2 *Cortical cholinergic denervation and cognitive impairment*

Early *in vivo* studies (Table 1.3) have provided support to post-mortem findings, showing greater cholinergic deficits alongside more severe cognitive impairment. For example, reduced cholinergic activity was observed in the neocortex and hippocampus in moderate–severe AD (Kuhl et al., 1999), whereas mild AD cohorts showed only selective temporal losses (15%) (Bohnen et al., 2003).

In PD cohorts, cholinergic losses were reported throughout the entire neocortex in people with PDD, while losses were confined to parietal and occipital cortices in PD without dementia (Kuhl et al., 1996). More severe deficits have also been reported in PDD when compared to AD with a similar degree of cognitive impairment AD (Bohnen et al., 2003). In that study, losses were also greater in PD without dementia compared to AD. These findings imply that cholinergic losses in PD without dementia are at least comparable to AD, and may progress linearly with cognitive decline.

Cortical cholinergic deficits have also been correlated with cognitive performance (Table 1.3). In AD, reduced cortical cholinergic activity was associated with worse performance on tests of attention and working memory, but not with short-term or long-term memory (Bohnen et al., 2005). In early and late-onset AD, cholinergic deficits have also been correlated with worse scores on the Mini-Mental State Examination (Shinotoh et al., 2000). A PET study in MCI localised cholinergic deficits to fronto-parietal, lateral temporal, and limbic regions (Haense et al., 2012). More prominent reductions in the lateral temporal cluster correlated with learning, executive, and language comprehension functions.

Cortical cholinergic deficits have also been reported in PDD and PD compared to controls (Bohnen et al., 2006). In both PD groups, cholinergic deficits correlated with working memory, attention, and executive function scores. The same relationship was not observed with either the duration *or* severity of motor symptoms. Taken together, the findings outlined above suggest that cortical cholinergic denervation may be particularly relevant for attention and executive dysfunction in AD and PD.

Different cholinergic projections have been associated with different aspects of executive-attentional functions in PD. For example, during a sustained attention task, cortical cholinergic denervation correlated with the susceptibility to distraction, independent of thalamic cholinergic or striatal dopaminergic integrity (Kim et al., 2019b). Furthermore, in a signal detection task, thalamic cholinergic denervation was specifically related to impaired signal detection, over and above cortical cholinergic and caudate dopaminergic deficits (Kim et al., 2017). These findings broadly corroborate the animal studies discussed previously (Section 1.5), demonstrating the potential role of cortical cholinergic function in top-down attentional control, and brainstem cholinergic function in bottom-up cue detection and salience processing (Bohnen et al., 2018b).

1.7.3 *Variable cholinergic denervation in PD without dementia*

The extent of cholinergic denervation appears to be variable in the pre-dementia stages of PD (Table 1.3). For example, normal range neocortical and thalamic AChE activity was reported in a large PD cohort without dementia (Bohnen et al., 2012). Among those with below normal range activity, 31% presented with neocortical dysfunction compared to 18% with thalamic dysfunction. Those with low thalamic AChE also had low neocortical function, while the opposite was not the case. No specific relationships were found between thalamic activity and cognitive test performance, whereas neocortical deficits were associated with worse scores on tests of verbal learning, attention, and executive function.

In line with the above observations, cholinergic deficits have been reported in the occipital cortex in very early PD (i.e., disease duration < 3 years, some of whom were *de novo*), while losses were more widespread and severe in advanced PD (Shimada et al., 2009). Several other studies have also shown support for mild cholinergic deficits in PD without dementia (Hilker et al., 2005; Bohnen et al., 2006; Klein et al., 2010).

Table 1.3 Sample of PET and SPECT imaging studies of cholinergic nerve terminal markers in Alzheimer's and Parkinson's disease

Ligand	Target	Method	Participants	Key findings	Reference
^{123}I-IBVM	VAChT	SPECT	AD early onset <65 years (13), AD late onset, (9), PD (9), PDD (6), HC (36)	Reduced VAChT binding in entire neocortex in PDD; only reduced in parietal and occipital cortex in PD. In early onset AD widespread reduction in neocortex and hippocampus (around 30%). In late onset AD, VAChT reductions only in temporal cortex and hippocampus.	(Kuhl et al., 1996)
^{11}C-PMP	AChE	PET	AD moderate-severe (14), HC (26)	Reduced (25-33%) AChE activity in neocortex and hippocampus in AD. No changes in AChE activity in caudate, putamen, thalamus, pons, or cerebellum.	(Kuhl et al., 1999)
^{11}C-PMP	AChE	PET	AD majority late onset (12), PD (11), PDD (14), HC (10)	Greater reduction in cortical AChE activity in PDD (−20 %), then PD (−12.9 %), least in AD (−9.1 %). Selective involvement of lateral temporal cortex in AD (−15 %).	(Bohnen et al., 2003)
^{11}C-PMP	AChE	PET	AD (15), HC (12)	Reduced cortical AChE activity in AD. Positive correlation between cortical AChE activity and attention and working memory scores, no correlation with short-term or long-term memory. Similar findings when limited to temporal cortex.	(Bohnen et al., 2005)
^{11}C-MP4A	AChE	PET	PD (17), PDD (10), HC (31)	Greater reduction in cortical AChE in PDD (-29.7%) vs moderate in PD (-10.7%). Close association between ^{18}F-DOPA binding and ^{11}C-MP4A binding in frontal and temporoparietal cortex in PDD.	(Hilker et al., 2005)
^{11}C-PMP	AChE	PET	PD (11), PDD (13), HC (14)	Reduced cortical AChE activity in PDD (-20.9%), and PD (-12.7%). Working memory-attention (WAIS III Digit Span), and attention-executive (Trail Making and Stroop Colour Word) performance correlated with cortical AChE activity in PD groups. No correlation with motor symptom severity.	(Bohnen et al., 2006)
^{11}C-MP4A	AChE	PET	PD early (<3 years) (9), PD (9), PDD (10), DLB (11), HC (26)	Reduced cortical AChE in PDD and DLB (-27%), more moderate loss in PD cortex particularly occipital cortex (-12%). No significant difference between early PD and advanced PD groups or between DLB and PDD.	(Shimada et al., 2009)
^{11}C-MP4A	AChE	PET	PD (9), PDD (8), DLB (6), HC (3)	Mild cortical AChE reduction in PD. Severe reductions in PDD and DLB (particularly in occipital cortex, in PDD/DLB, but not PD). No significant difference between PDD and DLB. Frontal-occipital gradient of increasing cortical cholinergic impairment in all disease groups, most severe in dementia subgroups.	(Klein et al., 2010)
^{11}C-PMP	AChE	PET	AD (13), PD (11), PDD (6), DLB (6), HC (14)	Greatest reduction in thalamic AChE activity in PDD (−19.8 %), then in DLB (−17.4 %), least in PD (−12.8 %). Spared thalamic AChE activity in AD.	(Kotagal et al., 2012)
^{11}C-PMP	AChE	PET	PD (101), HC	In PD, cortical AChE normal in 31%, thalamic AChE activity normal in 18%, cortical and thalamic binding values in 65%. No relationships between thalamic activity and cognitive scores. Neocortical deficits associated with worse scores on tests of verbal learning, attention, and executive function.	(Bohnen et al., 2012)
^{11}C-PMP	AChE	PET	PD (143)	Significant independent and interactive effects of cortical cholinergic denervation and caudate nucleus dopaminergic in predicting cognitive impairment category.	(Bohnen et al., 2015)

This table represents papers discussed in this thesis and is not the result of a systematic review.

1.7.4 *Topography of degeneration of cholinergic target pathways*

VAChT binding protocols have been used to assess the integrity of major cholinergic pathways, revealing differential denervation between target regions. When comparing DLB to controls, SPECT imaging has revealed binding reductions in Ch4 terminal regions of anterior cingulate cortex, superior and inferior parietal cortices, and thalamus (Haense et al., 2012). Terminals of striatal interneurons also showed bindings reductions, while Ch1 terminal regions of the hippocampus showed no differences.

In a serial VAChT PET imaging study in AD, reduced uptake was shown to correlate with global cognitive deficits, and to follow a topographic pattern of severity, from temporal-parietal extending to frontal cortices (Aghourian et al., 2017). This pattern corresponds with previous topographical findings of cholinergic nerve terminal losses accompanying disease progression (Kuhl et al., 1999; Bohnen et al., 2003), as well as the caudal-rostral pattern of degeneration of Ch4 and ensuing cortical projections (Liu et al., 2015). There was no evidence of uptake reductions in the hippocampus however (innervated by Ch1-2), indicating that Ch4 and Ch1-2 may be differentially involved AD (Aghourian et al., 2017).

VAChT PET imaging in PD has revealed a shared topography of cholinergic losses that correlated with deficits in multiple cognitive domains including memory, attention, and executive function (van der Zee et al., 2020). Affected regions included cingulate cortex, bilateral insula, hippocampal region, and visual thalamus. This topography of deficits suggests that cholinergic projections from both the basal forebrain and brainstem regions may underpin cognitive impairment in PD.

1.7.5 *Cholinergic denervation and PD mobility impairments*

PIGD symptoms are less responsive to dopaminergic replacement medications than other cardinal motor features in PD (Sethi, 2008; Vu et al., 2012). There is also evidence from randomised controlled trials for cholinergic treatments (i.e., cholinesterase inhibitor, rivastigmine) improving gait and reducing the number of falls compared to placebo (Chung et al., 2010; Li et al., 2015; Henderson et

al., 2016). Data derived from pharmacological interventions therefore highlights the potential role of cholinergic function in the pathophysiology of mobility issues in PD.

Compared to controls, reduced thalamic cholinergic activity has been reported in PDD, DLB, and PD. Each of these subgroups were statistically different from AD – who showed relatively spared thalamic activity (Kotagal et al., 2012). These findings therefore support post-mortem evidence for PD-specific thalamic cholinergic degeneration. Thalamic cholinergic losses, but not cortical cholinergic or striatal dopaminergic deficits, have also been correlated with impaired sensory processing during postural control in PD (Müller et al., 2013a) potentially owing to reduced integrity of PPN neurons.

Slower gait speed has been associated with combined neocortical cholinergic and nigrostriatal dopaminergic denervation in PD without dementia, compared to controls (Bohnen et al., 2013). Importantly, gait speed was not independently associated nigrostriatal or thalamic denervation. In a subsequent study, freezing of gait was more frequent among those with reduced neocortical AChE than thalamic AChE deficits (Bohnen et al., 2014). Freezing of gait was also common in the presence of neocortical β-amyloid deposition, as well as lower striatal dopaminergic activity. Together, these data that suggest the pathophysiological mechanisms contributing to PIGD features in PD involve neocortical and brainstem cholinergic deficits, and concomitant dopaminergic losses.

The contribution of cholinergic and nigrostriatal dopaminergic denervation to fall status has been assessed in PD without dementia. Specifically, reduced cortical AChE was reported in fallers and non-fallers compared to controls (Bohnen et al., 2009). Furthermore, lower thalamic AChE (but not cortical cholinergic, or nigrostriatal dopaminergic) activity was able to distinguish fallers from non-fallers. These findings were later replicated by the same group, who demonstrated greater prevalence of fall history among those with low thalamic AChE activity, compared to normal range (Bohnen et al., 2012).

VAChT PET imaging recently demonstrated that falls and freezing of gait may represent distinct entities with different pathophysiological substrates. ROI analysis revealed thalamic VAChT reductions in fallers compared to non-fallers,

which was complemented by changes in right visual thalamus (especially the right lateral geniculate nucleus), right caudate nucleus, and bilateral prefrontal regions in whole-brain voxel-based analyses (Bohnen et al., 2019). Freezing of gait status was associated with reduced VAChT expression in the striatum, hippocampus, and amygdala, which were more prominent in the bilateral striatum, temporal, and middle-frontal limbic regions. These observations identify thalamic and striatal cholinergic denervation as key contributors to falls and freezing of gait in PD, respectively.

1.8 *In vivo* structural imaging of cholinergic nuclei

1.8.1 *Magnetic resonance imaging (MRI): morphometric analysis*

MRI-based methods complement molecular imaging techniques, providing an *in vivo* surrogate marker of structural alterations in cholinergic neurons. Unlike molecular techniques, MRI-based methods are non-invasive and provide excellent soft tissue contrast and anatomical detail.

VBM is widely used in studies of PD and AD (Grothe et al., 2012; Kilimann et al., 2014; Ray et al., 2018; Schulz et al., 2018). VBM is an automated and unbiased technique that uses high-resolution T1-weighted images to extract volumetric measures on a voxel-by-voxel basis. This technique can therefore be combined with region of interest (ROI) analysis via stereotactic mapping (Kilimann et al., 2014) to extract cBF volumes.

For the PPN however, standard procedures for automated segmentation of grey and white matter are not appropriate, because the entire brainstem is usually segmented as white matter by standard techniques. As such, volumetric analysis of the brainstem is not possible without dedicated brainstem segmentation approaches and even these approaches do not yet allow segmentation of very small structures like the PPN. Instead, techniques combining stereotactic mapping and DTI have been used to assess microstructural changes in the PPN. These methods are discussed in more detail in Section 1.9.

1.8.2 *Volumetric assessment of cholinergic basal forebrain nuclei*

1.8.2.1 *Degeneration of substantia innominata and cognitive impairment*

Early work relied upon the manual delineation of the substantia innominanta (SI) – a larger anatomical landmark containing the nbM, revealing pronounced degeneration in Lewy body dementias (including DLB, and PDD) (Hanyu et al., 2002; 2005; Oikawa et al., 2004). These findings were replicated in a similar cohort including MCI disease stages, this time using volumetric analysis of the SI to achieve greater spatial resolution (Kim et al., 2011). SI volumes were smaller in PDD and DLB than AD, and correlated with global cognitive function across cognitively impaired PD groups (PD-MCI, PDD, DLB). In a later study, greater SI atrophy was also reported in PDD compared to PD-MCI and PD without cognitive impairments (the latter two did not differ), which also correlated with attention and object naming performance (Choi et al., 2012).

In a more recent exploratory analysis, a PD-MCI cohort were prospectively followed for a minimum of 2 years to evaluate dementia conversion (Lee et al., 2014). Converters showed more severe cognitive deficits in executive functions, immediate verbal memory, and visual recognition memory, compared with non-converters. Both converters and non-converters had smaller SI volumes compared to controls. Moreover, converters had a significantly smaller SI volumes than non-converters. These results provided initial support for cBF structural measures as a potential biomarker for PD cognitive impairment.

More recently, stereotactic information derived from combined post-mortem MRI and histology was used to create cytoarchitectonic maps of the cholinergic basal forebrain nuclei (Kilimann et al., 2014). These tools were developed in the context of AD research, with several studies reporting a topographical pattern of cholinergic basal forebrain atrophy, that corresponds with neuropathological findings (discussed next).

1.8.2.2 *Spatiotemporal pattern of cholinergic basal forebrain atrophy in AD*

Cross-sectional studies using VBM in combination with cytoarchitectonic maps have revealed cBF degeneration in AD and MCI cohorts (Teipel et al., 2005;

Grothe et al., 2010; 2012). In a large dataset including mild and manifest AD, cBF volumes declined from early adulthood onwards (Grothe et al., 2012). In mild AD, volume reductions were most prominent in the posterior region of the nbM, while atrophy was more extensive and included the whole cBF region in clinically manifest AD.

A similar pattern of posterior Ch4 degeneration was also observed in an independent MCI cohort, with no volume differences in Ch1–Ch2 compared to controls (Grothe et al., 2010). Ch1-3 correlated with MMSE scores, while Ch4p correlated with delayed recall scores. These findings suggest that subregional volumes of cholinergic nuclei may be differentially associated with global cognition and specific memory deficits in encoding and retrieval of episodic memories.

The above findings were replicated in a multicentre dataset using a newly developed cBF mask (Kilimann et al., 2014). Again, AD showed significant volume reductions of all subregions of the cBF, which were most prominent in Ch4p. MCI showed pronounced volume reductions in Ch4p, but preserved volumes of anterior-medial cBF regions. Diagnostic accuracy of Ch4p volume was superior to hippocampal volume (the most established structural imaging marker of AD) in both groups, suggesting that cBF morphometry may provide an emerging biomarker in AD (Kilimann et al., 2014). Findings from *in vivo* imaging studies therefore provide support to post-mortem studies demonstrating early vulnerability of the Ch4p region in MCI, which extends to more widespread regions of the cBF as cognitive symptoms worsen.

1.8.2.3 *Cholinergic basal forebrain atrophy in prodromal AD*

Early longitudinal data provided support for the use of cBF volumetry as a biomarker for AD cognitive symptoms. Among those who developed AD, significant cBF atrophy was present up to 4.5 years before the onset of clinical symptoms (Hall et al., 2008). Building on these findings, a later study assessed subregional cBF changes over a 3-year follow-up in initially healthy elderly individuals and mild AD (Grothe et al., 2013). In that study, people with mild AD showed reduced volumes at baseline compared to controls, which was more pronounced in Ch4p, and extended rostrally over time in those who declined

cognitively. These findings agree with cross-sectional data, showing spatially restricted atrophy at pre-dementia stages, with more widespread cBF involvement as the disease progresses.

AD pathology is characterised by amyloid and tau accumulation, which spreads across anatomically and functionally connected brain regions over time (Grothe et al., 2018; Hanseeuw et al., 2019). The prevailing staging model assumes AD pathology originates in the entorhinal cortex, spreading later to the neocortex (Braak and Braak, 1991). However, longitudinal data has shown that nbM volume loss precedes and predicts cognitive impairment and degeneration of the entorhinal cortex (Schmitz and Spreng, 2016), and CSF tau and amyloid ratios has been shown to moderate this prediction (Fernández-Cabello et al., 2020).

In asymptomatic AD, Ch4 atrophy has been observed in people with isolated tau (but not amyloid) pathology, while the spreading of atrophy to Ch1-2 was dependent on both proteinopathies in later disease stages (Cantero et al., 2020). Combined structural MRI and amyloid PET imaging has also revealed associations between basal forebrain atrophy and cortical amyloid load in people with AD and non-demented elderly participants (Grothe et al., 2014; Teipel et al., 2014). The same group also reported associations between basal forebrain atrophy and cortical amyloid and Lewy pathology, but not tau (Teipel et al., 2020). Furthermore, the nbM itself exhibited very little neuritic plaques. These findings point to the relevance of comorbid protein accumulation for regional cBF degeneration. Future longitudinal studies may provide more insight into the directionality of the interactions between cBF atrophy and cortical protein pathology.

1.8.2.4 *Cholinergic basal forebrain atrophy and PD cognitive decline*

Cross-sectional studies have highlighted an association between cBF atrophy and severity of cognitive impairment in PD. In a large cohort of mixed disease stages (228 *de novo*, 125 more advanced PD, 101 controls) larger Ch4 volumes correlated with scores on tests of global cognition, attention, and visuospatial function in both PD groups (Barrett et al., 2019).

Stereotactic mapping and VBM methods were more recently applied to assess longitudinal cholinergic basal forebrain changes in a large cohort of people with *de novo* PD (Ray et al., 2018). The authors demonstrated for the first time, that baseline Ch4 volumes could predict risk of MCI over a 5-year follow up period. Those with smaller volumes showed more severe and rapid decline on tests of memory and semantic fluency, but not on tests of executive function. In agreement with the AD literature, deterioration in the posterior Ch4 region may be characteristic of MCI in PD, as only volumes in this region were shown to differentiate PD with suspected MCI from controls (Ray et al., 2018). This finding was replicated in a study combining MRI and diffusion tensor imaging, discussed in more detail below (Schulz et al., 2018).

More recently, cBF volumes were assessed longitudinally over a 10-year period in people with PD, PDD, and controls (Pereira et al., 2020). Those with PDD at baseline, and those who later converted to PDD showed greater baseline Ch4 atrophy than controls, in addition to Ch1-2 atrophy over time compared to controls and PD. Longitudinal changes in Ch4 volumes predicted future PDD and were followed by changes in Ch1-2 – reflecting a posterior-anterior pattern of atrophy with disease progression – similar to that observed in AD.

In line with findings in *de novo* PD from the study by Ray et al (2018), a recent study in PD without dementia showed that cBF volumes were not significantly different to controls (Grothe et al., 2021). However, within the PD group, lower cBF volume correlated with worse performance on tests of global cognition, attention, executive function, and verbal memory (Grothe et al., 2021). These findings motive further cross-sectional work to better understand the extent of cBF degeneration across the disease spectrum and its relationship with cognitive performance.

1.8.2.5 *Cholinergic basal forebrain atrophy and PD gait impairment*

The structural integrity of the nbM has also been linked to PD posture and gait impairments. Cross-sectional data have demonstrated relationships between Ch4 grey matter density loss (but not Ch1-3), and slowing of gait speed and Timed Up and Go dual-task performance (Dalrymple et al., 2021). Longitudinal data collected from a PD cohort undergoing deep brain stimulation (DBS) of the

subthalamic nucleus revealed associations between nbM atrophy and increased swing time variability (a measure of gait variability), as well as decreased gait speed (Wilkins et al., 2020). Over three-years of follow-up, DBS (and dopaminergic medication) improved cardinal motor symptoms as well as gait speed, but had no beneficial effects on swing time variability.

Subregional changes in Ch4 have also been shown to predict the longitudinal progression of gait impairments (Wilson et al., 2020b). In that study, no relationships between cBF nuclei and gait changes were observed at baseline. However, in up to 3-years of follow-up, smaller Ch4 volume predicted increasing step time variability and shortening swing time, and smaller posterior Ch4 portions predicted shortening step length and increasing step time variability. These findings therefore suggest that higher order aspects of gait control may be reliant on the cortical cholinergic system.

1.9 *In vivo* microstructural imaging of cholinergic nuclei

1.9.1 *Diffusion tensor imaging*

Diffusion tensor imaging (DTI) is a powerful method for exploring microstructural changes within brain tissue. It is based upon the motion of water molecules, which varies in magnitude, direction, and degree of anisotropy, depending on the host tissue (Soares et al., 2013). With DTI analysis, it is possible to infer properties from each voxel in order to extract quantitative metrics, such as those listed below in Table 1.4. These methods have been used to index degeneration in cholinergic nuclei in people with PD (discussed below).

Table 1.4 Commonly used metrics derived from diffusion imaging

Diffusion MRI metric	Measures
Fractional anisotropy	Direction of water diffusion
Mean diffusivity	Overall magnitude of diffusivity
Axial diffusivity	Diffusion rate along main axis
Radial diffusivity	Diffusion rate along transverse axis

1.9.1.1 *Cholinergic basal forebrain microstructure and cognitive impairment*

In a multicentre retrospective study, increased mean diffusivity in the right basal forebrain has shown similar predictive power as cBF volumetry for the conversion from MCI to AD (Brueggen et al., 2015). Cross-sectional findings have also reported higher cBF mean diffusivity in MCI and AD compared to healthy controls, which again was comparable to volumetric assessments (Herdick et al., 2020).

Subregional changes to cBF microstructure have also been shown in cross-sectional studies of PD without dementia (Gargouri et al., 2019). Specifically, mean diffusivity and radial diffusivity were increased in Ch1-2 and Ch3-4 regions. In addition, reduced functional connectivity was observed between Ch1-2 and the medial temporal lobe, and between Ch3-4 and frontal, occipital, insular, and medial thalamic regions. Changes in diffusion measures and connectivity in Ch1-2 correlated with memory and visuospatial function, whereas changes in Ch3-4 correlated with global cognition and executive functions.

VBM and DTI measures were recently derived from a large drug-naïve PD cohort followed for 36 months (Schulz et al., 2018). Results revealed that in the nbM, both smaller volume and increased DTI mean diffusivity could predict the onset of cognitive impairment. Further analysis revealed that mean diffusivity was a stronger predictor than volumetric measures, suggesting that

microstructural changes in the nbM may precede macrostructural damage of the region, and may therefore be more sensitive to early changes related to PD cognitive decline.

In contrast to the above findings, a more recent study in PD without dementia revealed no significant associations between cBF mean diffusivity and cognitive function (diffusivity was not compared between groups) (Grothe et al., 2021). It is possible that analyses that account for the contribution of free water may provide different results (discussed in Section 1.9.2 below).

1.9.1.2 *PPN microstructure and PD mobility impairment*

In PD with FOG, altered mean diffusivity measures have been observed in subcortical structures connected with the PPN, including basal ganglia, thalamus, and cerebellum (Youn et al., 2015). The authors of this study manually delineated the PPN for region of interest analysis, demonstrating reduced fractional anisotropy, and increased mean diffusivity bilaterally in PD patients with FOG. Furthermore, fractional anisotropy in the right PPN was moderately correlated with FOG severity. Interestingly, a previous study assessing PPN structural connectivity with DTI also found degeneration in the right PPN in association with FOG (Fling et al., 2013).

More recently, a stereotactic map of the PPN (developed to improve targeting for DBS (Alho et al., 2017)) was used to extract DTI measures in a large cohort (n=147) of people with early-stage PD (diagnosed for 2 years or less), followed longitudinally over 72-months (Craig et al., 2020a). Survival analysis revealed that increased axial diffusivity in the PPN was able to predict future PIGD, independent from the risk incurred by dopamine reductions in the caudate. Findings discussed here therefore highlight the potential for measures of PPN microstructure to assess mobility impairments in PD.

In people with PD with FOG, larger dual-task interference has been corelated with more lateralised PPN structural connectivity (measured with DTI) (Peterson et al., 2015). This led to an inability to maintain stride length when participants were tasked with walking while simultaneously attending to an auditory tone to initiate a head turn. These observations suggest that the PPN

may play a role in attentional control in FOG, and provide further support to animal studies (outlined previously) underscoring the cognitive functions of the PPN (Steckler et al., 1994; Winn, 2008; Gut and Mena-Segovia, 2019). Further *in vivo* investigation may help to elucidate these relationships in the context of PD cognitive impairment.

1.9.2 *Free water imaging*

Despite their sensitivity, the traditional DTI metrics discussed above may not be tissue specific. The assumption of a single-tissue compartment per image voxel (Alexander et al., 2007) can lead to partial volume effects, such that the resulting diffusion metrics reflect multiple tissue components. Free water is present in CSF and can accumulate in extracellular space as a result of brain pathologies, such as neuroinflammation (Kamagata et al., 2020), and may therefore conflate DTI metrics. To overcome these effects, free water imaging techniques have been developed to quantify extracellular free water, which can be used to remove the contribution of free water, to provide a more specific estimate of tissue microstructure (Pasternak et al., 2009), *and/or* as potential marker of neuroinflammation (Kamagata et al., 2020).

Increased free water in the posterior substantia nigra is emerging as a marker of PD disease progression (Ofori et al., 2015b; Burciu et al., 2017) and has been correlated cross-sectionally with motor symptom severity (Ofori et al., 2015a). More recently, people with PD demonstrated elevated free water and free water-corrected DTI metrics in the cBF and PPN, respectively (Ray et al., under review). These measures were also associated with changes to cognitive performance during attentional reaction time tasks. Whether these methods are informative for mobility impairments when applied to the PPN remains open for investigation.

1.10 Resting-state quantitative EEG, cholinergic system changes, and PD symptoms

Molecular imaging continues to be instrumental to furthering our understanding of cholinergic system changes that accompany neurodegeneration. These methods are, however, expensive, and invasive, which can be a limiting factor in their clinical applicability. There is a need to identify and validate widely accessible, non-invasive markers of cholinergic system dysfunction that could be used to improve prognostic information and stratify people for targeted cholinergic drug treatments. In this regard, EEG is a potential candidate.

1.10.1 *Quantitative electroencephalography (qEEG)*

The summed electrical activity of populations of neurons can be measured from the scalp with electroencephalography (EEG). Quantitative EEG (qEEG) is a modern technique that involves recording digital EEG signals which are then processed, transformed, and analysed using mathematical algorithms. qEEG offers several key advantages over other neuroimaging techniques including its small, portable, non-invasive, and relatively low-cost setup that requires only a quiet space for acquisition. For such reasons, together with evidence outlined below, qEEG is increasingly recognised an attractive candidate for biomarker research (Klassen et al., 2011; McKeith et al., 2017).

Many studies have focused on the links between task-related EEG activity and behavioural performance, in attempt to better understand how neuronal activity organises action and cognition (Klimesch et al., 1998; Klimesch, 1999; Engel and Fries, 2010; Gola et al., 2012). However, during resting-state conditions, the brain remains functionally and metabolically active. Recording the spontaneous fluctuations in this activity has become an important research focus in recent years (Fox and Raichle, 2007), with functional imaging revealing considerable spatial and temporal organisation of brain networks (Yeo et al., 2011). Resting oscillatory activity may underpin some of the neurochemical and neuroanatomical changes associated with PD symptoms, and may therefore represent an important target for early disease monitoring.

Resting-state paradigms observe the brain in absence of active performance or stimulation, with eyes open or closed. This therefore represents a standardised procedure that can be easily carried out and repeated. In addition, the signal is not directly confounded by individual differences in behavioural performance or cognitive abilities. This makes resting-state studies particularly suitable for older populations, and those who may experience issues with cognitive and motor performance, such as people with PD.

1.10.2 *Resting-state qEEG in PD*

Power spectral analysis of resting-state qEEG may be a useful tool for predicting progression of PD (Geraedts et al., 2018). A range of spectral metrics have been studied for their relationship with PD clinical symptoms across the disease spectrum, among which, cognitive symptoms have been studied most extensively. Table 1.5 outlines qEEG metrics that have been used in the literature cited in this thesis, along with a summary of key findings (note, this table does not represent a systematic review of all PD-qEEG literature).

Studies assessing the relationship between PD motor function and qEEG have reported mixed findings (Geraedts et al., 2018). For example, no correlations between spectral power and Unified Parkinson's Disease Rating Scale III subscores or Hoehn & Yahr stage (H&Y) (Babiloni et al., 2011; Guner et al., 2016), while in other studies, H&Y stage was shown to correlate positively with theta (He et al., 2017a), alpha (Fonseca et al., 2009), and beta frequencies (He et al., 2017b).

The progression of PD cognitive decline has been associated with eyes-closed qEEG slowing, i.e., lower alpha and beta power, and higher delta and theta power. People with PDD generally exhibit markedly slower qEEG values than healthy controls and PD without cognitive impairment. Whereas slowing in PD-MCI has been shown to range somewhere in between PD and PDD (Caviness et al., 2007b; 2016). qEEG slowing has also been shown to correlate with global and domain specific cognitive impairment, although these findings are not universal (Caviness et al., 2007b; Babiloni et al., 2011; Caviness et al., 2015; Guner et al., 2016; Eichelberger et al., 2017). Follow-up data has also identified delta and theta power, slowing ratio (between slow and fast frequencies), and

peak background frequency as potential predictive biomarkers for dementia incidence in PD (Klassen et al., 2011; Caviness et al., 2015; Latreille et al., 2016).

Similar qEEG abnormalities have been observed in AD (Jeong, 2004; Babiloni et al., 2006; Vecchio et al., 2013; Babiloni et al., 2014), with some reports suggesting that qEEG slowing may be more pronounced in Lewy body disorders, both in the context of MCI (Benz et al., 2014; Massa et al., 2020; Schumacher et al., 2020a) and dementia states (Bonanni et al., 2008; Babiloni et al., 2011; Fonseca et al., 2013; van der Zande et al., 2018). However, attempts to reliably distinguish AD and PD in early MCI stages with qEEG measures have provided only moderate diagnostic accuracy (Babiloni et al., 2018; Schumacher et al., 2020a).

Lower alpha power has been reported in AD vs PDD (Bonanni et al., 2008; Babiloni et al., 2011). Furthermore, alpha power was reduced in AD compared to controls, but remained normal in Lewy body dementia (including PDD and DLB) (Schumacher et al., 2020b). In that study, eyes-open alpha power increases were shown to differentiate PDD and DLB from controls and AD. In addition, alpha reactivity (i.e., the magnitude of alpha power attenuation from eyes-closed to eyes-open) was more severely impaired in PDD and DLB compared to AD. The observations suggest that, in addition to eyes-closed data, eyes-open spectral changes may also be informative.

The above findings highlight a similar pattern of qEEG changes in AD and PD. Whether these changes arise from similar pathophysiological mechanisms has not yet been resolved. As outlined in previous sections, degeneration of the cortical cholinergic system represents a shared neuropathological feature of PD and AD. Furthermore, cholinergic input from the basal forebrain and PPN (via the thalamus) has been shown to influence cortical activity (discussed in section 1.5). Thus, a cholinergic contribution to the resting-state qEEG alterations reported here is therefore feasible.

Table 1.5 Summary of resting-state spectral quantitative EEG changes across the spectrum of PD cognitive impairment

Author	Cohort	qEEG metric	Main findings
(Caviness et al., 2007b)	PDD PD-MCI PD	Dominant frequency	PDD<PD-MCI<PD-NCOG
		Relative power	Delta: PDD>PD, PDD>PD-MCI Theta: PD-MCI>PD Alpha: PDD<PD Beta1 and Beta2: PDD<PD, PD-MCI<PD Across all groups: MMSE impairment correlated with higher delta and lower alpha power
(Bonanni et al., 2008)	DLB PDD-F PDD AD HC	Dominant frequency	DLB<AD, PDD-F<AD, DLB<PDD, PDD-F<PDD, DLB<CON, PDD-F<CON
		Variability	DLB<AD, PDD-F<AD, DLB<PDD, PDD-F<PDD, DLB<CON, PDD-F<CON
		Relative power	Delta: patient groups>CON, AD<DLB, AD<PDD-F Low theta: DLB and PDD-F>CON, DLB>AD, PDD Pre alpha: DLB>control and AD and PDD Alpha: AD>DLB and PDD-F
(Fonseca et al., 2009)	PDD PD-MCI PD HC	Absolute power	Posterior Delta: PD<PDD, PD-MCI<PDD, PDD>HC Posterior Theta: PDD>HC Alpha, Beta: ns
		Relative power	Posterior Delta: PD-MCI<PDD, PD<PDD, PD-MCI and PDD>HC Posterior Theta: PD<PD-MCI, PD<PDD, PD-MCI and PDD>HC Posterior Alpha: PD>PDD, PDD<HC Beta: ns
(Klassen et al., 2011)	PD	Dominant frequency Relative power	Lower than median dominant frequency and higher than median theta power predicted incidence of PDD
(Babiloni et al., 2011)	PDD AD HC	Relative power (source)	Central Delta: PDD>AD and HC Posterior Theta: PDD>AD and HC Posterior Alpha1: AD<PDD<HC (correlation in patient groups: lower alpha lower MMSE scores) Posterior Beta1: PDD>AD and HC
(Benz et al., 2014)	PD-MCI AD-MCI HC	Relative power	Theta: PD-MCI>HC, PD-MCI>AD-MCI Alpha2: PD-MCI<HC, AD-MCI<HC, PD-MCI<HC, PD-MCI<AD-MCI Delta, Beta: ns
(Caviness et al., 2015)	PD	Dominant frequency	ns
		Relative power	Delta: incident PDD>PD Theta, Alpha, Beta: ns Correlations with change in cognitive scores: Auditory verbal learning (-) Delta, Theta, (+) Beta Stroop Colour-Word (-) Delta, Theta, (+) Alpha Word association (-) Delta, (+) Alpha TMTB (+) Delta, (-) Alpha Clock drawing (-) Delta
		Dominant frequency	PDD<HC, PD>PDD

(Latreille et al., 2016)	PDD PD HC	Absolute power	Delta: PDD>HC, PD>HC Theta, Alpha, Beta: ns
		Slowing ratio	PDD>PD, PDD>HC. Slowing ratio and lower dominant occipital frequency predicted PDD
(Caviness et al., 2016)	ILB PD PD-MCI PDD HC	Dominant frequency	ILBD<HC, PDD<PD, PD-MCI<PD
		Relative power	Delta: PD<PD-MCI<PDD Theta: ILDB<PD, PD-MCI>PD Alpha: PD>PD-MCI>PDD Beta: ns
(Guner et al., 2016)	PD HC	Absolute power	Delta and theta: PD>CON Alpha: PD<CON Beta: ns
		Slowing ratio	PD>CON + correlation with MMSE impairment, no/weak correlations with domain specific tests
(Eichelberger et al., 2017)	PD	Slowing ratio	Low performance on clock drawing test and Rey copy test associated with greater slowing (parietal, all regions, respectively)
(Hassan et al., 2017)	PD PD-MCI PDD	Relative power	Delta: PDD>PD, PDD>PD-MCI Theta: PDD>PD, PDD>PD-MCI Alpha: none Beta: PDD<PD, PDD<PD-MCI Gamma: none
(He et al., 2017a)	PD PD-MCI HC	Relative power	Delta: none Theta: PDMCI>PD and HC Alpha: none Beta: PDMCI<CON
(Massa et al., 2020)	LBD-MCI PD AD-MCI HC	Slowing ratio	LB-MCI and PD>HC AD-MCI<LB-MCI No differences between AD-MCI and HC, LBD-MCI and PD, or PD and AD-MCI
(Schumacher et al., 2020a)	LB-MCI AD-MCI HC	Dominant frequency	LB-MCI<HC, LB-MCI<AD-MCI
		Relative power	Delta: none Theta: LB-MCI>HC Pre-alpha: LB-MCI>AD-MCI>HC Alpha: LB-MCI<HC Beta: LB-MCI<HC, LB-MCI<AD-MCI
		Slowing ratio	LB-MCI>HC
(Schumacher et al., 2020b)	DLB, PDD AD HC	Alpha reactivity	DLB/PDD<AD<HC
		Relative power	Eyes-open individual alpha: DLB/PDD>HC, DLB/PDD>AD Eyes-closed individual alpha: AD<HC

DLB = dementia with Lewy bodies; ILB = Incidental Lewy body disease (not meeting PD criteria); PDD-F = Parkinson's disease dementia with cognitive fluctuations; LBD = Lewy body disease HC = healthy controls; ns = not significant; TMTB = Trail Making Test..

1.1.1 Cholinergic system changes and EEG

Early animal and human studies using lesion, pharmacological, and post-mortem techniques provided evidence for the influence of cholinergic projections on EEG measures. In rats, selective excitotoxin lesions or destruction of the nbM were shown to increase slow wave EEG activity (increased delta, decreased alpha) (Buzsaki et al., 1988; Riekkinen et al., 1990).

In human studies conducted in AD, reduced CSF AChE correlated with delta power and alpha/delta slowing ratio (using prior *in vivo* recordings) (Riekkinen et al., 1989). At autopsy, those who demonstrated highest delta power presented with very low cortical ChAT and nbM cell count. In moderate-severe AD, those with prominent EEG slowing were shown to exhibit significantly lower ChAT in the frontal cortex than those without (Soikkeli et al., 1991). Furthermore, markers of dopamine, noradrenaline, or serotonin were not associated with EEG changes.

Pharmacological agents that influence cholinergic function have also been studied for their effects on resting-state spectral EEG markers. Administration of AChE inhibitors (i.e. donepezil, rivastigmine, tactrine, and galantamine), which sustain the availability of ACh by limiting its degradation in the synapse, have been shown to reduce EEG slowing (delta and theta rhythms) and increase dominant frequency (alpha rhythm), for reviews, see (Jeong, 2004; Babiloni et al., 2013). Furthermore, scopolamine – a muscarinic antagonist – is widely used as a pharmacological model of cognitive impairment (Klinkenberg and Blokland, 2010).

In humans, scopolamine administration has been shown to increase the power of delta and theta activity, and reduce alpha and beta frequency activity (Osipova et al., 2003), thereby mimicking the slowing effects of AD and PD on cortical signals. In turn, techniques combining scopolamine and quantitative EEG measures have been developed by way of monitoring cortical cholinergic activity (Johannsson et al., 2015).

Together, the research described above implies that EEG measures may be relevant for cholinergic system changes that underpin neurodegenerative conditions. Also apparent is the need to establish techniques to explore the

relationship between cholinergic system changes in those with disease-induced cholinergic loss and cortical EEG signals. To address this question, a small handful of studies have combined MRI-based methods with qEEG. Outcomes from these studies are discussed next.

1.11 Combining MRI and qEEG measures to index cholinergic system integrity

Given the heterogenous neurobiological substrates of cognitive and mobility impairments, and the overlapping themes from structural and functional imaging, cholinergic system alterations are likely to be expressed as changes to multiple properties of neuronal mechanisms. Consequently, multimodal measures may help to quantify/characterise distinct aspects of cholinergic changes more accurately than a single modality.

Previous attempts to study the relationship between cholinergic system deficits and qEEG have focused on quantifying white-matter hyperintensities along cholinergic fibre tracts. Using these methods, increased white matter lesion load has been shown to correlate with impaired alpha reactivity (an EEG metric that indexes a loss of the prominent alpha rhythm in response to eye-opening) in older adults (Wan et al., 2019), and with widespread reductions in alpha power in amnestic MCI (Babiloni et al., 2009).

In a more recent study using MRI-based volumetry in AD and Lewy body dementia (PDD and DLB), reduced alpha reactivity was shown to correlate with smaller nbM volumes, in PDD specifically (Schumacher et al., 2020b). In a more recent study by the same group, qEEG slowing (but not alpha reactivity) was associated with smaller nbM volumes in the MCI stages of AD and LBD. These findings highlight the potential for combined use of structural MRI and resting-state qEEG to index cholinergic system integrity in PD. Whether such changes can be identified in earlier, pre-dementia disease stages of PD remains largely unexplored. In addition, whether there are specific qEEG metrics that are more sensitive to cholinergic changes in different neurodegenerative diseases is still to be determined.

1.12 Thesis aims

The objective of this thesis was to combine clinical, electrophysiological, and neuroimaging data to explore relationships between the structural integrity of cholinergic nuclei in AD and PD. In particular however, the thesis will focus on how structural changes in these nuclei are associated with cognitive and mobility impairments in PD.

This was achieved through the following aims:

1. To assess cholinergic basal forebrain volumes and explore relationships with resting-state quantitative EEG spectral measures in people with PD, people with MCI associated with likely AD, and healthy controls (Chapter 3).
2. To assess pedunculopontine microstructural changes using DTI and explore relationships with resting-state quantitative EEG spectral measures in people with PD, people with MCI associated with likely AD, and healthy controls (Chapter 4).
3. To assess cholinergic basal forebrain volumes and explore relationships with cognitive and mobility performance in people with PD and healthy controls (Chapter 5).
4. To assess pedunculopontine microstructural changes using free-water corrected DTI as well as an estimation of free-water content, and explore relationships with cognitive and mobility performance in people with PD and healthy controls (Chapter 6).

Chapter 2. General methods

Data from two research studies were used in the different analyses comprising this thesis. The first study was previously conducted at the University Medical Centre Ljubljana, Slovenia, the second at Manchester Metropolitan University, UK.

Chapters 3 and 4 of this thesis focus on the analysis of data from the Slovenian study, while Chapters 5 and 6 focus on data from the UK study. General methods used in each of these studies are detailed in the following sections. Study specific methods are further outlined in the methods sections of respective chapters.

2.1 Slovenian study

2.1.1 *Study overview*

Data collection for the Slovenian study took place in 2013 and 2014, and constitutes an observational study with the broad aim of developing markers of cognitive decline and neurodegeneration. Standardised assessment included clinical history, neurological examination, and cognitive screening. Consecutive patients who were eligible and willing to take part were included.

2.1.2 *Participants and recruitment*

The study included a total of 73 participants. Thirty-one people with PD were recruited from the Movement Disorders Clinic of the Department of Neurology in Ljubljana. Diagnosis of PD was made by movement disorder specialists according to UK PD Society Brain Bank Criteria (Hughes et al., 1992).

Twenty-one people with MCI were recruited from the Memory Clinic of the Department of Neurology in Ljubljana. Diagnosis of MCI was based on established criteria (Albert et al., 2011). In brief, this included (i) evidence of a change in cognitive ability compared to previous levels – obtained from the individual or somebody who knew them well, or from an observing clinician; (ii)

two or more cognitive scores falling greater than 1.0 standard deviation below normative means (cognitive battery described below in Section 2.1.4); (iii) preservation of independence in functional abilities.

Twenty-one healthy control participants were recruited from the local community through word of mouth and advertisements in physician waiting rooms and newsletters.

No people with PD or MCI had characteristic clinical features to suggest other disorders. Exclusion criteria for all groups included: history of neurological disease or mental disorders (clinical disorders or acute medical conditions), previous large-artery stroke or cerebral haemorrhage, history of moderate to severe head injury, major depression, prior or current drug or alcohol abuse, and contraindications to MRI. People with PD and MCI were excluded if cognitive symptoms and examination suggested a diagnosis of dementia. Furthermore, healthy control participants were excluded if cognitive symptoms and examination suggested a diagnosis of MCI, or if they expressed concern about a persistent decline in cognitive function in comparison with their previous state, in order to exclude the presence of subjective cognitive decline.

2.1.3 *Ethical approval and consent*

Ethical approval for the study was provided by the Republic of Slovenia National Medical Ethics Committee. All participants provided informed written consent in accordance with the Declaration of Helsinki.

2.1.4 *Neuropsychological assessment*

Participants completed a battery of pen-and-paper cognitive tests assessing global and domain specific function. Details of these tests are outlined below.

2.1.4.1 *Montreal Cognitive Assessment (MoCA)*

Used to assesses global cognitive function (Nasreddine et al., 2005) via a 30-question test with a maximum score of 30, which is comprised of 7 subcomponents (max. score for each shown in parentheses):

Visuospatial/executive (/5) includes (i) shortened version of TMTB test (see below for description); (ii) copying a cube onto the page; (iii) drawing a clock face that reads a specified time, from memory.

Animal naming (/3) includes three pictures of animals that are to be named orally.

Delayed recall (/5) includes five words being read to the participant who is asked to repeat them (performed twice). On completion of the entire test, participant is asked to recall each of the five words.

Attention (/5) involves (i) repeating digits in forwards, and backwards, order; (ii) tapping hand when letter 'A' is heard from a list of letters that are read aloud; (iii) serially subtracting 7 from 100, until number 65 is reached.

Language (/3) includes (i) repeating two sentences exactly as they were read; (ii) naming maximum number of words in one minute beginning with letter 'F' (point given if >11 words reached).

Abstraction (/2) includes two pairs of words read orally to participant who is asked to disclose the conceptual similarity between the words.

Orientation (/6) involves naming the date, month, year, day, place, and city (i.e., current location).

Cut-off scores for screening cognitive impairment in PD have been identified as <21/30 for PDD and <26/30 for PD-MCI (Dalrymple-Alford et al., 2011).

2.1.4.2 *Trail making test (TMT)*

A commonly used measure of attention and executive function, comprising two parts – TMTA and TMTB (Reitan, 1958). TMTA requires a participant to draw a line on a page connecting numbers spanning from 1-25. In TMTB, the line connects a number, then corresponding letter, switching in this manner in ascending order. The numbers span from 1-13 and letters from A- L. The pen should be kept on the page throughout the duration of the tests. Tests are timed

and should be completed as quickly as possible. Participants are directed back to their last correct answer if a mistake is made.

2.1.4.3 *Colour-Word Test*

A subtest from the Delis-Kaplan Executive Function System used to assess response inhibition and cognitive flexibility (rule switching) (Delis et al., 2001). Participants are required to name the ink colour in which a word is printed, while inhibiting the reading of the word. For example, for the word 'blue' written in red ink – the answer would be 'red'. The list of words should be read as quickly as possible.

2.1.4.4 *Tower Test*

A subtest from the Delis-Kaplan Executive Function System, modified from older tower tasks e.g. (Shallice, 1982). It is used to assess executive functioning, including spatial planning, rule violations, and inhibition (Delis et al., 2001). The task involves transferring two-five disks of different sizes across three pegs to a match prespecified configuration, in as few moves as possible while (i) moving only one disk at a time and (ii) never placing a larger disk over a smaller disk. Completion time is recorded.

2.1.4.5 *Weschler Adult Intelligence Scale (WAIS) Similarities subtest*

Participants are orally presented with 19 pairs of words and asked to disclose the qualitative relationship between each of the pairs (Wechsler, 1997). For example, 'apple-pear', answer: fruit. The test is designed to assess verbal reasoning and the development of concepts.

2.1.4.6 *Letter Fluency*

A subtest from the Delis-Kaplan Executive Function System (Delis et al., 2001) used to assess executive retrieval of verbal information. Participants are given one-minute to generate as many unique words as possible starting with a given letter (letter fluency).

2.1.4.7 *Rey Osterreith Complex Figure Test (ROCFT: Copy trial and Delayed Recall trial)*

Participants are asked to draw the complex Rey figure, constructed of lines and geometric shapes, onto a piece of blank paper as accurately as possible. The copy trial is completed while viewing the original figure as a reference. For the Rey Long Delay trial, participants are asked to draw the figure from memory after a delay of approximately 30 minutes. The time to completion is recorded for each trial. The test provides a measure of visuo-constructional ability, planning, and visual memory (Osterrieth, 1944).

2.1.4.8 *California Verbal Learning Test (CVLT: Long Delay Free Recall trial).*

A list of 16 nouns, from four semantic categories, is read to the participant over five learning trials. After each trial the participant is asked to recall as many words as possible (free recall). An interference list of words is then presented. Free recall of the learned list is then tested after a period of 20 minutes. The CVLT provides a measure of verbal learning and memory (Delis et al., 2000).

2.1.5 ***Electroencephalography (EEG)***

EEG involves placing electrodes on the scalp that record the summation of postsynaptic dendritic potentials generated by populations of cortical pyramidal cells that are orientated to the scalp surface. The resulting signals are amplified and changes in voltage are plotted over time. The raw unit of measurement of EEG is volts (typically microvolts), which represents the change in the measured electrical potential between any given electrode and a reference electrode located elsewhere on the scalp.

Quantitative EEG (qEEG) methods were applied in this thesis, which involve the mathematical processing of digitally recorded EEG signals to derive waveform and oscillatory information. Neural oscillations contain a mix of multiple frequencies that simultaneously vary over time. These oscillations can be separated with processing techniques to represent the time series signal in the frequency domain (discussed in more detail in the following section 2.1.5.3).

2.1.5.1 *EEG acquisition*

Resting state EEG recordings were conducted between the hours of 09:00 and 11:00. Recordings were acquired from 32 active Ag/AgCl electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, TP9, CP5, CP1, CP2, CP6, TP10, P7, P3, Pz, P4, P8, PO9, O1, Oz, O2, PO10), mounted on an actiCAP (Brain Products GmbH, Germany), positioned in accordance with the extended 10-20 system (Jasper, 1958), with FCz serving as the physical reference and AFz as ground.

Recordings were amplified with a BrainAmp MR plus amplifier (Brain Products GmbH, Germany) and digitized at a sampling frequency of 512 Hz. Filtering of the raw EEG data was not used. Before recording, the participants' scalp was gel-abraded under each electrode to lower impedances under 5 kΩ homogenously across electrodes. Recordings were taken with eyes-closed and eyes-open, providing approximately 300 seconds of data for each condition.

2.1.5.2 *EEG pre-processing*

Pre-processing of EEG data was performed using the EEGLAB toolbox (Delorme and Makeig, 2004) and MATLAB 2019a (Mathworks, USA). Recordings were first band-pass filtered between 0.5 and 40 Hz. Noisy data segments and bad channels were removed using the default parameters of the *clean_artifacts* function (default parameters: Channel: 0.85, Line noise: 4, Burst: 5, Window: 0.25) within EEGLAB, which uses the artifact subspace reconstruction method to identify contaminated data. Bad channels were replaced using spherical spline interpolation and remaining data were recomputed against the average reference. Across all participants, an average of 2 channels were removed.

Data were then split into non-overlapping epochs of 2 seconds and subsequently decomposed using Independent Component Analysis (ICA, EEGLAB, Infomax algorithm; Makeig et al., 1996) to perform semi-automated and visual-inspection based rejection of epochs on the derived components (Delorme et al., 2007). To mark epochs for rejection, the following parameters were used in EEGLAB: abnormal values (+/- 25 SD), and abnormal spectra (+/- 50 dB in 0-2

Hz frequency range, +25 -100 dB in 20-40 Hz frequency range). Remaining epochs following automatic rejection underwent further visual inspection for artifacts. ICA was then performed on the remaining epoched data, to inspect and reject components contaminated with eye-movement and muscle artifacts, indicative of non-neural activity. The number of epochs accepted for further analysis ranged from 80-90, in each of the eyes-closed and eyes-open conditions.

2.1.5.3 *Spectral analysis*

The aim was to derive spectral information from participants during resting state conditions. The Fast Fourier transform (FFT) was used for this purpose, which is an important and widely used signal-processing technique for time series data analysis. The FFT is based on the principle that a time series signal can be represented as a weighted sum of sine waves, each with different amplitude, frequency, and phase characteristics. Thus, the time domain representation of the signal is deconstructed into the frequency domain, thereby revealing what frequencies are present and in what proportion. The magnitude squared of the FFT output reveals how much power the signal contains at a given frequency, the distribution of which is termed the power spectral density (PSD), expressed in milli-Volts^2.

The FFT assumes that the underlying signal is stationary (i.e., has properties that repeat over time) with an integer number of cycles. This of course is not the case with EEG data, which comprises a waveform that fluctuates over time with the dynamics of neural activity. Applying the FFT to non-stationary finite signal segments can introduce discontinuities, or truncations in the original waveform – leading to leakage of spectral energy across frequencies that where not present in the original data. These effects can be reduced with methods that compute the FFT in windows that reduce the discontinuities and average the outputs together to compute the PSD.

The Welch method (Welch, 1976) was used to estimate the PSD in this thesis. This represents a standard, widely used approach (Babiloni et al., 2018; Massa et al., 2020; Schumacher et al., 2020a) that involves cutting the EEG signal into shorter segments, multiplying the signal segments with a window function

(taper), obtaining individual PSD estimates for each segment, and finally averaging them together. This serves to increase the accuracy of the PSD estimate by reducing the variance introduced (as described above).

The sampling rate and the length of segments on which the FFT is performed determine the frequency resolution i.e., the spacing between frequency bins depicted in the PSD. In this thesis, data were sampled at 512 Hz, and the FFT was applied to 2 second epochs (i.e., FFT length 1024 points), thereby providing a frequency resolution of 0.5 Hz. This is adequate, given that the lowest frequency of interest was 2 Hz.

Windowing functions help to reduce the discontinuities at the boundaries of each segment by smoothly tapering the edges towards zero. The Hamming window was applied here, which is widely used (Schumacher et al., 2020a; 2020b; Zhang et al., 2021), and is the default setting in the EEGLAB *spectopo* function, which uses the MATLAB *pwelch* function for PSD estimation. Overlapping segments is a common approach that can increase the number individual PSD estimates for averaging (thereby reducing variance), and recover some of the attenuated signal lost at the tapered edges of the segments during windowing. A 50% overlap was applied, thereby obtaining twice as many PSD estimates (as compared to no overlap).

Further details of qEEG metrics that were extracted in each study are provided in the methods sections of respective chapters.

2.1.6 ***Structural neuroimaging***

2.1.6.1 *MRI acquisition*

MRI data were acquired using a 3T Philips Achieva MRI system. T1-weighted structural MRI data were acquired using a 3D-TFE sequence with the following parameters: repetition time = 9.9 ms; echo time = 4.5 ms; flip angle = 8°; matrix of 320 x 320 x 237 isotropic 0.8 mm resolution.

Diffusion-weighted data were acquired using a single-shot spin-echo planar imaging (EPI) sequence with the following parameters: 32 isotropically

distributed directions; SENSE parallel imaging; echo time = 73 ms, b = 1000 s/mm^2, matrix of 112 x 112 x 70; isotropic 2 mm resolution.

2.1.6.2 *MRI preprocessing*

T1-weighted images were automatically segmented into grey matter, white matter, and cerebrospinal fluid (CSF) partitions of 1.5 mm voxel size using the Statistical Parametric Mapping 12 toolbox (SPM, Wellcome Trust Centre for Neuroimaging, London, UK) implemented in MATLAB 2019a (Mathworks, USA). The resulting grey and white matter partitions of each participant were then high-dimensionally registered together using DARTEL (Diffeomorphic Anatomic Registration using Exponentiated Lie algebra) (Ashburner, 2007). DARTEL performs a diffeomorphic algorithm for intersubject registration, producing individual flow field maps – which provide parameters of the deformations of the image, in addition to average grey and white matter templates. The grey matter segments were then warped to MNI space using the individual flow fields from DARTEL registration, and voxel values were modulated for volumetric changes introduced by the high-dimensional normalisation. Therefore, the total amount of grey matter volume that was present before warping was preserved. Preprocessed grey matter images were subsequently visually inspected for segmentation and registration accuracy.

Diffusion-weighted images were preprocessed using the ExploredDTI toolbox (Leemans et al., 2009). Data were corrected for head motion, distortions induced by eddy currents, and EPI-induced geometrical distortions by registering each diffusion image to the corresponding T1-weighted anatomical image, with appropriate reorientation of the diffusion encoding vectors (Leemans and Jones, 2009). Diffusion tensors were estimated using the RESTORE approach (Chang et al., 2005) to create fractional anisotropy (FA), mean diffusivity (mD), axial diffusivity (aD), and radial diffusivity (rD) images.

2.1.6.3 *Stereotactic map of the cholinergic basal forebrain*

Cholinergic basal forebrain nuclei were identified using a stereotactic map, created via MRI and histological preparation of a post-mortem brain of a 56-

year-old male who died without any evidence of cognitive decline or psychiatric illness (Kilimann et al., 2014). Cholinergic nuclei were identified and delineated on digital pictures of the stained brain slices, following Mesulam's nonclamenture (Mesulam et al., 1983), then manually transferred into the corresponding magnetic resonance slices. Transformation of the delineations from the space of the dehydrated brain into the space of the *in situ* brain scan was performed using a high-dimensional non-linear registration between the two brain scans (Ashburner and Friston, 1999) before final transformation from *in situ* space into MNI space using the DARTEL registration method (Ashburner, 2007; Klein et al., 2009).

The stereotactic cBF map distinguishes different cholinergic subdivisions within the basal forebrain, including cell clusters corresponding to the medial septum, vertical and horizontal limb of the diagonal band, and nbM (Ch1–4 according to Mesulam's nomenclature) (Mesulam et al., 1983b) (Figure 2.1).

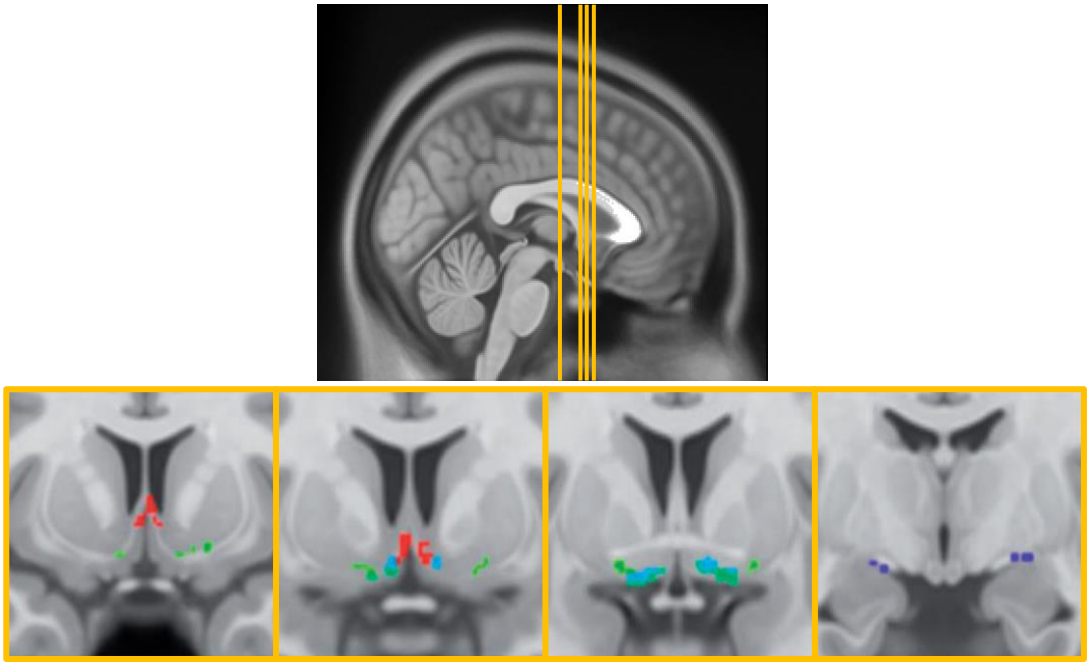


Figure 2.1 Cholinergic basal forebrain regions of interest

Subregional cBF map (Kilimann et al., 2014). Regions of interest have been superimposed on the T1-weighted MNI152 brain (Mazziotta et al., 2001). The four slices marked on (top) sagittal image correspond to the four (bottom) coronal images. Red represents the Ch1-2 region of interest corresponding to the medial septum and vertical limb of the diagonal band; green is the nucleus subputaminalis/anterior-lateral nbM and the horizontal limb of the diagonal band (not selected as a region of interest in the current thesis); light blue and purple are the Ch4 region of interest corresponding to the nbM, with purple representing Ch4p region of interest corresponding to the posterior nbM.

2.1.6.4 *Stereotactic map of the pedunculopontine nucleus*

The PPN was accessed via a stereotactic map that was developed to improve targeting of this region for DBS (Alho et al., 2017). Those who developed the map performed post-mortem MRI on the brain of a 66-year-old woman without parkinsonism or cognitive decline. Following autopsy, the brain was dehydrated, sectioned, Nissl stained, then digitised (Ashburner and Friston, 1999). PPN segmentation was performed on the digitised histological images using microscopy – over which a mask of the PPN was created. Finally, PPN images were registered with the postmortem MRI, and the PPN mask

transformed to MNI space using transformations that were generated during the normalisation of post-MRI to MNI space (Mazziotta et al., 2001) (Figure 2.2).

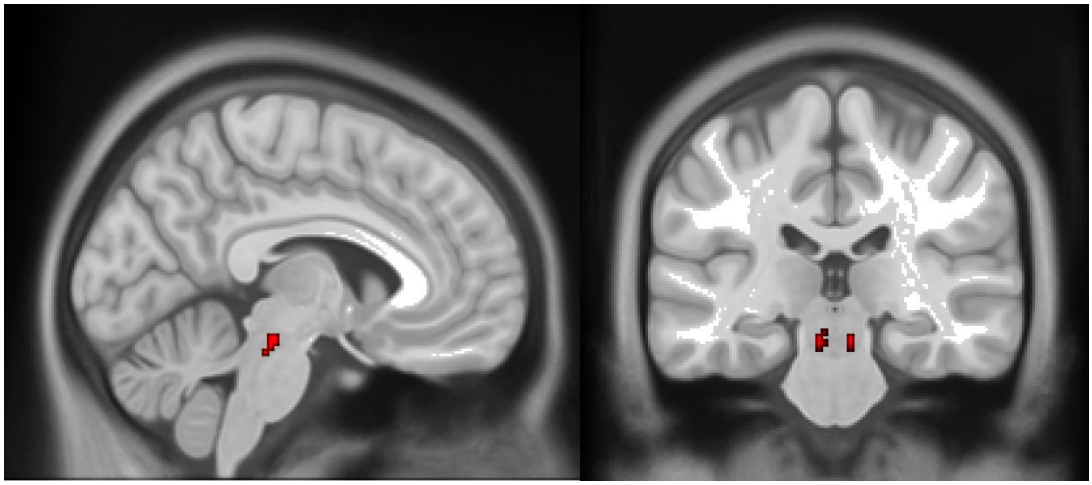


Figure 2.2 Pedunculopontine region of interest

Pedunculopontine map (Alho et al., 2017) superimposed on the T1-weighted MNI152 brain (Mazziotta et al., 2001). The PPN region of interest is displayed in red in sagittal view (left) and coronal view (right).

2.1.6.5 *Extraction of cBF grey matter volumes*

Grey matter volumes in cBF were calculated by summing the grey matter voxel values within the corresponding ROI masks in template space (Ashburner, 2009). Global grey matter and cBF volumes were further scaled via ANCOVA, using total intracranial volume (TIV) as a covariate, to extract unstandardised residual values which were then used as input variables in further analyses. Therefore, negative cBF values indicate smaller volumes than expected given head size.

Two distinct subdivisions of the cBF were studied, that can be separated anatomically, each with different cortical projections and behavioural functions (Mesulam et al., 1983b; Záborszky et al., 2018). These regions correspond to an anterior-medial portion of the cBF, combining the medial septum (Ch1) and the vertical limb of the diagonal band (Ch2), and a more posterior cBF region covering the nbM (Ch4). Analyses were also applied to a posterior section of the nBM (Ch4p), based on previous work showing particular involvement of this region in MCI/AD and PD (Grothe et al., 2012; 2013; Kilimann et al., 2014; Ray et al., 2018).

2.1.6.6 *Extraction of PPN diffusivity metrics*

For the extraction of diffusivity metrics, MNI-space PPN ROI images were first transformed to native space as follows: the MNI-space ICBM152 (Mazziotta et al., 2001) brain was DARTEL registered to the average template generated by the intersubject registration of T1 images. The resultant flow field was used to transform the MNI-space PPN ROI images into the average template space. Participant individual flow fields were then used to inverse warp the PPN ROI images into participant T1 native space. All warps of the ROI images used nearest-neighbour interpolation. Each ROI was visually checked for alignment with the PPN.

Next, voxels within PPN ROI images were conditioned on FA to remove voxels more likely to be dominated by white matter. Specifically, voxels with FA greater than 0.67 were removed, which represents the mean +1 SD of values of FA reported in (Alho et al., 2017). This step is relevant for the PPN, which has white

matter tracts from the brainstem coursing through it. Mean diffusivity values were then calculated from the remaining voxels within the PPN ROI. This resulted in a total of three metrics extracted from the PPN: mD, aD, and rD.

2.1.7 *Missing data*

Not all data was collected from all participants due to participant fatigue, technical issues, or time constraints during data collection. The number of missing datapoints for each measure is displayed in Table 2.1.

Table 2.1 Missing data

	PD	**MCI**	**CONTROL**
MoCA	2	1	1
TMT-A	4	6	7
Colour-Word	4	7	6
TMT-B	4	3	5
Tower	3	3	5
WAIS (S)	6	7	5
Letter Fluency	3	2	5
ROCFT (C)	2	2	4
CVLT (LDF)	3	3	4
ROCFT (D)	2	2	4
EEG	6	3	4
T1-MRI	1	1	1
DWI	2	1	2

Abbreviations: CVLT (LDF) = California Verbal Learning Test (Long Delay Free Recall); DWI = diffusion-weighted imaging; MRI = magnetic resonance imaging; MCI = mild cognitive impairment; MoCA = Montreal Cognitive Assessment; PD = Parkinson's disease; ROCFT (C) = Rey Osterreith Complex Figure Test (Copy); ROCFT (D) = Rey Osterreith Complex Figure Test (Delayed Recall); TMT = Trail Making Test; WAIS (S) = Weschler Adult Intelligence Scale (Similarities).

2.2 UK study

2.2.1 *Study overview*

Data for the UK study was collected throughout my PhD from 2019 to 2021. This constitutes an observational study in which clinical and neuroimaging data were collected from people with PD and healthy controls of a similar age to those with PD.

Clinical assessments (cognitive, gait and balance) were carried out at Manchester Metropolitan University, and brain imaging was conducted at Manchester Royal Infirmary. Participants were also given the option to volunteer for an additional cognitive study that took place online. Details of clinical and neuroimaging methods are outlined below.

2.2.2 *Participants and recruitment*

A total of 61 participants were enrolled onto this study. This included 39 people with PD and 22 healthy age-matched control participants.

PD specialists at Salford Royal NHS and Pennine Acute NHS Trust outpatient clinics were involved with the recruitment of people with PD. Healthy controls were identified from partners and friends of participants with PD, or from former participants who expressed interest in partaking in future research.

As expected, the majority (90%) of people with PD were taking dopaminergic mediations, 15% were also taking selective serotonin reuptake inhibitors, while none were taking acetylcholinesterase inhibitors or anticholinergics.

Inclusion criteria included: age greater than or equal to 50 years and (for people with PD) PD diagnosis for more than 1 year prior to study participation. Exclusion criteria included: inability to provide informed consent; inability to walk unaided; presence of alternate neurological conditions other than PD; presence of comorbidities known to affect gait or cognition including musculoskeletal disorders, dementia, or stroke; inability to walk unaided; and contraindications to MRI.

2.2.3 *Ethical approval and consent*

Ethical approval for the study was provided by the NHS Greater Manchester Research Ethics Committee (IRAS ID: 250729). All participants provided informed written consent prior to study participation in accordance with the Declaration of Helsinki.

2.2.4 *Neuropsychological assessment*

Participants completed a battery of cognitive assessments including pen-and-paper tests of global cognition (MoCA) and executive function (TMT and verbal fluency). Participants who volunteered to take part in the online cognitive study also completed computerised attentional tasks including simple reaction time, choice reaction time, and digit vigilance. Cognitive tests are described in more detail below.

2.2.4.1 *Montreal Cognitive Assessment (MoCA)*

A measure of global cognition. Described in more detail in section 2.1.4.1 above.

2.2.4.2 *Trail making test (TMT)*

A measure of attention and executive function. Described in more detail in section 2.1.4.2 above.

2.2.4.3 *Verbal Fluency*

A subtest from the Delis-Kaplan Executive Function System (Delis et al., 2001) used to assess executive retrieval of verbal information. Two core tasks are involved: letter fluency and semantic fluency. Participants are given one-minute to generate as many unique words as possible starting with a given letter (letter fluency) or within a semantic category (semantic fluency).

2.2.4.4 *Simple reaction time*

A computerised task in which participants are instructed to press the 'space bar' on a computer keyboard as quickly as possible when 'X' appears on screen (appears in only one location). First, 8 practice trials are completed. Followed by a total of 60 trials for the main task, including 3 sets of 20 trials, with randomised inter-stimulus intervals (ranging from 1-3 seconds). Response times are recorded (ms).

2.2.4.5 *Choice reaction time*

A computerised task in which participants are instructed to respond as quickly as possible when 'X' appears on screen. 'X' appears in two locations – left or right, with corresponding response keys – 'd' (left) and 'j' (right). First, 10 practice trials are completed. Followed by a total of 60 trials for the main task, including 3 sets of 20 trials, with randomised inter-stimulus intervals (ranging from 1-3 seconds). Response time (ms) and accuracy (units) are recorded.

2.2.4.6 *Digit vigilance*

A computerised task in which a single column of random numbers from 1-9 is presented on screen. Participants are instructed to respond as quickly as possible by identifying whether the number 6 is featured in the column of numbers – pressing 'd' (yes) or 'j' (no). First, 20 practice trials are completed. Followed by 120 trials for the main task, including 3 sets of 40 trials (comprised of an equal number of yes/no trials), with randomised inter-stimulus intervals (ranging from 0.8-1.2 seconds). Response time (ms) and accuracy (units) are recorded.

2.2.5 ***Functional mobility assessment***

Functional mobility was assessed with the Mini-Balance Evaluation System Test (Mini-BESTest) (Franchignoni et al., 2010). This scale contains 14-items each divided into one of four subdomains including (i) anticipatory postural adjustments, (ii) reactive postural control, (iii) sensory orientation, and (iv) dynamic gait. The maximum total score is 28, with each item scored from 0

(severe impairment, unable to perform) to 2 (normal function, successfully performed) on an ordinal scale. A summary of scale items is shown in Table 2.2.

The Mini-BESTest has been shown to be a reliable and valid measure of dynamic gait and balance in PD, demonstrating high inter-rater and test-retest reproducibility (Leddy et al., 2011). Furthermore, it was recently classified as a "recommended" scale in a critical review, conducted by the Movement Disorders Society Task Force, of existing clinical measurement scales used in PD to assess gait, balance, and posture (Bloem et al., 2016).

Table 2.2 Summary of the subdomains and items of the Mini-BESTest

Subdomains	**Items**
Anticipatory postural adjustments	1. Sit to stand
	2. Rise to toes (3 seconds)
	3. Stand on one leg (20 seconds)
Reactive postural control	4. Compensatory stepping - forward
	5. Compensatory stepping - backward
	6. Compensatory stepping – lateral, left/right
Sensory orientation	7. Stance – eyes open, firm surface (30 seconds)
	8. Stance – eyes closed, foam surface (30 seconds)
	9. Stance – eyes closed, incline ramp (30 seconds)
Dynamic gait	10. Change in gait speed
	11. Walk with head turns
	12. Walk with pivot turn
	13. Step over obstacle
	14. Timed Up and Go (single and dual-task)

2.2.6 ***Structural neuroimaging***

2.2.6.1 *MRI acquisition*

MRI acquisition was performed using a Philips Achieva 3T system (Philips Healthcare, Best, The Netherlands) using a 32-channel head-coil. T1-weighted images were acquired with the MPRAGE sequence: IR Method (voxel size 0.94 × 0.94 × 1 mm; FOV 240 (AP) × 192 (RL) mm; TR=8.4ms; TE=3.9ms; TI = 1150ms; FA = 8°). Diffusion images were acquired with the HARDI sequence which consisted of a cardiac-gated spin-echo echo-planar imaging sequence acquired axial-oblique and aligned with the anterior commissure/posterior commissure line (2 × 2 × 2 mm, 64 isotropically distributed diffusion-weighted directions with b = 1200 s/mm^2 and 6 non-diffusion-weighted volumes (B0); TE = 68 msec; TR = 24 sec; SENSE factor = 3.1). A reverse phase (b = 0 s/mm^2) reference scan with the opposite phase-encoding direction (posterior-to-anterior) was also acquired to correct for phase-encoding direction-induced distortions.

2.2.6.2 *MRI preprocessing*

The pipeline used for T1-weighted image preprocessing has been previously described above in section 2.1.6.2.

For diffusion-weighted images, eddy current-induced distortion correction and subject movements were corrected using Eddy FSL toolbox. Participants were removed if they had more than 3 mm absolute mean displacement, resulting in the removal of two people with PD. Fractional anisotropy (FA), mean diffusivity (mD), axial diffusivity (aD), and radial diffusivity (rD) images were then calculated. Free water-corrected versions of these images (FmD, FaD, FrD), in addition to free water images (FW), were created by fitting the bi-tensor model described by (Pasternak et al., 2009) to the raw diffusion data using custom Matlab scripts.

To align these images with participant T1 images, the B0 scan was extracted and affine registered with T1 anatomical images using antsRegistrationSyn.sh (Advanced Neuroimaging Tools (ANTs) (Klein et al., 2009). Diffusion images

were visually inspected for registration accuracy, resulting in removal of one person with PD.

2.2.6.3 *Stereotactic maps of the cholinergic basal forebrain and pedunculopontine nucleus*

Stereotactic maps were used to access structural measures of cBF (Kilimann et al., 2014) and PPN nuclei (Alho et al., 2017). The methods used to create these maps have been previously described above in sections 2.1.6.3 and 2.1.6.4.

The cBF map distinguishes different cholinergic subdivisions within the basal forebrain, including cell clusters corresponding to the medial septum, vertical and horizontal limb of the diagonal band, and nbM (Ch1–4 according to Mesulam's nomenclature) (Mesulam et al., 1983b). The PPN map includes the PPN region as a whole and therefore does not differentiate between different cell populations.

To provide an indication of the size and location of the cBF and PPN regions-of-interest, the stereotactic maps have been superimposed on the T1-weighted MNI152 brain (Mazziotta et al., 2001), displayed above in section 2.1.6.3 (Figure 2.1) and section 2.1.6.4, (Figure 2.2).

2.2.6.4 *Extraction of cBF grey matter volumes*

The methods used to extract cBF grey matter volumes have been previously described above in section 2.1.6.5.

2.2.6.5 *Extraction of PPN diffusivity metrics*

For the extraction of diffusivity metrics, MNI-space ROI images were first transformed to native space as follows: the MNI-space ICBM152 (Mazziotta et al., 2001) brain was DARTEL registered to the average template generated by the intersubject registration of T1 images. The resultant flow field was used to transform the MNI-space ROI images into the average template space. Participant individual flow fields were then used to inverse warp the ROI images into participant T1 native space. All warps of the ROI images used nearest-

neighbour interpolation. Each ROI was visually checked for alignment with the PPN.

Following the methods outlined in section 2.1.6.6, voxels within the PPN ROI images were then conditioned on FA to remove those contaminated by white matter. Specifically, voxels with FA greater than 0.67 were removed, which represents the mean + 1SD of values of FA reported in (Alho et al., 2017). Mean free water and diffusivity values were then calculated from the remaining voxels within the PPN ROI. This resulted in a total of seven metrics extracted from the PPN including traditional DTI metrics: mean diffusivity (mD), axial diffusivity (aD), radial diffusivity (rD) – and free water-corrected versions of these: FmD, FaD, FrD, as well as free water content (FW).

2.2.7 *Missing data*

Not all data was collected from all participants since not all were able to commit to the additional online study. In addition, some data points are missing due to participant fatigue, technical issues, or time constraints during data collection. The number of missing datapoints for each measure is displayed in Table 2.3.

Table 2.3 Missing data

	PD	**CONTROL**
MoCA	2	1
TMT-A	3	0
TMT-B	5	0
Letter fluency	3	0
Semantic fluency	3	0
Simple RT [a]	18	8
Choice RT [a]	18	8
Digit vigilance [a]	18	8
Mini-BESTest	1	0
TUG	3	0
TUG dual	4	0
T1-MRI [b]	3	1
DWI	4	1

[a] *These tasks were only administered to a subset of participants as part of the additional online study.*

[b] *Chapter 5 is missing two additional T1 images in PD. These participants had not yet been scanned at the time of analysis, due to a pause in data collection caused by national coronavirus lockdown restrictions and university closures. Data from these two participants are included in analyses in Chapter 6.*

Abbreviations: DWI = diffusion-weighted imaging; MoCA = Montreal Cognitive Assessment; MRI = magnetic resonance imaging; RT = reaction time; TMT = Trail Making Test; TUG = Timed Up and Go.

2.3 Data cleaning

Multimodal imaging techniques were used to acquire structural and functional metrics in the current thesis. This includes T1-weighted images, diffusivity values from diffusion-weighted images, and quantitative EEG. For each of these approaches, artefacts are frequently introduced during the acquisition (e.g., due to movement or noise from equipment) or preprocessing stages (e.g., due to segmentation or normalisation errors). Such artefacts are not of physiological interest to the current set of research questions and could have disproportionate impact on statistical outcomes. Thus, in order to standardise the data cleaning approach across the studies comprising this thesis and limit subjective assessment, all brain metrics that met the threshold for extreme outlier (i.e., exceeded 3 × the interquartile range) were removed prior to analyses (Gaetz et al., 2013; Orcioli-Silva et al., 2021; Sebastian et al., 2021). Details on the number of outliers that were removed, and the influence of data removal have been discussed in each chapter.

Chapter 3. Quantitative EEG and cholinergic basal forebrain atrophy in Parkinson's disease and mild cognitive impairment

3.1 Introduction

Cortical cholinergic denervation is widely reported in AD and Lewy body parkinsonian disorders, including PD and in particular PDD and DLB (Bohnen et al., 2018a; Craig et al., 2020b). Cortical cholinergic innervation originates primarily in the nbM of the basal forebrain (Mesulam et al., 1983b). Early neuropathological studies reported severe nbM neuronal loss in the advanced stages of these diseases (Candy et al., 1983; Liu et al., 2015). More recently, *in-vivo* structural MRI combined with stereotactic mapping of the cBF (Kilimann et al., 2014) revealed that nbM degeneration may occur early, and is associated with emerging cognitive deficits in AD (Kilimann et al., 2014; Schmitz and Spreng, 2016), PD (Ray et al., 2018; Schulz et al., 2018) and LBD (Schumacher et al., 2021).

Resting-state qEEG may index pathophysiological changes associated with cognitive neurodegenerative disorders. The dementia stage of AD and LBD is characterised by increased EEG slowing compared to healthy controls, which is typically more severe in LBD (Bonanni et al., 2008; Babiloni et al., 2011; van der Zande et al., 2018). Studies conducted in the MCI stage suggest that early qEEG abnormalities may be specific to LBD (Bonanni et al., 2015; Schumacher et al., 2020a).

Changes to alpha activity are frequently reported (Bonanni et al., 2008). Alpha reactivity represents the magnitude of alpha rhythm attenuation, from eyes-closed to eyes-open. Lower alpha reactivity may reflect EEG slowing (i.e., failure of alpha rhythms to emerge) and/or lack of neural desynchronization (i.e., failure to supress alpha rhythms). Reduced alpha reactivity has been reported in PDD and DLB (Schumacher et al., 2020b), AD (van der Hiele et al., 2007b; Babiloni et al., 2010a; Fonseca et al., 2011; Schumacher et al., 2020b), and MCI (Babiloni et al., 2010a).

The mechanistic underpinnings of these EEG abnormalities are not fully understood, but cholinergic system changes may play a role (Riekkinen et al., 1991; Babiloni et al., 2013). Recently, neuroimaging reports have suggested that cortical cholinergic pathways may mediate alpha reactivity. Wan et al. (2019) reported that in healthy individuals, increased functional connectivity (measured with functional MRI) between the nbM and the visual cortex was associated with greater alpha reactivity. In that study, reduced alpha reactivity was shown to correlate with white matter hyperintensity load along nbM-visual cortex fibre tracts in older participants. More recently, greater loss of alpha reactivity was reported in people with DLB and PDD in comparison to AD, which was also associated with smaller nbM volumes in PDD (Schumacher et al., 2020b).

The research described above implies that qEEG measures may be associated with cholinergic changes that underpin cognitive neurodegenerative conditions. Whether this relationship exists in predementia stages of PD and other dementias is still to be determined. The first aim of the current study was to assess qEEG measures in a clinically diverse predementia cohort including people PD (without dementia), MCI (associated with AD or vascular dementia), and age-matched controls. The second aim was to explore relationships between qEEG measures and volumetric changes within cBF nuclei.

3.2 Materials and methods

3.2.1 *Participants*

The participants for this study are described in more detail in the General Methods chapter (2.1). In brief, this study involved a total of 73 participants. Thirty-one were diagnosed with PD according to UK PD Society Brain Bank Criteria. Twenty-one had been diagnosed with MCI based on established criteria (Albert et al., 2011), described in General Methods (section 2.1). Twenty-one were healthy age-matched controls.

3.2.2 *Global cognition*

Global cognition was assessed with the MoCA (Nasreddine et al., 2005). This test has been previously described in General Methods (2.1.4.1).

3.2.3 *EEG acquisition and preprocessing*

Details of EEG acquisition and preprocessing steps have been outlined in the General Methods chapter (sections 2.1.5.1 and 2.1.5.2). In brief, resting-state EEG recordings were collected from each participant during eyes-closed and eyes-open conditions. This chapter focused on data from the eyes-closed condition for global frequency analysis (described below). Chapter 4 focused on data from the eyes-open condition for global frequency analysis.

3.2.4 *Global EEG frequency analysis*

Global (over all electrodes) eyes-closed relative power was estimated for standard EEG frequency bands including delta: 2–4 Hz, theta: 4–5.5 Hz, pre-alpha: 5.5-8 Hz, alpha: 8–13 Hz, beta: 13-30 Hz, calculated as the sum of EEG power in each frequency band divided by the total EEG power between 2-30Hz. The pre-alpha band was included based on previous studies in LBD (Bonanni et al., 2008; Schumacher et al., 2020a).

Using global relative power, two ratios were also calculated: (i) slow/fast frequencies = sum of delta, theta, and pre-alpha power, divided by the sum of alpha and beta power (Latreille et al., 2016); (ii) theta/alpha = theta power divided by alpha power (Schumacher et al., 2020a).

3.2.5 *EEG alpha reactivity analysis*

EEG data from three occipital electrodes (O1, O2, Oz) were used for alpha reactivity analysis, following (Wan et al., 2019; Schumacher et al., 2020b). The PSD for each of these electrodes was averaged for eyes-open and eyes-closed conditions, separately. Alpha reactivity was then calculated as:

$$alpha\ reactivity = \frac{eyes\ closed\ alpha\ power - eyes\ open\ alpha\ power}{eyes\ closed\ alpha\ power}$$

Using the above formula, alpha power was calculated as the relative power within a frequency bin (± 2 Hz) around the individual alpha peak frequency (Schumacher et al., 2020b). Individual alpha peak frequency corresponds to the peak in the PSD in an extended alpha frequency band from 6-14 Hz, using the eyes-closed data (Babiloni et al., 2010a). Individual alpha peak frequency was used to obtain alpha power, in place of the standard alpha boundaries, to account for the shift of the alpha peak to slower frequencies in cognitive neurodegenerative conditions (Babiloni et al., 2010a). Accordingly, a larger alpha reactivity value indicates greater attenuation of alpha power from eyes-closed to eyes-open.

3.2.6 *MRI acquisition and preprocessing*

Details of MRI acquisition and preprocessing methods have been previously described in the General Methods chapter (sections 2.1.6.1 and 2.1.6.2).

3.2.7 *Stereotactic map of the cholinergic basal forebrain*

A stereotactic map of cBF nuclei was used to extract volumetric data (Kilimann et al., 2014). The methods used to create this map have been outlined in General Methods (2.1.6.3). The cBF map distinguishes different cholinergic subdivisions within the basal forebrain, including cell clusters corresponding to the medial septum, vertical and horizontal limb of the diagonal band, and nbM (Ch1–4 according to Mesulam's nomenclature) (Mesulam et al., 1983b) (displayed in General Methods, section 2.1.6.3, Figure 2.1).

3.2.8 *Extraction of cBF grey matter volumes*

Grey matter volumes in cBF were calculated by summing the grey matter voxel values within the corresponding cBF ROI mask in template space (Ashburner, 2009). Global grey matter and cBF volumes were further scaled via ANCOVA, using total intracranial volume (TIV) as a covariate, to extract unstandardised residual values which were then used as input variables in further analyses. Therefore, negative cBF values indicate smaller volumes than expected given head size.

Two distinct subdivisions of the cBF were studied, that can be separated anatomically, each with different cortical projections and behavioural functions (Mesulam et al., 1983b; Záborszky et al., 2018). These regions correspond to an anterior-medial portion of the cBF, combining the medial septum (Ch1) and the vertical limb of the diagonal band (Ch2), and a more posterior cBF region covering the nbM (Ch4). Analyses were also applied to a posterior section of the nBM (Ch4p), based on previous work showing particular involvement of this region in MCI/AD and PD (Grothe et al., 2012; 2013; Kilimann et al., 2014; Ray et al., 2018).

3.2.9 ***Statistical analyses***

Statistical analyses were conducted in IBM SPSS statistics 26. Kolmogorov-Smirnov tests and boxplot and histogram inspections were used to assess the distribution of continuous variables. Since relative qEEG power was not normally distributed in all groups, these variables were log-transformed to achieve normal distribution.

3.2.9.1 *Data cleaning*

The approach taken for data cleaning has been described in the General Methods chapter (section 2.3). This resulted in the removal of alpha reactivity data from one person with PD, in whom this measure fell more than 3 × below the IQR. The boxplot below shows that this alpha reactivity datapoint is substantially lower than other observations (Figure 3.1). The scatterplot also shows that the inclusion of this datapoint (when plotted against cBF volume, as in the current analyses) results in a pattern that is inconsistent with the majority of the data. Given that alpha reactivity incorporates data from two separate recording conditions (i.e., during eyes open, and eyes closed conditions), it is possible that either of these data files were contaminated with artefacts during the acquisition or preprocessing stages, and is thus influencing the outcome measure when incorporated into the alpha reactivity formula.

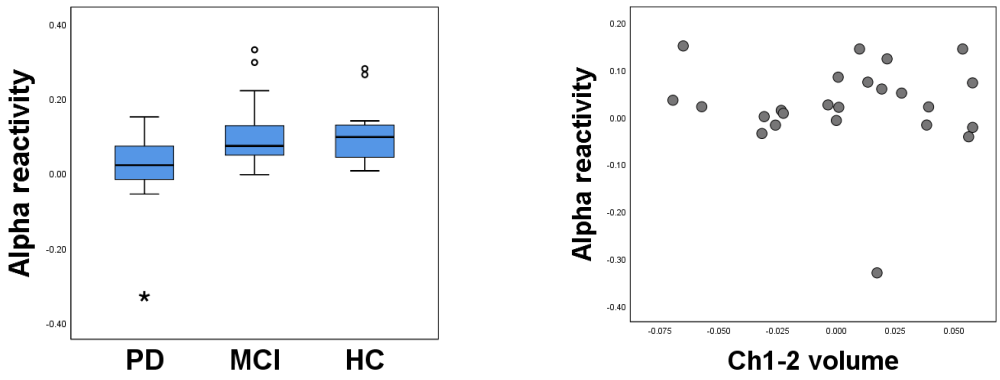


Figure 3.1 Boxplot and scatterplot showing outlier data for alpha reactivity in PD

3.2.9.2 *Group comparisons*

Univariate ANOVAs (controlling for age, sex, and global grey matter (GM) volume (for volumetric data only)) were used to compare demographics, MoCA scores, qEEG metrics, and cBF volumes between groups (PD, MCI, controls). Significant group level differences were followed up with post-hoc tests, with Bonferroni correction for multiple comparisons.

3.2.9.3 *Within-group relationships between cBF volumes, qEEG, and global cognition*

Partial correlations were conducted in each group separately to determine the relationship between subregional cBF volumes and qEEG metrics. These were controlled for age, sex, and global GM volume to ensure relationships were independent of demographic characteristics and degeneration across the wider brain.

Partial correlations, with the same control variables as those described above, were also conducted to in each group separately to explore relationships between MoCA scores and cBF volumes and qEEG metrics. All *P*-values from partial correlation analyses were false discovery rate (FDR)-corrected.

3.3 Results

EEG, MRI, and cognitive data were not collected from all participants due to participant fatigue, technical issues, or time constraints. The number of missing datapoints prior to analyses is reported in General Methods (section 2.1.72.2.7). For clarity, df are also reported throughout the results section where appropriate. Besides the (outlier) removal of alpha reactivity data from one person with PD (described above in section 3.2.9.1), there were no other exclusions.

3.3.1 *Demographics and global cognition*

There were no significant differences in age among the groups. The proportion of male participants was smaller in MCI (24%) and controls (33%), and equal in PD (52%). Univariate comparisons, controlling for age and sex, revealed group differences in MoCA scores (Table 3.1), post-hoc comparisons showed that people with people with MCI scored lower than controls (P = 0.002). There were no differences between PD and MCI (P = 0.187) or controls (P = 0.122).

3.3.2 *Group differences: qEEG metrics and cholinergic basal forebrain volumes*

Univariate ANOVAs (controlling for age and sex) revealed group differences in pre-alpha power (Table 3.1). Post-hoc tests showed that pre-alpha power was higher in people with PD compared to controls (P = 0.001), with a trend-level difference in people with MCI compared to controls (P = 0.07). There were no differences between PD and MCI (P = 0.71) (Figure 3.2).

Group differences (Table 3.1) in the ratio of power in slow to fast frequencies showed greater slowing in people with PD compared to controls (P = 0.037), but no differences between people with MCI and controls (P = 0.78), or PD and MCI (P = 0.55). There were no group differences in delta, theta, alpha, or beta power, or theta/alpha ratio (Figure 3.2).

There was an overall effect of group on alpha reactivity (Table 3.1), post-hoc tests revealed that alpha reactivity was significantly reduced in people with PD compared to controls (P = 0.04), with an additional trend level difference in PD

compared to MCI (P =0.07). There were no differences in in MCI compared to controls (P = 0.98) (Figure 3.2).

Finally, ANOVAs (controlling for global GM, age, and sex) revealed no significant group differences in Ch1-2, Ch4, or Ch4p volumes (Table 3.1, Figure 3.2).

Table 3.1 Participant characteristics, qEEG metrics, and cholinergic basal forebrain volumes

	PD	MCI	CONTROL	ANOVA
N (Male %)	31 (52%)	21 (24%)	21 (33%)	$\chi^2 = 4.40$, $P = 0.110$
Age	67.8 (6.31)	70 (7.43)	66.3 (7.27)	F(2,71) = 1.55, p = 0.22
Disease duration	6.62 (2.14)	–	–	
MoCA	26.03 (2.08)	24.75 (2.12)**	27.45 (2.11)	**F(2, 64) = 7.70, p = 0.002**
Delta power	2.33 (0.41)	2.20 (0.60)	2.14 (0.52)	F(2,55) = 0.77, p = 0.466
Theta power	1.98 (0.41)	1.82 (0.55)	1.64 (0.41)	F(2,55) = 6.30, p = 0.110
Pre-alpha power	2.44 (0.56)**	2.14 (0.55)	1.75 (0.43)	**F(2,55) = 6.98, p = 0.002**
Alpha power	3.76 (0.29)	3.77 (0.47)	3.76 (0.40)	F(2,55) = 0.12, p = 0.885
Beta power	3.08 (0.35)	3.08 (0.54)	3.34 (0.49)	F(2,55) = 2.28, p = 0.112
Theta/alpha ratio	0.54 (0.15)	0.50 (0.19)	0.45 (0.15)	F(2,55) = 1.12, p = 0.334
Slowing ratio	0.79 (0.16)*	0.72 (0.16)	0.65 (0.14)	**F(2,55) = 3.36, p = 0.042**
Alpha reactivity	0.04 (0.06) *	0.10 (0.09)	0.10 (0.08)	**F(2,54) = 4.10, p = 0.022**
Ch1-2 [mm^3]	0.001 (0.04)	-0.011 (0.03)	0.011 (0.04)	F(2,64) = 0.57, p = 0.571
Ch4 [mm^3]	-0.003 (0.06)	-0.005 (0.04)	0.008 (0.05)	F(2,64) = 2.78, p = 0.070
Ch4p [mm^3]	-0.001 (0.05)	-0.011(0.04)	0.014 (0.04)	F(2,64) = 1.30, p = 0.279

*Mean (standard deviation). Metrics in bold were significantly different at P < 0.05 between groups in a whole-sample univariate analysis (controlling for age, sex, and global grey matter volume (for volumetric data only)). * and ** indicate groups who were significantly different to controls at P < 0.05 and P < 0.01, respectively (Bonferroni adjusted); Abbreviations: Ch1-2, region corresponding to the medial septum and vertical limb of the diagonal band; Ch4, region corresponding to the nucleus basalis of Meynert; Ch4p, region corresponding to the posterior nucleus basalis of Meynert; MCI, mild cognitive impairment; MoCA = Montreal Cognitive Assessment; PD, Parkinson's disease.*

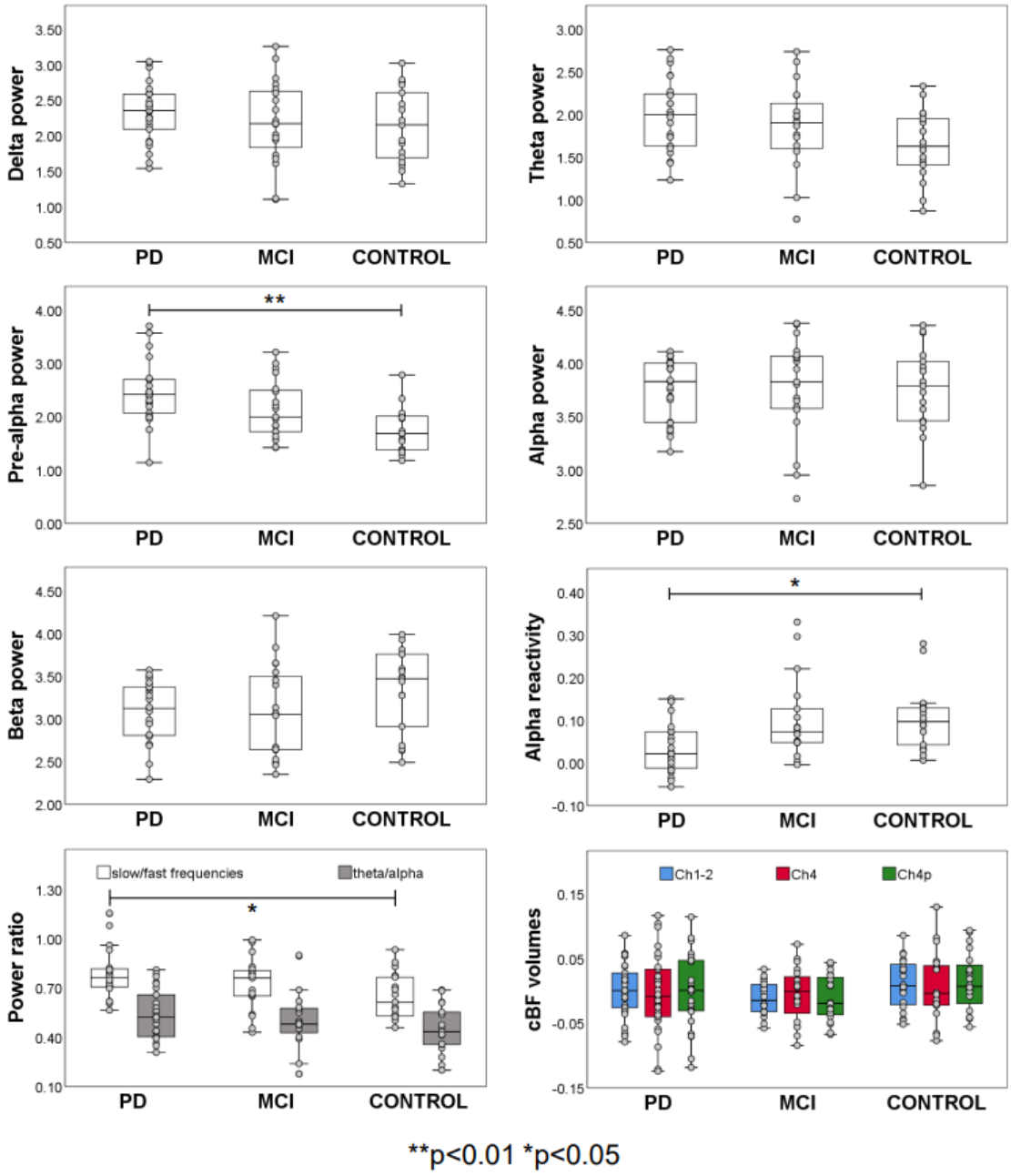


Figure 3.2 Comparison of qEEG metrics and cholinergic basal forebrain volumes between groups

Abbreviations: cBF, cholinergic basal forebrain; Ch1-2, region corresponding to the medial septum and vertical limb of the diagonal band; Ch4, region corresponding to the nucleus basalis of Meynert; Ch4p, region corresponding to the posterior nucleus basalis of Meynert; MCI, mild cognitive impairment; PD, Parkinson's disease.

3.3.3 *Group-specific relationships between qEEG metrics and cholinergic basal forebrain volumes*

In people with PD, partial correlations (controlling for global GM, age, and sex) revealed significant positive correlations (Table 3.2) between alpha reactivity and Ch1-2 (P = 0.003) and Ch4p (P = 0.009) volumes (Figure 3.3, A). In the MCI group, pre-alpha power was positively correlated with Ch1-2 (P = 0.02) and Ch4p (P = 0.04) volumes (Figure 3.3, B). There were no significant correlations between qEEG metrics and cBF volumes in controls (P > 0.17).

Table 3.2 Partial correlations between cholinergic basal forebrain volumes and qEEG metrics

	PD			**MCI**			**CONTROL**		
	Ch1-2	Ch4	Ch4p	Ch1-2	Ch4	Ch4p	Ch1-2	Ch4	Ch4p
Delta	-0.10	0.06	-0.18	0.27	-0.08	0.13	-0.34	-0.11	-0.15
Theta	-0.27	0.02	-0.25	0.45	0.06	0.29	-0.41	-0.04	-0.25
Pre-alpha	-0.29	0.12	-0.07	0.67*	0.29	0.64*	-0.25	0.03	0.01
Alpha	0.13	-0.06	0.14	-0.20	-0.02	-0.29	0.54	0.31	0.20
Beta	0.15	-0.32	0.01	-0.06	-0.17	-0.07	-0.24	-0.34	-0.02
Theta/alpha	-0.22	0.06	-0.22	0.40	0.09	0.38	-0.50	-0.15	-0.27
Slowing. ratio	-0.20	0.28	-0.13	0.57	0.15	0.49	-0.50	0.02	-0.29
Alpha reactivity	0.69**	0.33	0.63**	-0.19	0.47	0.23	0.23	-0.27	0.13

*Pearson correlation coefficient (controlling for age, sex and, TIV-normalised global grey matter (GM) volume). * indicates significant correlation at P < 0.05; ** indicates significant correlation at. P < 0.01 (false discovery rate corrected for multiple comparisons). Abbreviations: Ch1-2, region corresponding to the medial septum and vertical limb of the diagonal band; Ch4, region corresponding to the nucleus basalis of Meynert; Ch4p, region corresponding to the posterior nucleus basalis of Meynert; MCI, mild cognitive impairment; PD, Parkinson's disease.*

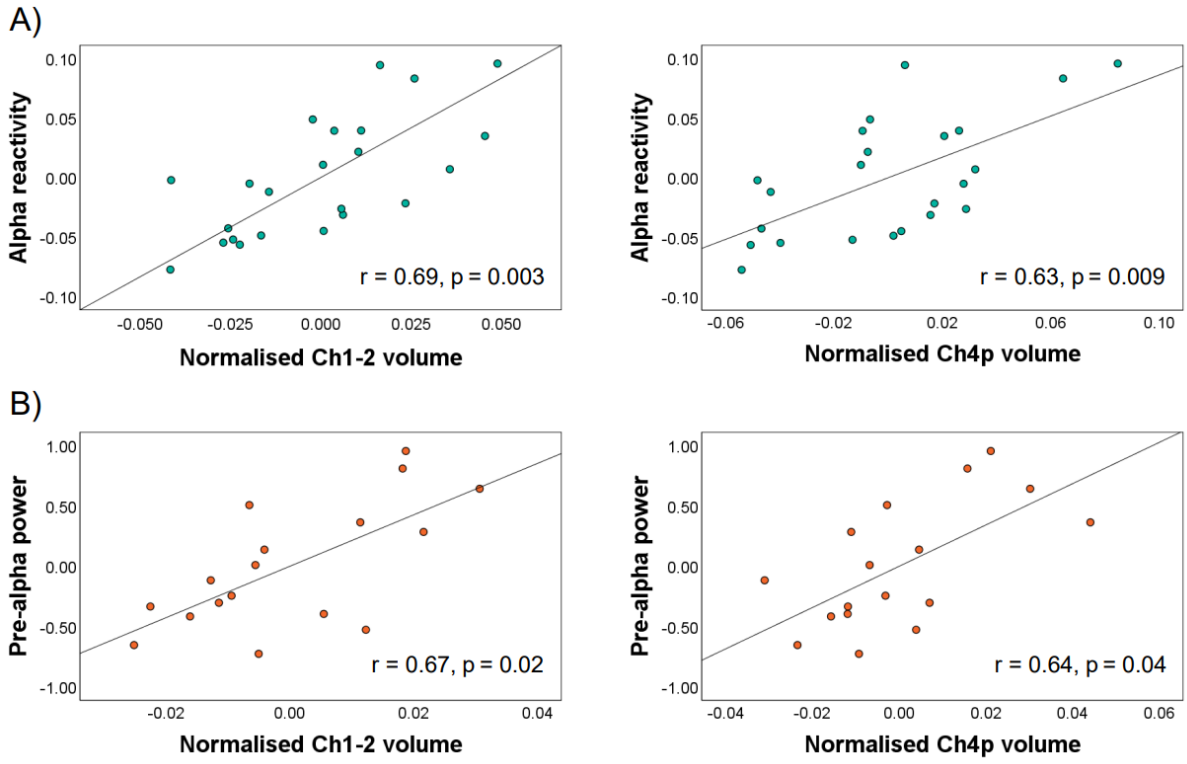


Figure 3.3 Partial correlations between qEEG metrics and cholinergic basal forebrain volumes in (A) Parkinson's disease (PD) and (B) mild cognitive impairment (MCI)

Abbreviations: Ch1-2, region corresponding to the medial septum and vertical limb of the diagonal band; Ch4p, region corresponding to the posterior nucleus basalis of Meynert.

3.3.4 *Correlations with MoCA performance*

There were no significant partial correlations between cBF volumes and MoCA scores within any of the groups (PD: $r < 0.11$, $P > 0.59$; MCI: $r < 0.24$, $P > 0.35$; controls: $r < 0.48$, $P > 0.09$). There were also no significant partial correlations between qEEG metrics and MoCA scores within any of the groups (PD $r < 0.30$, $P > 0.17$; MCI: $r < 0.31$, $P > 0.27$; controls: $r < 0.34$, $P > 0.23$).

3.4 Discussion

This study explored the relationship between regional cBF atrophy and qEEG changes in a heterogenous sample comprising individuals with PD (without dementia), people with MCI, and age-matched controls. There were four main outcomes: (i) people with PD and MCI showed increased power in slower frequencies compared to controls; (ii) people with PD showed an impairment in EEG alpha reactivity from eyes-closed to eyes-open conditions; (iii) alpha reactivity correlated positively with Ch1-2 and Ch4p volumes in people with PD; and (iv) pre-alpha power correlated positively with Ch1-2 and Ch4p volumes in people with MCI. These outcomes are discussed in more detail below.

3.4.1 *Increased power in slow EEG frequencies in PD*

People with PD demonstrated higher pre-alpha power and a larger slowing ratio compared to controls. These findings are consistent with previous studies which generally report an increase in slow wave activity, even early in PD (for review see: Geraedts et al., 2018).

No differences in eyes-closed qEEG metrics were observed when directly comparing PD and MCI groups. Few studies have compared early changes in qEEG characteristics between these clinical groups, but some differences have been reported. For example, in the MCI disease stage, LBD patients presented with increased pre-alpha, and decreased beta and dominant frequency, compared to AD (Schumacher et al., 2020a). However, attempts to classify MCI-LBD from MCI-AD with EEG measures have been unreliable, providing only moderate-no diagnostic accuracy (Babiloni et al., 2018; Schumacher et al., 2020a). Group level differences between MCI-LBD and MCI-AD have also been shown for the alpha/theta ratio, however, MCI-AD did not differ from PD without cognitive impairment (Massa et al., 2020). Taken together with results from the current study, these findings suggest that qEEG changes appear early, and are more pronounced in LBD compared to AD, but with some degree of overlap. This is also consistent with previous reports showing less pronounced slowing in the dementia stage of AD compared to LBD (Bonanni et al., 2008; 2016; van der Zande et al., 2018; Schumacher et al., 2020b).

A trend-level increase in pre-alpha power was also observed in people with MCI compared to controls, which could indicate the emergence of early qEEG alterations in this group. Indeed, longitudinal studies in MCI have shown a decrease in power of posterior alpha sources over time (Babiloni et al., 2014). However, in line with the current findings, a number of cross-sectional studies have revealed no significant differences between MCI and controls in resting-state qEEG measures (Stam et al., 2003; van der Hiele et al., 2007a; Massa et al., 2020).

3.4.2 *Reduced alpha reactivity in PD*

A reduction in alpha reactivity was shown among people with PD compared to controls, indicating a smaller suppression of alpha power across occipital electrodes upon opening the eyes. A non-significant trend for lower alpha reactivity was also observed in PD when compared to MCI. This is consistent with a previous study in the dementia stage of LBD (including DLB and PDD) in which alpha reactivity impairments distinguished LBD from AD (Schumacher et al., 2020b). Observations from the current study therefore extend the findings from dementia studies to the earlier, predementia stage of PD.

Similar to previous studies, no differences in alpha reactivity were shown between MCI and controls (van der Hiele et al., 2007b; Kurimoto et al., 2008), though see (Babiloni et al., 2010a). People with MCI have heterogeneous underlying pathology (i.e., AD or vascular) (Dong et al., 2017). It is possible that alpha reactivity changes in MCI are obscured by such heterogeneity.

3.4.3 *Reduced alpha reactivity was associated with smaller Ch1-2 and Ch4p volumes in PD*

No between-group differences were revealed in sub-regional volumes of the cBF. More substantial cholinergic deficits are observed in PDD compared to earlier predementia stages of PD (Bohnen et al., 2018a; Craig et al., 2020b) – when cholinergic degeneration can be variable (Bohnen et al., 2012). Consistent with the current findings, Ray et al. (2018) observed no cross-sectional differences in cBF volumes between controls and people with PD.

Significant associations between alpha reactivity and cBF volumes were revealed in people with PD. More specifically, smaller volumes of cholinergic cell clusters corresponding to the medial septum, and the vertical and horizontal limb of the diagonal band (Ch1-2), as well as the posterior nbM region (Ch4p), were related to a loss of alpha reactivity. Previous work suggests that deficits in cholinergic signalling may contribute to EEG alterations (Riekkinen et al., 1991), which is supported by the reversal of EEG slowing with acetylcholinesterase inhibitors in PD and AD (Bosboom et al., 2009; Babiloni et al., 2013). More recently, a combined MRI and qEEG study revealed that loss of alpha reactivity was correlated with volume loss within the nbM in PDD (Schumacher et al., 2020b). The current findings therefore extend this work to early PD stages, and provide additional evidence for the possible role of cholinergic system changes and in alpha reactivity impairments.

In combined EEG-fMRI studies, greater alpha activity has been associated with reduced neuronal activation in visual cortices (Feige et al., 2005). Thus impairments in suppressing alpha activity upon opening the eyes may reflect a lack of neuronal desynchronisation (Adrian and Matthews, 1934). Animal studies have provided direct evidence for the modulatory role of the cBF on cortical desynchronisation and visual processing (Pinto et al., 2013). Specifically, cBF activation was shown to desynchronise cortical activity and improve visual responses, while inactivation of cBF had the opposite effect.

More recent work in older adults provided evidence for the role of cholinergic system changes in modulating alpha reactivity (Wan et al., 2019). Specifically, greater alpha reactivity was linked to increased functional connectivity between the nbM and primary visual cortex, while increased burden of white matter hyperintensities along nbM-visual cortex tracts was associated with alpha reactivity deficits. When considered with the above evidence, the results from the current study could imply that alpha reactivity impairments, as an index of cortical activation in occipital regions, may be underpinned by cholinergic changes arising from structural degeneration in anterior and posterior cBF regions in people with PD.

In the context of lack of group-level differences in cBF volumes, the associations with qEEG measures observed here may relate to functional changes in cortical

cholinergic pathways occurring without significant neuronal degeneration. Ch1-2 projects to limbic regions including the hippocampus, while Ch4 projects to the entire cortical mantle (Mesulam et al., 1983b). In PD without dementia, reduced neocortical and hippocampal ChAT activity has been reported in absence of Ch4, and Ch1-2 degeneration, respectively (Hall et al., 2014). Interestingly, these participants also presented with alpha synuclein inclusions in Ch1-2 and Ch4 neurons, suggesting that Lewy depositions may play a role in early cholinergic dysfunction.

3.4.4 *Increased pre-alpha power was associated with larger Ch1-2 and Ch4p volumes in MCI*

In people with MCI, pre-alpha power was increased in those with larger Ch1-2 and Ch4p volumes. Given the small sample size, the lack of confirmation re: diagnostic cause of MCI symptoms, and the cross-sectional nature of the study, this finding will need further corroboration. While speculatory, it could suggest that those with MCI not associated with cholinergic system loss have more preserved cBF volumes, but pathology in the wider brain results in increased pre-alpha rhythms and cognitive decline. The lack of relationship between this rhythm and cBF volumes in PD, despite those participants having more prominent increases in pre-alpha, are consistent with this speculation. However, studies with confirmed diagnoses that distinguish pathological causes in MCI participants are needed to test these ideas.

3.4.5 *Lack of associations with MoCA performance*

Global cognitive performance (measured with the MoCA) did not show any significant associations with cBF volumes or qEEG metrics within any group. Previous studies in which these relationships have been investigated have reported mixed findings. For example, cross-sectional relationships between Ch4 and MoCA scores have been reported in more advanced PD (Barrett et al., 2019). In a more recent study, Ch4 volume was also shown to correlate positively with MMSE scores in MCI-LB but not MCI-AD (Schumacher et al., 2021). However, an earlier study by the same group failed to detect any significant relationships in a more advanced disease cohort including people with AD, PDD, and DLB (Schumacher et al., 2020b).

Inconsistencies within the qEEG literature also make it difficult to draw accurate conclusions regarding the relevance of eyes-closed resting-state qEEG changes to cognitive performance in neurodegenerative conditions. For example, more severe global cognitive performance has been correlated with higher pre-alpha power and lower alpha power in MCI-AD and MCI-LB (Schumacher et al., 2020a). Meanwhile, and consistent with the current findings, other studies have not observed any significant relationships in PD and AD cohorts (Babiloni et al., 2010a; Schumacher et al., 2020b). It is possible that cBF volumetry and qEEG may be more sensitive to domain-specific cognitive performance, rather than global measures.

3.4.6 *Strengths and limitations*

A key strength of the current study is the application of multimodal techniques to study the early disease stages of a clinically diverse sample. Both MRI and EEG are widely available, non-invasive, and relatively low-cost, making them attractive candidates for use in clinical settings. Few studies have combined these tools to explore their relationship in the context of cholinergic changes in different neurodegenerative conditions. Results are derived from resting-state qEEG metrics that are relatively straightforward to implement, do not require advanced analysis or expertise, and can be easily replicated.

However, the nature and extent of the relationship between qEEG and cBF volumes, and its clinical relevance, require further exploration. Future work will therefore aim to extend the current analysis to investigate additional measures of cholinergic system degeneration, and their relationship with cortically measured EEG. From a clinical perspective, further development of accessible markers of cholinergic system changes in different neurodegenerative conditions has the potential to help determine those who may benefit most from cholinergic medications in the early disease stages.

There are some limitations to the current study. The small sample size and relatively large number of statistical comparisons increases the statistical uncertainty of findings. The range of metrics included in the current analysis was limited in attempt to constrain the number of statistical comparisons. However, it is possible that additional spectral EEG features that are not

included here may also be sensitive to early changes within these diseases. Relatedly, analyses included only spectral features. While beyond the scope of this study, connectivity and network features are likely to be of relevance to cholinergic system changes, and may provide greater group-level discriminative power (Gratwicke et al., 2015; Hassan et al., 2017). Finally, although participants showed no clinical characteristics to suggest alternative neurodegenerative diagnoses, the lack of biomarker evidence means that concomitant AD, LB, or vascular pathology cannot be fully ruled out.

In conclusion, this study demonstrates that qEEG abnormalities are present in the early, predementia stages of PD when compared to controls. Specifically, alpha reactivity was reduced, and power in slow frequencies (pre-alpha in particular) was increased. Furthermore, the results suggest that early EEG changes may be related to changes occurring within cortically projecting cholinergic nuclei. In MCI and PD (without dementia), smaller Ch1-2 and Ch4p volumes were associated with increased pre-alpha power, and decreased alpha reactivity, respectively. In these neurodegenerative conditions, structural and functional changes within the cBF system occur against a backdrop of disease related pathology. Additional data with control for pathologies in the wider brain are necessary to confirm the nature of the relationship between cBF integrity and qEEG measures, particularly in the early disease stages.

Chapter 4. Pedunculopontine nucleus microstructure and quantitative EEG in Parkinson's disease and mild cognitive impairment

4.1 Introduction

The link between cholinergic system degeneration and cognitive decline is well established in neurodegenerative conditions as such as PD and AD (Bohnen et al., 2018a; Craig et al., 2020b), in which cBF atrophy has been shown to predict the onset of cognitive impairment (Kilimann et al., 2014; Schmitz and Spreng, 2016; Ray et al., 2018; Schulz et al., 2018; Grothe et al., 2021). In comparison to basal forebrain nuclei, however, the brainstem PPN, another major source of cholinergic projections in the brain, has received little attention with respect to its involvement in cognitive functions.

Traditionally, functions of the PPN have been assigned to wakefulness (as a key component of the reticular activating system) and locomotion (as part of the functionally defined mesencephalic locomotor region) (Moruzzi and Magoun, 1949; Whelan, 1996). However, more recent accounts derived largely from animal studies suggest that the role of the cholinergic PPN is more complex than originally thought, and may involve both motor *and* cognitive functions (Steckler et al., 1994; Gut and Winn, 2016; Gut and Mena-Segovia, 2019).

Post-mortem studies have reported significant loss of cholinergic PPN neurons in people with PD (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989; Gai et al., 1991). PPN deep brain stimulation (DBS) has been shown to relieve gait and falls in PD (Moro et al., 2010; Thevathasan et al., 2012; Welter et al., 2015), however, positive outcomes have not been consistently reported (Ferraye et al., 2010). Furthermore, PPN-DBS has been shown to influence cognitive performance, with limited benefit for postural and gait symptoms (see Stefani et al., 2013). For example, improvements in delayed recall, working memory, executive functions, and verbal fluency have been observed (Alessandro et al., 2010; Ceravolo et al., 2011). A better understanding of the relationship between

changes occurring in the PPN region and cognitive symptoms is therefore warranted.

Ascending PPN projections have the potential to generate distinct effects on cortical oscillatory activity (Steriade, 2004; Roš et al., 2010; Mena-Segovia and Bolam, 2011; Li and Zhang, 2015). Thus, PPN impairment (as a consequence of neurodegeneration) may result in changes to cortically measured EEG. Consistent with this idea, PPN local field potentials have revealed theta, alpha, beta, and gamma oscillations that were coupled to cortical EEG (Androulidakis et al., 2008; Tsang et al., 2010; Thevathasan et al., 2012; Fraix et al., 2013).

In support of findings from Chapter 3, volumetric changes in the cBF have shown associations with qEEG activity, including alpha power changes from eyes-closed to eye-open states (Schumacher et al., 2020b). Whether resting-state qEEG holds physiological relevance for structural measures of the PPN remains to be determined.

Methods to assess PPN volume *in vivo* are not widely available due to difficulties in accurately segmenting grey and white matter in the brainstem. However, diffusion tensor imaging (DTI) provides a complementary approach to assess microstructural changes in subcortical grey matter structures (Deng et al., 2018). In a recent longitudinal study combing stereotactic mapping and DTI, elevated PPN diffusivity measures were shown to be useful for predicting which people with PD who were at risk of developing PIGD (Craig et al., 2020a). Whether these measures can be used to distinguish people with PD from controls or people with different neurodegenerative conditions, and whether they are associated with cognitive impairment or qEEG, is not currently known.

The aims of current study are therefore to a) combine DTI and stereotactic mapping to determine whether people with PD show PPN microstructural degeneration compared to people with MCI and controls, and b) explore relationships between PPN diffusivity metrics, resting-state qEEG, and cognitive performance.

4.2 Materials and methods

4.2.1 *Participants*

The participants for this study are described in more detail in the General Methods chapter (section 2.1.2). In brief, this study involved a total of 73 participants. Thirty-one were diagnosed with PD according to UK PD Society Brain Bank Criteria. Twenty-one had been diagnosed with MCI based on established criteria (Albert et al., 2011), described previously in General Methods (section 2.1.2). Twenty-one were healthy controls of a similar age to those with a neurodegenerative diagnosis.

Cognitive scores were used to assign people with PD into two clinical subgroups for further analyses (those with and without PD-MCI), based on established criteria (Litvan et al., 2012). Specifically, PD-MCI was determined as two or more cognitive scores falling more than 1.0 standard deviation below normative means. Details of cognitive assessments that were conducted are outlined below.

4.2.2 *Cognitive assessment*

Participants completed a battery of nine neuropsychological tests to assess domain-specific cognition. Tests included: Trail Making Test (TMT, Part A and B), Colour-Word Test, Tower Test, Weschler Adult Intelligence Scale (WAIS, Similarities), Letter Fluency, Rey Osterreith Complex Figure Test (ROCFT, Copy trial and Delayed Recall trial), California Verbal Learning Test (CVLT, Long Delay Free Recall trial). Descriptions of each of these tests have been provided in General Methods (section 2.1.4).

4.2.3 *EEG acquisition and pre-processing*

Details of EEG acquisition and pre-processing steps have been outlined in General Methods (sections 2.1.5.1 and 2.1.5.2). In brief, resting-state EEG recordings were collected from each participant during eyes-closed and eyes-open conditions. This chapter focused on data from the eyes-open condition for global frequency analysis (described below). Chapter 3 focused on data from the eyes-closed condition for global frequency analysis.

4.2.4 *Global EEG frequency analysis*

Global (over all electrodes) eyes-open relative power was estimated for standard EEG frequency bands including delta: 2–4 Hz, theta: 4–5.5 Hz, pre-alpha: 5.5-8 Hz, alpha: 8–13 Hz, beta: 13-30 Hz, gamma: 30-40 Hz, calculated as the sum of EEG power in each frequency band divided by the total EEG power between 2-40 Hz. The pre-alpha band was included based on previous studies in Lewy body diseases (Bonanni et al., 2008; Schumacher et al., 2020a).

4.2.5 *MRI acquisition and preprocessing*

Details of MRI acquisition and preprocessing have been outlined in General Methods (sections 2.1.6.1 and 2.1.6.2).

4.2.6 *Stereotactic map of the pedunculopontine nucleus region of interest*

The PPN region of interest was accessed via a stereotactic map that was developed to improve targeting of this region for DBS (Alho et al., 2017). The methods that were used to create this map have been outlined in General Methods (section 2.1.6.4).

4.2.7 *Extraction of PPN diffusivity metrics*

Full details of the pipeline used to extract PPN diffusivity metrics have been outlined in General Methods (section 2.1.6.6).

Mean diffusivity values were calculated from voxels within the PPN ROI. This resulted in a total of three metrics extracted from the PPN: mean diffusivity (mD), axial diffusivity (aD), and radial diffusivity (rD).

4.2.8 *Statistical analyses*

Statistical analyses were conducted in IBM SPSS statistics 26. Kolmogorov-Smirnov tests and boxplot and histogram inspections were used to assess the distribution of continuous variables. Since relative qEEG power was not

normally distributed in all groups, these variables were log-transformed to achieve normal distribution.

4.2.8.1 *Data cleaning*

The approach taken for data cleaning has been described in the General Methods chapter (section 2.3). This resulted in the removal of PPN diffusivity data from one person with MCI, in whom these measures fell more than 3 × above the IQR. As shown in the boxplots below (Figure 4.1), these datapoints are substantially higher than most of the observations, and do not appear to accurately represent true physiological data. DWI is particularly susceptible to movement-related artefacts. Although correction was made for such confounds (i.e., eddy current distortion), it is possible that other motion artifacts may have been introduced (e.g., vibration-induced signal loss) that corrupted the voxel-wise correspondence across DWIs during acquisition, ultimately leading to an error in tensor estimation.

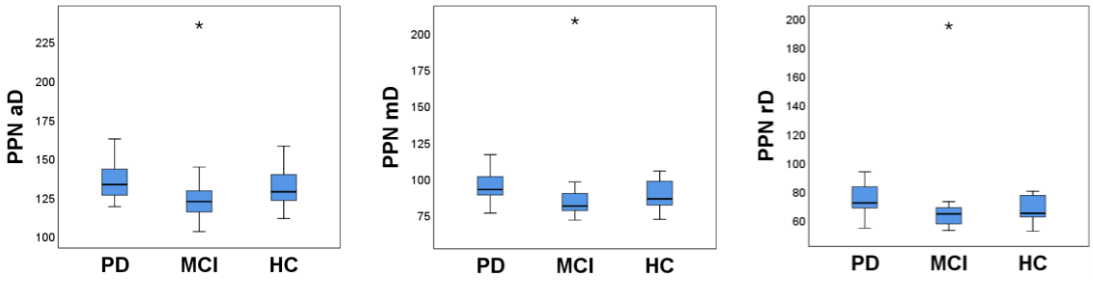


Figure 4.1 Boxplots showing outlier data for PPN diffusivity in people with MCI

4.2.8.2 *Group comparisons*

Univariate ANOVAs (controlling for age, sex, and global grey matter diffusivity (for diffusivity data only)) were used to compare demographics, cognitive scores, qEEG measures, and PPN diffusivity metrics between groups (PD, MCI, controls). Significant group level differences were followed up with post-hoc tests, with Bonferroni correction for multiple comparisons.

4.2.8.3 *Within-group relationships between PPN diffusivity, qEEG, and cognitive performance*

Partial correlations were subsequently conducted in each clinical group separately (PD (without MCI), PD-MCI, MCI, controls) to explore the relationship between cognitive scores and PPN diffusivity metrics, controlling for age, sex, and global grey matter diffusivity. The same analysis was used to explore the relationship between qEEG and PPN diffusivity metrics. Given the exploratory nature of these investigations, partial correlations were considered significant at an uncorrected threshold of $P < 0.05$.

4.3 Results

EEG, MRI, and cognitive data were not collected from all participants due to participant fatigue, technical issues, or time constraints. The number of missing datapoints prior to analyses is reported in General Methods (section 2.1.7). For clarity, df are also reported throughout the results section where appropriate. Besides the (outlier) removal of PPN diffusivity data from one person with MCI (described above in section 4.2.8.1), there were no other exclusions.

4.3.1 ***Demographics and cognitive scores***

Participant demographics are reported in Table 4.1. Groups were well matched with respect to age and sex.

Group comparisons between cognitive scores are reported in Table 4.1. Post-hoc comparisons showed that cognitive performance was impaired in people with PD compared to controls on several tests including TMTB ($P = 0.001$); WAIS Similarities ($P = 0.008$); ROCF delayed recall ($P = 0.048$); in addition to a trend-level difference for letter fluency ($P = 0.073$).

A similar pattern of impaired cognitive performance was reported in people with MCI compared to controls, including TMTB ($P < 0.001$); Tower ($P < 0.040$); WAIS Similarities ($P = 0.023$); CVLT long delay free recall ($P = 0.007$);

ROCF delayed recall (P = 0.010); in addition to trend-level differences for letter fluency (P = 0.059) and TMTA (P = 0.069).

Within the PD sample, 16 participants satisfied criteria for PD-MCI, with two or more cognitive scores falling greater than 1.0 standard deviation below normative means (Litvan et al., 2012).

Table 4.1 Participant demographics and clinical characteristics

	PD	**MCI**	**CONTROL**	**ANOVA**
N (Male %)	31 (52%)	21 (24%)	21 (33%)	χ^2 = 4.40, P = 0.110
Age	67.8 (6.31)	70 (7.43)	66.3 (7.27)	F(2,71) = 1.55, P = 0.22
Disease duration	6.62 (2.14)	–	–	
TMT-A	-0.38 (1.28)	-0.58 (1.18)	0.44 (0.48)	**F(2,51) = 3.336, *P* = 0.043**
Colour-Word	-0.95 (0.76)	-0.99 (0.89)	-0.66 (0.62)	F(2,51) = 0.601, P = 0.552
TMT-B	-0.35 (0.95)**	-0.80 (0.99)**	0.71 (0.45)	**F(2,56) = 11.427, *P* < 0.001**
Tower	0.04 (1.07)	-0.52 (1.19)*	0.46 (0.79)	**F(2,57) = 3.278, *P* = 0.045**
WAIS (S)	-0.52 (0.86)**	-0.62 (0.68)*	0.19 (0.68)	**F(2,50) = 5.889, *P* = 0.005**
Letter Fluency	-0.61 (1.09)	-0.72 (1.03)	0.19 (1.20)	**F(2,58) = 3.542, *P* = 0.035**
ROCFT (C)	0.02 (1.43)	0.33 (1.47)	0.18 (0.69)	F(2,60) = 0.884, P = 0.418
CVLT (LDF)	0.21 (0.96)	-0.56 (1.10)**	0.62 (0.82)	**F(2,58) = 5.182, *P* = 0.009**
ROCFT (D)	0.55 (1.65)*	-0.16 (1.24)*	1.28 (0.87)	**F(2,60) = 5.117, *P* = 0.009**

*Mean (standard deviation) z-scores. Metrics in bold were significantly different at P<0.05 between groups in a whole-sample univariate analysis. * indicates groups who were significantly different to controls at P < 0.05; ** indicates groups who were significantly different to controls at P < 0.01 (Bonferroni adjusted). Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; TMT = Trail Making Test; WAIS (S) = Weschler Adult Intelligence Scale (Similarities); ROCFT (C) = Rey Osterreith Complex Figure Test (Copy); CVLT (LDF) = California Verbal Learning Test (Long Delay Free Recall); ROCFT (D) = Rey Osterreith Complex Figure Test (Delayed Recall).*

4.3.2 *Group differences: qEEG relative power and PPN diffusivity*

Univariate ANOVAs (controlling for age and sex) revealed between group differences in eyes-open relative pre-alpha and beta power (Table 4.2). Post-hoc tests showed that pre-alpha power was higher in people with PD group compared to controls ($P = 0.01$), while beta power was lower ($P = 0.03$). There were no differences between PD and MCI ($P > 0.50$) or MCI and controls ($P > 0.51$) (Figure 4.2). Between group comparisons for alpha reactivity have been previously reported in Chapter 3, in summary, alpha reactivity was significantly reduced in people with PD compared to controls ($P = 0.04$).

ANOVAs controlling for age, sex, and global grey matter diffusivity revealed significant group differences across all PPN diffusivity metrics (Table 4.2). Post-hoc tests revealed that PPN aD ($P = 0.009$), PPN mD ($P = 0.005$), and PPN rD ($P = 0.003$) measures were significantly greater in people with PD compared to people with MCI. There were no differences between PD and controls ($P > 0.27$), or MCI and controls ($P > 0.10$) (Figure 4.3).

Table 4.2 Pedunculopontine nucleus diffusivity and qEEG relative power

	PD	**MCI**	**CONTROL**	**ANOVA**
Delta	2.38 (0.40)	2.42 (0.39)	2.42 (0.48)	F(2,55) = 0.194, *P* = 0.824
Theta	2.05 (0.34)	1.98 (0.32)	1.84 (0.39)	F(2,55) = 1.054, *P* = 0.356
Pre-alpha	2.37 (0.53)*	2.07 (0.44)	1.88 (0.38)	**F(2,55) = 4.438, *P* = 0.016**
Alpha	3.62 (0.28)	3.49 (0.40)	3.43 (0.46)	F(2,55) = 1.298, *P* = 0.281
Beta	3.29 (0.41)*	3.48 (0.42)	3.66 (0.35)	**F(2,55) = 3.525, *P* = 0.036**
Gamma	1.46 (0.53)	1.80 (0.66)	1.65 (0.63)	F(2,55) = 0.194, *P* = 0.824
PPN aD	135.23 (12.71)♦	121.65 (10.59)	130.90 (14.69)	F(2,61) = 1.215, *P* = 0.305
PPN mD	94.24 (11.46)♦♦	82.67 (7.38)	88.83 (10.49)	**F(2,61) = 5.415, *P* = 0.007**
PPN rD	73.75 (11.28)♦♦	62.13 (6.28)	67.80 (8.81)	**F(2,61) = 6.12, *P* = 0.004**

*Mean (standard deviation). Metrics in bold were significantly different at P < 0.05 between groups in a whole-sample univariate analysis (controlling for age, sex, and global grey matter diffusivity (for diffusivity data only)). * indicates significantly different to controls at P<0.05; ♦ indicates significantly different to MCI at P < 0.05; ♦♦ indicates significantly different to MCI at P < 0.01 (Bonferroni adjusted). Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; PPN = pedunculopontine nucleus, aD = axial diffusivity; mD = mean diffusivity; rD = radial diffusivity. PPN metrics multiplied by 10000. Units for qEEG power values = micro-Volts-squared per Hz ($\mu V^2/Hz$).*

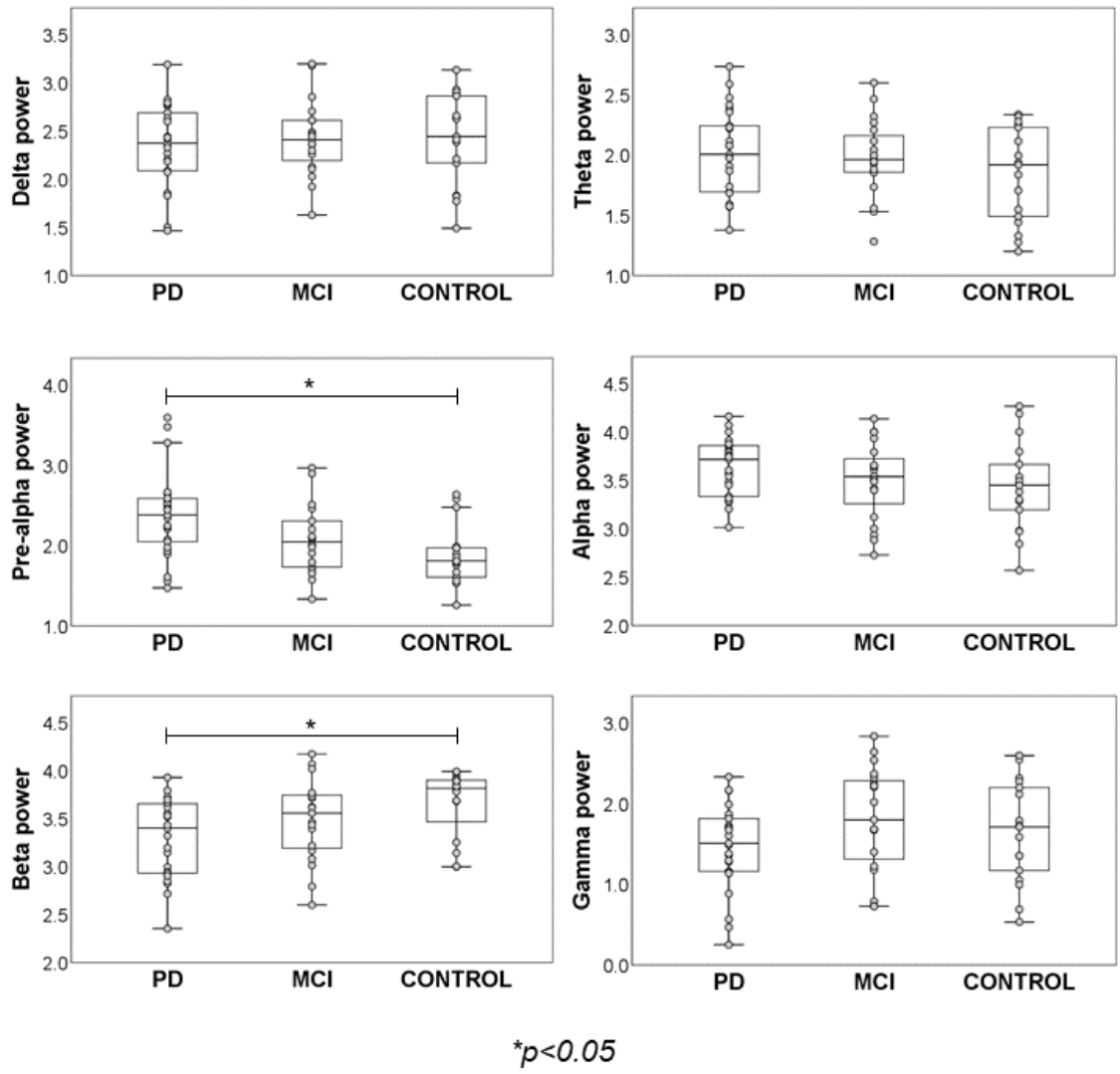


Figure 4.2 Comparison of eyes-open resting state qEEG power between groups

Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment.

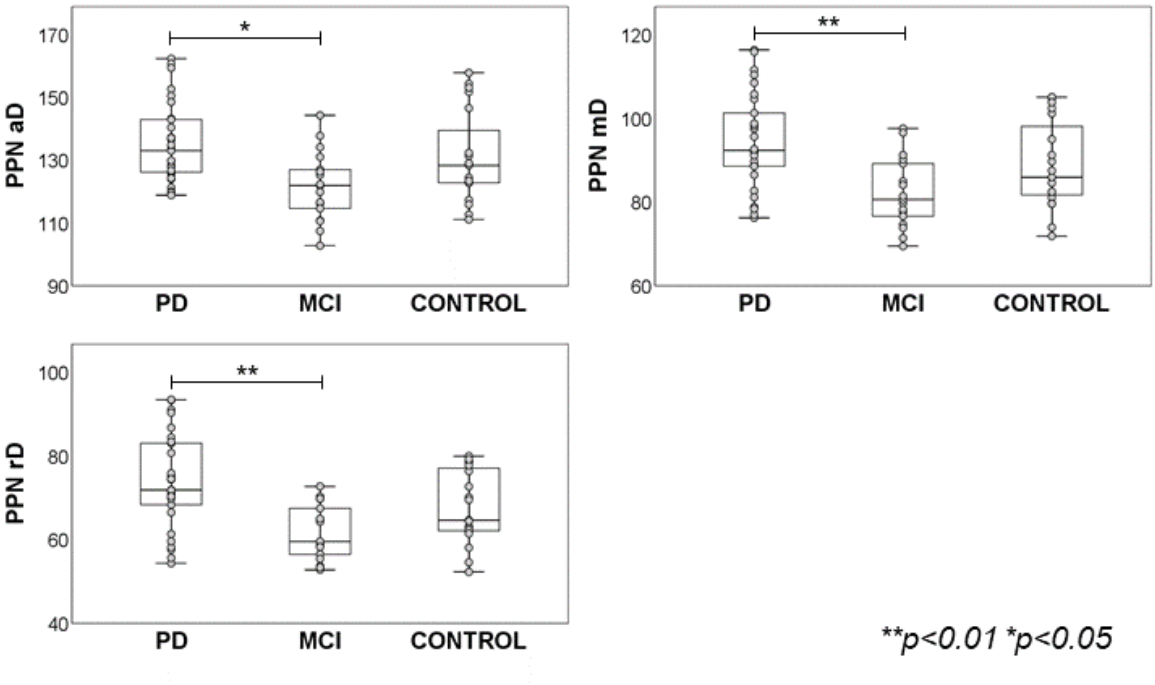


Figure 4.3 Comparison of pedunculopontine nucleus diffusivity metrics between groups

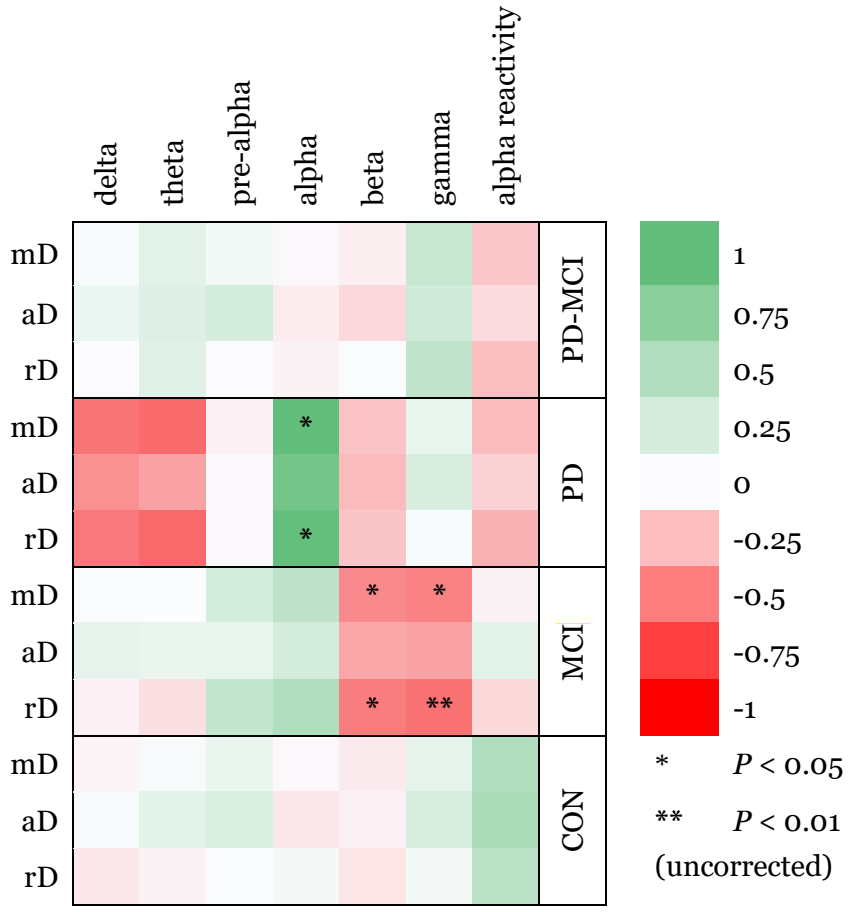
Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; PPN = pedunculopontine nucleus; aD = axial diffusivity; mD = mean diffusivity; rD = radial diffusivity. PPN metrics multiplied by 10000.

4.3.3 *Relationships between qEEG power and PPN diffusivity*

In the PD (without MCI) group, alpha power was positively correlated with PPN mD and PPN rD (r = 0.71, *P* = 0.04; r = 0.75, *P* = 0.03), controlling for age, sex, and global grey matter diffusivity (Table 4.3, Figure 4.4, A).

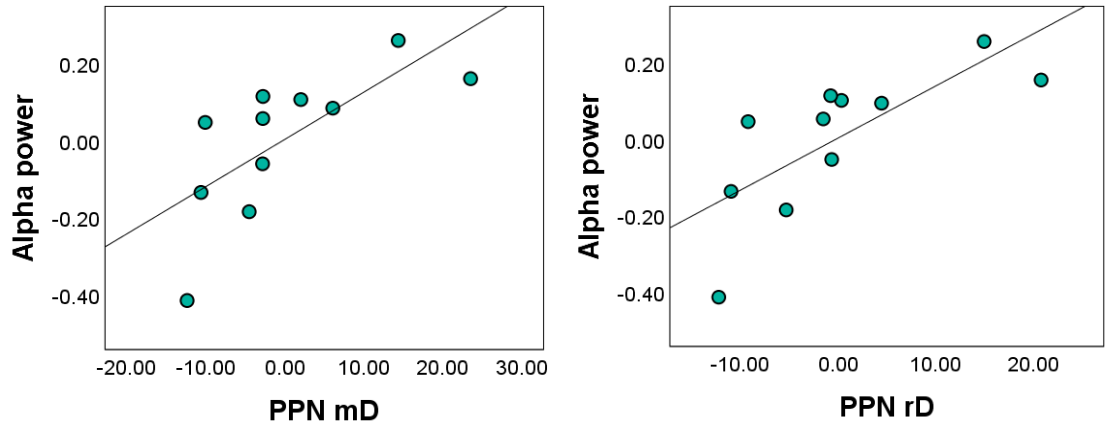
In the MCI group, PPN mD and PPN rD correlated negatively with beta (r = -0.62, *P* = 0.03; r = -0.67, *P* = 0.02) and gamma (r = -0.67, *P* = 0.02; r = -0.72, *P* = 0.008) power (Table 4.3, Figure 4.4, B). There were no significant correlations in PD-MCI (r < 0.33, *P* > 0.39) or controls (r < 0.16, *P* > 0.54).

Table 4.3 Partial correlations between pedunculopontine nucleus diffusivity metrics and qEEG measures



Pearson's partial correlations controlling for age, sex, and global grey matter diffusivity. Legend shows colour intensity proportional to Pearson's correlation coefficient. Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; aD = axial diffusivity; mD = mean diffusivity; rD = radial diffusivity.

(A) PD (without MCI)



(B) MCI

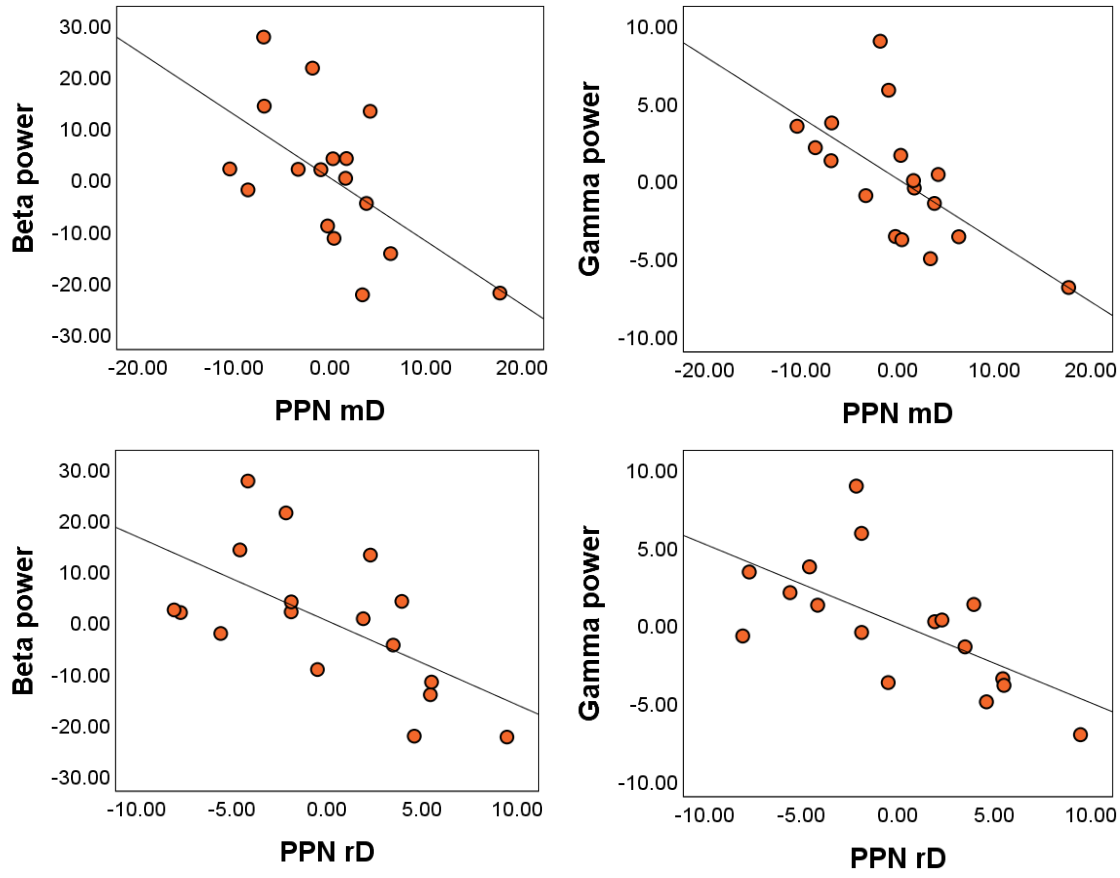


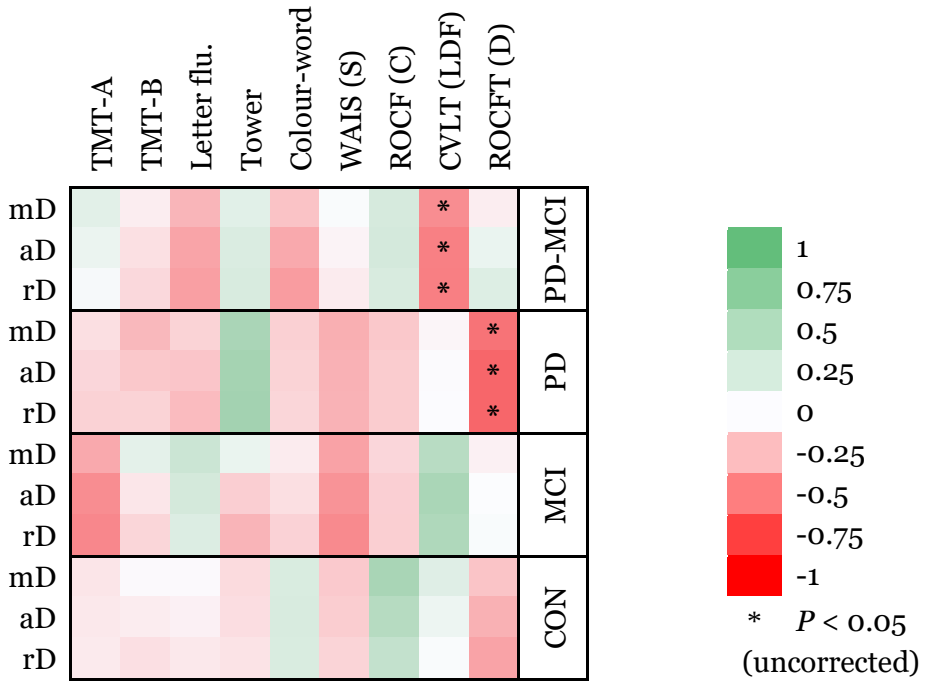
Figure 4.4 Partial correlations between pedunculopontine nucleus diffusivity metrics and qEEG relative power

(controlling for age, sex, and global grey matter diffusivity) in (A) PD (without MCI), and (B) MCI. Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; aD = axial diffusivity; mD = mean diffusivity; rD = radial diffusivity.

4.3.4 *Relationships between PPN diffusivity and cognitive scores*

In people with PD (without MCI), ROCF delayed recall performance was negatively correlated with PPN mD, aD, and rD (r = -0.70, *P* = 0.03; r = -0.76, *P* = 0.02; r = -0.77, *P* = 0.01). In people with PD-MCI, CVLT long delay free recall performance correlated negatively with PPN mD, aD, and rD (r = -0.56, *P* = 0.04; r = -0.63, *P* = 0.02; r = -0.64, *P* = 0.02). In people with MCI, there was a negative trend-level association between TMTA and PPN mD (r = -0.57, *P* = 0.08). There were no significant correlations in the control group (r < 0.46, *P* > 0.12) (Table 4.4).

Table 4.4 Partial correlations between pedunculopontine nucleus diffusivity metrics and cognitive scores



Pearson's partial correlations controlling for age, sex, and global grey matter diffusivity (for diffusivity data only)). Legend shows colour intensity proportional to Pearson's correlation coefficient. Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; aD = axial diffusivity; mD = mean diffusivity; rD = radial diffusivity; TMT = Trail Making Test; WAIS (S) = Weschler Adult Intelligence Scale (Similarities); ROCFT (C) = Rey Osterreith Complex Figure Test (Copy); CVLT (LDF) = California Verbal Learning Test (Long Delay Free Recall); ROCFT (D) = Rey Osterreith Complex Figure Test (Delayed Recall).

4.3.5 *Relationships between qEEG power and cognitive scores*

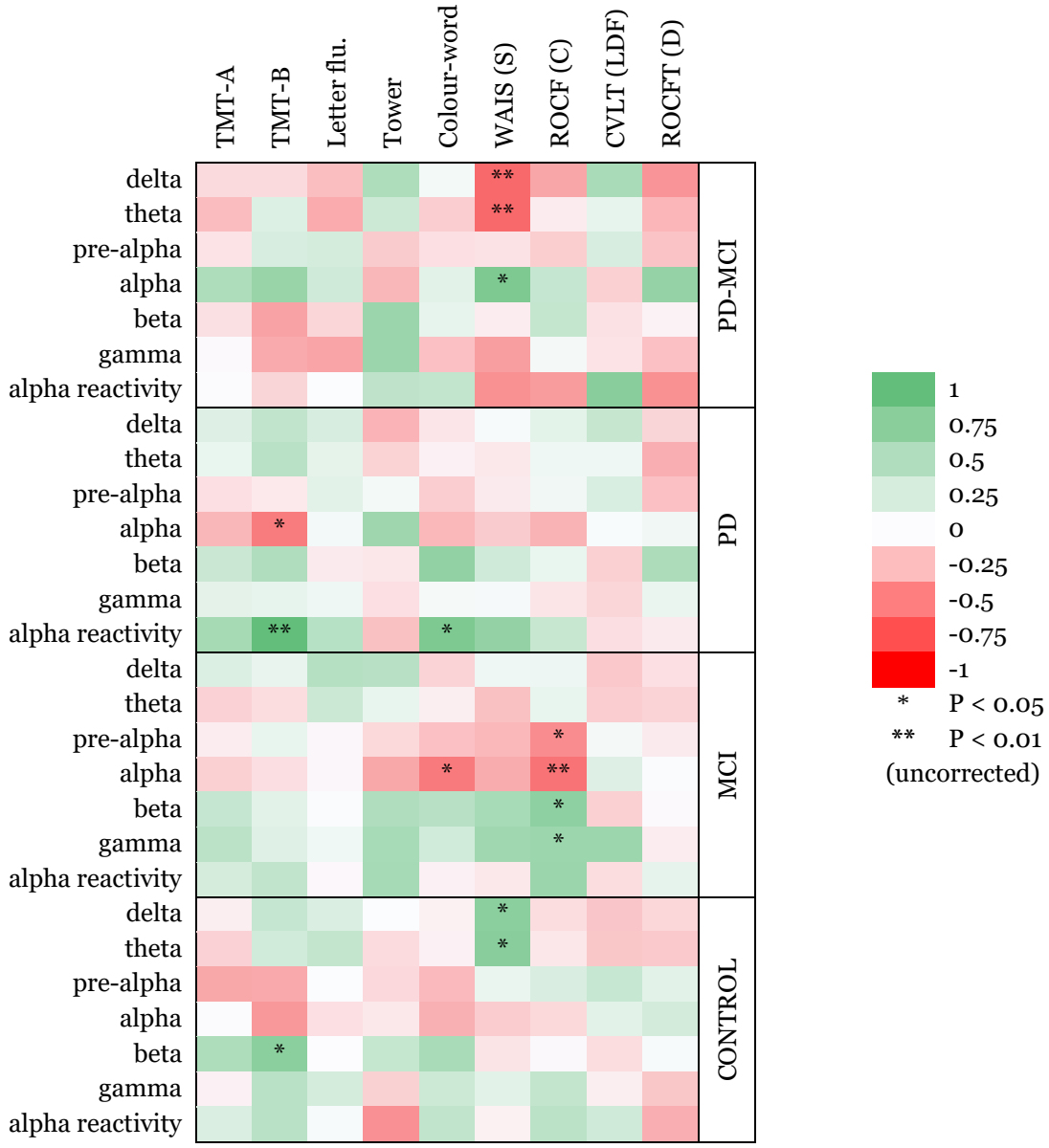
In people with PD-MCI, partial correlations controlling for age and sex revealed a negative association between WAIS similarities performance and delta (r = -0.84, *P* = 0.002) and theta (r = -0.84, *P* = 0.002) power, and a positive association with alpha power (r = -0.73, *P* = 0.02) (Table 4.5).

In PD (without MCI), TMTB scores correlated negatively with alpha power (r = -0.784, *P* = 0.02). Furthermore, better TMTB and colour-word performance was also associated with higher alpha reactivity (r = 0.88, *P* = 0.001; r = 0.727, *P* = 0.03) (Table 4.5).

In people with MCI, colour-word scores correlated negatively with alpha power (r = -0.74, *P* = 0.02). In addition, Rey Osterreith Copy scores correlated negatively with pre-alpha and alpha power (r = -0.64, *P* = 0.02; r = -0.78, *P* = 0.001), and positively with beta and gamma power (r = 0.63, *P* = 0.02; r = 0.55, *P* = 0.04) (Table 4.5).

In controls, higher WAIS similarities scores were associated with higher delta and theta (r = 0.65, p = 0.02; r = 0.67, *P* = 0.02) power, while TMTB scores associated positively with beta power (r = 0.65, *P* = 0.03) (Table 4.5).

Table 4.5 Partial correlations between qEEG measures and cognitive scores



Pearson's partial correlation controlling for age and sex. Legend shows colour intensity proportional to Pearson's correlation coefficient. Abbreviations: PD = Parkinson's disease; MCI = mild cognitive impairment; aD = axial diffusivity; mD = mean diffusivity; rD = radial diffusivity; TMT = Trail Making Test; WAIS (S) = Weschler Adult Intelligence Scale (Similarities); ROCFT (C) = Rey Osterreith Complex Figure Test (Copy); CVLT (LDF) = California Verbal Learning Test (Long Delay Free Recall); ROCFT (D) = Rey Osterreith Complex Figure Test (Delayed Recall).

4.4 Discussion

This study explored the relationship between PPN diffusivity, resting-state qEEG, and cognitive performance in a heterogenous sample comprised of people with PD (both with and without MCI), people with MCI, and controls of a similar age.

There were six main outcomes: (i) PPN diffusivity metrics were increased in PD compared to people with MCI; (ii) eyes-open relative power was higher in the pre-alpha range and lower in the beta range, in people with PD compared to controls; (iii) there were within-group relationships between qEEG metrics and PPN diffusivity; (iv) PPN diffusivity correlated with cognitive performance in both PD and MCI groups; (v) cognitive performance correlated with qEEG power within each group.

4.4.1 *Increased PPN diffusivity in PD*

PPN diffusivity measures were significantly elevated in people with PD compared to people with MCI. This finding is consistent with previous post-mortem reports of PPN degeneration in PD (Hirsch et al., 1987; Jellinger, 1988; Rinne et al., 2008; Schmeichel et al., 2008), and preserved/no loss in AD (Woolf et al., 1989). Importantly, this finding also suggests that combined stereotactic mapping and DTI can be used to distinguish people with PD in from other neurodegenerative conditions.

To date, very few studies have assessed structural changes within the PPN *in vivo*. The methods applied in Chapter 3 to assess cBF volumes are not appropriate for the PPN since the entire brainstem is typically segmented as white matter using standard automated techniques. That said, a recent study combining volumetry and DTI measures in the nBM showed that mD was a stronger predictor of cognitive impairment than volumetric measures (Schulz et al., 2018). This could suggest that microstructural changes in cholinergic nuclei may precede macrostructural degeneration, thereby providing a more sensitive marker of early and more subtle changes related to PD symptoms.

A recent *in vivo* study used similar methods to those applied in the current study and showed that PPN diffusivity was elevated in people with PD who later

developed postural and gait disturbances (Craig et al., 2020a). The results presented here imply that stereotactic mapping alongside DTI measures, as an *in vivo* surrogate marker of microstructural degeneration, may be informative for understanding early degenerative processes and related symptomology in people with PD.

In the current study, PPN diffusivity metrics were elevated in people with PD compared to controls, but this difference was not significant. It is possible that larger sample sizes could serve to reduce the (relatively large) degree of variability among controls, and allow group-level differences to emerge.

4.4.2 *EEG spectral changes in PD compared to controls*

Pre-alpha power was higher and beta power was lower, in people with PD compared to controls. Resting-state qEEG studies conducted in PD and AD typically record during eyes-closed conditions, while eyes-open conditions are not widely utilised. In a recent study, however, eyes-open resting alpha power was shown to be increased in Lewy body dementias (including DLB and PD dementia, PDD) compared to AD and healthy controls (Schumacher et al., 2020b). The current study therefore extends these findings to earlier disease stages, providing evidence for changes to eyes-open oscillatory activity in the predementia stages of PD (including PD with and without MCI).

The aforementioned study by Schumacher et al. (2020b) used an extended alpha frequency band (from 4 to 14 Hz) to account for the age (and neurodegeneration) – related shift of the alpha peak to slower frequencies (Klimesch, 1999). In the current study, the pre-alpha frequency range sits within this extended alpha range. It is therefore possible that enhanced power within the pre-alpha range is indexing alpha frequency alterations. Furthermore, mean power values in the standard alpha range were increased in PD in the current study, but did not reach statistical significance. Together, these findings may suggest that alterations in the alpha range during eyes-open resting conditions appear early in PD.

Previous studies have reported lower beta activity in early Lewy body diseases using eyes-closed resting-state conditions (He et al., 2017a; Schumacher et al.,

2020a). Results from the current study extend these findings to eyes-open conditions. The functional role of beta oscillations is not well understood. Top-down visual processing (which occurs during eyes-open rest (Feige et al., 2005)) and maintenance of current cognitive state have been shown to modulate beta activity (Wróbel, 2000; Engel and Fries, 2010; Gola et al., 2012). Such that lower activity has been associated with impairments to attention activation (alertness) and sustaining attention (vigilance) (Gola et al., 2012). Furthermore, top-down beta activity has been shown to enhance bottom-up gamma activity, potentially subserving attentional control and perceptual processing (Richter et al., 2017). Findings from the current study may imply that eyes-open resting beta provides a functional measure of impairments to attentional and perceptual control processes in people with PD.

4.4.3 *PPN diffusivity was positively associated with alpha power in PD (without MCI)*

In PD (without MCI), those with greater PPN microstructural degeneration demonstrated higher alpha power. This finding supports previous studies demonstrating cortical alpha band alterations in association with the PPN. Coherent alpha between the cortex and caudal (i.e. cholinergic) PPN region has been reported in people with PD (undergoing DBS) (Androulidakis et al., 2008; Thevathasan et al., 2012). Caudal PPN neurons express predominantly cholinergic (and glutamatergic) neurons (Martinez-Gonzalez et al., 2011) and have widespread ascending connections with the thalamus and basal forebrain (and hence cortex) (Mena-segovia et al., 2008). These findings suggest a possible channel of communication through which the cortex interacts with cholinergic PPN neurons via alpha oscillations.

Alpha oscillations are considered to play a key role in attention and allocation of processing resources (Klimesch, 1999; Palva and Palva, 2007). Increased alpha activity is thought to be indicative of inhibition of cortical processes related to distraction i.e., from external sensory input (Ward, 2003; Jensen and Mazaheri, 2010). Consistent with this idea, during eyes-open resting conditions (as in the current study) when the visual system is actively processing external stimuli, alpha power is typically low (Barry and De Blasio, 2017). Moreover, alpha activity has been shown to increase when attention is directed internally (e.g.,

using mental imagery) (Cooper et al., 2003), potentially reflecting a state of intention – which has been ascribed to directing ongoing perception (Shaw, 1996).

Previous work suggests that top-down (voluntary) control of attention is supported by basal forebrain-cortical cholinergic pathways, whereas bottom-up (externally driven) sensory processing involves PPN-thalamo-cortical cholinergic pathways (Kim et al., 2018). In experimental work, prevention of bottom-up sensory processing was accompanied by an increase in cortical alpha power (Benedek et al., 2014). When taken together with findings from the current study, disruption to thalamocortical cholinergic projections (as a consequence of PD neurodegeneration of the PPN) may be indexed by resting-state eyes-open alpha synchronisation at the cortex. This may indicate a mechanism by which external input is supressed, and the cortex remains in a more internally orientated state.

PPN and alpha activity changes that relate to attention impairments may also be relevant for PD mobility impairments. Attentional impairments are common in PD, even in early disease stages (Aarsland et al., 2010; Weintraub et al., 2015). The link between postural instability, gait impairment, falls, and attention deficits is well recognised (Yarnall et al., 2011). PPN alpha activity (coherent with the cortex) has been shown to correlate with gait speed in people with PD undergoing DBS (Thevathasan et al., 2012). Furthermore, reduced structural connectivity of the PPN (with cerebellum, thalamus, and multiple regions of the frontal cortex) has been associated with poorer attentional control in people with PD with freezing of gait (Fling et al., 2013; Peterson et al., 2015). A more detailed understanding of the relationship between cortical oscillatory activity, PPN changes, and PD cognitive and motor symptoms may therefore offer clinical relevance for determining/assessing PPN-DBS therapeutic outcomes.

No specific associations between PPN diffusivity and qEEG measures were observed in the PD-MCI group, the reason for this is not obvious. The neurobiological substrate for PD-MCI appears to be heterogenous, implicating neurotransmitter deficits, proteinopathies (i.e., Lewy body and AD), and vascular pathology, reviewed in (Halliday et al., 2014). The modulatory effect of such processes on resting-state qEEG has been recognised (Caviness et al., 2017;

Gaubert et al., 2019; Tanabe et al., 2020). In this study, PD without MCI may therefore provide a 'cleaner' window into PPN-qEEG associations. However, additional data with control for pathologies in the wider brain are necessary to confirm the nature of the PPN-qEEG relationships presented here.

4.4.4 *PPN diffusivity was negatively associated with beta and gamma power in MCI*

In the MCI group (not related to PD), lower PPN diffusivity was associated with higher beta and gamma power. In the context of preserved PPN diffusivity metrics and normal qEEG in this group, these findings might imply that the observed relationship between PPN microstructure and beta/gamma is physiological in nature, and lost in PD.

In early studies, PPN stimulation (as part of the reticular activating system) was shown to influence cortical states associated with EEG desynchronisation (i.e. transition from slow to fast oscillations in the beta/gamma range) (Moruzzi and Magoun, 1949; Steriade et al., 1990; 1991). Thus, PPN degeneration may disturb beta/gamma oscillations at the cortex. The associations between increased PPN diffusivity and lower beta/gamma power observed in the current study broadly follows this proposed pattern of altered activity. Furthermore, coherent activity between the cortex and PPN has been reported in beta and gamma ranges, indicating a possible channel of communication through which the PPN and cortex interact (Mena-segovia et al., 2008; Tsang et al., 2010; Fraix et al., 2013; Valencia et al., 2014).

DBS studies conducted in PD have been informative for revealing the potential physiological role of PPN beta/gamma activity. For example, beta band coherence between the PPN and midline prefrontal cortex has been reported during movement preparation (in ON state) (Tsang et al., 2010). Midline prefrontal cortex has been shown to support attention towards the external environment during low demand/arousal conditions that do not require extensive processing (Gilbert et al., 2006). Beta and gamma oscillations were also induced in the PPN during visual stimulation and imagined gait and object movement, suggesting a potential role in attention control (in addition to its motor role) (Lau et al., 2015). Relatedly, PPN stimulation in the beta range (10-

25 Hz) has been shown to promote alertness (Arnulf et al., 2010). Thus, in the current study, PPN projections may be influencing cortical processes associated with alertness/attention and visual processing, reflected in beta and gamma oscillatory activity.

4.4.5 *Increased PPN diffusivity was associated with poorer delayed recall performance in PD*

In people with PD, elevated PPN diffusivity was associated with poorer memory performance, including Rey Osterrieth Complex Figure Test Delayed Recall in PD (without MCI) and CVLT Long Delay Free Recall scores in PD-MCI. There was also a non-significant negative trend between TMT-A and PPN mD and rD in people with MCI.

The above relationships between PPN structure and memory performance were not expected given that the PPN has been most associated with behavioural flexibility and attention in animal literature (discussed in section 1.5.5). That said, previous studies have also reported improvements to delayed recall performance (as well as working memory, executive functions, and verbal fluency) following PPN-DBS in people with PD (Alessandro et al., 2010; Ceravolo et al., 2011). A possible explanation could be that attentional resources are important for various cognitive control processes. In this instance, attention may be contributing to selecting, storing, and updating representations in memory (Oberauer, 2019). In support, previous work has suggested that impaired verbal memory performance in people with PD may be attributable to deficits in attention allocation, formulation of retrieval strategies, and effortful learning (Ivory et al., 1999). Thus, the observed correlations between PPN structure and memory performance in the current study may be mediated by the relationship with attentional processes.

4.4.6 *Associations between qEEG power and cognitive performance*

In people with PD-MCI, higher delta and theta, and lower alpha power, correlated with poorer WAIS Similarities scores. In contrast, these associations were in the opposite direction in control participants, who showed a positive

relationship between delta and theta power and WAIS performance. Studies focusing on pathological ageing (including PD and AD), generally agree that enhanced qEEG slowing is associated with cognitive impairment (Caviness et al., 2007a; 2015; Babiloni et al., 2010b; 2018; Klassen et al., 2011; Guner et al., 2016). Thus, the shift to slower frequencies in association with poorer WAIS performance in people with PD-MCI in the current study is broadly consistent with these findings.

For healthy ageing, reports of qEEG alterations are less consistent. Some studies report that age-related increases in slow wave activity are indicative of subsequent cognitive deterioration (Prichep et al., 2006; Stomrud et al., 2010). Whereas other studies show positive associations between slow wave activity and cognitive performance (Finnigan and Robertson, 2011; Vlahou et al., 2014; Torkamani-Azar et al., 2020). Thus, observations from the current study corroborate these latter findings. It has been suggested that inconsistent results may reflect distinct processes. Specifically, enhanced slowing may be related to a transition of the alpha peak to lower frequencies – which is frequently observed in older adults (Klimesch, 1999) – whereas when alpha slowing is not present, higher delta-theta may be a marker of healthy neurocognitive function (Finnigan and Robertson, 2011). This conclusion is consistent with the findings reported in the current study within PD-MCI and control groups.

In people with PD (without MCI) higher alpha power was associated with poorer TMTB performance. A similar relationship was observed between alpha power and Colour-Word and Rey Osterreith Copy scores in people with MCI. As discussed above, higher alpha power during eyes-open conditions may reflect a more internally orientated state. Lower alpha power has been associated with increased activity in attention networks using combined EEG and fMRI (Sadaghiani et al., 2010), while higher eyes-open resting alpha has been associated with poorer sustained visual attention performance (Torkamani-Azar et al., 2020). Correlations between higher eyes-open alpha power and deficits in cognitive performance reported in the current study may imply that people with PD and MCI fail to appropriately engage attentional processes – that are subserving various cognitive functions (Oberauer, 2019) including those required for executive and visuospatial performance.

In support of the above point, loss of alpha reactivity was associated with poorer TMTB and colour-word performance in people with PD (without MCI). In the healthy brain, alpha reactivity (i.e., the magnitude of alpha power attenuation upon opening the eyes) is thought to reflect neuronal desynchronisation (Adrian and Matthews, 1934). Thus, the current finding may imply that deficits in cognitive performance were underpinned by a lack of cortical responsiveness in people with PD, in whom the cortex remained in a more synchronised state.

Beta power correlated positively with TMT-B scores in controls. Beta and gamma power also correlated positively with Rey Osterreith Copy scores in MCI. Previous work using eyes-closed qEEG have failed to demonstrate associations between domain specific neuropsychological assessments and beta/gamma activity (Babiloni et al., 2010b). Given the associations between beta/gamma activity and attention maintenance and perceptual processing discussed above (Wróbel, 2000; Engel and Fries, 2010; Gola et al., 2012; Richter et al., 2017), it is possible that eyes-open resting state qEEG may be more sensitive for probing these relationships than eyes-closed data. However, this remains speculatory, and would need to be corroborated in larger samples with correction for multiple comparisons.

4.4.7 ***Strengths and limitations***

A key strength of the current study lies in the application of multimodal non-invasive techniques to study the early disease stages of a clinically diverse sample. Few studies have assessed structural changes in the PPN region *in vivo*. This is also the first study to combine DTI microstructural measures of the PPN with resting-state qEEG.

The seeming specificity of PPN mD and rD relationships presented in both the PD and MCI groups (rather than aD) is open for interpretation. Although, there was a tendency for aD metrics to follow the same pattern. In a previous study, future mobility impairments in PD were associated with PPN aD in particular, but not mD or rD (Craig et al., 2020a). The relevance of each of these diffusivity metrics to neurodegenerative processes is therefore still to be determined.

Results from the current study provide evidence for associations between PPN microstructure and cortical qEEG during eyes-open rest. Given that partial correlations were not corrected for multiple comparisons (and would not survive correction), the observed relationships could be artefactual. In addition, the sample size included in each group is small which also increases the statistical uncertainty of findings herein. It must therefore be stressed that the associations between PPN microstructure and cortical oscillatory activity remain speculative and require future corroboration.

For purposes of discussion, the potential influence of PPN microstructural changes and resting-state oscillatory activity on cognitive/behavioural function have also been discussed in the context of existing knowledge, however this too is speculative. That said, previous work in PD using simultaneous magnetencephalography and local field recordings have characterised widespread resting functional connectivity between the PPN and distant brain regions (including cortex) in the alpha and beta ranges at rest (Jha et al., 2017).

In conclusion, elevated PPN diffusivity distinguished people with PD from people with MCI. Cortically measured qEEG activity may hold physiological relevance to structural changes occurring in the PPN region, particularly in the alpha, beta, and gamma ranges. Alpha band alterations in association with PPN microstructural degeneration may be relevant for attentional dysfunction, which occurs frequently in PD and has been linked to postural instability, gait impairments, and falls. Degeneration of the cBF and PPN represents a shared substrate for these deficits, but the specifics of their contribution is still to be determined. Future imaging, clinical, and electrophysiological studies are therefore required to further understand the interplay between changes occurring in the cholinergic system, and cognitive and mobility impairments in PD.

Chapter 5. Cholinergic basal forebrain atrophy influences the relationship between cognition and mobility in Parkinson's disease

5.1 Introduction

Gait and balance impairments are a common and early manifestation of PD (Galna et al., 2015), contributing to fall risk (Lord et al., 2016), cognitive decline (Morris et al., 2017), disability, and reduced quality of life (Muslimović et al., 2008). Importantly, gait and balance impairments often show limited improvement with dopamine-replacement medications (Sethi, 2008; Curtze et al., 2015), suggesting that non-dopaminergic systems may be involved. Furthermore, randomised controlled trials have demonstrated beneficial effects of cholinergic treatments (i.e. cholinesterase inhibitor, rivastigmine) for improving gait and reducing falls (Chung et al., 2010; Li et al., 2015; Henderson et al., 2016). However, our current understanding of the role and contribution of cholinergic system changes remains incomplete.

There is evidence from animal and human literature that the cholinergic PPN is involved in balance and gait deficits (Karachi et al., 2010). Imaging studies using positron emission tomography (PET) conducted in people with PD have revealed relationships between thalamic cholinergic denervation and falls, freezing of gait, and postural control (Bohnen et al., 2009; 2014; 2019; Müller et al., 2013a). In addition, microstructural changes in the PPN, measured using a stereotactic map of the region and diffusion tensor imaging (DTI) have been associated with increased risk for the development of postural instability and gait disturbance (PIGD) symptoms in PD (Craig et al., 2020a).

However, there is also evidence that the cortical cholinergic system may be involved in mobility impairment in PD. For example, PET imaging has demonstrated a relationship between reduced gait speed and cortical (but not thalamic) cholinergic denervation (Bohnen et al., 2013). More recently, volumetric measures of the nbM (using stereotactic mapping and VBM) were able to predict longitudinal gait impairments in early PD, although cross-

sectional relationships in that study were non-significant (Wilson et al., 2020b). Using similar methods, cross-sectional relationships between nbM atrophy and slower walking speed were reported in people with PD approximately 10 years after diagnosis (Dalrymple et al., 2021).

A critical finding leading to the assumption that the cortex is involved in gait and balance is that dual-tasking interferes with these mobility measures. For example, dual-tasking (counting backwards) during quiet stance has been shown to increase postural sway in people with PD (Marchese et al., 2003). Furthermore, dual-task walking studies highlight a slower, asymmetric, and more variable gait in people with PD compared to normal walking (Yogev et al., 2005; Plotnik et al., 2011a; Rochester et al., 2014) and deficits in attention and executive function have been shown to exacerbate these effects (Yogev et al., 2005; Plotnik et al., 2011a).

Emerging evidence suggests that dual-task walking deficits may be associated with a loss of cortical cholinergic activity. Short latency afferent inhibition (SAI) – a transcranial magnetic stimulation technique that is thought to index cholinergic function (Di Lazzaro et al., 2000; 2002) has been associated with gait speed and attentional impairments (Rochester et al., 2012). More recently, older adult and PD fallers showed a reduction in gait speed while simultaneously performing a verbal fluency task (compared to walking without a task), and this change correlated with reduced SAI (Pelosin et al., 2016). These findings suggest that deterioration of walking during dual-task conditions may be underpinned by a loss of attention-executive function via cortical cholinergic deficits.

The Timed Up and Go (TUG) test is widely used in PD clinics to measure balance and mobility (Morris et al., 2001). Importantly, TUG performance has been correlated with functional mobility, gait speed, and falls in older adults and people with PD (Balash et al., 2005; Viccaro et al., 2011; Nocera et al., 2013; Lopes et al., 2020). The test also includes a dual-task condition. A recent study in people with PD showed that smaller Ch4 volumes were associated with performance decline on the TUG dual-task (which involved counting backwards in threes) (Dalrymple et al., 2021). However, that study did not assess whether TUG dual-task interference was associated with executive deficits.

The aims of the current study are therefore to a) determine whether there are relationships between executive function and TUG dual-task interference in a group of healthy controls and people with PD; b) to determine if cortical cholinergic system integrity is associated with TUG dual-task interference, and c) to determine if any relationship between executive performance and TUG dual-task performance is mediated by cholinergic basal forebrain integrity.

5.2 Materials and methods

5.2.1 *Participants*

The participants for this study are described in more detail in the General Methods chapter (section 2.2.2). At the time of analysis, data had not been collected from all participants (two people with PD) due to a pause in data collection caused by national coronavirus lockdown restrictions and university closures. One person with PD and one control participant were also excluded prior to analyses due to identification of (previously undisclosed) neurological conditions (stroke and encephalitis) identified on MRI. Thus, this study included 36 people with PD and 21 controls.

5.2.1.1 *Clinical assessments*

People with PD were tested 'on' dopaminergic medication for all assessments. See General Methods chapter (section 2.2.2) for a description of medications. Global cognition was assessed with the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005). Executive function was assessed with the Trail Making Test (TMT) (Reitan, 1958), semantic fluency, and letter fluency tests (Delis et al., 2001). Following (Periáñez et al., 2007), a TMT task-switching measure was calculated as the ratio between TMT Part A (TMTA, i.e. numeric only condition) and TMT Part B (TMTB, i.e. alternating number-letter condition) using the following formula: (TMTB-TMTA)/TMTA. A higher value indicates lower task switching capacity.

Dynamic balance and functional mobility was assessed with the Mini Balance Evaluation System Test (Mini-BESTest) (Franchignoni et al., 2010). The Mini-

BESTest is a 14-item balance scale comprised of 4 subcomponents measuring anticipatory postural adjustments, postural responses, sensory orientation, and dynamic gait. Each task is rated on a 2-point ordinal scale from 0 to 2. The maximum score for the Mini-BESTest is 28 (described previously in General Method, section 2.2.5).

The TUG test, which forms the final item of the Mini-BESTest, was also used in isolation as a measure of functional gait and balance (Podsiadlo and Richardson, 1991). Participants were assessed on both the TUG single-task and TUG dual-task. The TUG single-task requires a person to stand up from an armless chair, walk 3 m, turn around, walk back to the chair, and sit down. The TUG dual-task follows the same procedure, with the addition of a cognitive task – counting backwards, out loud, in threes. TUG dual-task interference, defined as the change in performance relative to an individual's TUG single-task, was calculated as defined by (Rochester et al., 2014), with the following formula: (TUG dual-task – TUG single-task)/TUG single-task. A higher value indicates worsened performance.

5.2.2 *MRI acquisition and preprocessing*

Details of MRI acquisition and preprocessing methods have been previously described in the General Methods (sections 2.1.6.1 and 2.1.6.2).

5.2.3 *Stereotactic map of the cholinergic basal forebrain*

A stereotactic map of cBF nuclei was used to extract volumetric data (Kilimann et al., 2014). The methods used to create this map have been outlined in General Methods (section 2.1.6.3). The cBF map distinguishes different cholinergic subdivisions within the basal forebrain, including cell clusters corresponding to the medial septum, vertical and horizontal limb of the diagonal band, and nbM (Ch1–4 according to Mesulam's nomenclature) (Mesulam et al., 1983b) (displayed in General Methods, section 2.1.6.3, Figure 2.1).

5.2.4 *Extraction of cBF grey matter volumes*

Grey matter volumes in cBF were calculated by summing the grey matter voxel values within the corresponding cBF ROI mask in template space (Ashburner, 2009). Global grey matter and cBF volumes were further scaled via ANCOVA, using total intracranial volume (TIV) as a covariate, to extract unstandardised residual values which were then used as input variables in further analyses. Therefore, negative cBF values indicate smaller volumes than expected given head size.

Two distinct subdivisions of the cBF were studied, that can be separated anatomically, each with different cortical projections and behavioural functions (Mesulam et al., 1983b; Záborszky et al., 2018). These regions correspond to an anterior-medial portion of the cBF, combining the medial septum (Ch1) and the vertical limb of the diagonal band (Ch2), and a more posterior cBF region covering the nbM (Ch4). Analyses were also applied to a posterior section of the nBM (Ch4p), based on previous work showing particular involvement of this region in MCI/AD and PD (Grothe et al., 2012; 2013; Kilimann et al., 2014; Ray et al., 2018).

5.2.5 *Statistical analyses*

Statistical analyses were conducted in IBM SPSS statistics 26. The distributions of continuous variables were tested for normality through Kolmogorov-Smirnov tests and boxplot and histogram inspections.

5.2.5.1 *Data cleaning*

The approach taken for data cleaning has been described in the General Methods chapter (section 2.3). This resulted in the removal of Ch4 volumetric data from one control participant, in whom this measure was more than 3 × above the IQR (Figure 5.1). The effects of maintaining this datapoint are also reported in the results section.

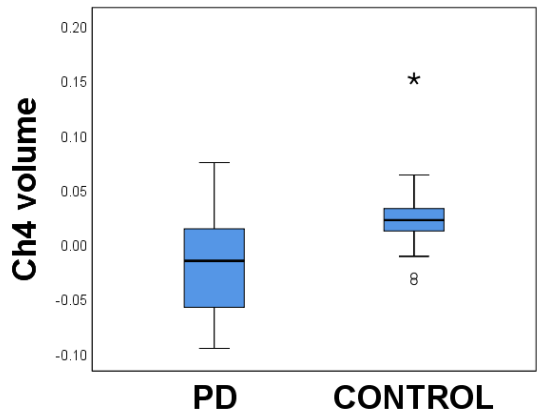


Figure 5.1 Boxplot showing outlier datapoint for Ch4 volume in controls

5.2.5.2 *Group comparisons*

Student's t-tests were used to assess group differences in cognitive and mobility performance and cBF volumes, between controls and people with PD. T-tests significant at $P < 0.05$ were further evaluated for significance after correction for age and sex using ANCOVA. The above contrasts were FDR-corrected for multiple comparisons.

5.2.5.3 *Relationships between TUG dual-task interference, cognitive performance, and cBF volumes*

Pearson's partial correlations (one-tailed) were conducted to examine within-group relationships between TUG dual-task interference and cBF volumes, and their associations with cognitive performance, controlling for age and sex.

5.2.5.4 *Mediation analysis*

Cognitive scores and cBF volumes demonstrating significant ($P < 0.05$ uncorrected) partial correlations with TUG dual-task interference were carried forward into mediation analysis. Separate linear regression models were constructed including (i) cognitive scores alone; and (ii) both cognitive scores and cBF volumes. Analyses were controlled for participant age and sex. Thus,

relationships between cognition and cBF volumes and TUG dual-task interference were established, and the influence of cognitive performance on TUG dual-task interference was assessed while controlling for variations in cBF structure.

The mediation effect was evaluated as a decrease in the value of the standardised regression coefficient (β) for the association between cognitive performance and TUG dual-task interference after inclusion of cBF volume in the model. Variance inflation factors (VIF > 3) were used to monitor multicollinearity (Pieruccini-Faria et al., 2019). To test the specificity of the investigated relationships, a similar analysis was performed using global grey matter (GM) volume as a mediator.

5.3 Results

MRI and cognitive data were not collected from all participants due to participant fatigue, technical issues, or time constraints. The number of missing datapoints prior to analyses is reported in General Methods (section 2.2.7). For clarity, *N* and df are also reported throughout the results section where appropriate. Besides the (outlier) removal of Ch4 volumetric data from one control participant (described above in section 5.2.5.1), there were no other exclusions.

Variance inflation factor for global grey matter was 4.359 (above the predefined > 3 threshold), demonstrating high multicollinearity with Ch4 volume, thus this variable was not controlled for in the following analyses.

5.3.1 *Demographic and clinical variables*

Demographic and clinical characteristics are shown in Table 5.1. PD and control groups were well matched with respect to age and sex. The mean disease duration in people with PD was 82.49 (± 77.41) months.

People with PD had greater global cognitive impairment than controls demonstrated by lower MoCA scores (Table 5.1). TMT switching scores were also lower in PD compared to controls, while semantic and letter fluency scores

were similar. These contrasts also survived additional correction for age and sex (MoCA: $P = 0.022$; TMT switching: $P = 0.001$).

Gait and balance were impaired in people with PD compared to controls, demonstrated by significantly lower Mini-BESTest scores, and longer completion time for both the TUG single-task and TUG dual-task (Table 5.1). TUG dual-task interference was also greater in people with PD compared to control participants. These contrasts also survived additional correction for age and sex (TUG single: $P = 0.001$; TUG dual: $P < 0.001$; TUG dual interference: $P = 0.05$).

Table 5.1 Demographic and clinical characteristics

	PD	**CONTROL**	**Group difference**
N	36	21	
Age	66 (7.5)	64.7 (7.8)	$t = 0.612$, $P = 0.543$
Sex [M:F]	25:11	11:10	$\chi^2 = 1.66$, $P = 0.20$
Disease duration [months]	82.49 (77.41)	–	–
MoCA [/30]	27.27 (2.58)	29.0 (1.03)	**$t(51) = -2.854$, $P = 0.012$***
(TMTB-TMTA)/TMTA	1.51 (1.05)	0.94 (0.71)	**$t(51) = 2.171$, $P = 0.035$***
Semantic fluency [total words]	23.85 (6.29)	27.19 (6.87)	$t(52) = -1.836$, $P = 0.096$
Letter fluency [total words]	53.79 (17.57)	51.90 (16.64)	$t(52) = 0.392$, $P = 0.697$
Mini-BESTest [/28]	20.0 (6.6)	26.5 (1.7)	**$t(54) = -4.400$, $P < 0.001$***
TUG-single [seconds]	9.66 (3.13)	6.97 (1.24)	**$t(52) = 3.739$, $P < 0.001$***
TUG-dual [seconds]	13.06 (5.22)	8.30 (1.79)	**$t(51) = 4.016$, $P < 0.001$***
TUG-dual interference	0.36 (0.32)	0.20 (0.20)	**$t(51) = 2.059$, $P = 0.045$***

*Mean (standard deviation). P-values for t-test contrasts are FDR-corrected for multiple comparisons. * indicates contrasts that survived additional correction for age and sex with ANCOVA at P < 0.05. Abbreviations: F = female; M = male; Mini-BESTest = Mini-Balance Evaluation System Test; MoCA = Montreal Cognitive Assessment; PD = Parkinson's disease; TMT = Trail Making Test; TUG = Timed Up and Go*

5.3.2 *Cholinergic basal forebrain volumes*

Ch4 and Ch4p volumes were significantly smaller in PD compared to controls (

Figure 5.2), while Ch1-2 volumes were not significantly different (statistics reported in Table 5.2). These contrasts also remained significant after correction for age and sex with ANCOVA. As expected, including the outlier datapoint that was removed for Ch4 in controls resulted in a larger effect size, and smaller *P* value ($F(1,47) = 11.259$, $P = 0.002$), but the overall result remained the same (data cleaning described above in section 5.2.5.1).

Table 5.2 Cholinergic basal forebrain volumes

	PD	CONTROL	*t*-test	ANCOVA[a]
Ch1-2 [mm^3]	-0.009 (0.06)	0.016 (0.04)	$t(49) = -1.648$, $P = 0.106$	–
Ch4 [mm^3]	-0.016 (0.04)	0.019 (0.03)	**$t(48) = -3.012$, $P = 0.012$**	**$F(1,46) = 8.132$, $P = 0.012$**
Ch4p [mm^3]	-0.015 (0.06)	0.026 (0.05)	**$t(49) = -2.517$, $P = 0.022$**	**$F(1,46) = 1.771$, $P = 0.014$**

Mean (standard deviation). P-values for contrasts are FDR corrected for multiple comparisons. [a] Age and sex added as covariates. Abbreviations: Ch1-2, Ch4, Ch4p = cell clusters corresponding to the medial septum, nbM, and posterior nbM region, respectively.

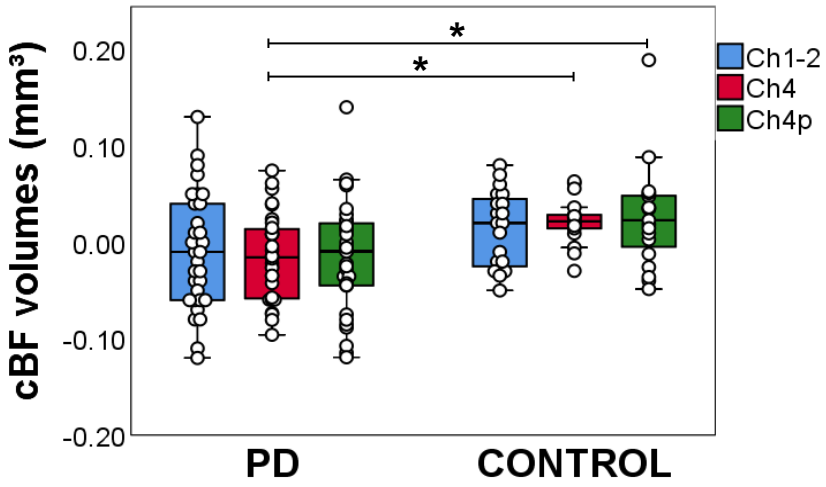


Figure 5.2 Boxplot of cholinergic basal forebrain (cBF) volumes in people with Parkinson's disease (PD) and control participants

** indicates significant group difference at* P < *0.05. Abbreviations: Ch1-2, Ch4, Ch4p = cell clusters corresponding to the medial septum, nbM, and posterior nbM region, respectively.*

5.3.3 *Relationships between cognitive scores and TUG dual-task interference*

In people with PD, partial correlations controlling for age and sex revealed a significant negative association between letter fluency test scores and TUG dual-task interference (Table 5.3, Figure 5.3 – A). Correlations between other cognitive scores and TUG dual-task interference were non-significant.

In controls, significant negative correlations were revealed between TUG dual-task interference and MoCA and semantic fluency performance (Table 5.3, Figure 5.3 – B). Correlations with letter fluency and TMT switching were non-significant.

5.3.4 *Relationships between cBF volumes and TUG dual-task interference*

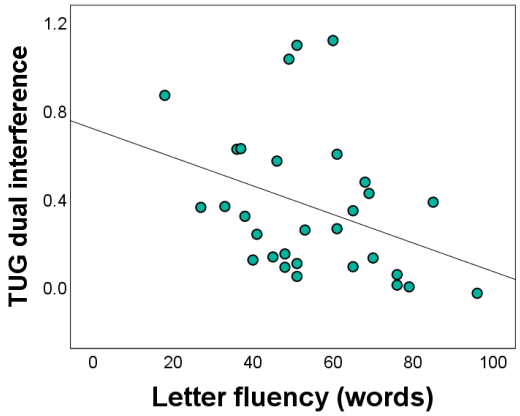
For cBF correlations with TUG dual-task interference, only Ch4 volume showed a significant negative relationship in people with PD (Table 5.3, Figure 5.3 – C). Associations with Ch1-2 and Ch4p volumes were non-significant. In controls, no significant correlations were revealed between TUG dual-task interference and cBF volumes. Including the outlier datapoint that was removed for Ch4 in controls did not alter these results (r(15) = 0.071, *P* = 0.393) (data cleaning described above in section 5.2.5.1).

Table 5.3 Partial correlations between cognitive scores and cholinergic basal forebrain volumes, and Timed Up and Go dual-task interference

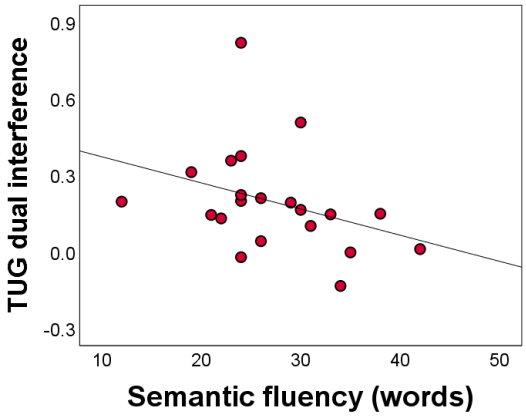
	TUG dual-task interference	
	PD	Control
MOCA	r(26) = -0.077, *P* = 0.348	**r(16) = -0.427, *P* = 0.038**
Semantic fluency	r(26) = -0.114, *P* = 0.281	**r(17) = -0.620, *P* = 0.002**
Letter fluency	**r(24) = -0.319, *P* = 0.048**	r(17) = -0.296, *P* = 0.109
TMTB-TMTA/TMTA	r(25) = 0.162, *P* = 0.210	r(17) = 0.226, *P* = 0.176
Ch1-2	r(25) = -0.135, *P* = 0.251	r(15) = -0.371, *P* = 0.071
Ch4	**r(25) = -0.466, *P* = 0.007**	r(14) = 0.040, *P* = 0.441
Ch4p	r(25) = -0.254, *P* = 0.100	r(15) = 0.025, *P* = 0.462

Pearson's partial correlation coefficient r(df). Controlling for age and sex covariates.
Abbreviations: Ch1-2, Ch4, Ch4p = cell clusters corresponding to the medial septum, nbM, and posterior nbM region, respectively; MoCA = Montreal Cognitive Assessment; TMT = Trail Making Test; TUG = Timed Up and Go.

(A) Parkinson's disease:



(B) Controls:



(C) Parkinson's disease:

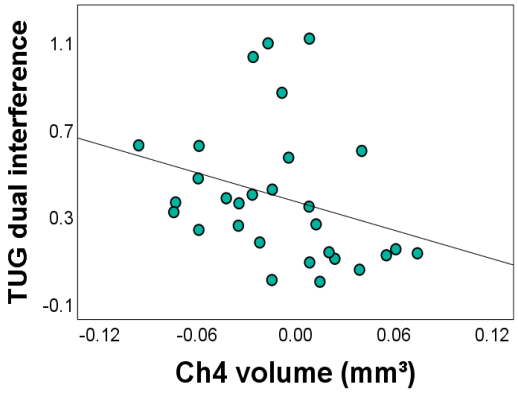


Figure 5.3 Scatterplots of Timed Up and Go (TUG) dual-task interference and verbal fluency performance and Ch4 volumes in people with Parkinson's disease (A, C), and control participants (B)

5.3.5 *Mediation analysis*

The following mediation analysis is restricted to people with PD, as this was the only group to demonstrate associations between TUG dual-task interference and *both* executive function (letter fluency) and cBF volumes (Ch4). Letter fluency was significantly associated with TUG dual-task interference in the one-tailed partial correlations described above controlling for age and sex (reaching trend level in regression; Table 5.4, Model 1). Inclusion of Ch4 volume in the model led to an attenuation of this association as demonstrated by a reduction of the standardised β coefficient for letter fluency, and only Ch4 volume

remained as a significant predictor in this combined model (Table 5.4, Model 2, Figure 5.4). A mediation effect was not demonstrated for global GM volume (Table 5.4, Model 3, Figure 5.4).

Table 5.4 Regression models for TUG dual-task interference in people with Parkinson's disease

	Standardised β coefficient (*P* value)
Model 1	
Letter fluency	-0.317 (0.097)
Model 2	
Letter fluency	-0.037 (0.856)
Ch4	**-0.534 (0.029)**
Model 3	
Letter fluency	-0.217 (0.329)
Global GM volume	-0.139 (0.669)

Age and sex added as covariates to regression models. Abbreviations: Ch4 = cell clusters corresponding to the nbM; GM = grey matter.

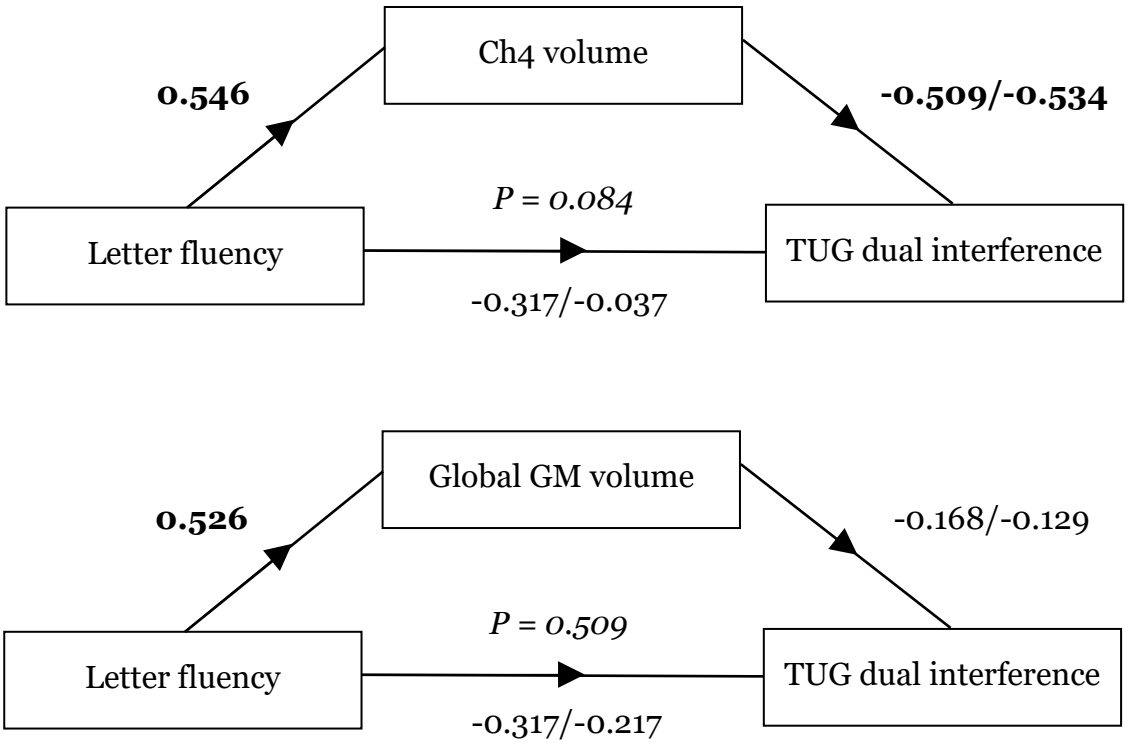


Figure 5.4 Mediation models for the effect of Ch4 and global grey matter volume on the influence of executive function on Timed Up and Go dual-task interference in people with Parkinson's disease.

Figure shows standardised regression coefficients for each path in the model (controlling for age and sex). Coefficients after the forward slash represent values adjusted for the mediation effect. Coefficients in bold correspond to significant associations (P < .05). Central P-value represents significance of combined model. Abbreviations: GM = grey matter; TUG = Timed Up and Go.

5.4 Discussion

Four key findings emerge from this study. First, Ch4 and Ch4p volumes were significantly lower in people with PD compared to healthy controls, after correction for age and sex. Second, executive function (in controls and PD) and Ch4 volume (only in PD) were associated with TUG dual-task interference. Fourth, the relationship between executive function (letter fluency) and TUG dual-task interference in people with PD was mediated by Ch4 atrophy. These observations suggest that the contribution of executive deficits to dual-task performance changes are mediated by structural degeneration of the Ch4 region.

5.4.1 *Ch4 atrophy in people with Parkinson's disease*

The finding of lower Ch4 volumes in people with PD is consistent with previous *in vivo* cross-sectional studies (Schumacher et al., 2020b; 2021). Volume loss in the posterior Ch4 region was also significantly greater in people with PD compared to controls in the current study. Previous studies assessing subregional cBF atrophy have also reported on the early vulnerability of the posterior Ch4 region in the early stages of PD and AD, in association with emerging cognitive and gait deficits (in PD) (Grothe et al., 2013; Kilimann et al., 2014; Ray et al., 2018; Wilson et al., 2020b). Taken together, these findings imply that more detailed analysis of cBF structure could provide an early marker for people with PD who may experience cognitive and mobility deficits. Finally, Ch1-2 volumes were not significantly different between people with PD and controls, which agrees with previous findings showing earlier vulnerability of the Ch4 region (Ray et al., 2018; Schulz et al., 2018), with Ch1-2 degeneration following later, as the disease progresses (Pereira et al., 2020).

5.4.2 *Associations between verbal fluency performance and TUG dual-task interference*

In both healthy older adults and people with PD, TUG dual-task interference – indicating the degree of performance decline prompted by performing a secondary (cognitive) task, was associated with verbal fluency performance. Verbal fluency is associated with executive control and relies on sustained

attention, working memory, inhibition, and cognitive flexibility (Schwartz et al., 2003; Robinson et al., 2012). Previous work in older adults has shown associations between TUG performance (without dual-task) and executive functions, including verbal fluency (Callil Voos et al., 2011; Herman et al., 2011; Gothe et al., 2014). The current study therefore extends these relationships to TUG dual-task performance in PD populations.

The TUG is a widely used clinical tool for the assessment of functional mobility (Podsiadlo and Richardson, 1991; Morris et al., 2001; Nocera et al., 2013; Lopes et al., 2020). Functional mobility is a term used to reflect balance and gait manoeuvres used in everyday life (e.g., sit-to-stand, walking, turning) (Podsiadlo and Richardson, 1991). Performing such balance and gait tasks with a concurrent cognitive task has been shown to exacerbate mobility impairments. For example, people with PD display greater gait asymmetry and variability, and reduced speed and coordination during dual-task walking, compared to normal walking (Yogev et al., 2005; Plotnik et al., 2009; 2011a; 2011b; Rochester et al., 2012). Dual-task interference on postural instability (increased postural sway) has also been reported in PD (Marchese et al., 2003). A recent study showed that mild cognitive impairment status and TUG dual-task performance were independently associated with dual-task changes to gait speed and step time variability in people with PD (Johansson et al., 2021). Thus, while not directly measured, it is possible that such balance and gait changes were implicated during TUG dual-task performance in the current study.

Executive function – including the ability to plan, initiate and inhibit behaviour, and control attentional resources – is central to the simultaneous performance of tasks (Yogev-Seligmann et al., 2008). Executive deficits have been shown to account for dual-task mobility changes described above. For example, changes in gait speed during dual-task walking have been associated with TMT performance in older adults (Coppin et al., 2006). Similarly, in PD, dual-task walking changes to gait have been associated with performance on various tests assessing executive function (Yogev et al., 2005; Rochester et al., 2008; Lord et al., 2010; Plotnik et al., 2011a). In an earlier study, executive dysfunction was shown to predict variance in walking speed in people with PD during functional dual-tasks, including sit-to-stand, walking, turning, and carrying a tray (i.e., similar to the components of the TUG), while performing a concurrent cognitive

(memory) task (Rochester et al., 2004). Together with results from the current study, these observations suggest that a decline in executive function may exacerbate the effects of dual-tasking on functional mobility.

5.4.3 *Ch4 mediates the relationship between letter fluency and TUG dual-task interference*

Mediation analysis suggested that the relationship between executive function and TUG dual-task interference was mediated by Ch4 structural degeneration in people with PD. No mediation effect was observed when global grey matter was substituted in the model for Ch4, implying that the mediation effect was specific to Ch4 volumetric changes.

Significant associations between executive function and TUG dual-task interference were observed in one-tailed partial correlations. However, these relationships were not sufficiently powered to detect a significant main effect when taken into mediation models, owing to the two-tailed nature of regression analyses. However, it was deemed appropriate to proceed to investigate indirect effects of Ch4 on this relationship, given the observations from correlational analyses and the proposed hypothesis of Ch4 mediating the link between executive function and dual-task TUG interference. Thus, the intention was to shed light on the interplay between these variables which have previously demonstrated independent associations in the literature.

Theoretical frameworks proposed to explain dual-task interference, including capacity theory and bottleneck theory (Ruthruff et al., 2001; Tombu and Jolicoæur, 2003), converge on the idea that inappropriate or ineffective allocation of attentional resources are key to dual-task deficits. Attentional control, as a core component of executive function, is thought to rely on prefrontal cholinergic function (Sarter et al., 2001). In animal studies, selective cholinergic lesions of the nbM have been shown to impair performance during sustained attention tasks (McGaughy et al., 1996), with larger deficits emerging when responding to multimodal cues (i.e., visual and/or auditory cues) compared to unimodal (Turchi and Sarter, 1997). Furthermore, lesions of cholinergic-prefrontal projections have been shown to impair inhibition and resistance to distraction (Dalley et al., 2004; Newman and McGaughy, 2008).

These observations may underpin the mediating effect of Ch4 (and its cortical projections) on the link between dual-task performance and executive control.

It is now well recognised that executive control and attention are important not only for cognition but also for mobility (Woollacott and Shumway-Cook, 2002; Yarnall et al., 2011; Morris et al., 2016). Reduced automaticity during mobility functions in PD is thought to reflect dysfunction within networks involving the prefrontal cortex, PPN, thalamus, and basal ganglia (Bohnen and Jahn, 2013). This places additional burden on (already impaired) executive-attentional control processes (Yogev-Seligmann et al., 2008; Wu et al., 2015), such that motor-cognitive deficits are compounded during dual-tasks – in which concurrent motor-cognitive demands compete for limited and impaired resources. Results from the current study provide further support to growing evidence for the role of cortical cholinergic system changes in the cognitive control of functional mobility in PD (Yarnall et al., 2011; Morris et al., 2019).

Few studies have assessed the relationship between cholinergic system changes and dual-task performance. Pharmacological intervention with acetylcholinesterase inhibitors (increasing circulating levels of acetylcholine), have shown beneficial effects on gait speed and variability under dual-task walking conditions in people with PD (performing concurrent verbal fluency task) compared to controls (Henderson et al., 2016). A previous study reported an association between greater dual-task gait change (speed) and reduced cholinergic function measured with SAI in older adult and PD fallers (Pelosin et al., 2016). More recently, greater TUG dual-task interference was associated with Ch4 atrophy in a PD cohort diagnosed for approximately 10 years (Dalrymple et al., 2021). The current result therefore extends these findings, demonstrating that this relationship is present in earlier disease stages of PD.

Acetylcholinesterase interventions have also shown improvements for balance deficits and falls in PD (Chung et al., 2010; Henderson et al., 2016) – which have been linked to attention-executive impairment (Woollacott and Shumway-Cook, 2002; Lord et al., 2010; Nocera et al., 2010), and to PPN-thalamic cholinergic degeneration (Bohnen et al., 2009; 2013). Greater dual-task interference has also been correlated with asymmetry of PPN structural connectivity and tests of inhibition (specifically, Go-NoGo target accuracy)

(Peterson et al., 2015). More recently, PPN microstructural degeneration was reported in people with PD with impaired behavioural flexibility during attentional reaction time tasks (Ray et al., under review). Thus, it is possible that the TUG dual-task interference effects reported in the current study may be also underpinned by motor and/or cognitive functions of the PPN in addition to Ch4.

5.4.4 *Limitations*

Some limitations to the current study should be acknowledged. Firstly, although functional mobility was assessed with a reliable clinical scale, it is possible that specific relationships between PD mobility symptoms and cBF volumes would be more readily detected with quantitative gait and balance measures (Wilkins et al., 2020; Wilson et al., 2020b). The same could also be said for the inclusion of a more comprehensive cognitive battery. Furthermore, only one type of dual-task was used in the current study. Using other secondary tasks, for example, incorporating both motor and cognitive elements (Peterson et al., 2015), may uncover additional/alternative dual-task effects.

Volumetric assessment of cBF volumes provide a measure of structural alterations, however it does not index cholinergic activity directly. These methods are also not suitable for the assessment of brainstem structures, and therefore cannot be used to investigate the contribution of the PPN volumetric changes. That said, recent work (including the results from Chapter 4) has shown utility in diffusion-weighted imaging measures for assessing microstructural changes in this region (Craig et al., 2020a; Ray et al., under review). Diffusivity measures may therefore provide insight into the potential contribution of PPN structural changes to PD cognitive and mobility impairments.

Time to completion was used as the outcome measure for TUG conditions. It is possible that a direct measure of counting performance may have provided additional insight with regards to resource allocation. It would also have allowed for a calculation of change between cognitive-only and dual-task conditions. Furthermore, the cross-sectional nature of the study provides only a

narrow window of insight into the evidently complex functional interplay of pathophysiological processes involved in PD symptoms.

While not the focus of the current study, it is possible that combined dopaminergic system changes also contributed to the link between executive function and dual-task interference. Molecular imaging studies have reported interactive effects of nigrostriatal dopaminergic and basal forebrain cortical cholinergic denervation on gait slowing in people with PD (Bohnen et al., 2013). Similar effects have also been reported for executive function and attention (Bohnen et al., 2015). Relatedly, participants in the current study were tested in the "on" medication state. Levodopa therapy has been shown to influence postural stability (Rocchi et al., 2002) and executive functions (Cools, 2006). Future multimodal imaging studies may therefore benefit from combined assessment of dopaminergic and cholinergic changes to uncover their mediating effects in the context of dual-task mobility in PD.

5.4.5 *Conclusion*

In conclusion, this study used the TUG dual-task to probe the interplay between cognitive and mobility functions, and cBF integrity. Results show that executive function was associated with greater TUG dual-task interference in people with PD, and that this relationship may be mediated by Ch4 structural integrity. These findings strengthen the evidence for the contribution of cortical cholinergic system changes in PD cognitive and mobility symptoms.

Chapter 6. **Free water imaging of the pedunculopontine nucleus in Parkinson's disease**

The PPN has been implicated in the pathophysiology of PD. Cholinergic and noncholinergic cell loss has been reported in this region (Hirsch et al., 1987; Jellinger, 1988; Rinne et al., 2008; Schmeichel et al., 2008). In primate models of PD, PPN lesions have been shown to induce postural and gait deficits (Karachi et al., 2010). Cholinergic PPN cell loss and decreases in thalamic cholinergic activity have also shown associations with fall history and impaired postural control in people with PD (Bohnen et al., 2009; Karachi et al., 2010; Müller et al., 2013a). More recently, microstructural changes in the PPN were able to predict the development of PIGD in people with early PD (Craig et al., 2020a).

In addition to its role in movement, accumulating evidence from clinical and experimental data suggests that the PPN also has a role in cognitive functions and adaptive behaviour (Stefani et al., 2013; Gut and Winn, 2016; Mena-Segovia and Bolam, 2017; Gut and Mena-Segovia, 2019). The PPN is a key interface between basal ganglia, thalamic, and brainstem circuitry and is thus well positioned to influence a range of functions (Winn, 2006; Gut and Winn, 2016). However, the role of PPN degeneration in PD symptomology remains unclear, and objective and accessible imaging markers that can be applied *in vivo* are lacking.

Diffusion tensor imaging can be used to assess structural changes in the PPN in people with PD. As noted above, these methods have been useful for discriminating people with PD at risk of developing PIGD (Craig et al., 2020a). Altered diffusivity metrics have also been reported in the PPN and connected subcortical structures, including basal ganglia, thalamus, and cerebellum, in people with PD with freezing of gait compared to non-freezers (Youn et al., 2015). The above observations suggest that PPN diffusion measures may be relevant for PD symptoms, however, few studies have investigated their relationship with cognition.

A potential caveat to conventional diffusivity metrics used in the studies above is that they are derived from single tensor models, that assume a single-tissue

compartment per voxel. Free water, which is present in cerebrospinal fluid and also accumulates in extracellular space due to neuroinflammation, can bias these diffusion indices (Kamagata et al., 2020). To address this issue, bi-tensor analyses have been developed that separate the diffusion properties of water in brain tissue from those in extracellular space (Pasternak et al., 2009). These so called 'free water imaging' methods can be used to remove the contribution of free water from diffusion metrics, whilst also providing a free water metric as a potential marker of neuroinflammation (Kamagata et al., 2020).

Elevated free water values have been reported in the substantia nigra of people with PD, which also correlated with motor symptom severity (Ofori et al., 2015a). Whether free water imaging methods can also be used to reveal changes in the PPN of people with PD remains to be determined. Recent work suggests that free water measures of the PPN may be useful for distinguishing PD from controls, and may also be relevant for performance on attentional tasks (Ray et al., under review). These free water imaging methods have not yet been assessed for their relationship with functional mobility in people with PD.

To this end, the aims of the current study were to (i) evaluate whether PPN DTI metrics (including traditional and free water-corrected diffusion metrics, and free water values) can distinguish people with PD from controls, and (ii) explore relationships between these measures and performance on tests of attention and executive function, and functional mobility.

6.1 Materials and methods

6.1.1 *Participants*

The participants for this study are described in more detail in the General Methods chapter (section 2.2.2). As previously described in Chapter 5, one person with PD and one control participant were excluded prior to analyses due to identification of (previously undisclosed) neurological conditions (stroke and encephalitis) identified on MRI. Thus, this study involved a total of 59 participants. Thirty-eight were diagnosed with PD – all of whom had received their diagnosis more than 1 year prior to the study. Twenty-one participants

were age-matched healthy controls. Following exclusions, neither group had any neurological conditions beside PD.

6.1.2 *Clinical assessments*

Global cognition was assessed with the MoCA (Nasreddine et al., 2005). Executive function was assessed with semantic fluency and letter fluency tests (Delis et al., 2001), and the Trail Making Test (TMT) (Reitan, 1958) – from which TMT task-switching performance was determined as the following ratio: (TMTB-TMTA)/TMTA (Periáñez et al., 2007), a higher value indicating lower task switching capacity.

A subset of 20 people with PD and 13 control participants also completed a battery of attention tasks including simple reaction time, choice reaction time, and digit vigilance. Following the methods outlined in (Allcock et al., 2009), four cognitive measures were derived from these tasks: power of attention (POA) sustained attention, cognitive reaction time (RT), and fluctuating attention – a description of these measures is provided in Table 6.1.

Dynamic balance and functional mobility was assessed with the Mini Balance Evaluation System Test (Mini-BESTest) (Franchignoni et al., 2010). The Timed Up and Go (TUG) test, which forms the final item of the Mini-BESTest, was also used in isolation as a measure of functional gait and balance. This included TUG-single task and TUG-dual-task. TUG dual-task interference was also calculated from these tests, defined as the change in performance relative to an individual's TUG single task, with the following formula: (TUG dual-task – TUG single task)/TUG single task (Rochester et al., 2014). A higher value indicates worsened performance.

Table 6.1 Attention measures derived from reaction time tasks

Task	Description	Measure
Power of attention (POA)	Sum of mean choice, simple, and digit vigilance reaction times. This reflects levels of effortful concentration.	Response time (ms)
Sustained attention	Number of digit vigilance errors subtracted from the sum of correct responses from choice and digit vigilance tasks. Higher value reflects greater ability to sustain attention.	Units
Cognitive reaction time (Cognitive RT)	Choice reaction time minus simple reaction time. A higher value reflects longer processing speed.	Response time (ms)
Fluctuating attention	Sum of coefficients of variance simple, choice, and digit vigilance reaction times. A higher value reflects increased fluctuations in attention.	Coefficient of variance (%)

Adapted from (Allcock et al., 2009).

6.1.3 *MRI acquisition and preprocessing*

Details of MRI acquisition and preprocessing have been outlined in General Methods (sections 2.2.6.1 and 2.2.6.2).

6.1.4 *Region of Interest: pedunculopontine nucleus*

The PPN region of interest was accessed via a stereotactic map that was developed to improve targeting of this region for DBS (Alho et al., 2017). The methods that were used to create this map have been outlined in General Methods (section 2.1.6.4).

6.1.5 *Extraction of PPN diffusivity metrics*

Full details of the pipeline used to extract PPN diffusivity metrics have been outlined in General Methods (2.2.6.5).

Mean free water and diffusivity values were calculated from the voxels within the PPN ROI. This resulted in a total of seven metrics extracted from the PPN including traditional DTI metrics: mean diffusivity (mD), axial diffusivity (aD),

radial diffusivity (rD) – and free water-corrected versions of these: FmD, FaD, FrD, as well as free water content (FW).

6.1.6 ***Statistical analyses***

Statistical analyses were conducted in IBM SPSS statistics 26. Kolmogorov-Smirnov tests and boxplot and histogram inspections were used to assess the distribution of continuous variables.

6.1.6.1 *Data cleaning*

The approach taken for data cleaning has been described in the General Methods chapter (section 2.3). This resulted in the removal of PPN free water content from one control participant in whom this measure was more than 3 × above the IQR (Figure 6.1). The effects of retaining this datapoint are also reported in the results section.

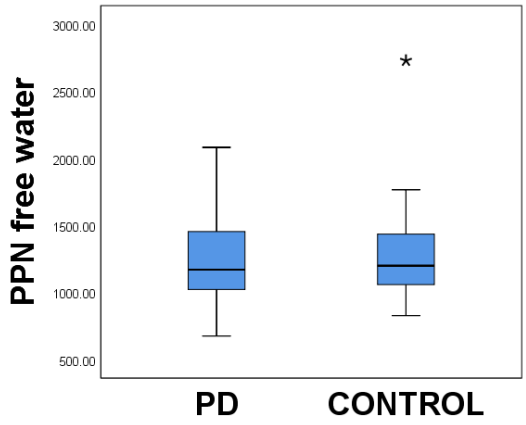


Figure 6.1 Boxplot showing outlier datapoint for PPN free water content in controls

6.1.6.2 *Group comparisons*

Student's t-tests were used to assess group differences in PPN diffusivity metrics and performance on tests of cognition and functional mobility, between controls and people with PD. T-tests significant at $P < 0.05$ were further evaluated for

significance after correction for age, sex, and global grey matter diffusivity (for PPN data) using ANCOVA. The above contrasts were FDR-corrected for multiple comparisons.

6.1.6.3 *Relationships between PPN diffusivity and cognitive and mobility performance*

Pearson's partial correlations (two-tailed) were first conducted across the whole sample to explore associations between PPN diffusivity measures and cognitive and mobility performance, controlling for group, age, sex, and global grey matter diffusivity.

Correlations significant at $P<0.05$ (uncorrected) across the whole sample were further evaluated within each group separately with partial correlations, controlling for age, sex, and global grey matter diffusivity.

6.2 Results

MRI and cognitive data were not collected from all participants due to participant fatigue, technical issues, or time constraints. The number of missing datapoints prior to analyses is reported in General Methods (section 2.2.7). For clarity, *N* and df are also reported throughout the results section where appropriate. Besides the (outlier) removal of PPN free water content data from one control participant (described above in, section 6.1.6.1), three additional people with PD were excluded during preprocessing of diffusion images (described in General Methods, sections 2.2.6.2). Specifically, two people with PD demonstrated more than 3 mm absolute mean displacement (movement), and the diffusion image for one additional person with PD did not accurately align with their T1 image during affine registration.

6.2.1 ***Group comparisons***

Comparisons between demographic, cognitive and mobility performance, and PPN diffusivity measures are reported in Table 6.2. Both groups were well

matched with respect to age and sex. Mean disease duration among people with PD was 6.97 (±6.39) years.

People with PD showed greater global and executive impairment, indicated by significantly lower MoCA and TMT switching scores compared to controls. These results also survived correction for age and sex covariates (MoCA: $F(1,52) = 5.529$, $P = 0.023$; TMT switching: $F(1,50) = 4.258$, $P = 0.044$).

As expected, people with PD also showed greater functional mobility deficits demonstrated by lower MINI-BESTest scores, longer TUG (single and dual) completion times, and increased TUG dual-task interference. These results also survived correction for age and sex covariates (MINI-BESTest: $F(1,54) = 17.186$, $P < 0.001$; TUG: $F(1,52) = 13.274$, $P = 0.001$; TUG dual-task: $F(1,51) = 15.134$, $P < 0.001$; TUG dual-task interference: $F(1,51) = 4.114$, $P = 0.048$).

None of the diffusivity metrics in the PPN were significantly different between people with PD and controls. This result remained the same when the PPN free water outlier datapoint in controls was included ($t(49) = -0.260$, $P = 0.796$) (data cleaning described above in section 6.1.6.1).

Table 6.2 Demographic, clinical, and PPN diffusivity measures

	PD	**CONTROL**	**Group difference**
N	38	21	
Age	65.6 (7.6)	64.7 (7.8)	t = 0.415, P = 0.680
Sex [M:F]	27:11	11:10	χ^2 = 2.06, P = 0.17
Disease duration [months]	83.65 (76.71)	–	–
MoCA [/30]	26.64 (3.80)	29.0 (1.03)	***t*(54) = -2.717, *P* = 0.009***
(TMTB-TMTA)/TMTA	1.54 (1.06)	0.94 (0.71)	***t*(52) = 2.309, *P* = 0.025***
Semantic fluency [words]	23.8 (7.8)	27.19 (6.87)	t(54) = -1.644, P = 0.106
Letter fluency [words]	53.74 (18.6)	51.90 (16.64)	t(54) = 0.374, P = 0.710
POA [ms]	2115.5 (524.65)	1890.77 (229.0)	t(31) = 1.457, P = 0.155
Fluctuating attention	0.47 (0.15)	0.45 (0.06)	t(31) = 0.301, P = 0.766
Cognitive RT [ms]	160.0 (168.59)	163.85 (56.95)	t(31) = -0.079, P = 0.938
Sustained attention	167.35 (18.86)	174.77 (3.81)	t(31) = -1.392, P = 0.174
Mini-BESTest [/28]	19.7 (7.1)	26.5 (1.7)	***t*(56) = -4.336, *P* = 0.001***
TUG single [seconds]	9.72 (3.14)	6.97 (1.24)	**t(54) = 3.820, *P* = 0.001***
TUG dual [seconds]	13.18 (5.28)	8.30 (1.79)	**t(53) = 4.090, *P* = 0.001***
TUG dual interference	0.36 (0.31)	0.20 (0.20)	**t(53) = 2.132, *P* = 0.038***
PPN aD	117.63 (5.88)	116.29 (5.90)	t(49) = 0.795, P = 0.699
PPN mD	68.68 (2.81)	68.39 (2.16)	t(49) = 0.389, P = 0.699
PPN rD	44.21 (2.17)	45.39 (4.00)	t(49) = -1.369, P = 0.413
PPN FaD	97.69 (3.73)	96.30 (2.89)	t(49) = 1.419, P = 0.413
PPN FmD	59.30 (0.74)	59.42 (0.43)	t(49) = -0.661, P = 0.699
PPN FrD	40.10 (2.14)	40.98 (1.43)	t(49) = -1.616, P = 0.413
PPN FW	121.55 (32.88)	119.55 (25.64)	t(48) = 0.509, P = 0.699

Mean (standard deviation). P-values for t-test contrasts are FDR-corrected for multiple comparisons.

** indicates contrasts that survived additional correction for age and sex with ANCOVA. Diffusivity metrics multiplied by 10000, FW multiplied by 100. Abbreviations: aD, mD, rD = axial, mean, radial diffusivity, respectively; FaD, mD, rD = free water-corrected axial, mean, radial diffusivity, respectively; FW = free water; Mini-BESTest = Mini-Balance Evaluation System Test; MoCA = Montreal Cognitive Assessment; PD = Parkinson's disease; POA = power of attention; PPN = pedunculopontine nucleus; RT = reaction time; TMT = Trail Making Test; TUG = Timed Up and Go.*

6.2.2 *Relationships between PPN diffusivity and cognitive and mobility performance across the whole sample*

Partial correlations across the whole sample controlling for group, age, sex, and global grey matter diffusivity showed that elevated free water-corrected rD and mD metrics in the PPN were associated with faster response times as measured by POA and cognitive RT, respectively. All other correlations between PPN diffusivity metrics and cognitive and mobility measures were non-significant (statistics reported in Table 6.3). Including the rejected PPN free water content outlier datapoint did not alter the overall results (data cleaning described above in section 6.1.6.1).

Table 6.3 Partial correlations between PPN diffusivity and cognitive and mobility measures across the whole sample

	aD	mD	rD	FaD	FmD	FrD	FW
MoCA	r(43) = .028, *P* = .853	r(42) = .122, *P* = .432	r(43) = .198, *P* = .193	r(43) = .104, *P* = .497	r(43) = -.084, *P* = .584	r(43) = -.138, *P* = .365	r(42) = .128, *P* = .409
TMT switiching	r(41) = -.003, *P* = .987	r(40) = -.021, *P* = .894	r(41) = -.096, *P* = .540	r(41) = .090, *P* = .568	r(41) = .066, *P* = .672	r(41) = -.051, *P* = .745	r(40) = -.084, *P* = .596
Semantic fluency	r(43) = -.090, *P* = .558	r(42) = .007, *P* = .962	r(43) = .101, *P* = .507	r(43) = -.273, *P* = .070	r(43) = -.123, *P* = .419	r(43) = .193, *P* = .203	r(42) = .053, *P* = .733
Letter fluency	r(43) = .267, *P* = .076	r(42) = .292, *P* = .064	r(43) = .160, *P* = .293	r(43) = -.053, *P* = .729	r(43) = .011, *P* = .941	r(43) = .055, *P* = .719	r(42) = .213, *P* = .166
POA	r(24) = .348, *P* = .081	r(24) = .228, *P* = .262	r(24) = -.009, *P* = .967	r(24) = .350, *P* = .079	r(24) = -.286, *P* = .157	**r(24) = -.417, *P* = .034**	r(23) = .246, *P* = .227
Cognitive RT	r(24) = -.023, *P* = .910	r(24) = -.145, *P* = .481	r(24) = -.174, *P* = .397	r(24) = -.019, *P* = .925	**r(24) = -.465, *P* = .017**	r(24) = -.182, *P* = .374	r(23) = -.194, *P* = .342
Sustained attention	r(24) = -.348, *P* = .068	r(24) = -.364, *P* = .069	r(24) = -.280, *P* = .166	r(24) = -.147, *P* = .475	r(24) = -.317, *P* = .114	r(24) = -.012, *P* = .955	r(23) = -.331, *P* = .098
Fluctuating attention	r(24) = -.093, *P* = .650	r(24) = -.229, *P* = .261	r(24) = -.223, *P* = .274	r(24) = .094, *P* = .649	r(24) = -.287, *P* = .155	r(24) = -.201, *P* = .325	r(23) = -.122, *P* = .552
Mini-BESTest	r(45) = .084, *P* = .574	r(44) = .231, *P* = .123	r(45) = .163, *P* = .274	r(45) = .070, *P* = .641	r(45) = -.107, *P* = .473	r(45) = -.116, *P* = .437	r(44) = .193, *P* = .199
TUG single	r(43) = -.045, *P* = .769	r(42) = -.142, *P* = .358	r(43) = .-011, *P* = .942	r(43) = -.067, *P* = .660	r(42) = .287, *P* = .056	r(43) = .201, *P* = .185	r(42) = -.099, *P* = .524
TUG dual	r(42) = -.088, *P* = .571	r(41) = -.158, *P* = .312	r(42) = -.059, *P* = .704	r(42) = .027, *P* = .861	r(42) = .284, *P* = .062	r(42) = .114, *P* = .462	r(41) = -.187, *P* = .230
TUG dual interference	r(42) = -.053, *P* = .733	r(41) = -.022, *P* = .888	r(42) = -.044, *P* = .777	r(42) = .116, *P* = .452	r(42) = .076, *P* = .622	r(42) = -.067, *P* = .664	r(41) = -.106, *P* = .500

Controlling for group, age, sex, and global grey matter diffusivity. Bold values indicate P < 0.05 (uncorrected). Abbreviations: aD, mD, rD = axial, mean, radial diffusivity, respectively; FaD, mD, rD = free water-corrected axial, mean, radial diffusivity, respectively; FW = free water; POA = power of attention; TMT = Trail Making Test; RT = reaction time; TUG = Timed Up and Go.

6.2.3 *Group-specific relationships between PPN diffusivity and cognitive and mobility performance*

Follow-up analyses using partial correlations within each group separately (controlling for age, sex, and global grey matter diffusivity) revealed that the relationships between PPN diffusivity and attentional tasks identified across the whole sample were driven by associations within people with PD specifically. In particular, elevated FmD was associated with less time allocated to cognitive choice (cognitive reaction times) and elevated FrD correlated with faster attentional reactions times (POA). The same relationships were not observed in controls (Table 6.4, Figure 6.2).

Table 6.4 Partial correlations between PPN diffusivity and attentional measures

	FmD		FrD	
	PD	Control	PD	Control
POA	–	–	r(11) = -.517, *P* = .060	r(8) = -.153, *P* = .673
Cognitive RT	**r(11) = -.658, *P* = .020**	r(8) = -.163, *P* = .652	–	–

Controlling for age, sex, and global grey matter diffusivity. Abbreviations: FmD, FrD = free water-corrected mean and radial diffusivity, respectively; POA = power of attention; RT = reaction time.

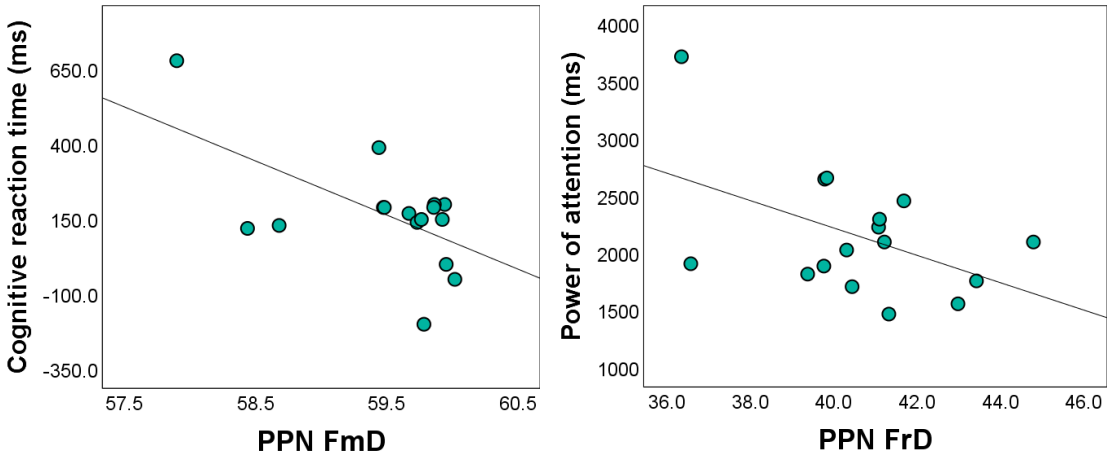


Figure 6.2 Scatterplots of free water-corrected diffusivity in the PPN and attentional reaction times in people with Parkinson's disease

Abbreviations: FmD, FrD = free wate- corrected mean and radial diffusivity, respectively.

6.3 Discussion

This study used traditional DTI metrics and free water imaging methods (to capture free water content, and to correct DTI metrics for free water), to assess changes in PPN microstructure and associations with cognitive and mobility performance in people with PD and healthy older controls.

No significant group differences were shown for any of the PPN diffusivity metrics assessed in the current study. Therefore, these methods were not able to distinguish people with PD from controls in this instance. In contrast to current observations, traditional DTI metrics (aD, mD, and rD) were previously shown to be elevated in people with PD compared to people with mild cognitive impairment (not related to PD) in Chapter 4. Furthermore, people with PD with freezing of gait have previously shown increased PPN mD compared to non-freezers (Youn et al., 2015). However, other work from our lab has also failed to detect group differences between PD and controls using these traditional

measures (Craig et al., 2020a). It is likely that PD heterogeneity in cholinergic system degeneration can at least partly explain these inconsistent findings.

More recently, free water-corrected metrics (namely, aD and mD), but not free water content, in the PPN were found to be significantly increased in people with PD compared to controls (Ray et al., under review). Earlier work however, using hand-drawn ROIs, showed that free water and free water-corrected fractional anisotropy in the PPN were not significantly different in PD compared to controls, whereas these measures were altered in other atypical parkinsonian disorders (including multiple system atrophy and progressive supranuclear palsy) (Planetta et al., 2016). Taken together, these observations suggest that free water-corrected DTI metrics may not be reliably useful by themselves for distinguishing people with PD from controls, and that any group differences may be study specific.

6.3.1 *Relationships between PPN diffusivity and cognitive performance in people with PD*

In people with PD, elevated free water-corrected mD and rD measures in the PPN were associated with faster response times as measured by POA and cognitive reaction time. Specifically, in those with greater PPN microstructural degeneration, reaction times were shorter, and less time was allocated to the processing of cognitive choice. While not significantly different to controls, performance in people with PD was less accurate and fluctuated more (as shown by smaller sustained attention and larger fluctuating attention measures). These findings suggest that faster responses came at a cost of less accurate and more variable performance in people with PD.

The above findings are consistent with recent work conducted in a larger cohort of people with PD (n=97) using free water imaging methods (Ray et al., under review). In that study, elevated free water-corrected PPN aD was associated with a reduction in cognitive reaction time, while elevated PPN free water content correlated with shorter reaction times as measured by POA. Furthermore, performance accuracy also became significantly worse over time in those demonstrating greater diffusivity.

These results can be considered in the context of animal literature, in which the PPN has been implicated in rapid decision making (Gut and Winn, 2016). For example, during a radial maze task, PPN-lesioned rats performed quicker than controls (i.e., in moving to different maze arms), but made significantly more errors (i.e., in deciding which arm to enter) (Keating and Winn, 2002). In line with results from the current study (i.e., faster, but more variable and erroneous responses), this could suggest that loss of PPN integrity is associated with a loss of motor inhibition necessary for behavioural flexibility, resulting in faster reaction times that do not allow for accurate selection of motor responses. That said, it must be noted that this study did not specifically measure behavioural flexibility. Rather, the attentional reaction time tasks showing relationships with PPN integrity allow us to draw parallels with the animal literature cited.

The stereotactic map used in the current study does not dissociate between different subpopulations of PPN neurons. However, for purposes of discussion, structural changes to cholinergic PPN neurons are considered for their potential relevance for the observed relationships with cognitive performance. Selective cholinergic PPN lesions have been shown reduce the number of correct responses and increase the variability in response times during a serial reaction time task in rats (Cyr et al., 2015). Furthermore, cholinergic PPN neurons have been shown to fire transiently during sensory stimulation, while non-cholinergic neuronal firing is prolonged and seemingly unrelated to sensory input (Petzold et al., 2015). Thus, it is possible that the attentional tasks used in the current study were reliant on PPN cholinergic neurons for processing of task related visual cues.

Molecular imaging studies have also provided evidence for the role of PPN-thalamic cholinergic losses in sensory processing in people with PD. For example, greater thalamic cholinergic losses were associated with greater vulnerability to distraction during a sustained attention task (using perceptual noise as a distractor) (Kim et al., 2017). Furthermore, thalamic cholinergic losses have also been correlated with impaired sensory processing during postural control in people with PD (Müller et al., 2013a). When taken with findings from the current study, this could suggest that difficulties in integrating sensory information (i.e., visual cue) into motor output (i.e., reaction response)

may be related to the integrity of PPN cholinergic neurons and their thalamic efferents.

Relatedly, animal studies have also demonstrated a key role of cholinergic PPN neurons in mediating motor inhibition. These neurons are intricately connected with the basal ganglia, and have descending projections to lower brainstem motor structures (Martinez-Gonzalez et al., 2011). Stimulation of cholinergic PPN neurons has been shown to reduce motor activity via descending projections, by increasing the extensor muscles without affecting flexors (Josset et al., 2018). Ascending PPN cholinergic projections have also been shown to interrupt striatal output by inhibiting its projection neurons (Gut and Mena-Segovia, 2019). It is possible that in the current study, microstructural changes to PPN cholinergic neurons may have impacted on the ability of people with PD to inhibit movement in order to produce controlled responses.

Contrary to previous work (Karachi et al., 2010; Craig et al., 2020a), no associations between PPN integrity and mobility performance were detected in people with PD. In the study by Craig et al (2020a), increased PPN aD was associated with the development of postural instability and gait difficulties over a 72-month follow-up period, thus suggesting that longitudinal clinical changes more informative than cross-sectional analyses. It is also possible that the mobility measures used in the current study were not sensitive to PPN structural changes.

Analyses conducted in the previous chapter showed associations between Ch4 volumetric changes and dual-task interference and executive function in people with PD. Similar associations have been reported for PPN structural connectivity in people with PD with freezing of gait (Peterson et al., 2015). Given the well-recognised link between mobility impairments and attention-executive function in PD (Yarnall et al., 2011; Morris et al., 2016), probing cognitive-motor functions and assessing the relative contribution of Ch4 and PPN changes to these symptoms in PD represents an important focus of future work.

In the current study, relationships with attentional tasks were observed for free water-corrected DTI metrics only, while traditional DTI metrics were non-

significant. While these results will need to be corroborated in larger cohorts, it could suggest that free water-corrected metrics provide a more sensitive measure. Relatedly, free water-corrected metrics have also shown greater sensitivity than traditional metrics for detecting grey matter abnormalities in the wider brain in people with PD compared to controls (Andica et al., 2019). Similarly, elevated free water-corrected DTI metrics have been reported in the substantia nigra, while traditional uncorrected metrics remained unchanged (Ofori et al., 2015a).

6.3.2 *Strengths*

A key strength of the current study lies in the application of non-invasive imaging methods to assess structural changes in the PPN region *in vivo*. Few studies have accessed this region with diffusion MRI methods. Another strength relates to the inclusion of both cognitive and mobility measures. It will be important to incorporate these measures in future investigations, in light of the new conceptual framework proposing the PPN as a critical mechanism for assessing incoming sensory data and making swift responses (Gut and Winn, 2016).

6.3.3 *Limitations*

Results from partial correlations conducted in the current study would not survive correction for multiple comparisons. The observed relationships could therefore indicate false positive results. In addition, the sample size included is small, particularly for attentional tasks, given that only a subset of participants completed these. Together, these factors increase the statistical uncertainty of findings herein. Thus, the reported associations between PPN microstructure and cognitive measures require future corroboration in larger cohorts.

6.3.4 *Conclusion*

This study demonstrates a tentative relationship between elevated free water-corrected PPN diffusivity metrics (mD and rD) and faster reaction times, including taking less time to consider choices between actions, in people with PD. PPN diffusivity measure were not significantly different between people

with PD and controls. Further work is required to determine to utility of these measures for detecting structural alterations in the PPN, and assessing relationships with cognitive and mobility symptoms in people with PD.

Chapter 7. General discussion

7.1 Thesis objective

The main objective of this thesis was to combine clinical and multimodal neuroimaging data to study the relationship between cholinergic system integrity and cognitive and mobility symptoms in people with PD.

Cognitive and mobility impairments are a leading concern for people living with PD (Deane et al., 2015). These symptoms often coexist and are intricately linked, such that cognitive deficits (namely attention-executive function) can implicate aspects of mobility (Yarnall et al., 2011; Morris et al., 2016) and postural and gait difficulties are associated with increased risk for cognitive decline (Burn et al., 2006; Morris et al., 2017). Neuropathological and recent *in vivo* imaging research has implicated cholinergic system degeneration in PD cognitive and mobility symptoms (Bohnen and Albin, 2011; Yarnall et al., 2011; Bohnen et al., 2018a; Morris et al., 2019). However, current understanding of the physiological role of the cBF and PPN (as major cholinergic projection neurons) in the human brain and specific contributions to PD symptomology is limited.

There is a need to identify accessible markers of cholinergic system degeneration to better understand its contribution to cognitive and mobility symptoms across the course of PD. The methods used in the current thesis address this research problem by combining MRI and recently developed stereotactic maps of the cBF and PPN, providing detailed morphometric and diffusivity assessments as *in vivo* surrogate markers of structural integrity. These techniques were also combined with EEG measures in a novel approach to explore relationships between structural alterations and cortical activity.

In addressing the thesis objective, measures of cBF volume and PPN diffusivity were used to index the structural health of these nuclei in people with PD without dementia and healthy older controls (Chapter 5 and 6), and people with mild cognitive impairment likely related to AD (Chapter 3 and 4). These

structural measures were subsequently assessed for their relationship with resting-state qEEG (Chapter 3 and 4) and cognitive and functional mobility performance (Chapter 5 and 6).

7.2 Summary of findings

7.2.1 *Structural changes to cBF and PPN were observed in people with PD*

Measures of cBF volume distinguished between groups in one of the two studies in which these methods were applied. In Chapter 3 (Slovenian cohort), cBF volumes did not differ significantly between people with PD, MCI, or healthy controls. Meanwhile, in Chapter 5 (UK cohort), Ch4 volumes were significantly reduced in people with PD (indicating greater atrophy) compared to controls. The mean duration of PD was similar across both studies but deviated more in Chapter 5. Given that group-level differences in cBF volumes are more readily detected in later disease stages (Pereira et al., 2020), the inclusion of people with more advanced PD in Chapter 5 could account for these differences.

In line with observations from Chapter 3, several recent studies have failed to distinguish people with PD and MCI from controls with cBF volumetric measures (Ray et al., 2018; Schulz et al., 2018; Pereira et al., 2020; Wilson et al., 2020b; Grothe et al., 2021; Schumacher et al., 2021), while others *have* revealed Ch4 degeneration in LB-MCI and AD-MCI cohorts compared to controls (Grothe et al., 2010; Kilimann et al., 2014; Schumacher et al., 2021). Taken together, these findings suggest that structural changes in the cBF region appear to be variable in earlier predementia disease stages and motivate further work to better understand the extent of degeneration across the disease spectrum.

Like cBF volumetry, measures of PPN diffusivity were able to distinguish between groups in one of the two studies in which these techniques were used. In Chapter 4 (Slovenian cohort), traditional DTI metrics were significantly elevated in people with PD compared to people with MCI, thus indicating greater microstructural degeneration. This finding broadly confirms post-

mortem reports of PPN degeneration in PD and preserved/no loss in AD (Hirsch et al., 1987; Jellinger, 1988; Woolf et al., 1989; Rinne et al., 2008; Schmeichel et al., 2008). Importantly, these observations also imply that combined stereotactic mapping and DTI can be used to distinguish people with PD in earlier predementia disease stages. This raises the potential that measures of PPN microstructure could be used to improve prognostic information, given that diffusivity measures in this region have been implicated in longitudinal changes in mobility and cognitive performance in people with early PD (Craig et al., 2020; Ray et al., under review). The methods reported in the current study will therefore enable further investigation (including cross-sectional studies) of the role of the PPN in PD symptoms.

In Chapter 6 (UK cohort), neither traditional DTI metrics *or* free water imaging metrics (including free water-corrected DTI metrics and free water content) in the PPN showed significant differences in people with PD compared to controls. As per the above cBF discussion, the lack of group-level differences in PPN microstructure in this study may be a result of variable degeneration in predementia disease stages. In line with the results presented here, findings have been mixed among the few studies in which PPN diffusivity has been assessed cross-sectionally. For example, the aforementioned study by Craig et al (2020a), did not show any cross-sectional differences in traditional PPN metrics in people with PD compared to controls. A later study of parkinsonian disorders reported elevated free water-corrected FA and free water content in the PPN in multiple system atrophy, and progressive supranuclear palsy, but not in PD (Planetta et al., 2016). Meanwhile, in the recent study by Ray et al (under review), free water-corrected DTI metrics (aD and mD) in the PPN (but not free water content) were shown to distinguish people with PD from controls in early disease stages. Further work is therefore needed to help determine the most sensitive diffusivity measures in the PPN in people with PD.

7.2.2 *People with PD displayed qEEG alterations compared to healthy controls*

In comparison to controls, people with PD displayed qEEG alterations in both eyes-closed (Chapter 3) and eyes-open (Chapter 4) resting-state conditions. This was marked by an increase in power in slower frequencies, and impairments to

alpha reactivity (i.e., the suppression of alpha power from eyes-closed to eyes-open, across occipital electrodes). This shift to slower frequencies is frequently reported in PD and AD literature, and has shown a linear relationship with cognitive decline (Vecchio et al., 2013; Babiloni et al., 2018; Geraedts et al., 2018). Previous work also suggests that qEEG slowing is more pronounced in LB disorders compared to AD (Schumacher et al., 2020a). The current findings provide support to this claim, as people with MCI demonstrated only a trend-level increase in slow wave activity compared to controls. The mechanisms underpinning qEEG slowing remain unclear, however, results from Chapter 3 and 4 imply that cholinergic degeneration may play a role (discussed next).

7.2.3 *Changes to cBF volume and PPN diffusivity were associated with qEEG alterations in people with PD and people with MCI*

Findings from Chapter 3 and 4 suggest that qEEG alterations may be relevant for cBF and PPN structural changes in people with PD and MCI. Specifically, Ch1-2 and Ch4p volume loss was associated with alpha reactivity impairments in people with PD, and lower (eyes-closed) pre-alpha power in people with MCI (Chapter 3). Meanwhile, elevated PPN diffusivity was associated with higher (eyes-open) alpha power in people with PD, and lower beta and gamma power in people with MCI (Chapter 4). These results will be elaborated on below.

People with PD with Ch1-2 and Ch4p atrophy displayed impairments in desynchronising the cortex upon opening their eyes (demonstrated by alpha reactivity deficits). Studies in animals have implicated cBF projections in rapidly modulating the cortex and in visual processing (Pinto et al., 2013). Work in older adults has linked alpha reactivity with increased functional connectivity between the nbM and primary visual cortex (Wan et al., 2019). In that study, increased burden of white matter hyperintensities along nbM-visual cortex tracts was associated with alpha reactivity deficits. While the current study was not designed to measure cholinergic activity directly, the results could imply that alpha reactivity, as an index of cortical activation across occipital regions, may provide a functional measure of cholinergic changes arising from structural degeneration in anterior and posterior cBF regions in people with PD.

In assessing subregional cBF volumetric changes, previous work has implicated the Ch4p region in predicting PD-MCI (Ray et al., 2018), with degeneration extending to anterior Ch1-2 regions as the disease progresses and PDD ensues (Pereira et al., 2020). Earlier work also reported reduced hippocampal and neocortical ChAT activity in absence of frank neuronal loss in Ch1-2 and Ch4, respectively (i.e., cells that provide cholinergic innervation to these regions) (Hall et al., 2014). While the specificity of alpha reactivity impairments to changes in these cBF subregions will need to be corroborated in future work, it is worth speculating that this qEEG metric could be sensitive to cholinergic changes that may herald PDD, even before significant structural changes can be detected at the group level.

Ch1-2 and Ch4p volume loss was associated with lower pre-alpha power in people with MCI. Previous work has shown associations between greater white matter hyperintensities in cholinergic pathways and alpha power reductions in MCI (Babiloni et al., 2009). The alpha peak has been shown to shift to slower frequencies in ageing and neurodegeneration (Klimesch, 1999), thus the cBF-pre-alpha relationship reported here may be indexing pathological alpha frequency alterations (in an extended alpha range). Alternatively, it could reflect a physiological cBF-theta relationship, given that Ch1-2 regulates hippocampal theta (Buzsaki, 2002), and lesions of the nbM increase slow wave EEG activity (Buzsaki et al., 1988; Riekkinen et al., 1990). Regardless, these findings warrant further investigation as theta-alpha qEEG changes appear to be relevant for cBF structural changes in MCI.

Findings from Chapter 4 showed that greater PPN microstructural degeneration was associated with higher eyes-open alpha power in people with PD (without MCI). Higher eyes-open alpha activity is thought to reflect a state in which attention is directed internally (Cooper et al., 2003). Moreover, when externally driven sensory processing is disrupted, cortical alpha power increases (Benedek et al., 2014). This 'bottom-up' control of attention is thought to rely on PPN-thalamocortical cholinergic pathways (Kim et al., 2018). Thus, in the current study, microstructural changes in the PPN, and consequential disruptions to thalamo-cortical projections, may be indexed by cortical alpha changes in people with PD.

In people with MCI, increased PPN diffusivity correlated with lower beta and gamma power. Coherent activity between the cortex and PPN has been reported in beta and gamma ranges, indicating a possible channel of communication through which the PPN and cortex interact (Mena-segovia et al., 2008; Tsang et al., 2010; Fraix et al., 2013; Valencia et al., 2014). As a key component of the reticular activating system, the PPN is capable of influencing cortical states associated with EEG desynchronisation (i.e., transition from slow to fast oscillations in the beta/gamma range) (Moruzzi and Magoun, 1949; Steriade et al., 1990; 1991). Furthermore, PPN stimulation within the beta range has been shown to promote alertness (Arnulf et al., 2010). Thus, in the current study, PPN structural changes may be influencing cortical processes associated with alertness.

7.2.4 *Ch4 integrity influenced the relationship between executive function and mobility in people with PD*

In Chapter 6, the TUG dual-task was used to probe the interplay between cognitive and mobility functions, and cBF integrity. Results revealed an association between executive function (namely verbal fluency performance) and TUG dual-task interference in people with PD, which was mediated by Ch4 structural integrity. That is, during dual-tasking, people with PD relied upon greater cognitive control of mobility functions – thought to reflect reduced automaticity (Wu et al., 2015), and this relationship was influenced by the extent of Ch4 atrophy.

Animal models of PD have provided direct evidence for the role of cBF in the cognitive control of mobility. Specifically, combined cortical cholinergic and striatal dopaminergic losses (but not dopaminergic losses alone) were shown to impair gait and postural control and increase falls during tasks designed to tax the attentional control of movement (Kucinski et al., 2013). cBF losses are thought to indirectly deprive the cortico-striatal processing of movement related cues (Sarter et al., 2014). In the current study the attentional system was taxed during TUG dual-tasking. In the context of Ch4 atrophy in people with PD, this may have further impaired attentional-motor integration, resulting in a failure to appropriately update the motor system with cues to select and update

movement output, with consequences for mobility performance, reflected in longer time to completion.

This study is one of few to assess the relationship between cholinergic system changes and dual-task performance in people with PD. It is also the first to demonstrate the mediating effects of Ch4 volumetric changes on the executive function-mobility relationship. Results presented here therefore provide further support to growing evidence for the role of cortical cholinergic system changes in the cognitive control of functional mobility in people with PD (Yarnall et al., 2011; Morris et al., 2019).

7.2.5 *PPN diffusivity may be linked to behavioural flexibility*

Results from Chapter 6 indicated a potential link between PPN microstructural changes and faster attentional responses. In particular, elevated PPN diffusivity correlated with shorter reaction times (measured by POA) and less time allocated to processing cognitive choice (measured by cognitive reaction time). These relationships were specific to people with PD, and were limited to free water-corrected DTI metrics, insofar as traditional DTI metrics and free water content did not show any significant associations with attentional performance.

In the context of lack of group level differences in PPN diffusivity, these results may imply that the application of free water-correction improves the sensitivity of DTI metrics for detecting changes in cognitive performance related to changes in PPN structure. Interestingly, the aforementioned study by Ray et al (under review) reported similar associations between free water-corrected PPN diffusivity and faster reaction times in people with PD. The larger PD cohort and increased statistical power in that study aided in the interpretation of the current results (discussed next).

While not significantly different to controls, people with PD demonstrated more variable attentional performance overall. Mean values showed that the responses of people with PD were less accurate and fluctuated more (as shown by smaller sustained attention and larger fluctuating attention measures). While

tentative, this could suggest that faster responses came at a cost of less accurate and more variable performance in people with PD.

The PPN is well positioned to influence motor output via its widespread connections with basal ganglia, lower brainstem structures, and motor cortex (Jenkinson et al., 2009; Martinez-Gonzalez et al., 2011; Gut and Winn, 2016), and has also been shown to modulate cortical activity via the thalamus (Steriade et al., 1990; Mena-Segovia and Bolam, 2011), and to respond to sensory input (Petzold et al., 2015). PPN lesions in rats have been shown to impair behavioural adaptation and sensorimotor integration (MacLaren et al., 2013; Maclaren et al., 2014). This translated as a failure to update motor output in response to changing task demands. Thus, the current data could imply that people with PD with PPN degeneration show impairments in behavioural adaptation. More specifically, the control of the PPN over motor output structures may be weakened as a result of degeneration, thereby limiting the ability to update and integrate motor responses in the context of attentional cues.

As previously discussed, PPN microstructure has previously been implicated in the risk of developing PIGD in people with PD (Craig et al., 2020a). However, in the current study (Chapter 6), performance on measures of dynamic gait and balance (Mini-BESTest), and functional mobility (TUG), did not show any significant associations with PPN diffusivity in people with PD. Previous work using quantitative gait measures has shown associations between reduced PPN structural connectivity and gait impairments arising from freezing of gait (Fling et al., 2013) and dual-task interference (Peterson et al., 2015). It is possible that the clinical scales used in the current study were not sufficiently sensitive to detect associations between PD mobility impairments and PPN microstructural changes. Future use of more fine-grained, quantitative measures of posture and gait are therefore needed to enable more detailed investigations of the role of PPN microstructural changes for these functions in people with PD.

7.3 Strengths and implications

This thesis used clinical and imaging data from two independent predementia cohorts who were well matched with healthy control participants. This allowed for disease-related changes in clinical and imaging measures to be characterised against/alongside normal healthy ageing processes. Overall, the reported outcomes provide further understanding of cholinergic system degeneration in PD and MCI.

The fact that the structural imaging methods used in the current study were able to distinguish people with PD without dementia from controls and people with MCI, using cross-sectional data in small cohorts is of merit. Combining these methods with additional clinical and neuroimaging markers could therefore improve the accuracy of prognostic information. Future work could apply these techniques to investigate the spatial and temporal relationships between cBF/PPN degeneration and wider brain pathology to better understand the contribution to cognitive and mobility symptoms across the course of PD.

The multimodal approach applied in Chapter 3 and 4 data made it possible to identify a possible link between the structural health of cholinergic nuclei and changes to cortical activity. The evidence presented here suggests that early qEEG changes in PD and MCI may be driven by changes occurring in cholinergic nuclei. Thus, resting-state qEEG may provide a potential target for early disease monitoring in clinical settings. Recording resting oscillatory activity offers a standardised procedure that can be easily implemented and repeated. These paradigms are conducted in the absence of a specific task and do not depend on cognitive/motor functions, making them particularly suitable for people with PD and MCI – in whom these functions may be compromised. Future work should therefore be directed towards assessing the validity of EEG as a marker of cholinergic system changes in neurodegenerative conditions.

These results not only provide a better understanding of PD and MCI related changes at both cortical and subcortical levels, but may also have wider implications for other diseases that exhibit cholinergic dysfunction including PDD/DLB, progressive supranuclear palsy, and multiple systems atrophy

(Bohnen and Albin, 2011). This is one of only a handful of studies to combine *in vivo* MRI-based structural measures of cBF structure with qEEG, and is the first to do so for the PPN. If replicated in future studies, this information could be used to stratify people who may benefit from cholinergic medications or to assess the response to these medications.

Chapter 5 highlighted the utility of the TUG dual-task for probing the influence of cortical cholinergic system degeneration on the cortical control of mobility. This task can be administered quickly and easily with minimal equipment. It also includes balance and gait manoeuvres that typically occur in everyday life and therefore holds ecological merit. Future work could combine the TUG with quantitative measures of posture and gait to examine these relationships with greater fidelity (discussed below).

7.4 Limitations and future work

There are several limitations of the work presented in this thesis. Many of the limitations specific to each study have been outlined in the relevant chapters, therefore the current discussion will provide an overview of general methodological issues, with consideration of directions for future work.

The sample size in each study was small, such that the results should be viewed as exploratory rather than as confirmatory evidence, particularly given the large number of comparisons that were performed. Relatedly, data was missing across all studies, particularly for cognitive tests, resulting in further reductions in statistical power and degrees of freedom in these analyses. An important goal of future work should involve the replication of these results in larger cohorts, to increase the power and statistical certainty of reported outcomes.

EEG analyses were largely limited to global (across all electrodes) power, providing a relatively rudimentary measure of cortical dynamics. While this approach is widely used in the literature, it neglects spatial information that may be of relevance given the proposed topographical organisation of cBF projections. More refined techniques, such as source localisation or more

complex connectivity parameters, could offer greater insight into the topography of frequency changes across the scalp in relation to cholinergic system changes.

Relatedly, resting-state protocols were used for the acquisition of EEG data. Like global power analyses, these techniques benefit from being easy to implement and replicate, making them attractive candidates for application in clinics. However, the study of resting-state data presents some challenges. For example, in contrast to the controlled nature of task-based experiments, resting-state designs are unconstrained, such that the emergent data may be more variable across participants, thus making analysis and interpretation more elusive. Future work could benefit from the combined use of resting-state and task-based data to make more detailed inferences regarding the relationship between cortical dynamics, behavioural state, and alterations in cBF and PPN structure.

It is important to acknowledge that measures of motor function were absent from the Slovenian dataset. As a consequence, associations between motor symptoms and brain measures could not be investigated in the studies involving this cohort (Chapter 3 and 4). Future work that incorporates measures of motor function would also be useful in determining whether the reported relationships between cBF/PPN structure and resting-state EEG exist independently from measures of motor disease severity.

The MiniBESTest and TUG (used in Chapter 5 and 6) provide a gross indication of functional mobility and therefore do not offer the same degree of granularity as quantitative measures, such as those derived from 3D motion tracking, wearable sensors, and pressure-sensitive walkways and platforms. Given that specific posture and gait domains may be more dependent on cortical control than others (Morris et al., 2019), future work may benefit from incorporating quantitative measures into the TUG assessment and/or investigating the effects of different dual-tasks (i.e., cognitive/motor), to detect subtle deviations in posture and gait. These methods could be combined with measures of cBF and PPN to reveal more specific relationships that would otherwise go undetected with the clinical scales used here.

Cognitive tests were limited to attention-executive domains in the UK cohort. Furthermore, the cognitive battery used in the Slovenian cohort was not large enough to reach the more detailed level 2 MDS criteria for PD-MCI, and there were also several missing datapoints within the tests that were administered. Thus, more complete data and additional tests would serve to improve the accuracy with which people with PD can be categorised as having PD-MCI. Future work including more comprehensive cognitive assessments may help to capture more domain specific relationships with mobility and cholinergic system changes.

While the cBF stereotactic map used in current analyses corresponds to cholinergic neurons in this region, there are also other neurochemical cell populations located within the basal forebrain that may have contributed to the reported outcomes. Similarly, the PPN map does not dissociate between different cell populations, therefore the current observations cannot be assigned to changes to cholinergic cells specifically. Moreover, the relationships between cBF/PPN structural alterations and qEEG were discussed in the context of potential changes to cholinergic activity but these interpretations remain speculative without a direct measure of cholinergic function (i.e., with PET). Future work that incorporates multimodal imaging approaches is required to fully understand the effects of cBF volume loss and PPN diffusivity on cholinergic activity.

In extension of the work conducted in this thesis, future studies could incorporate tractography to explore the influence that structural changes in the cBF and PPN have on their connectivity with the wider brain. Given the intricate connections between the cBF and cortex, and PPN and basal ganglia, these methods could be particularly informative for determining specific pathways implicated in the cortical control of posture and gait in people with PD. As in the current study, EEG could also be applied to assess whether changes to structure and connectivity are reflected in cortical activity.

Mixed pathologies are common in people with PD (and AD), and each could result in subtle physiological changes. While participants showed no clinical characteristics to suggest alternative neurodegenerative diagnoses, the lack of

biomarker evidence means that mixed LB, AD, or vascular pathology cannot be fully ruled out. Studies combining post-mortem examination with ante-mortem imaging techniques will help to determine the influence of these processes. Such methods would also be informative for understanding specific degenerative changes that underpin alterations in cBF volumetric and PPN diffusion metrics, thus improving the interpretation of these measures in the context of disease and symptom progression.

Another potential limitation relates to the influence of medication on outcome measures. The majority of people with PD were taking dopaminergic medications, and a small number were also taking selective serotonin reuptake inhibitors (UK cohort, data not available for Slovenian cohort). These medications have been shown to modulate EEG dynamics, particularly in the alpha range (Bruder et al., 2008; Babiloni et al., 2013), and cognitive functions, including executive function (Cools, 2006; Skandali et al., 2018). From a research standpoint, further work is therefore required to understand the potential influence of medication on the results presented in this thesis. Nevertheless, this is likely to be a more accurate representation of clinical practice, where patients will be taking various medications.

Finally, the current thesis focused on cross-sectional data, providing only a snapshot of the complex disease course that characterises PD. Correlational analyses were also largely conducted without adjustment for multiple comparisons to allow for exploratory-level insight into what is still an emerging area of *in vivo* research. These outcomes therefore provide foundational evidence that warrants further investigation. Future work assessing longitudinal changes with more stringent statistical parameters will therefore provide valuable information with regards to the nature of symptom progression and the predictive power of the brain measures employed herein.

7.5 Conclusion

Results from this thesis reveal that structural changes in the cBF and PPN can be detected in people with PD without dementia. Volumetric loss in cBF may be

relevant for changes in cortically measured EEG activity, and cognitive functions involved in the cortical control of mobility. Elevated PPN diffusivity may also be associated with cortical dynamics and behavioural flexibility during attentional tasks.

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