Predicting cognitive impairment in Parkinson's disease using neurophysiology and biochemical parameters as biomarkers

Dr Alison Jane Yarnall

Doctor of Philosophy thesis submission

Institute for Ageing and Health

July 2013

Contents

List of tables

List of figures

Abstract

Parkinson's disease (PD) is a common neurodegenerative condition, with multiple associated non-motor symptoms. Of these, dementia is a frequent debilitating complication of the disorder, with significant morbidity and mortality. Some forms of mild cognitive impairment in PD (PD-MCI) may represent a pre-dementia state and certain clinical, laboratory and neurophysiological parameters may increase the accuracy of prediction of cognitive decline. If validated, these markers would offer the opportunity for disease modification and therapeutic intervention at a critical early stage of the illness, when the viable neuronal population is greater. The key aim of this thesis was to characterise cognitive impairment in PD in a cohort of newly diagnosed cases, and evaluate how a panel of biomarkers correlated with cognitive phenotypes to predict risk of future cognitive decline.

The main findings were that PD-MCI was common, and was associated with a distinct clinical phenotype. Memory impairment was the most common single domain affected, although the majority of those with PD-MCI were classified as nonamnestic single domain subtype. A significant correlation was found between pattern recognition memory, sensitive to temporal lobe impairments, and cerebrospinal amyloid-β 1-42 levels, thought to represent amyloid-β metabolism and deposition. Both amyloid-β 1-42 and 1-40 levels were significantly lower in those with impaired cognition. In addition, short latency afferent inhibition, a neurophysiological *in vivo* non-invasive measurement of cholinergic function, was also reduced in participants with mild cognitive impairment. These findings suggest that cholinergic dysfunction and amyloid deposition may contribute to the underlying pathophysiology of early PD-MCI.

The major conclusion from this thesis is that PD-MCI is heterogeneous and more frequent than previously reported in early disease. This is associated with abnormalities of amyloid processing and cholinergic dysfunction, and may highlight those at risk of developing dementia. Longitudinal assessment of these individuals will enable us to determine and better model those measures predictive of cognitive decline at an early disease stage.

Acknowledgements

I would like to acknowledge all those who have ensured that this project was successful. Special thanks go to my supervisors, Professor David Burn and Professor Lynn Rochester for their inspiration, vision and guidance over the last three years. In addition, Dr Mark Baker provided neurophysiological expertise and input for part of this project. Full analysis of my dataset would not have been possible without the work of my colleagues Dr Tien Khoo, Dr Gordon Duncan, Dr Jonathan Evans and Dr David Breen, who all assisted in the large amount of data collection from participants both here in Newcastle and also in Cambridge. Collaborators on the ICICLE project have included Professor Roger Barker, Professor Keith Wesnes and Professor Trevor Robbins, who have provided valuable feedback and insight on cognitive aspects of the study. I am indebted to Professor Brit Mollenhauer and Dr Niels Kruse, who analysed the cerebrospinal fluid specimens.

The Clinical Ageing Research Unit, part of the Institute for Ageing and Health, provided an ideal working environment for undertaking this research. Recruitment of Parkinson's disease participants was largely through the Movement Disorders clinic at the RVI, Newcastle, and I am grateful to the doctors and nurses within the clinic for referring patients to the study. A minority of patients were also referred from surrounding areas, and therefore I am also thankful to the wider PD team in the North East and to DeNDRoN for their assistance in recruitment.

My husband, family and friends and fellow researchers all provided invaluable support during my PhD. I would like to dedicate this thesis to my husband, who over the years has listened tirelessly to various presentations, read my manuscripts and provided good humour plus good cooking to keep me going.

Lastly, this study was funded by Parkinson's UK and The Michael J Fox Foundation. My salary was through the Newcastle University Lockhart Fund and Michael J Fox grant. The research was also supported by the National Institute for Health Research (NIHR) Newcastle Biomedical Research Unit based at Newcastle Hospitals Foundation Trust and Newcastle University.

Statement of work undertaken

The study design was prior to my starting as a PhD student and was conceived by Professor David Burn. The Parkinson's disease patient clinical and neuropsychological assessments plus lumbar punctures were performed by me, Dr Tien Khoo and Dr Gordon Duncan at the Clinical Ageing Research Unit in Newcastle and by Dr Jonathan Evans and Dr David Breen at the Cambridge Centre for Brain Repair. The majority of clinical, neuropsychological and lumbar punctures were from the Newcastle team. Control clinical and neuropsychological assessments were undertaken by me and Dr Gordon Duncan, with the assistance of the nursing team at the Clinical Ageing Research Unit. Analysis of the cerebrospinal fluid was kindly provided by Dr Brit Mollenhauer and her team in Gottingen, Germany, who have international expertise in this area. I undertook the majority of the short latency afferent inhibition assessments, with a few of the initial measurements performed by a medical student who was undertaking an MRes project (Rachel David). The short latency afferent inhibition protocol was established by Dr Mark Baker, in the Institute of Neuroscience at Newcastle University, who also programmed the appropriate software. After Dr Baker trained me to run the protocol, I ran the experiments independently to collect the raw data, although technical assistance was provided if required.

The data cleaning and checking was completed by me, Dr Duncan and Dr Khoo in Newcastle. I analysed all the data independently and performed the statistical analysis, with some advice from Dr Shirley Coleman, Industrial Statistics Research Unit, Newcastle University. Data management was in conjunction with data managers as part of the ICICLE team.

Abbreviations

a = Amnestic (MCI)

Aβ = Amyloid-β

Aβ40 = Amyloid-β 1-40

Aβ42 = Amyloid-β 1-42

ACh = Acetylcholine

AChE = Acetylcholinesterase

AD = Alzheimer's disease

APB = Abductor pollicis brevis

APOE = Apolipoprotein E

APP = Aβ precursor protein

αsyn = Total α-synuclein

CANTAB = Cambridge Neuropsychological Test Automated Battery

CDR = Cognitive Drug Research computerised battery

ChEI = Cholinesterase inhibitor

CN = Cognitively normal

DLB = Dementia with Lewy bodies

EMG = Electromyogram

FDI = First dorsal interosseous muscle

FTD = Frontotemporal dementia

GABA = Gamma-aminobutyric acid

GDS-15 = Geriatric Depression Scale-15

ICICLE-PD = Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation-Parkinson's disease

ISI = Interstimulus interval

LB = Lewy bodies

LEDD = Levodopa equivalent daily dose

LICI = Long interval cortical inhibition

MCI = Mild cognitive impairment

md = Multiple-domain (MCI)

MDS = Movement Disorder Society

MEP = Motor evoked potential

MMSE = Mini mental state examination

MNMT = Median nerve motor threshold

MoCA = Montreal cognitive assessment

MRI = Magnetic resonance imaging

na = nonamnestic

NART = National Adult Reading Test

NbM = Nucleus basalis of Meynert

NMS = Non-motor symptoms

NPI = Neuropsychiatric Inventory

OTS = One Touch Stockings of Cambridge test

PAL = Paired associates learning

PD = Parkinson's disease

PDD = Parkinson's disease dementia

PDND = Parkinson's disease non-demented

PDQ-39 = Parkinson's disease Quality of Life Questionnaire

PIGD = Postural instability gait difficulty

PoA = Power of attention

P(181)-tau = Tau phosphorylated at threonine 181

PPN = Pedunculopontine nucleus

PRM = Pattern recognition memory

QSBB = Queen Square Brain Bank

RBD = Rapid eye movement behaviour disorder

RMT = Resting motor threshold

SAI = Short latency afferent inhibition

sd = Single-domain (MCI)

SEP = Somatosensory evoked potential

SICI = Short-interval intracortical inhibition

SN(pc) = Substantia nigra (pars compacta)

SRM = Spatial recognition memory

TD = Tremor dominant

TMS = Transcranial magnetic stimulation

ToL = Tower of London test

T-tau = Total tau

UPDRS = Unified Parkinson's Disease Rating Scale

VH = Visual hallucination

Chapter 1 Parkinson's disease in context

Parkinson's disease (PD) is the second commonest neurodegenerative disorder affecting patients in the UK after Alzheimer's disease (AD). Although historically classified as a motor system disorder, it is increasingly recognised that the majority of the morbidity and mortality associated with PD is related to non-motor aspects. Thus, the focus of research in recent years has turned towards an improved understanding of the non-motor symptoms (NMS), of which cognitive impairment forms a significant burden.

1.1 Epidemiology

The annual incidence of PD has been estimated at 8 - 22/100,000 per year in both Europe and the USA (Twelves *et al.*, 2003; Foltynie *et al.*, 2004; von Campenhausen *et al.*, 2005; Taylor *et al.*, 2006; Alves *et al.*, 2008; Winter *et al.*, 2010), ranging from 9 per 100,000 in Eastern Europe to 22 per 100,000 in Aberdeen, with a comparable local annual incidence rate in the Newcastle-Gateshead area of 15.9/100,000 (Duncan *et al.*, 2013b). Reasons for differences between studies include differences in case ascertainment, the population denominator and case definition. The greatest risk factor for development of PD is age, with prevalence figures estimated at 0.3% in those aged over 55, 1% in those over 65, 3.1% over 75 and 4.3% in the oldest age group of over 85 (de Rijk *et al.*, 1995). The risk of PD is also increased in men, who are approximately 1.5 times more likely than women to develop the disease; however, this may only apply to Western populations and in older age groups (Taylor *et al.*, 2007). Possible explanations for the excess in male cases include the protective effect of female hormones, gender-specific exposure to occupational or environmental risk factors or genetic influences (Alves *et al.*, 2008), although the exact mechanisms remain unclear.

PD is a chronic progressive neurodegenerative disorder for which at present there is no known cure. Median age of disease onset is 60 years (Lees *et al.*, 2009), with median time from symptom onset to death of just over 12 years (Hely *et al.*, 2005; Hely *et al.*, 2008). The standardised mortality ratio is comparable to that of the general population in early disease (Marras *et al.*, 2005), but rises to between 1.5 and 3.1 as disease progresses (Hely *et al.*, 1999; Herlofson *et al.*, 2004; Hely *et al.*, 2005;

Hely *et al.*, 2008). The commonest cause of death in patients with PD is pneumonia (Beyer *et al.*, 2001; Hely *et al.*, 2005).

1.2 Aetiology of PD

The precise cause of PD remains the subject of intense research, but is likely to be a combination of genetic and environmental factors. Although the vast majority of cases are sporadic in nature, a monogenic aetiology is more likely in those aged under 45 years old, while recent studies have identified at least eight significant loci (Nussbaum RL, 2003; Gasser *et al.*, 2011) (Table 1-1). After advancing age, family history of PD is the biggest risk factor for developing the disease, with early reports suggesting that patients with PD are four times more likely to have a first-degree relative affected than controls (Payami *et al.*, 1994). Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) are the most frequent cause of inherited PD, with the commonest mutation producing a glycine to serine amino acid substitution at codon 2019 (G2019S), thought to be present in 1-2% of sporadic PD patients of European descent (Gilks *et al.*, 2005). This figure is much higher in the southern Mediterranean population, in African Arabs and in Ashkenazi Jews. The clinical presentation of *LRRK2* is indistinguishable from that of idiopathic Parkinson's disease, although dementia tends to be less prominent and the disease course is generally milder (Healy *et al.*, 2008). An age-related reduced penetrance is observed, with the risk of PD in those with G2019S mutations being 28% at 59 years, 51% at 69 years and 74% at 79 years (Healy *et al.*, 2008).

Genetic factors may still be important in so-called sporadic disease, but the impact of functional polymorphisms, for example, is less apparent than loss of function or toxic gain of function in monogenic forms, and thus less easy to establish. The pathological hallmark of PD is the presence of Lewy bodies (LB) and Lewy neurites within the substantia nigra pars compacta (SNpc) with consequent degeneration of dopaminergic neurones (Braak *et al.*, 2003). LBs consist of insoluble α–synuclein, ubiquitin, neurofilament plus other proteins and aggregate in the neuronal cytoplasm or axons (when the protein aggregation is identified as Lewy neurites) in the central and autonomic nervous system (Braak *et al.*, 2004). Growing evidence argues for the role of altered intracellular protein handling in PD, with either an increase in abnormal protein production, impaired protein clearance, or both (Olanow and McNaught, 2011).

Table 1-1 Monogenetic causes of Parkinson's disease

Locus	Gene	Mode of inheritance	Pathology	Comments	References
PARK1	α-Synuclein (*SNCA*)	Autosomal dominant	Widespread Lewy bodies	Rare; different point mutations correspond to different phenotypes, from early, severe, rapidly progressive plus dementia to mild late-onset PD	(Polymeropoulos *et al.*, 1997; Spira *et al.*, 2001)
PARK2	*Parkin*	Autosomal recessive	Substantia nigra degeneration ; occasional LB	Commonest AR form of PD; early onset, slow progression	(Kitada *et al.*, 1998; Farrer *et al.*, 2001)
PARK4	α-Synuclein (*SNCA*)	Autosomal dominant	Widespread α-syn accumulation	Duplications- late onset typical PD; triplications- early onset, rapid progression, dementia	(Singleton *et al.*, 2003; Fuchs *et al.*, 2007; Ikeuchi *et al.*, 2008)
PARK6	*PINK1*	Autosomal recessive	Unknown (1 case – LB pathology, nigral neuronal loss & gliosis)	Early onset, slow progression	(Valente *et al.*, 2001; Valente *et al.*, 2004a; Valente *et al.*, 2004b; Samaranch *et al.*, 2010)
PARK7	*DJ-1*	Autosomal recessive	Unknown	Rare; early onset, slow progression	(van Duijn *et al.*, 2001; Bonifati *et al.*, 2003)
PARK8	*LRRK2*	Autosomal dominant	Variable; usually Lewy bodies	Commonest AD form of PD; clinical features as for sporadic PD	(Zimprich *et al.*, 2004; Di Fonzo *et al.*, 2005)
PARK9	*ATP13A2* (Kufor-Rakeb disease)	Autosomal recessive	Iron deposition in basal ganglia	Early onset parkinsonism, spasticity, dementia, supranuclear gaze palsy	(Ramirez *et al.*, 2006; Schneider *et al.*, 2010)
PARK15	*FBX07*	Autosomal recessive	Unknown	Rare; early onset, LD-responsive, spasticity	(Di Fonzo *et al.*, 2009)

Within cells, abnormal protein components are cleared via the autophagic-lysosomal system if insoluble (Ventruti and Cuervo, 2007), and largely through the ubiquitin-proteasome system if soluble protein degradation is required (Pickart, 2001). Defects in the ubiquitin-proteasome system (such as those caused by *Parkin* mutations) or excessive abnormal protein production (for example, due to *SNCA* mutations) precipitate accumulation of abnormal proteins within the neurone, leading to proteolyic stress and ultimately cell death (Olanow and McNaught, 2011). Some researchers hypothesise that in fact LBs may be a form of aggresome – an intracellular inclusion formed in response to proteolyic stress – and hence have a cytoprotective function (Olanow *et al.*, 2004; Tanaka *et al.*, 2004). Recent post-mortem work challenges these views and suggests that neuronal cell dysfunction and cell death may actually precede the formation of LBs and Lewy neurites, when it was shown that substantia nigra (SN) cell dysfunction was greater in those with incidental Lewy bodies than those with PD or controls, with an intermediate number of LBs in the incidental LB group (Milber *et al.*, 2012). Further work is required to delineate the exact mechanisms underlying the function and formation of Lewy pathology and its relation to neurodegeneration in PD.

The concept of epigenetics in neurodegeneration has received attention in recent years, and may go some way to bridging our understanding of the interplay between genetic and environmental factors (Urdinguio *et al.*, 2009; Qureshi and Mehler, 2011). Epigenetics refers to heritable changes in gene expression that are not due to changes in the underlying DNA sequence (Urdinguio *et al.*, 2009). Examples of such epigenetic mechanisms include DNA methylation, histone modifications and non-coding RNA regulation, although few studies have specifically addressed this in PD.

Environmental factors that may contribute to the pathophysiology of PD include smoking, caffeine, head trauma and exposure to solvents and other toxins. The inverse relationship between smoking and risk of PD (Hernan *et al.*, 2001; Allam *et al.*, 2004) and caffeine intake and risk of the disease (Ross *et al.*, 2000) was initially thought to be related to pre-morbid low sensation seeking personality traits rather than neuroprotective effects of these substances *per se* (Evans *et al.*, 2006). However, evidence from animal model studies has demonstrated that some compounds in tobacco reduce the breakdown of dopamine via inhibition of monoamine oxidase

(MAO) A and B, and that nicotine may enhance dopaminergic release within the striatum by actions on nicotinic acetylcholine (ACh) receptors (Quik *et al.*, 2012). Similarly, caffeine is an adenosine A2 receptor antagonist: These compounds normally reduce excessive motor activity through actions on the inhibitory gamma-aminobutyric acid (GABA) basal ganglia pathways (Jankovic, 2008). Phase III trials are currently on going to determine whether an adenosine A2 receptor antagonist can delay the onset of motor symptoms of PD and reduce striatal neuronal loss.

The concept of repeated severe head trauma as a cause of parkinsonism is not new; however, the association between head injury and idiopathic PD is disputed. One reason why studies have found conflicting results as to whether head injury precedes the disease is that history of trauma is difficult to assess due to recall bias. The most recent and largest study to date on the subject avoided the problem of recall bias by using hospital records of head injury in PD and control cases to determine the relationship between the two (Fang *et al.*, 2012). Although there was an increased risk of hospitalisation due to head injury in PD compared to control cases (odds ratio 1.42), the injuries generally occurred shortly before the index date (within a year), and the authors concluded that there was insufficient evidence to conclude that head trauma later in life increases the risk of PD. Possible explanations as to why this may have been biologically plausible include disruption to the blood-brain barrier with subsequent exposure to neurotoxins and the inflammatory response associated with brain injury (Morley and Duda, 2012).

More controversial in the aetiology of PD is exposure to solvents and subsequent risk of the disease. Trichloroethylene has been recognised as a potential risk factor for many years (Guehl *et al.*, 1999), and a recent case-control study using twin pairs discordant for PD added to this body of evidence (Goldman *et al.*, 2012b). In this study, 99 twin pairs were interviewed using a structured occupational questionnaire with respect to six specific solvents. Twins with PD were six times more likely to have been exposed to trichloroethylene than their unaffected co-twin, and there was also a trend towards significance for exposure to perchloroethylene and carbon tetrachloride and PD risk. Again this is biologically plausible and supported by rodent experiments demonstrating that these solvents trigger mitochondrial dysfunction and oxidative

stress, leading to degeneration of dopaminergic neurones in the SN (Sherer *et al.*, 2007; Liu *et al.*, 2010).

Finally, there is evidence that some lifetime-related exposures may protect against the development of PD. In a large longitudinal cohort study in Finland, individuals with adequate vitamin D status (vitamin D levels of at least 50 nmol/L) had a 65% lower risk than those with values less than 25 nmol/L after adjustment for several potential confounders including age, sex, physical activity and month of blood draw (Knekt *et al.*, 2010). Vitamin D receptors and 1-α-hydroxylase are widespread throughout the human brain in neurons and glial cells, and are found in high density in the hypothalamus and large neurons of the SN (Eyles *et al.*, 2005). Vitamin D may exert a neuroprotective effect through antioxidant activities, immunomodulation, and neuronal calcium regulation or by improving nerve conduction (Newmark and Newmark, 2007; Evatt, 2010).

In conclusion, the exact aetiology of Parkinson's disease in the majority of individuals remains unknown, but environmental, occupational, genetic and epigenetic factors may contribute. Determining the interplay of these factors is important in establishing the underlying disease mechanisms and in developing potential targets for treatment.

1.3 Pathological correlates of PD

The pathological diagnosis of PD is typified by the loss of neuromelanin containing dopaminergic neurones in the SNpc, with LBs and Lewy neurites seen in surviving neurones. It is clear, however, that Lewy pathology is not limited to dopaminergic neurones, and also affects the cholinergic neurons of the nucleus basalis of Meynert (nbM), serotonergic neurones within the raphe nucleus and noradrenergic neurones in the locus coeruleus (Forno, 1996; Braak *et al.*, 2003). A major component of both LBs and Lewy neurites is an abnormally modified and aggregated form of α–synuclein, normally a presynaptic protein. The precise mechanisms triggering fibrillization and aggregation of α-synuclein remains the subject of debate, as does the inter-cellular transfer of α-synuclein. One hypothesis, proposed by Braak and colleagues, is that transfer of Lewy pathology is not random but spreads from the medulla oblongata and olfactory nucleus in a caudo-rostral direction to further susceptible structures within the brainstem, limbic system and finally neocortical regions (Braak *et al.*, 2003). This corresponds with the presumed preclinical and premotor stages of PD (Figure 1-1).

Braak stages 1 and 2, where pathology is confined to the medulla, pontine tegmentum and olfactory nucleus correlates with a premotor phase, with autonomic features and hyposmia. Stages 3 and 4, when disease becomes symptomatic causing sleep and motor deficits, is associated with parallel neuropathology in the SN, other vulnerable areas of the midbrain and the basal forebrain. Braak stages 5 and 6, with the occurrence of cortical Lewy pathology, are associated with cognitive and other neuropsychiatric deficits (Braak *et al.*, 2004). Against this hypothesis is work demonstrating widespread LB pathology in dementia with Lewy bodies (DLB) and Alzheimer's disease (AD) without motor features consistent with PD, and also the absence of correlation between Braak staging and disease severity (Burke *et al.*, 2008). In addition, a large clinicopathological study established that over half of subjects with Braak stage 5 or 6 disease actually had no clinical signs of an extrapyramidal syndrome or dementia (Parkkinen *et al.*, 2008). A further contentious issue is where Lewy pathology begins within the brain: one theory is that the process of PD actually begins in the gut through exposure to toxic or infectious agents and spreads trans-synaptically via the dorsal motor nucleus to the central nervous system (Braak *et al.*, 2006; Hawkes *et al.*, 2007; Olanow, 2012; Shannon *et al.*, 2012a; Shannon *et al.*, 2012b). Lastly, the question of how α-synuclein pathology propagates remains unanswered, and indeed how allografts of foetal ventral mesencephalic tissue transplanted into the striatum of PD patients can develop LB pathology (Kordower *et al.*, 2008; Li *et al.*, 2008). Theories include trans-synaptic spread through inflammation, oxidative stress or excitotoxic mechanisms (Li *et al.*, 2008; Lees *et al.*, 2009) or a prion-like process, where α-synuclein misfolding initiates aggregation of the abnormal protein in adjacent cells by so-called "permissive templating" (Olanow and McNaught, 2011).

1.4 Diagnosis of PD

The diagnosis of PD remains based on clinical features, and depends critically on the demonstration of bradykinesia, defined as progressive fatiguing and decrement of repetitive alternating movements during finger or foot tapping (Abdo *et al.*, 2010), plus at least one of rigidity, 4-6Hz rest tremor and postural instability. Exclusion criteria include early severe autonomic involvement, neuroleptic treatment at onset of symptoms and early marked dementia, as these may point to alternative diagnoses (Hughes *et al.*, 1992). Three or more supporting features are required for a definite

diagnosis, comprising asymmetrical onset and progression, excellent response to levodopa and more recently, hyposmia and visual hallucinations (Lees *et al.*, 2009) (Table 1-2). The Queen Square Brain Bank (QSBB) criteria have been found to be highly accurate for the diagnosis of PD by movement disorder specialists, with a positive predictive value of 98.6%, negative predictive value of 90%, sensitivity 91.1% and specificity 98.4% in a longitudinal clinicopathological study (Hughes *et al.*, 2002).

The European Federation of Neurological Societies (EFNS) and Movement Disorder Society-European Section (MDS-ES) have recently published guidelines for the diagnosis of PD, and recommend the use of the QSBB criteria in the clinical diagnosis as Level B evidence (probably effective) (Berardelli *et al.*, 2013). In cases of diagnostic uncertainty, neuroimaging may be supportive (Berardelli *et al.*, 2013). Specifically, the use of single photon emission tomography (SPECT) with selective pre-synaptic radioligands targeting the dopamine transporter (DaTscan) can be a cost-effective method to differentiate degenerative parkinsonism and atypical tremor disorders or drug-induced parkinsonism (Level A evidence; effective). A newer recommendation is the use of transcranial sonography (TCS), as hyperechogenicity of the substantia nigra is associated with a classification accuracy of nearly 90% (Berg *et al.*, 2008; Gaenslen *et al.*, 2008). It is recommended that TCS can be used for the differentiation of PD from atypical and non-degenerative parkinsonian disorders, for the early diagnosis of PD and in the detection of subjects at risk for PD in conjunction with other screening tests. TCS is highly operator-dependent, however, and its use is still not widespread. Magnetic resonance imaging (MRI) at 1.5 Tesla can assist in discriminating PD and atypical parkinsonian disorders (Level B evidence), and lastly, olfactory testing can be used in confirming the diagnosis of PD (Level A evidence).

Table 1-2 Queen Square Brain Bank criteria for the diagnosis of Parkinson's disease

Step 1 Diagnosis of parkinsonian syndrome

Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude or repetitive actions) and at least one of the following:

- Muscular rigidity
- 4- to 6-Hz rest tremor
- Postural instability not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction

Step 2 Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language and praxis
- Babinski sign
- Presence of a cerebral tumour or communicating hydrocephalus on CT scan
- Negative response to large doses of L-dopa (if malabsorption excluded)
- MPTP exposure

Step 3 Supportive prospective positive criteria of Parkinson's disease. Three or more required for the diagnosis of definite Parkinson's disease:

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting the side onset most
- Excellent response (70–100%) to L-dopa
- Severe L-dopa-induced chorea
- L-dopa response for 5 years or more
- Clinical course of 10 years or more
- Hyposmia
- Visual hallucinations

1.5 Clinical features of PD

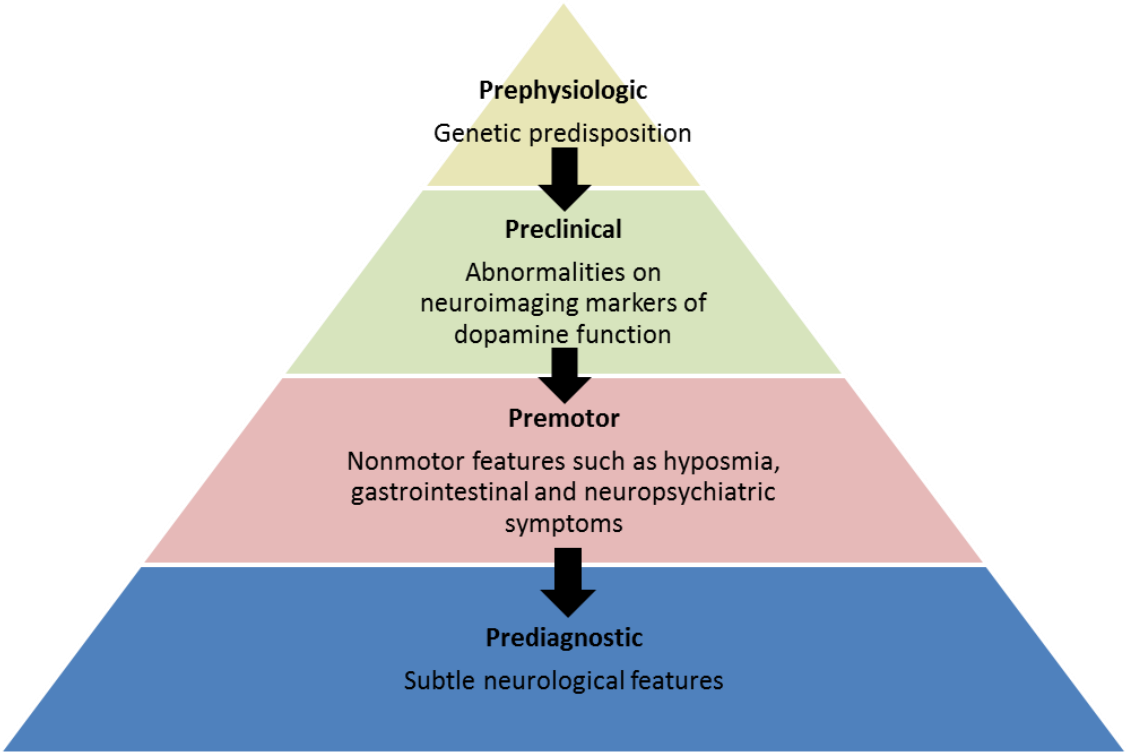
1.5.1 Motor features

The motor features of PD usually begin insidiously after the loss of up to 60% of dopaminergic SN neurones (Fearnley and Lees, 1991; Damier *et al.*, 1999; Bohnen *et al.*, 2006a). Postural instability develops later in the disease and its presence early on should prompt consideration of an alternative diagnosis. Patients may complain of a loss of dexterity in tasks such as dressing or shaving, with or without associated micrographia, which may be noted more if the dominant hand is affected. Indeed handedness is likely to be associated with side of onset of the disease: A recent meta-analysis concluded that PD patients more often have symptoms affecting their dominant side (van der Hoorn *et al.*, 2012). Overall, 59.5% of right-handed patients had right-side dominant disease, with 59.2% of left-handed patients having predominantly left-sided symptoms (odds ratio 2.13; 95% confidence interval 1.71 - 2.66). Extrapyramidal rigidity may manifest as stiffness or non-specific muscular pains, with difficulty turning in bed or getting out of a low chair. Tremor may be noticed by family and is typically present at rest initially. Other motor features include hypomimia, hypophonia and dystonia.

1.5.2 Non-motor symptoms

The morbidity and mortality associated with non-motor symptoms (NMS) in PD is increasingly recognised. NMS include gastrointestinal, urinary, cognitive, behavioural, sleep and cardiovascular symptoms. Previously thought of in the context of established disease (Parkinson, 1817), it is now recognised that NMS such as constipation, rapid eye movement (REM) behaviour disorder (RBD), depression and hyposmia often precede the motor features by many years and may represent a "Parkinson's At Risk Syndrome" (PARS), allowing earlier diagnosis and a potential opportunity for more effective use of neuroprotective therapies (Stern and Siderowf, 2010). The PARS study developed by Stern and collaborators in the USA is currently in progress, with the aim of recruiting over 15,000 individuals, starting by screening for hyposmia (see 'Premotor' phase in Figure 1-1).

Figure 1-1 Parkinson's disease at risk syndrome. Modified from Stern and Siderowf, 2010



Other studies have demonstrated that NMS are common even early in the disease process. A large retrospective clinicopathological study of 433 subjects with pathologically-proven PD established that 21% of patients actually presented with NMS such as pain, urinary symptoms and neuropsychiatric features; these patients were also more likely to have had a delayed diagnosis than those presenting with motor symptoms (O'Sullivan *et al.*, 2008). A more recent study of 159 newly diagnosed PD participants and 99 age-matched controls in Newcastle demonstrated that even in those with a median disease duration of only 4.4 months, NMS were significantly more common in PD patients, with a mean number of 8.4 NMS experienced compared with 2.8 in controls (Khoo *et al.*, 2013). The commonest symptoms experienced were sialorrhea, urinary urgency, hyposmia, anxiety, and constipation (Table 1-3). Increasing motor severity was associated with a greater number of NMS.

NMS are a significant cause of disease burden in early PD and are associated with reduced functional status and sense of wellbeing. NMS with the greatest negative impact on health related quality of life, as measured by the Parkinson's Disease Quality of Life Questionnaire (PDQ-39) (Peto *et al.*, 1995), were depression, anxiety, RBD, sensation of incomplete bowel emptying, hyperhidrosis, leg swelling, diplopia,

impaired concentration and forgetfulness in the same study group (Duncan *et al.*, 2013a). In combination, these symptoms predicted 49% of the variance in quality of life.

As PD progresses, the frequency of NMS increases, with neuropsychiatric burden (depression, anxiety, apathy, psychosis and cognition) having a greater impact on quality of life than motor symptoms (Schrag *et al.*, 2000b; Schrag *et al.*, 2000a; Weintraub *et al.*, 2004b; Slawek *et al.*, 2005; Barone *et al.*, 2009; Martinez-Martin *et al.*, 2011). Despite this, NMS are under-recognised by clinicians and patients: the under-reporting of NMS ranged from 31.8% (diplopia) to 65.2% (delusions) in one cross-sectional international study (Chaudhuri *et al.*, 2010). Recognition of these symptoms is important as many are amenable to treatment, including dopaminergic therapy (Chaudhuri and Schapira, 2009; Zesiewicz *et al.*, 2010; Seppi *et al.*, 2011). Examples of this include pramipexole in the treatment of depression (Barone *et al.*, 2010; Seppi *et al.*, 2011) and clozapine in the treatment of psychosis (Morgante *et al.*, 2004; Merims *et al.*, 2006; Seppi *et al.*, 2011), both of which have been considered to be efficacious in an MDS evidence based review of treatments for NMS (Seppi *et al.*, 2011).

The heterogeneity of NMS experienced by people with PD indicates that they are not all caused by the same underlying pathological substrate and suggests that dopamine alone is unlikely to be the sole contributor. There is evidence that cholinergic dysfunction is important in the pathophysiology of cognitive impairment (Tiraboschi *et al.*, 2000; Bohnen *et al.*, 2003; Yarnall *et al.*, 2011; Yarnall *et al.*, 2013), depression (Bohnen *et al.*, 2007; Meyer *et al.*, 2009), hyposmia (Bohnen *et al.*, 2010) and falls (Bohnen *et al.*, 2009; Bohnen and Albin, 2011) in PD. In addition to ACh loss, depression in PD has been attributed to complex interactions between dopaminergic loss in the SN via mesolimbic circuits, serotonergic loss from the raphé nucleus and noradrenergic deficits due to degeneration of neurones in the locus coeruleus (Aarsland *et al.*, 2012). Changes in inflammatory and neurotrophic factors and psychosocial elements may also contribute to mood disorder (Aarsland *et al.*, 2012).

In conclusion, NMS are common in PD, even early in the disease process. As the disease progresses, the occurrence of NMS increases and impacts negatively on quality of life. Symptoms may not be recognised by patients or clinicians caring for them, despite the fact that they may be successfully treated. The underlying

pathophysiology is heterogeneous and likely to involve strategic neuronal loss and complex interactions between neurotransmitters throughout cortical and subcortical systems.

Table 1-3 Frequency of non-motor symptoms in PD and controls (Khoo et al, 2013); *****denotes significant differences after Bonferroni correction for multiple tests

Non-motor symptom	**PD N (%)**	**Control N (%)**	***p*-value**
Gastrointestinal tract, n (%)			
Sialorrhea	89 (56.0)	6 (6.1)	**<0.001***
Dysphagia	32 (20.1)	3 (3.0)	**<0.001***
Nausea	4 (2.5)	15 (15.2)	0.142
Constipation	67 (42.1)	7 (7.1)	**<0.001***
Bowel incontinence	9 (5.7)	5 (5.1)	1.000
Incomplete bowel emptying	51 (32.1)	12 (12.1)	**<0.001***
Hyposmia	71 (44.7)	10 (10.1)	**<0.001***
Weight change (unexplained)	36 (22.6)	19 (19.2)	0.536
Urinary tract, n (%)			
Urinary urgency	74 (46.5)	19 (19.2)	**<0.001***
Nocturia	42 (26.4)	17 (17.2)	0.095
Sexual function, n (%)			
Sexual dysfunction	33 (20.8)	10 (10.1)	0.026
Impaired libido	28 (17.6)	7 (7.1)	0.016
Cardiovascular, n (%)			
Orthostatic symptoms	53 (33.3)	11 (11.1)	**<0.001***
Falls	37 (23.3)	4 (4.0)	**<0.001***
Lower limb swelling	29 (18.2)	11 (11.2)	0.157
Neuropsychiatric & Cognitive, n (%)			
Forgetfulness/ memory	88 (55.3)	41 (41.4)	0.040
Impaired concentration	47 (29.6)	2 (2.0)	**<0.001***
Anxiety	68 (42.8)	10 (10.1)	**<0.001***
Low mood	59 (37.1)	10 (10.1)	**<0.001***
Loss of interest/ apathy	44 (27.7)	3 (3.0)	**<0.001***
Delusions	1 (1.0)	0 (0.0)	1.000
Visual hallucinations	35 (22.0)	0 (0.0)	**<0.001***
Sleep, n (%)			
Daytime somnolence	59 (37.1)	18 (18.2)	**0.001***
Insomnia	28 (17.6)	13 (13.1)	0.385
Dream re-enactment	55 (34.6)	8 (8.1)	**<0.001***
Vivid dream imagery	48 (30.2)	5 (5.1)	**<0.001***
Restless legs	44 (27.7)	11(11.1)	0.002
Pain, n (%)			
Pain (unexplained)	60 (37.7)	3 (3.0)	**<0.001***
Miscellaneous, n (%)			
Diplopia	16 (10.1)	3 (3.0)	0.048
Hyperhidrosis	16 (10.1)	6 (6.1)	0.360

1.5.3 Subtypes of PD

As discussed above, patients with PD present with a diverse range of motor and non-motor symptoms, leading to considerable phenotypic heterogeneity. This has led researchers to hypothesise that idiopathic PD comprises specific subtypes that may be associated with distinct underlying pathophysiological processes. Cluster analysis of clinical data on prevalent and early stage PD participants recruited from separate centres in the UK identified three (Graham and Sagar, 1999) or four (Lewis *et al.*, 2005) main subgroups. Graham and Sagar divided participants into 'motor only', 'motor and cognitive' and 'rapidly progressive' clusters (Graham and Sagar, 1999), whilst Lewis and colleagues categorised those with early stage disease into: a) younger disease onset; b) tremor dominant; c) non-tremor dominant with cognitive impairment; and d) rapid disease progression without cognitive impairment (Lewis *et al.*, 2005). Certain characteristics differed across the subgroups described in the latter study, with those in the younger disease onset group (mean age at onset 50 years) experiencing slow disease progression, mild motor symptoms, motor fluctuations and less cognitive impairment or affective symptoms than the non-tremor dominant and rapidly progressive subtypes. Tremor dominant patients were similar to the former group, although had more prominent tremor and were older. Those with non-tremor dominant presentations displayed cognitive impairment, most notable within the executive domain, and more advanced motor disease, whilst those with rapid disease progression displayed a more aggressive motor disease course but minimal cognitive decline. Clinical classification of PD into motor phenotypes is useful and has consistently demonstrated that those with postural instability gait difficulty (PIGD) phenotype have more rapid disease progression than those who persistently have tremor dominant symptoms (Foltynie *et al.*, 2002; Post *et al.*, 2007). Importantly, this phenotype is also associated with accelerated cognitive decline and risk of dementia (Alves *et al.*, 2006; Burn *et al.*, 2006; Taylor *et al.*, 2008), in agreement with Lewis and colleagues (Lewis *et al.*, 2005), and may be due in part to loss of cholinergic neurons (Tiraboschi *et al.*, 2000).

These clinical findings were corroborated in part by a large retrospective clinicopathological study of 242 pathologically confirmed PD cases from QSBB (Selikhova *et al.*, 2009). Selikhova and colleagues confirmed that those with early onset shared similar characteristics to those classified by Lewis *et al*, with greater time

to falls and cognitive decline but pathologically similar LB burden to tremor dominant phenotypes. Interestingly, those with tremor dominant disease did not have significantly longer disease duration than those with non-tremor dominant presentations. The latter group was strongly associated with the development of cognitive impairment, had a more extensive LB disease and had greater amyloid-β plaque plus neurofibrillary tangle deposition than other subtypes. Participants with rapid disease progression tended to be older at disease onset, were more likely to have early midline motor deficits plus early depression, and to present with tremor dominant disease. The burden of LB disease was lower than in those who were non-tremor dominant, but similar to those with early onset and tremor dominant disease.

These clinicopathological findings from this group were generally consistent with those from an earlier study (Halliday *et al.*, 2008), where three subtypes of PD were defined pathologically. The group with younger onset had longer disease duration and conformed to the Braak staging hypothesis (Braak *et al.*, 2003). Around a quarter of cases were classified as having a dementia dominant syndrome, with severe neocortical LB pathology at disease onset, and the final subtype consisted of older age at onset, with shorter disease duration and a high cortical LB burden. The latter group also had significant co-existent AD and cerebrovascular pathology.

Despite these classifications, the course of clinically advanced PD seems to follow a typical trajectory regardless of age at death or age at disease onset, with a similar pathological outcome (Kempster *et al.*, 2010). The milestones of visual hallucinations (VH), frequent falls, cognitive impairment and care home placement consistently occurred approximately at 5, 4, 3.5 and 3 years before death, respectively. The presence of VH and dementia were associated with high cortical LB burden, with dementia also being associated with significant amyloid-β plaque and neurofibrillary tangle deposition.

In summary, there is considerable phenotypic heterogeneity within the PD diagnostic "umbrella". Clinical and pathological studies indicate that those with persistent non-tremor dominant symptoms are at greater risk of motor progression and cognitive decline, and this may be due to high cortical LB counts, Alzheimer-type pathology and cholinergic dysfunction.

1.6 Parkinson's disease dementia

As discussed above, cognitive impairment is an important non-motor symptom in PD and has a considerable impact on both patients and their carers. The development of PD dementia (PDD) is a frequent and distressing complication of the disease, with a cumulative incidence approaching 80% in community based studies (Aarsland *et al.*, 2003a; Buter *et al.*, 2008; Hely *et al.*, 2008). PDD is associated with a twofold increase in mortality (Levy *et al.*, 2002c), a loss of independence (Aarsland *et al.*, 2000), increased caregiver strain (Aarsland *et al.*, 2007a) and increased healthcare costs (Vossius *et al.*, 2011), the latter largely due to the cost of institutionalisation. Within PDD, neuropsychiatric symptoms including depression, anxiety, apathy and hallucinations affect up to 90% of patients (Aarsland *et al.*, 2007a) and also significantly contribute to caregiver burden (Schrag *et al.*, 2006).

PDD shares many clinical, neuropsychological and neuropathological features with DLB; these diagnoses are separated by the timing of cognitive symptoms to motor onset. Those who develop cognitive dysfunction within a year of motor onset or prior to motor symptoms are classified as DLB, whilst those with extrapyramidal features for longer than one year before cognitive decline develops are designated as PDD (McKeith *et al.*, 2005; Lippa *et al.*, 2007). PDD and DLB are likely to represent different phenotypes of the same underlying disease process: indeed the characteristics of visual hallucinations (Mosimann *et al.*, 2004; Mosimann *et al.*, 2006), fluctuating cognition, attentional-executive plus visuoperceptual deficits (Noe *et al.*, 2004), neurotransmitter deficits (Klein *et al.*, 2010), α-synuclein and Alzheimer-type pathology (Ballard *et al.*, 2006), neuroleptic sensitivity (Galvin *et al.*, 2006), REM sleep behaviour disorder (RBD) (Boeve *et al.*, 1998) and autonomic dysfunction (Allan *et al.*, 2006) are similar across both diseases. The major differences seen, aside from the temporal course, include the excess of amyloid deposition in DLB (Gomperts *et al.*, 2008), superior motor response to levodopa in PDD (Molloy *et al.*, 2005) and older age at onset in DLB.

1.6.1 Epidemiology of PDD

PDD has an associated mortality risk (Levy *et al.*, 2002c), and therefore cumulative prevalence is a more accurate representation of dementia frequency than cross-sectional assessments, which may underestimate the risk of dementia. As stated

above, the cumulative risk varies from 78% at eight years (Aarsland *et al.*, 2003a), to 60% at 12 years (Buter *et al.*, 2008) and 83% at 20 years (Hely *et al.*, 2008). The point prevalence of dementia in patients with PD is between 24 and 31%, with 3 to 4% of dementia in the population attributable to PDD (Aarsland *et al.*, 2005b). The prevalence of PDD in the general population aged 65 and over is approximately 0.2 to 0.5% (Aarsland *et al.*, 2005b). Compared to subjects without PD, those with the disease have between a two- and six-fold increased risk of dementia (Marder *et al.*, 1995; Aarsland *et al.*, 2001; Hobson and Meara, 2004; de Lau *et al.*, 2005). The mean age at diagnosis of PDD is between 71 and 78 years, with an average PD disease duration at that time of between 10 and 14 years (Hughes *et al.*, 2000; Aarsland *et al.*, 2003a; Hely *et al.*, 2008). Considerable heterogeneity exists in the timescale to develop dementia from disease onset; some patients develop cognitive impairment within a few years of diagnosis, whilst others remain cognitively intact for 10 to 20 years. Although the risk of dementia increases as disease progresses, a degree of cognitive impairment may be present at diagnosis in over a third of people with PD (Foltynie *et al.*, 2004). Over a follow-up of five years, 17% of these incident cases developed dementia, with an incidence of 38.7 per 1000 person-years (95% confidence interval 23.9 – 59.3) (Williams-Gray *et al.*, 2009a).

1.6.2 Risk factors for PDD

The greatest risk factor for the development of PDD is increasing age (Hughes *et al.*, 2000; Aarsland *et al.*, 2001; Hobson and Meara, 2004; Aarsland *et al.*, 2007b; Williams-Gray *et al.*, 2007a; Uc *et al.*, 2009; Williams-Gray *et al.*, 2009a). Severity of parkinsonism increases risk of cognitive decline (Hughes *et al.*, 2000; Aarsland *et al.*, 2001; Green *et al.*, 2002; Uc *et al.*, 2009), and acts synergistically with age (Levy *et al.*, 2002b). Other established risk factors include the presence of hallucinations (Aarsland *et al.*, 2003a; Hobson and Meara, 2004; Galvin *et al.*, 2006), symmetry of motor symptoms and PIGD phenotype (Aarsland *et al.*, 2003a; Alves *et al.*, 2006; Burn *et al.*, 2006; Uc *et al.*, 2009), RBD (Vendette *et al.*, 2007; Postuma *et al.*, 2012), male gender (Galvin *et al.*, 2006; Uc *et al.*, 2009) and mild cognitive impairment at baseline (Hobson and Meara, 2004; Janvin *et al.*, 2006; Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). The presence of apathy (Dujardin *et al.*, 2009; Butterfield *et al.*, 2010; Ziropadja *et al.*, 2012), depression (Starkstein *et al.*, 1992), disease duration (Green *et*

al., 2002; Hobson and Meara, 2004), orthostatic hypotension (Allcock *et al.*, 2006), reduced educational level (Green *et al.*, 2002), hyposmia (Bohnen *et al.*, 2010; Baba *et al.*, 2012) and gastrointestinal or urological symptoms (Uc *et al.*, 2009) in the context of cognitive decline are additional potential risk factors that require further evaluation.

The use of anticholinergic drugs to treat bladder dysfunction, mood and pain is still widespread, especially amongst older patients, and over-the-counter medications which antagonise muscarinic receptors, such as diphenyhydramine (an antihistamine), are also freely available. Many PD patients with urinary urgency, low mood or pain may be treated with these drugs, and evidence suggests that they may accelerate cognitive decline (Ehrt *et al.*, 2010). Thus, chronic use (more than two years) of antimuscarinic drugs in patients with PD has been associated with more than twice the number of cortical plaques or tangles compared to those who had used the drugs for less than two years or not at all (Perry *et al.*, 2003). Although no difference was noted between those with documented confusion, it suggests that anticholinergic drugs increase Alzheimer pathology and hence may contribute to cognitive impairment in PD. It should, however, be noted that this work has not yet been replicated and therefore should be treated with some caution. Additionally, pharmacological studies have shown a deficit in memory in those with Parkinson's disease treated with anticholinergic drugs when compared to those who had not received this class of drugs (Dubois *et al.*, 1987; Cooper *et al.*, 1992), and scopolamine, a drug with potent antimuscarinic activity, caused a transient reversible subcortico-frontal syndrome when administered at subclinical doses to those with PD but not control participants (Bedard *et al.*, 1999). More recently, in a longitudinal cohort study, those PD subjects who had taken drugs with proven anticholinergic activity had a significant median decline in their Mini Mental State Examination (MMSE) score of 6.5 points over 8 years, compared to just 1 point in those who had not used those drugs (Ehrt *et al.*, 2010). This class effect of drugs is not just limited to PD patients. In older adults, it has long been recognised that anticholinergic activity is associated with delirium (Han *et al.*, 2001; Tune, 2001), cognitive decline (Mulsant *et al.*, 2003) and even slowing of gait speed (Nebes *et al.*, 2007). Hence, anticholinergic drug use is likely to be an additional risk factor for cognitive decline in PD.

The majority of the risk factors discussed above have been assessed in prevalent PD cohorts. In a longitudinal community-based incident PD cohort in the UK, age of greater than or equal to 72 combined with posterior cortical deficits (impaired semantic fluency and pentagon copying) had an odds ratio of 88 for dementia within the first 5 years of diagnosis (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). The strongest independent risk factor, however, for cognitive decline was the presence of the microtubule-associated protein tau (*MAPT)* H1/H1 genotype (Goris *et al.*, 2007; Williams-Gray *et al.*, 2009a), with an estimated odds ratio of 12.1. This is supported by a more recent study demonstrating that the *MAPT* H1 haplotype was found in considerably more patients with PDD than controls (Seto-Salvia *et al.*, 2011). The mechanism of action is speculated to be that of protein aggregation.

Other genes that may confer an increased risk of cognitive decline in PD have been investigated. Whilst apolipoprotein E (*APOE*) has been established as a risk factor for the development of Alzheimer's disease (AD), studies in cognitive decline in PD have produced conflicting results. The three common alleles of *APOE* (ε2, ε3 and ε4) produce differing risks for AD, with ε2 being protective and ε4 both increasing the risk and reducing the age at onset of AD (Farrer *et al.*, 1997). Mechanisms by which *APOE* ε4 may operate include promoting the deposition of amyloid-β (Aβ) throughout the cortex, accelerating the configuration of amyloid from benign diffuse amyloid accumulation to neurotoxic β-pleated sheet aggregations, and increasing the frequency of neuritic plaque formation with associated cholinergic loss (Cummings *et al.*, 1998). Given the growing body of evidence implicating Aβ in the pathophysiology of PDD (Masliah *et al.*, 2001; Pletnikova *et al.*, 2005; Lashley *et al.*, 2008; Clinton *et al.*, 2010; Siderowf *et al.*, 2010; Compta *et al.*, 2011b), it would seem likely that the *APOE* ε4 genotype would increase the risk of PDD. However, the largest case-control study to date in 528 PD patients and 512 controls did not confirm this theory, with no association found between *APOE* ε4 carriers and cognitive decline (Williams-Gray *et al.*, 2009b). The same paper included an updated meta-analysis of 4,198 PD cases and 10,066 controls, where only a modestly increased risk of cognitive decline was found in *APOE* ε4 carriers (odds ratio 1.74, 95% confidence interval 1.36 - 2.23). However, the authors cautioned against interpreting this as a positive result, citing small sample sizes, heterogeneous odds ratios and possible publication bias as confounding factors, with further longitudinal studies of several thousand participants required to draw

reliable conclusions (Williams-Gray *et al.*, 2009b). Although a more recent longitudinal study did find that PD patients who were *APOE* ε4 carriers experienced more rapid cognitive decline (Morley *et al.*, 2012), the number of PD participants was relatively small (212) and the follow up was relatively short, with no control group included. Therefore, at present, there is insufficient evidence to ascertain whether *APOE* influences cognition in PD.

Mutations in the glucocerebrosidase (*GBA*) gene increase the risk of PD (Sidransky *et al.*, 2009) and are over-represented in other Lewy body diseases (Clark *et al.*, 2009). There is also growing evidence that carriers of *GBA* mutations are at increased risk of cognitive decline (Neumann *et al.*, 2009; Alcalay *et al.*, 2012; Seto-Salvia *et al.*, 2012; Winder-Rhodes *et al.*, 2013). Biological plausibility for this theory originates in mouse models of Gaucher's disease (caused by homozygous loss-of-function *GBA* alleles), where an accumulation of α–synuclein/ubiquitin aggregates within the hippocampal neurones was noted, with a corresponding memory deficit (Sardi *et al.*, 2011). This is corroborated by post-mortem work in PD patients demonstrating more widespread cortical Lewy body deposition in those with *GBA* mutations than matched PD controls (Neumann *et al.*, 2009).

Other genes implicated in cognitive impairment in PD include butyrylcholinesterase-K (*BuChE-K*), catechol-*O*-methyltransferase (*COMT*), brain-derived neurotrophic factor (*BDNF*) and *SNCA* (Foltynie *et al.*, 2005; Williams-Gray *et al.*, 2007b; Ikeuchi *et al.*, 2008; Lane *et al.*, 2009; Williams-Gray *et al.*, 2009a). Duplications and triplications of *SNCA* are associated with parkinsonism and dementia (Ikeuchi *et al.*, 2008), and variations in *BuChE-K* act synergistically with *APOE* in Lewy body dementias (Lane *et al.*, 2009). Polymorphisms in the *COMT* (val^{158}met) genotype produce differing levels of dopamine release in the dorsolateral prefrontal cortex and exert differing cognitive influences during the PD time course, with met homozygotes performing worse on tests of executive function in early disease and better as the disease progresses; the pattern is reversed for those with val/val polymorphisms (Williams-Gray *et al.*, 2007b; Williams-Gray *et al.*, 2009a). However, COMT genotype does not influence overall cognitive decline and risk of dementia (Williams-Gray *et al.*, 2009a; Morley *et al.*, 2012).

In summary, many risk factors have been postulated for cognitive decline in PD. The most robust are increasing age, male gender, visual hallucinations, motor severity and

phenotype, cognitive impairment at baseline and *MAPT* H1/H1 haplotype. Variations in risk factors are likely to partly explain the heterogeneity of cognitive impairment seen in PD.

1.6.3 Profile of cognitive impairment in PDD

Although heterogeneous in nature (Verleden *et al.*, 2007), the cognitive profile of PDD differs from that of AD, with deficits in attention being one of the strongest predictors of PDD (Bronnick *et al.*, 2007). Furthermore, attentional deficit is an important determinant of inability to perform instrumental and physical activities of daily living, even after controlling for age, sex, motor function and other cognitive profiles (Bronnick *et al.*, 2006). Scores of attention also predict falls in PD (Allcock *et al.*, 2009). In addition to impairments in attention, visuospatial and executive dysfunction are characteristic, and cognitive symptoms typically fluctuate (Troster, 2008). Patients with PDD tend to perform worse than AD patients on demanding attentional tasks and in tasks requiring executive skills; these differences seen between the disorders become more pronounced as dementia progresses. Executive problems are a common feature of PDD (Aarsland *et al.*, 2003b) and lead to disturbances in adaptive, goal-directed behaviour. Executive dysfunction can manifest as difficulties in planning, set-shifting, mental flexibility, abstraction, solving multiple step problems, selection of information, resisting cognitive interference and in the retrieval of information (Kehagia *et al.*, 2010). Although many patients with PDD have a cognitive profile resembling that of DLB with a fronto-striatal executive syndrome and visuospatial deficits, a proportion may display more mnemonic and other temporo-parietal disturbances (Kehagia *et al.*, 2010): this spectrum is likely to represent differing contributions of underlying pathologies. The characteristics of the cognitive deficits seen in PDD and neuropsychological tests that may be useful are shown in Table 1-4.

Table 1-4 Pattern of cognitive deficits in PDD and example of tests used in diagnosis

Cognitive Domain	Description of cognitive deficit	Examples of neuropsychological tests
Executive	↓↓ verbal fluency, ↓ set-shifting, planning & concept formation; may influence memory functions	Verbal fluency (semantic & phonemic); ToL/SoC (CANTAB); WCST; Stroop
Attention & Working Memory	Fluctuating alertness; auditory and visual attentional deficits	Digit span; Trail Making Test
Memory	Episodic memory less affected than AD; ↓ visual & verbal memory; recall > recognition affected early on	Free and cued recall, e.g. CVLT or RAVLT
Visuospatial & constructural function	Globally impaired visual perception (predisposes to VH); impaired size and form discrimination; ↓ face recognition; marked impairment on clock-drawing test	Clock-drawing test; Benton's judgement of line orientation
Language	Less impairment than AD; less affected until severe disease	Boston naming test

ToL Tower of London test; SoC Stocking of Cambridge (both from CANTAB [Cambridge Neuropsychological Test Automated Battery]); WCST Wisconsin Card Sorting Test; CVLT California Verbal Learning Test; RAVLT Rey's Auditory Verbal Learning Test; VH visual hallucinations

1.6.4 Clinical features of PDD

The onset of cognitive decline in PDD is insidious, with an associated slow progression of disease. In a prospective study of prevalent PD in Norway, participants were followed longitudinally for up to eight years and compared with AD and control subjects (Aarsland *et al.*, 2004). The mean annual decline in MMSE score in all PD patients was 1.1 but varied widely; those who developed PDD had a mean annual decline of 2.3, which was similar to that seen in AD (2.6). A comparable decline in MMSE score was seen in a later study of PDD and DLB, where a mean fall of 4.5 points (3.9 for DLB) was seen over a two-year period (Burn *et al.*, 2006).

Neuropsychiatric features are ubiquitous in PDD, and indeed their presence forms supportive criteria for the diagnosis of dementia in PD (Emre *et al.*, 2007). In a multi-centre study of 537 PDD patients, 89% of subjects had at least one symptom on the Neuropsychiatric Inventory (NPI) and 77% had two or more symptoms (Aarsland *et al.*, 2007a). Symptom burden was associated with more advanced PD and more severe

dementia. Visual hallucinations (VH) occur more commonly in PDD than PD, with frequencies of between 30-50% for PD (Fenelon *et al.*, 2000; Holroyd *et al.*, 2001; Williams and Lees, 2005) but 45-65% in PDD (Aarsland *et al.*, 2007a; Goetz *et al.*, 2008a). VH are more common than auditory hallucinations, which in turn occur more frequently than tactile or olfactory hallucinations (Fenelon and Alves, 2010). More benign visual phenomena such as a sense of 'passage' or 'presence' may occur early in PD, with subsequent illusions, simple visual hallucinations then complex formed VH as PDD progresses (Fenelon and Alves, 2010). Delusions including paranoid ideation and phantom boarder may be present in over a quarter of patients (Goetz *et al.*, 2008a). Other neuropsychiatric features commonly encountered in PDD are depression, anxiety and apathy (Aarsland *et al.*, 2007a).

Lastly, other clinical characteristics of PDD include an over-representation of the PIGD motor phenotype (Burn *et al.*, 2006), autonomic dysfunction (Allan *et al.*, 2007) and sleep disturbance. RBD is a risk factor for the development of dementia in PD (Postuma *et al.*, 2012), and both excessive daytime somnolence and poor sleep quality are commonly reported (Boddy *et al.*, 2007).

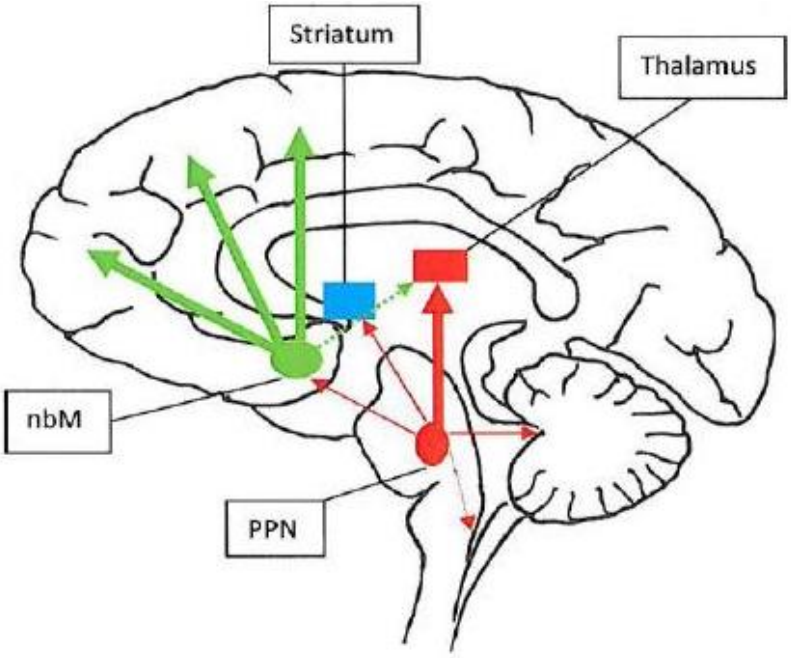
1.6.5 Pathogenesis

The pathophysiology of PDD is poorly understood and may vary between individuals, with post-mortem studies producing conflicting findings. Potential contributors to the underlying pathology include LBs and α-synuclein, Aβ plaque deposition, tau pathology, neuronal and synaptic loss, diffuse cerebrovascular disease and neurotransmitter changes, including degeneration of the cholinergic systems. Braak hypothesised that as PD progresses to stages 5 and 6, LB pathology reaches the limbic and neocortical regions (Braak *et al.*, 2004): indeed there is evidence that the development of dementia occurs with this disease progression (Braak *et al.*, 2005). Several studies have implicated these limbic and cortical LBs as the main substrate for driving cognitive decline in PD (Hurtig *et al.*, 2000; Mattila *et al.*, 2000; Apaydin *et al.*, 2002; Aarsland *et al.*, 2005a). However, recent studies highlight the importance of Aβ deposition in the development of PDD (Sabbagh *et al.*, 2009; Halliday and McCann, 2010; Compta *et al.*, 2011b). Furthermore, a synergistic interaction between α-synuclein and Aβ accumulation has been found both *in vitro* (Pletnikova *et al.*, 2005; Lashley *et al.*, 2008) and in transgenic mice models (Masliah *et al.*, 2001; Clinton *et al.*,

2010), with evidence that Aβ promotes α-synuclein fibrillization and hence may influence the progression of cognitive decline (Masliah *et al.*, 2001). The observation that Aβ plaque burden, specifically diffuse plaque load, correlates with overall cortical LB burden strengthens the concept of a synergistic effect between these major pathological proteins (Lashley *et al.*, 2008). Age at disease onset and disease duration also contributes to the pathological findings associated with PDD (Kempster *et al.*, 2010), with older age at disease onset and cortical Aβ score predicting shorter time to dementia onset (Compta *et al.*, 2011b). Furthermore, older age increases the likelihood of age-associated pathologies; even in older subjects without PD, LB pathology is common and is associated with AD-type changes (Zaccai *et al.*, 2008). Age can also be associated with increased vascular burden, which in turn may contribute to PDD in some patients (Jellinger and Attems, 2008; Sonnen *et al.*, 2010). In addition, synaptic dysfunction in LB diseases could further influence the pathological process, as illustrated in a study of DLB demonstrating a post-synaptic loss of dendritic spines due to aggregation of pre-synaptic α-synuclein (Kramer and Schulz-Schaeffer, 2007).

The variability and fluctuations seen in the clinical aspects of PDD suggests that at least some of the cognitive deficits may be the result of functional neurotransmitter imbalance or synaptic malfunction, rather than a structural substrate: In this context ACh loss almost certainly contributes to PDD. Through modulation within open circuits connecting structures to the basal ganglia, the cholinergic system has widespread influence upon a number of functions, including cognition, attention, gait and postural stability (Yarnall *et al.*, 2011). In the brain, ACh is supplied by three main sources (Figure 1-2). Neuronal loss within the pedunculopontine nucleus (PPN) and nucleus basalis of Meynert (nbM), in particular, are likely to be important in the pathogenesis of PDD. The PPN is located in the caudal mesopontine tegmentum and comprises a significant population of cholinergic neurons, although other neurotransmitters in this structurally heterogeneous nucleus include glutamate, gamma amino-butyric acid (GABA) and dopamine (Jenkinson *et al.*, 2009). The cholinergic neurones of the PPN and the nbM are known loci of degeneration in PD, with the striatal cholinergic system remaining relatively preserved (Calabresi *et al.*, 2006). The nbM undergoes profound degeneration in PD (Arendt *et al.*, 1983; Nakano and Hirano, 1984), with more severe cholinergic loss being associated with cognitive impairment (Zweig *et al.*, 1993; Jellinger, 2006).

Figure 1-2 Schematic representation of the cholinergic output in the cortex



Cholinergic interneurons in the striatum are shown in blue. The pedunculopontine nucleus (PPN; shown in red) provides the majority of cholinergic input to the thalamus, with other projections to the nucleus basalis of Meynert (nbM), striatum, substantia nigra, subthalamic nucleus, globus pallidus interna, cerebellum and spinal cord. The nbM (shown in green) sends cholinergic projections to the cerebral cortex, and also to thalamic nuclei.

Further support for a cholinergic basis for cognitive impairment in PD originates from autopsy studies indicating that a midfrontal cholinergic deficit is more severe in patients with Lewy bodies than in AD (Tiraboschi *et al.*, 2000). Additionally, in AD with diffusely distributed Lewy bodies, cholinergic deficits in the caudate, frontal, temporal and parietal cortex are more pronounced than in pure AD (Langlais *et al.*, 1993). Although previously much of the evidence for altered cholinergic transmission was derived *in vitro*, the advent of positron emission tomography (PET) offers the opportunity of measuring acetylcholinesterase (AChE), a surrogate marker for cortical cholinergic activity, *in vivo*. AChE PET imaging in AD and PDD of equal global dementia severity demonstrated a greater reduction in cortical AChE activity in PDD compared to AD (Bohnen *et al.*, 2003). The reduction in cortical AChE activity was also greater in non-demented PD subjects compared to those with AD. More specifically, a reduction in AChE activity correlated with poorer performance in tests of executive and attentional function (Bohnen *et al.*, 2006b), hallmarks of the type of cognitive impairment seen in PD. Thus forebrain cholinergic system degeneration seems to

occur early in PD, with deteriorating cognition associated with more severe cortical cholinergic loss (Bohnen *et al.*, 2003; Shimada *et al.*, 2009; Bohnen and Albin, 2011).

In addition to ACh, other neurotransmitters have been implicated in the pathophysiology of cognitive impairment and may interact with the cholinergic system. Calabresi and colleagues proposed a convergent neurochemical model to explain cognitive deficits that occur in PD (Calabresi *et al.*, 2006). They suggested that noradrenergic and serotonergic deficits in PD (supported by post-mortem work (Scatton *et al.*, 1983)) may influence dopamine and ACh release, which in turn affects synaptic plasticity and has a detrimental effect on the storage of neural information (Calabresi *et al.*, 2006). Neurotransmitters may also interact with other pathophysiological processes and influence cognition. Evidence of a neuromodulatory influence of Aβ on cholinergic function has been demonstrated, with concentrations of Aβ negatively affecting ACh synthesis and release (Kar *et al.*, 2004). Serotonin neurotransmission has been shown to alter cortical Aβ levels, with a reduction in plaque load demonstrated in both a transgenic mice model of AD and in healthy older adults following administration of a selective serotonin reuptake inhibitor (Cirrito *et al.*, 2011). In PD, a recent small imaging study demonstrated an inverse correlation between serotonin and amyloid binding (Kotagal *et al.*, 2012b).

In summary, the pathophysiological process underlying PDD is heterogeneous and is likely to differ between individuals. The co-existence of α-synuclein, Aβ and tau deposition; neuronal loss; and neurochemical dysfunction has similarities with AD and suggests there may be common mechanisms triggering neurodegeneration and fibrillary protein aggregation (Jellinger, 2010a). Understanding this pathogenic process in PDD is important for prognostication and developing appropriate therapeutic agents in the future.

1.6.6 Biomarkers in PDD

A biomarker is defined as a biological characteristic that is objectively measured and evaluated as an indicator of normal biological or pathologic processes or of pharmacologic responses to a therapeutic intervention. There are many biomarkers that have been proposed as possible candidates for the development of PDD; these cover different modalities and are shown in Table 1-5 (Svenningsson *et al.*, 2012).

Table 1-5 Studies of potential biomarkers in PDD (adapted from Svenningsson 2012)

Biomarker	Biomarker subtype	Findings	Reference(s)
Imaging	Structural MRI	Atrophy temporal, parietal & occipital cortices in PDD	(Burton *et al.*, 2004; Beyer *et al.*, 2007; Song *et al.*, 2011; Weintraub *et al.*, 2011; Melzer *et al.*, 2012)
		Hippocampal plus parieto-temporal atrophy predicted cognitive impairment	(Weintraub *et al.*, 2012)
	^{18}FDG-PET	Widespread cortical metabolism in PDD; especially temporal, parietal & occipital areas	(Huang *et al.*, 2007; Jokinen *et al.*, 2010; Klein *et al.*, 2010)
		↓ perfusion occipital & posterior cingulate cortices predicted dementia	(Bohnen *et al.*, 2011)
	PiB PET	No difference in PiB amyloid binding in PDD vs. PD-CN	(Foster *et al.*, 2010; Gomperts *et al.*, 2012)
		PiB binding at baseline predicted ↓ cognition during longitudinal follow-up	(Gomperts *et al.*, 2013)
	AChE PET	Widespread ↓AChE activity in PDD: this correlated with attention/ executive function	(Bohnen *et al.*, 2003; Bohnen *et al.*, 2006b; Shimada *et al.*, 2009)
	SPECT perfusion	Hypoperfusion parietal/ occipital regions in cognitive impairment	(Firbank *et al.*, 2003; Nobili *et al.*, 2009)
CSF	Aβ42	↓ PDD>PD-MCI>PD-CN; Aβ correlated with memory impairment	(Mollenhauer *et al.*, 2006b; Compta *et al.*, 2009; Alves *et al.*, 2010; Montine *et al.*, 2010)
		↓ Aβ42 predicted cognitive decline during longitudinal follow-up	(Siderowf *et al.*, 2010)
	tau	Mixed results	(Mollenhauer *et al.*, 2006b; Compta *et al.*, 2009; Alves *et al.*, 2010; Montine *et al.*, 2010)
	α-synuclein	↓ total α-syn in PD & DLB cf controls/AD	(Hong *et al.*, 2010; Mollenhauer *et al.*, 2011b)
Plasma	Epidermal growth factor	↓ EGF levels predicted cognitive decline	(Chen-Plotkin *et al.*, 2011)
Neuro-physiology	EEG	Low background rhythm frequency predicted cognitive decline	(Klassen *et al.*, 2011)
	SAI	Abnormal SAI in PDD and PD-MCI cf controls	(Celebi *et al.*, 2012; Yarnall *et al.*, 2013)

Neuroimaging methods used to predict PDD have included both structural and functional techniques. These are discussed in some detail in section 1.7.4, but in brief, structural imaging has demonstrated that atrophy of the parietal, temporal and occipital cortices occur in cross-sectional studies of those with PDD or mild cognitive impairment (PD-MCI) compared with those with normal cognition (PD-CN) (Burton *et al.*, 2004; Beyer *et al.*, 2007; Song *et al.*, 2011; Weintraub *et al.*, 2011; Melzer *et al.*, 2012). In a recent longitudinal study, an AD-type pattern of atrophy on MRI predicted long-term cognitive decline in PD subjects (Weintraub *et al.*, 2012). Cortical hypometabolism and hypoperfusion were noted in similar areas using positron emission tomography (PET) and single photon emission computed tomography (SPECT), respectively, in those with PD and cognitive impairment (Firbank *et al.*, 2003; Huang *et al.*, 2007; Nobili *et al.*, 2009; Jokinen *et al.*, 2010; Klein *et al.*, 2010); with a longitudinal study indicating that cerebral hypometabolism in the visual association and posterior cingulate cortices may predict the development of dementia (Bohnen *et al.*, 2011). PET imaging using radiolabelled metabolites of acetylcholinesterase (AChE) as a marker of ACh activity has demonstrated widespread reductions in AChE in those with PDD compared with AD and control participants (Bohnen *et al.*, 2003; Shimada *et al.*, 2009). Lastly, in terms of imaging, a recent study of amyloid burden detected using Pittsburgh Compound B (PiB) PET has shown promise in predicting cognitive decline in PD, with a higher baseline PiB retention and a diagnosis of PD-MCI associated with worsening cognition at follow-up (Gomperts *et al.*, 2013). Greater amyloid deposition at baseline also predicted executive impairment over time.

Other biomarkers that may be used to predict PDD include cerebrospinal fluid (CSF), plasma proteins and neurophysiological techniques. CSF and short latency afferent inhibition (SAI) as biomarkers are discussed in further detail in Chapter 3 and 4, respectively. Epidermal growth factor (EGF) is a plasma protein that has been postulated to support dopaminergic neurons as a neurotrophic factor, and has been associated with both baseline cognition and an eight-fold risk of progression to dementia for those with EGF levels in the lowest quartile (Chen-Plotkin *et al.*, 2011). In a smaller study of *de novo* PD subjects followed over two years, baseline EGF levels predicted poorer performance in frontal and temporal cognitive function (Pellecchia *et al.*, 2013). Finally, quantitative EEG may be used as a predictive biomarker for the development of dementia (Klassen *et al.*, 2011). In a prospective study of 106 non-

demented PD participants, those with lower than median background rhythm frequency were 13-times more likely to develop dementia than those with normal rhythms over a mean duration of 3.3 years. The authors hypothesised that disruption in the default network (defined as the network of brain region activity that occurs during the cognitive resting state), perhaps due to amyloid deposition, could explain these findings (Klassen *et al.*, 2011).

In conclusion, many biomarkers have been studied as possible predictors of dementia in PD. Due to the complexity and heterogeneity of the underlying pathophysiological processes it is unlikely that a single biomarker will predict PDD. But, taken together, these laboratory, imaging and clinical risk factors may allow clinicians to predict which patients are most likely to progress to this state, thus allowing better use of targeted interventions and improved prognostication.

1.6.7 Diagnosis of PDD

Based on a comprehensive literature review of the clinical, psychological and behavioural features of PDD, the Movement Disorder Society (MDS) Task Force developed clinical criteria for the diagnosis of probable and possible PDD in 2007 (Table 1-6 and Table 1-7) (Emre *et al.*, 2007). The diagnosis of PDD should occur within the context of PD, with impairment in two or more cognitive domains (attention, executive, visuospatial or memory) that is severe enough to affect activities of daily living. Behavioural features such as apathy, depression, anxiety, excessive daytime somnolence, hallucinations and delusions are supportive features but are not necessary for the diagnosis. The MDS Task Force also produced recommendations in the same year for the operationalization of the diagnostic criteria based on two levels (Dubois *et al.*, 2007). Level I assessment can be used by any clinician and does not require neuropsychological expertise (Table 1-8). MMSE score should be < 26, with impairment in two or more cognitive functions measured by: counting months backward or serial 7's (attention); phonemic fluency or clock-drawing test (executive function); pentagon copying (visuospatial); or 3-word recall from the MMSE (memory). The presence of apathy, depressed mood, delusions or excessive daytime sleepiness, as measured by the four-item Neuropsychiatric Inventory, may support the diagnosis of probable PDD. Level II assessment requires detailed neuropsychological testing, and

is recommended when there is uncertainty regarding the diagnosis or in the context of research or pharmaceutical trials.

Table 1-6 Features associated with dementia in PD (Emre *et al.,* 2007)

I. Core features
1. Diagnosis of PD according to QSBB criteria
2. A dementia syndrome with insidious onset and slow progression, developing within the context of established Parkinson's disease and diagnosed by history, clinical, and mental examination, defined as:
• Impairment in >1 cognitive domain
• Representing a decline from premorbid level
• Deficits severe enough to impair daily life (social, occupational, or personal care), independent of the impairment ascribable to motor or autonomic symptoms
II. Associated clinical features
1. Cognitive features:
• *Attention*: Impaired. Impairment in spontaneous and focused attention, poor performance in attentional tasks; performance may fluctuate during the day and from day to day
• *Executive functions*: Impaired. Impairment in tasks requiring initiation, planning, concept formation, rule finding, set shifting or set maintenance; impaired mental speed (bradyphrenia)
• *Visuospatial functions*: Impaired. Impairment in tasks requiring visual-spatial orientation, perception, or construction
• *Memory*: Impaired. Impairment in free recall of recent events or in tasks requiring learning new material, memory usually improves with cueing, recognition is usually better than free recall
• *Language*: Core functions largely preserved. Word finding difficulties and impaired comprehension of complex sentences may be present
2. Behavioural features:
• Apathy: decreased spontaneity; loss of motivation, interest, and effortful behaviour
• Changes in personality and mood including depressive features and anxiety
• Hallucinations: mostly visual, usually complex, formed visions of people, animals or objects
• Delusions: usually paranoid, such as infidelity, or phantom boarder, delusions
• Excessive daytime sleepiness
III. Features which do not exclude PDD, but make the diagnosis uncertain
• Co-existence of any other abnormality which may by itself cause cognitive impairment, but judged not to be the cause of dementia, e.g. presence of relevant vascular disease in imaging
• Time interval between the development of motor and cognitive symptoms not known
IV. Features suggesting other conditions or diseases as cause of mental impairment, which, when present make it impossible to reliably diagnose PD-D
• Cognitive and behavioural symptoms appearing solely in the context of other conditions such as: - Acute confusion (due to systemic diseases or abnormalities; drug intoxication) - Major Depression according to DSM IV
• Features compatible with "Probable Vascular dementia" criteria

Table 1-7 Criteria for diagnosis of probable and possible PDD (Emre *et al.,* 2007)

Probable PD-D
A. Core features: Both must be present
B. Associated clinical features:
• Typical profile of cognitive deficits including impairment in at least two of the four core cognitive domains
• The presence of at least one behavioural symptom (apathy, depressed or anxious mood, hallucinations, delusions, excessive daytime sleepiness) supports the diagnosis of Probable PDD
C. None of the group III features present
D. None of the group IV features present
Possible PD-D
A. Core features: Both must be present
B. Associated clinical features:
• Atypical profile of cognitive impairment in one or more domains, such as prominent or receptive-type (fluent) aphasia, or pure storage-failure type amnesia (memory does not improve with cueing or in recognition tasks) with preserved attention
• Behavioural symptoms may or may not be present
OR
C. One or more of the group III features present
D. None of the group IV features present

Table 1-8 Algorithm for diagnosing PDD at Level I (Dubois *et al.,* 2007)

1 A diagnosis of PD based on the QSBB criteria
2 PD developed prior to the onset of dementia
3 MMSE < 26
4 Cognitive deficits severe enough to impact daily living (based on caregiver interview or Pill Questionnaire)
5 Impairment in ≥2 of the following tests:
Months reversed or Serial 7's backward from 100 (≥2 incorrect responses)
Phonemic fluency (<10 words in 60 sec) or Clock drawing
MMSE Pentagons
3-Word recall

1.6.8 Treatment of PDD

The initial management of PDD, after careful explanation of the diagnosis, starts with the withdrawal of potential contributing medications such as non-selective anticholinergic drugs. Rationalisation and simplification of PD treatment by the gradual removal of dopamine agonists, monoamine oxidase inhibitors then catechol-*O*-methyltransferase inhibitors should be done slowly to avoid worsening motor

symptoms. Co-existent depression, anxiety and RBD should be treated, although no randomised controlled trials in these non-motor symptoms in PDD specifically have been reported. If psychotic symptoms predominate, clozapine has been shown to be efficacious (Seppi *et al.*, 2011), although in practice quetiapine may be used more commonly due to the potential risk of agranulocytosis and subsequent monitoring required with clozapine.

Cholinesterase inhibitors (ChEIs) form the mainstay of treatment of PDD: A recent Cochrane review supports their use and showed a positive overall effect on global assessment, cognitive function, behavioural disturbance and activities of daily living (Rolinski *et al.*, 2012). The only two adequately powered randomised controlled trials of ChEIs in PDD are the EXPRESS (Emre *et al.*, 2004) and the EDON (Dubois *et al.*, 2012) study. The EXPRESS study enrolled 541 participants with mild to moderate PDD (mean MMSE 19/30), who were randomised to receive either rivastigmine (3-12mg) or placebo for 24 weeks. Those who received the active drug had a moderate improvement in the primary and secondary outcomes, with a mean improvement of 2.1 points (out of 70) in the Alzheimer's Disease Assessment Scale (ADAS-cog), compared with a 0.7 point worsening in the placebo group. Improvements in executive and attentional functions, behavioural symptoms and activities of daily living were significantly better in the treatment group. There were, however, marked variations within treatment responses. These improvements were maintained up to 48 weeks in an open-label extension of the study (Poewe *et al.*, 2006). The more recent EDON study recruited 550 PDD subjects (mean MMSE 21/30) and randomised to placebo or donepezil (5 or 10mg) for 24 weeks. Although no difference in the primary endpoint, the ADAS-cog, was seen in the intention-to-treat population by the pre-defined statistical model, when the treatment-by-country interaction term was removed from the model the improvements observed were similar to the EXPRESS study. Secondary end-points of global cognition using MMSE score and tests of executive and attention were significantly better in the donepezil group, although no difference was seen in activities of daily living or behavioural symptoms. Adverse events in both the EXPRESS and EDON study were mild, but largely compromised gastrointestinal side-effects and increased tremor: there is some evidence that these may be reduced with administration of a rivastigmine patch instead of capsules (Emre *et al.*, 2011).

Randomised controlled studies have investigated the effect of the partial NMDA-receptor antagonist memantine in PDD, with generally negative results (Aarsland *et al.*, 2009a; Leroi *et al.*, 2009; Emre *et al.*, 2010). Some improvement in cognition was noted and the drug was well tolerated, but the MDS Task Force have judged that as yet, there is insufficient evidence to recommend its use in this context (Seppi *et al.*, 2011).

Improving knowledge of the underlying pathogenesis of PDD should translate to identifying novel targets and developing new treatments for PDD. As the disease is heterogeneous, different patients may benefit from different treatments. The development of disease-modifying treatments that may halt or limit the progression of the pathology is vital to prevent the surge of dementia cases that is predicted in the future. In view of the overlap of pathology between PDD and AD, work in AD may have direct translational benefit to PDD: One example of this is anti-amyloid strategies. A recent study in triple transgenic mouse models of AD demonstrated that subcutaneous injections of apomorphine improved memory function and decreased intraneuronal Aβ and phosphorylated tau levels compared with controls (Himeno *et al.*, 2011). It is therefore conceivable that apomorphine, which may inhibit Aβ fibrillization (Lashuel *et al.*, 2002), could be used as a modifier of amyloid deposition in PDD. Other potential targets for treatment include prevention of α-synuclein aggregation, trophic factors and anti-inflammatory strategies.

Lastly, in parallel with the involvement of the multidisciplinary team, the management of PDD should include a discussion of the cognitive and psychological benefits of exercise (Tanaka *et al.*, 2009; Cruise *et al.*, 2011) and cognitive training (Paris *et al.*, 2011). These certainly deserve consideration in future clinical trials, and are likely to be instituted in addition to therapeutic approaches.

1.7 Mild cognitive impairment in PD

Mild cognitive impairment (MCI) is defined as a transitional state between normality and dementia, with little or no impairment in daily functioning and subjective or objective cognitive impairment (Petersen, 2004). Data from epidemiological studies demonstrates that 10-15% of subjects with MCI per year progress to dementia, largely of the Alzheimer's-type (Petersen *et al.*, 2009).

The concept of mild cognitive impairment in PD (PD-MCI) has received increasing attention recently. Although not analogous to the field of AD, where the significance of MCI may be better understood, it is now recognised that PD-MCI may represent a pre-dementia state and is associated with increased healthcare costs (Vossius *et al.*, 2011), poorer self-reported quality of life (Klepac *et al.*, 2008; Leroi *et al.*, 2012) plus subtle impairments in instrumental activities of daily living (Rosenthal *et al.*, 2010; Leroi *et al.*, 2012).

1.7.1 Epidemiology of PD-MCI

The prevalence of PD-MCI varies widely depending on the study population (community- or hospital-based, incident or prevalent cases, age and disease severity, use of controls), the neuropsychological tools used and the definition of MCI applied (Table 1-9). Not surprisingly, studies of incident, drug-naïve patients have demonstrated the lowest prevalence of PD-MCI (14.8 (Poletti *et al.*, 2012) and 18.9 % (Aarsland *et al.*, 2009b)), with longer disease duration such as the work from Janvin and colleagues (Janvin *et al.*, 2006) or retrospective studies (Sollinger *et al.*, 2010) producing higher figures (52.8% for both). However, the presence of PD-MCI was 57% in a longitudinal study of patients with relatively short disease duration (Williams-Gray *et al.*, 2007a). In an attempt to overcome methodological variation, Aarsland and colleagues performed a meta-analysis of over 1,000 patients encompassing eight centres in Europe and the USA (Aarsland *et al.*, 2010). A mean of 25.8% (95% CI, 23.5–28.2) were classified as PD-MCI, based on age- and education-corrected z scores of less than 1.5 SD below normative values in one or more cognitive domains, although significant variations were seen across the separate centres studied.

Table 1-9 Demographic and clinical features of studies in PD-MCI

Reference	PD	Controls	Mean age PD	Disease duration (years)	Type of study	Country of origin	MCI definition	Cognitive domains assessed	%MCI	Cognitive profile	Other comments
1. (Pai and Chan, 2001)	102	0	68	NA	Cross-sectional; hospital clinic; prevalent cases	Taiwan	<1.5 SD below age- and educationally-matched groups; Cognitive Ability Screening Instrument (CASI) used (max score 100)	Remote memory, recent memory, attention, mental manipulation, orientation, abstract thinking, language, drawing, & verbal fluency	38.2	Recent memory, verbal fluency, abstract thinking, & orientation all impaired to a high degree	1. Higher educational level did not protect against cognitive decline 2. CASI has not been validated in PD
2.(Foltynie *et al.*, 2004)	159	0	70.6	NA Incident	Cross-sectional; community; incident	UK	<1 SD below normative values for PRM and TOL	Temporal lobe plus frontostriatal impairment tested with PRM and TOL, respectively; MMSE also assessed	30.1*	9.6% fronto-striatal impairment; 8.2% temporal lobe deficit; 11% global deficits*	*1.Note 159 patients but 13 had MMSE<24; in total 36% therefore had some cognitive impairment 2. Patients with global or frontal impairments were significantly older, had higher UPDRS motor scores, & lower premorbid IQs than CN
3.(Muslimovic *et al.*, 2005)	115	70	66.2	1.6	Cross-sectional; hospital clinic; early PD	Nether-lands	<2 SD below mean score of matched controls in ≥3 neuropsychological tests	Psychomotor speed, attention, language, memory, executive functions, & visuospatial/constructive (28 tests administered)	23.5 (4.3 control group)	Attention, executive & memory function most impaired cf controls	1. Age at disease onset independent predictor of cognitive ↓ 2. Impaired were older, male, had later disease onset, ↑ disease severity, ↑depression scores, & more severe axial & speech symptoms
4.(Janvin *et*	72	38	71.0	aMCI	Longitudi	Norway	≤1.5 SD below	Short-time	52.8	naMCI-sd	1. 62% MCI demented at 4

***al.*, 2006)**				10.6; naMCI-sd 11.4; multiple domains 11.6; CN 12.2	nal; community; prevalent cases		mean score of controls in 1, 2 or 3 neuropsychological tests; DRS and MMSE also used	visual memory (BVRT), visuospatial (JLO) & attention/ executive (SWT) domains		44.7%, multiple domains slightly impaired 39.7%, amnestic 15.8%	years cf 20% cognitively normal (OR 4.8; 95% CI 1.58–14.8) 2. Single domain nonamnestic MCI only associated with development dementia (OR 8.3; 95% CI 1.8 –37.5)
5.(Caviness *et al.*, 2007)	86	0	CN 75.2; MCI 74.6; PDD 79.9	CN 5.4; MCI 9.2; PDD 16.8	Cross-sectional; prevalent cases	USA	≤1.5 SD below age corrected mean score consistently in 1 of 5 cognitive domains, plus subjective memory complaint	Frontal/executive (Stroop, TMT A&B), amnestic (RAVLT), visuospatial (CDT, JLO), attention (WAIS III forward and backwards) & language (COWA, category fluency)	20.9 (25.3 non-demented PDs)	sdMCI-frontal/ executive 39%; naMCI-md 22%; aMCI-sd 22%; aMCI-md 11%; sdMCI-language 6%	Language dysfunction rarely seen; domains of visuospatial and attention did not reach threshold of dysfunction in PD-MCI group
6.(Williams-Gray *et al.*, 2007a)	126	0	NA	NA ('3.5 years' for whole cohort)	Longitudinal; community	UK	<1 SD below matched normative values	Frontal (TOL, SRM, phonemic fluency), temporal (PRM, semantic fluency) & parietal function (pentagon copying)	57	NA	Predictors of cognitive decline independent of age were non-tremor dominant phenotype, impaired semantic fluency & impaired pentagon copying
7.(Kim *et al.*, 2009)	141	0	CN 57; MCI 64	CN 3.6; MCI 4.0	Cross-sectional	Korea	<1.5 SD below the mean score for the age- and education-matched control group in ≥1 of 5 cognitive domains tested	Attention (forward digit span); language (BNT); visuospatial (RCFT); memory (SVLT) & executive function (phonemic word association test)	40.4	aMCI-sd 25.9%; sd language 12.3%; sd visuospatial 10.5%; sd executive 3.5%; md 47.4%	Age was significant predictor of all subtypes of MCI; domain most influenced by age was executive function
8.(Elgh *et al.*, 2009)	86	30	68.1	CN 2.1; MCI 1.4	Cross-sectional	Sweden	<1.5 SD below control means	Episodic memory; working memory;	30	30% had deficits in ≥1	1.Education was the only independent significant

					; newly diagnosed; drug naive		(matched for age/ sex/educational level if possible) for >50% of single test results within a domain	visuospatial function; verbal fluency; naming and executive function		domain, 16% deficits in ≥2 domains	predictor of severe cognitive impairment 2.Included 5 patients in 'PD' group with normal DaT
9.(Mamikonyan *et al.*, 2009)	106	0	64.6	6.5	Cross-sectional	USA	≤1.5 SD below normative means on ≥2 tests (for memory and attention) or a single test (attention)	Memory (HVLT-R – 3 components), executive function (Stroop, semantic fluency) and attention (digit span)	29.2	17.9% sd (attention 8.5, memory 5.7, executive 3.8%); 11.3% md	Predictors of of MCI were ↑age, ↑ Hoehn & Yahr stage,↑ UPDRS motor score, anti-anxiety medication use and a trend towards ↑ ESS score
10.(Aarsland *et al.*, 2009b)	196	171	67.7	2.3	Cross-sectional; incident; drug naive	Norway	<1.5 SD below mean for corrected z score for at least one of the cognitive domains	Verbal memory (CVLT-II); visuospatial (VOSP silhouettes); attentional/ executive (serial 7's, Stroop, semantic fluency)	18.9	62.2% of MCI group had naMCI-sd; 24.3% aMCI-sd; 10.8% aMCI-md & 2.7% naMCI-md	1.The largest effect size was found for verbal memory 2.No clinical or demographic differences found between those with PD-MCI cf PD-CN
11.(Sollinger *et al.*, 2010)	72	0	CN 63.7; MCI 66.0	CN 5.8; MCI 8.7	Retro-spective record review	USA	Deficits in ≥2 tests within a domain; plus subjective & objective cognitive impairment (strict cut-offs not used)	Visuospatial (JLO, pentagons); language (BNT, phonemic & semantic fluency); attention (digits forwards, TMT A); executive (TMT B, 'WORLD' backwards) & memory (CERAD word list or HVLT-R)	52.8	naMCI-sd 36.8%, aMCI-sd 23.7%, aMCI-md 23.7% & naMCI-md 15.8% (memory>executive>visuospatial>language>attention deficits)	Those with MCI had ↑ duration of PD and ↑ PIGD subscale scores
12.(Aarsland	1,34	0	NA	NA	Meta-	8	<1.5 SD below	Attention/	25.8	11.3% naMCI-	MCI associated with ↑age

***et al.*, 2010)**	6				analysis	centres	mean for corrected z score on ≥ 1 cognitive domain	executive; visuospatial; memory (variety of tests used)		sd; 8.9% aMCI-sd; 4.8% aMCI-md & 1.3% naMCI-md. Memory (13.3%)>visuospatial (11%)>attention/executive dysfunction 10.1%)	at assessment & at disease onset, male gender, depression, more severe motor symptoms, and advanced disease stage
13.(Poletti *et al.*, 2012)	121	100	66.6	1.2	Cross-sectional; early drug naive	Italy	<1.5 SD scores in ≥ 2 tests	Memory (RAVLT, Rey Figure Recall); language (BNT, semantic & phonemic fluency); executive (Stroop, FAB, TMT A&B, digit span, Corsi test, MCST, CPM 47); praxis (Rey figure copy) & visuospatial function (JLO)	14.8 (control group 7)	5% naMCI-md; 4.1% naMCI-sd; 4.1% aMCI-md & 1.7% aMCI-sd	1.Bradykinesia, axial impairment & absence of tremor associated with ↑ risk of MCI 2.PIGD patients had higher proportion of MCI than TD (23.2 v 6.3%)
14.(McColgan *et al.*, 2012)	132	0	NA	1.1	Incident; commmunity	UK	<1 SD in ≥2 tests plus subjective or objective decline	NA (included ACE-R, TOL, PRM, SRM, PAL, semantic & phonemic fluency)	22	NA	1.ACE-R good screening test of MCI (score <89) 2.MCI older & less educated than CN
15.(Yu *et al.*, 2012)	94	84	61.6	4.0	Cross-sectional; early; mild disease	Taiwam	<1.5 SD in ≥1 cognitive domain	Executive; category fluency; memory function; psychomotor speed; visuospatial; attention; language	46.8 (PDD 9.6)	naMCIsd 25.5%; aMCImd 7.4%; naMCImd 7.4%; aMCIsd 6.4%	1.Executive most common domain affected (29.5%), then visuospatial then memory (15.9 & 13.6%). 2.MCST greatest effect size of cognitive tests

PRM pattern recognition memory; SRM spatial recognition memory; TOL tower of London task; PAL paired associates learning; BVRT Benton visual retention test; JLO judgement of line orientation test; SWT Stroop word test; TMT Trails making test; RAVLT Rey auditory verbal learning test; CDT clock drawing test; WAIS Wechsler Adult Intelligence Scale; COWA controlled oral word association; BNT Boston naming test; RCFT Rey Complex Figure Test; SVLT Seoul Verbal Learning Test; HVLT-R Hopkins verbal learning test-revised; CVLT-2 California verbal learning test 2; VOSP visual object and space perception battery; FAB frontal assessment battery; MCST modified card sorting test; CPM 47 Raven coloured progressive matrices; ACE-R Addenbrooke's Cognitive Examination-Revised; na amnestic; a amnestic; sd single domain; md multiple domain; CN cognitively normal; ESS Epworth sleepiness scale; PIGD postural instability gait difficulty; TD tremor dominant

Prior to 2012, there were no consensus criteria for the diagnosis of PD-MCI. Of the 15 studies in Table 1-9, ten used neuropsychological test scores of less than 1.5 standard deviations (SD) below control values or normative means, three used a cut-off of 1 SD (all three studies by the same group), one used 2 SD and the last used no strict cut-off. This explains in part the variation in frequencies of MCI reported. As per the definition, 16% and 2.5% of subjects will fall below 1 and 2 SD of the mean, respectively, of a normally distributed test, and hence a less stringent cut-off could lead to a higher likelihood of false positive MCI cases. This was succinctly demonstrated by researchers in New Zealand, who evaluated 143 PD participants and 50 matched controls on 20 neuropsychological measures (Dalrymple-Alford *et al.*, 2011). The prevalence of PD-MCI was 14% when a cut-off of two test scores within one domain less than 2 SDs below normative values was used, but increased to 89% when one score less than 1 SD was used. The authors recommended that two scores less than 1.5 SD either within a single domain or in two separate domains should be used as criteria for PD-MCI (Dalrymple-Alford *et al.*, 2011). Variations are also attributable to whether subjective cognitive impairment was included as part of the diagnostic criteria; there is however, evidence that this may not be a reliable discriminator of MCI, with subjective memory complaints commonly reported in those who are cognitively normal (PD-CN) and under-reported in those with impairments (Caviness *et al.*, 2007). In addition, the differences in frequencies of MCI may be due to the number of neuropsychological tests used by study participants, with larger numbers of tests increasing the probability of false-positives. This can be avoided to some extent by defining MCI using impairment in more than one test, or standardising impairments within tests within separate cognitive domains.

1.7.2 Profile of cognitive impairment in MCI

As different neuropsychological tests cannot be fully separated on their neuroanatomical basis, it can be difficult to classify cognitive deficits based on impairments in each test, and therefore discrepancies are seen in the cognitive deficits reported in PD-MCI. Furthermore, researchers' interpretations can mean that tests may be categorised in different domains, which affects study analysis. For example, semantic fluency has been categorised as a test of executive (Aarsland *et al.*, 2009b; Mamikonyan *et al.*, 2009) and language function (Sollinger *et al.*, 2010; Poletti *et al.*,

2012). These differences and the cognitive heterogeneity of PD mean that it is difficult to generalise findings to the PD-MCI population overall. However, a significant number of studies have found that nonamnestic single-domain MCI (naMCI-sd) is the most common subtype (Janvin *et al.*, 2006; Caviness *et al.*, 2007; Aarsland *et al.*, 2009b; Mamikonyan *et al.*, 2009; Aarsland *et al.*, 2010; Sollinger *et al.*, 2010; Goldman *et al.*, 2012a; Yu *et al.*, 2012). This is in contrast to the general population, where amnestic MCI is the most common subtype.

Early studies, largely from researchers in Cambridge, in cognitively impaired but non-demented PD participants focussed on a fronto-striatal dysexecutive syndrome that was driven by dopaminergic deficits, but which may also be precipitated by medications due to dopamine 'overdosing' in the caudate nucleus and ventral striatum (Kehagia *et al.*, 2010). Prominent frontal/executive deficits have been found in a number of studies in PD-MCI (Foltynie *et al.*, 2004; Muslimovic *et al.*, 2005; Caviness *et al.*, 2007; Mamikonyan *et al.*, 2009; Yu *et al.*, 2012). There is evidence that these deficits may impact on other neuropsychological domains, with one study demonstrating that executive deficits explained a significant proportion of memory deficits (Bronnick *et al.*, 2011). Some studies have suggested that executive dysfunction may predict cognitive decline (Levy *et al.*, 2002a; Woods and Troster, 2003; Janvin *et al.*, 2005); however, findings from a larger, less heterogeneous and well-characterised cohort in the UK established that fronto-striatal deficits at baseline were associated with a better prognosis and did not increase the likelihood of progression to dementia (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). Mnemonic deficits (in both retrieval and encoding) were prominent findings for a number of groups and signifies an important aspect of early cognitive dysfunction (Aarsland *et al.*, 2009b; Elgh *et al.*, 2009; Kim *et al.*, 2009; Aarsland *et al.*, 2010; Sollinger *et al.*, 2010). Amnestic impairments were present even after controlling for attention/executive dysfunction (Aarsland *et al.*, 2010). There is considerable heterogeneity within the memory deficits in PD, which have traditionally been thought to comprise deficits in retrieval; that is, problems with recall of learned information but with sparing of encoding, storage and recognition (Poletti *et al.*, 2011). However, recent studies indicate that there is diversity in memory profiles affected, which include encoding and retention in addition to retrieval (Weintraub *et al.*, 2004a; Whittington *et al.*, 2006; Bronnick *et al.*, 2011). Other domains affected in PD-MCI are psychomotor speed,

attention and visuospatial function (Muslimovic *et al.*, 2005; Mamikonyan *et al.*, 2009; Aarsland *et al.*, 2010; Aarsland *et al.*, 2011; Barone *et al.*, 2011; Goldman *et al.*, 2012a), with language function less likely to be affected (Caviness *et al.*, 2007).

Therefore, within PD-MCI there are substantial variations in the cognitive construct. Deficits are present in early disease, even in *de novo* patients, and the differences seen may represent differing underlying pathological mechanisms.

1.7.3 Risk factors and course

As discussed above, the heterogeneity of PD-MCI suggests that cognitive correlates may not be generalizable to the whole population. Nevertheless, increased age (Foltynie *et al.*, 2004; Muslimovic *et al.*, 2005; Williams-Gray *et al.*, 2007a; Kim *et al.*, 2009; Mamikonyan *et al.*, 2009; Aarsland *et al.*, 2010; McColgan *et al.*, 2012), motor disease severity (Foltynie *et al.*, 2004; Muslimovic *et al.*, 2005; Mamikonyan *et al.*, 2009; Aarsland *et al.*, 2010), non-tremor-dominant motor phenotype (Muslimovic *et al.*, 2005; Williams-Gray *et al.*, 2007a; Sollinger *et al.*, 2010; Poletti *et al.*, 2012) and lower educational levels (Foltynie *et al.*, 2004; Elgh *et al.*, 2009; McColgan *et al.*, 2012) all seem to be robustly associated with MCI risk. Other possible associations include presence of depression (Muslimovic *et al.*, 2005; Aarsland *et al.*, 2010), male gender (Aarsland *et al.*, 2010) and anti-anxiety medication use (Mamikonyan *et al.*, 2009). In addition, one recent study examined the predictors of separate MCI subtypes in PD (Goldman *et al.*, 2012a). In keeping with previous studies, naMCI-sd was the most common subtype in 128 PD-MCI participants, but within the naMCI-md participants, significantly greater scores on axial functioning/gait subset of the Unified PD Rating Scale (UPDRS) motor score were observed, compared with the aMCI-sd subtype (Goldman *et al.*, 2012a). The authors postulated that non-dopaminergic deficits may link these specific motor and cognitive phenotypes.

To date, only three studies have published data on longitudinal outcomes in PD-MCI. Janvin and colleagues studied 72 non-demented PD participants with a mean age at baseline of 71 years and a disease duration of between 10 and 12 years, of whom 59 completed assessments four years later (Janvin *et al.*, 2006). Presence of MCI at baseline assessment was significantly associated with cognitive decline at follow-up, with 62% of those with PD-MCI versus 20% of those who were cognitively normal developing dementia. After controlling for age, sex, disease stage and education in a

logistic regression model, MCI at baseline was strongly associated with development of dementia (odds ratio 5.1; 95% CI, 1.51–16.24). The only subtype that was associated with later dementia was naMCI-sd (odds ratio 8.3; 95% CI, 1.8 –37.5); however, the small numbers within the subgroups means that definitive conclusions cannot be drawn.

The CamPaIGN cohort is another longitudinal study which has explored the evolution of cognitive dysfunction (Foltynie *et al.*, 2004; Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). Although MCI was not studied specifically, over a mean of 5.2 years, 17% of incident PD cases developed PDD, with increasing age, impairments in semantic fluency and visuospatial function and *MAPT* H1/H1 genotype all significantly associated with a more rapid cognitive decline (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). *COMT* genotype and impairments in frontally-based tasks were not associated with deteriorating cognition, lending support to the hypothesis that more posteriorly based cognitive deficits increase the risk of subsequent decline due to a more posterior deposition of Lewy bodies, combined with the ageing process plus cholinergic dysfunction. Most recently, follow-up of MCI cases from the ParkWest Study (Aarsland *et al.*, 2009b) demonstrated that a diagnosis of MCI *vs*. non-MCI at baseline visit had a relative risk for dementia of 39.2 at three years (Pedersen *et al.*, 2013). 27% of those with MCI progressed to dementia over follow-up compared to 0.7% of those who were cognitively normal, although it should be noted that 21.6% of those with MCI reverted to normal cognition.

1.7.4 Pathogenesis

The exact underlying pathophysiology of PD-MCI remains the subject of debate, largely due to the scarcity of neuropathological data. Structural and functional imaging, neurophysiological techniques and CSF analysis provides some *in vivo* evidence of the mechanisms underlying MCI. Only one neuropathological study in well-characterised PD-MCI in which participants were followed prospectively has been reported (Adler *et al.*, 2010). Eight cases were examined, of who four were classified as amnestic and four as nonamnestic MCI. The neuropathology was heterogeneous, with five cases exhibiting limbic or neocortical Lewy body (LB) pathology and the remainder predominantly consisting of brainstem LB pathology. Diffuse amyloid plaques were seen in the majority of cases (Adler and Beach, 2010), with two of the amnestic MCI

subgroup meeting neuropathological criteria for AD. Cerebrovascular pathology was frequently seen. In correspondence relating to this study, Kurt Jellinger reported on a further eight cases of MCI, where four cases were defined as aMCI-sd, three were naMCI-sd and one MCI multiple-domains (Jellinger, 2010b). Again the neuropathology was heterogeneous, with most patients having limbic or neocortical LB deposition, and some displaying Alzheimer-type pathology. These studies provide evidence that the neuropathology underlying PD-MCI may be similar to, but less advanced than, that found in PDD.

Changes seen in structural and functional imaging provide further evidence of the possible underlying pathogenesis of PD-MCI. Extensive grey matter loss on structural magnetic resonance imaging (MRI) is a consistent finding in PDD (Burton *et al.*, 2004; Melzer *et al.*, 2012); in MCI, a more selective loss has been observed (Bruck *et al.*, 2004; Beyer *et al.*, 2007; Song *et al.*, 2011; Melzer *et al.*, 2012). Atrophy was noted in frontal (Beyer *et al.*, 2007; Song *et al.*, 2011; Melzer *et al.*, 2012), prefrontal (Bruck *et al.*, 2004; Song *et al.*, 2011; Weintraub *et al.*, 2011), temporal (Beyer *et al.*, 2007; Melzer *et al.*, 2012), hippocampal (Bruck *et al.*, 2004; Weintraub *et al.*, 2011; Melzer *et al.*, 2012), amygdala (Melzer *et al.*, 2012), parietal (Weintraub *et al.*, 2011) and occipital (Song *et al.*, 2011; Weintraub *et al.*, 2011) regions, and may be attributable to neuronal and synaptic loss from LB and/or AD-type pathology. It has been postulated that hippocampal atrophy may be a biomarker of early cognitive decline in PD (Weintraub *et al.*, 2011), with longitudinal follow-up indicating that hippocampal plus parieto-temporal atrophy predict cognitive impairment (Weintraub *et al.*, 2012). However, a study in *de novo* incident PD cases did not demonstrate significant grey matter loss, perhaps due to the shorter disease duration, arguing for functional neurotransmitter loss rather than structural grey matter loss as a pathological basis for MCI (Dalaker *et al.*, 2010).

Functional imaging using 18F-fluorodeoxyglucose (FDG) PET has demonstrated metabolic abnormalities associated with PD-MCI, with metabolic reductions demonstrated in frontal and parietal association areas plus relative increases in the cerebellar vermis and dentate nuclei (Huang *et al.*, 2007). This pattern predicted performance in memory and visuospatial domains, with a more recent PET study by the same authors revealing a difference in parietal and prefrontal metabolism in those

with multiple-domain MCI compared to PD-CN participants (Huang *et al.*, 2008). Other studies have demonstrated cerebral hypometabolism in posterior cortical regions in participants with PD-MCI compared to those with PD and normal cognition (Hosokai *et al.*, 2009; Pappata *et al.*, 2011). Taken together, neurotransmitter deficits in PD-MCI may explain the PET findings, with dopaminergic dysfunction accounting for frontal hypometabolism and subcortical cholinergic loss leading to posterior changes. The former presupposition is strengthened by a functional imaging study which used functional MRI (fMRI) and dopamine transporter binding SPECT scanning to assess brain responses during a working memory task in early drug naïve PD-MCI participants (Ekman *et al.*, 2012). Compared to PD-CN subjects, those with PD-MCI had markedly reduced recruitment of the right caudate nucleus, bilateral anterior cingulate cortex and to a lesser extent the right dorsolateral prefrontal cortex. These findings correlated with working memory function, and lend support to dopaminergic dysfunction as a basis for the functional changes seen.

Other potential mechanisms that may underpin the pathophysiology of MCI in PD include cholinergic dysfunction and abnormal processing of the amyloid precursor protein. Cholinergic loss is an established feature of PDD (Tiraboschi *et al.*, 2000; Bohnen *et al.*, 2003) and may contribute to PD-MCI: Evidence from a PET study demonstrating a reduction in nicotinic ACh receptors in the midbrain, pons, and cerebellum in PD subjects with MCI support this hypothesis (Meyer *et al.*, 2009). In addition, short latency afferent inhibition (SAI) is abnormal in PD-MCI (Yarnall *et al.*, 2013). SAI is a non-invasive neurophysiological technique that relies on cholinergic excitability in the cerebral cortex, and hence can be used as a proxy measure of cholinergic activity. This theory of a cholinergic basis to MCI has biological plausibility in terms of the Braak hypothesis: at Braak Stage 3, where the motor disease may become apparent, there is already destruction of the basal forebrain cholinergic nuclei and consequent ACh loss. Lastly, abnormal Aβ deposition and fibrillization due to altered amyloid precursor processing may contribute to PD-MCI. Reduced CSF levels of Aβ42, a marker of amyloid deposition and aggregation, were found in those with PD and who were cognitively impaired but not demented (Montine *et al.*, 2010). Reduced CSF Aβ42, 40 and 38 levels also correlated with memory function in early *de novo* PD participants (Alves *et al.*, 2010). However, detection of amyloid-β deposition using PET imaging with Pittsburgh Compound B (PiB) in PD-MCI has been less convincing. In two

small cross-sectional studies, PiB retention did not differ between groups with PD and normal cognition, PD-MCI or PDD (Foster *et al.*, 2010; Gomperts *et al.*, 2012), although in more recent longitudinal work, amyloid burden at baseline predicted cognitive decline during follow-up (Gomperts *et al.*, 2013). In a further cross-sectional study of 40 PD participants with risk factors for the development of dementia (30 defined as MCI), cortical PiB binding was below that expected for older individuals and for those at risk of AD; PiB retention was, however, correlated with global cognitive function and a tests of executive function (Petrou *et al.*, 2012).

In conclusion, the pathogenesis of PD-MCI is heterogeneous and may differ between individuals and between subtypes. Lewy body deposition, amyloid deposition and neurotransmitter deficits are all likely to contribute, although to a lesser degree than those changes seen in PDD. Further *in vivo* and post-mortem studies will facilitate future work.

1.7.5 Definition of PD-MCI

Due to the increasing research in PD-MCI and a lack of standardization in defining the disorder, the Movement Disorder Society (MDS) commissioned a Task Force to evaluate the literature (Litvan *et al.*, 2011) and propose criteria (Litvan *et al.*, 2012) for the diagnosis of PD-MCI. The criteria are shown in Table 1-10. They require the diagnosis of PD, subjective or objective cognitive decline and the demonstration of cognitive deficits that do not interfere with functional independence. The Task Force defined both a level I category, for an abbreviated assessment, and a more comprehensive level II assessment for more diagnostic certainty and for use in a research setting. Strict cut-offs were not defined, with impairment in performance in neuropsychological tests of 1 to 2 SDs. The Task Force also gave examples of global cognitive scales and neuropsychological tests that could be used. With time these criteria may be refined further, although it should be noted that PD-MCI is a contentious term and may not necessarily be useful in terms of prognostication. Reasons to contend this include the fact that MCI was extrapolated from the AD literature, and this may not be analogous to PD; that there is considerable heterogeneity in PD-MCI; and that PD-MCI may represent a fluid state, with some returning to normal cognition over time in addition to converting to dementia.

Table 1-10 Diagnostic criteria for PD-MCI

I. Inclusion criteria • Diagnosis of PD • Cognitive decline, in context of established PD, reported by patient/carer/treating physician • Cognitive deficits on either formal neuropsychological testing or a scale global cognitive abilities • Cognitive deficits not severe enough to interfere with functional independence, although subtle impairments me be present
II. Exclusion criteria • PDD • Other primary explanation for cognitive impairment (e.g. stroke, major depression, delirium) • Other PD-associated comorbid conditions (e.g. motor impairment, severe anxiety, psychosis) that may significantly influence cognitive testing
III. Specific guidelines for PD-MCI level I and level II categories **A. Level I (abbreviated assessment)** • Impairment on a scale of global cognitive abilities validated for use in PD (MoCA, SCOPA-COG, PD CRS, MDRS)* *or* • Impairment on at least two tests, when a limited battery of neuropsychological tests is performed **B. Level II (comprehensive assessment)** • Neuropsychological testing that includes 2 tests within each of the 5 cognitive domains (*attention and working memory, executive, language, memory, and visuospatial*) • Impairment on ≥ 2neuropsychological tests (either 2 impaired tests in 1 cognitive domain or 1 impaired test in 2 different cognitive domains) • Impairment on neuropsychological tests may be demonstrated by: o Performance approximately 1 to 2 SDs below appropriate norms or o Significant decline demonstrated on serial cognitive testing or o Significant decline from estimated premorbid levels
IV. Subtype classification for PD-MCI (optional, requires two tests for each of the five cognitive domains assessed) - PD-MCI single-domain—abnormalities on two tests within a single cognitive domain (specify the domain), with other domains unimpaired *or* - PD-MCI multiple-domain—abnormalities on at least one test in two or more cognitive domains (specify the domains)

*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

1.7.6 Treatment

To date, there have been no randomised controlled trials in PD-MCI. A small study of Atomoxetine, a selective norepinephrine reuptake inhibitor, produced an improvement in global cognition in non-demented PD patients, although cognition was only a secondary outcome measure (Weintraub *et al.*, 2010). Trials of cholinesterase

inhibitors in PD-MCI and mild dementia, such as the MUSTARDD-PD study (www.clinicaltrials.gov: NCT01014858), are on-going and should inform future practice. The continued investigation of the pathogenesis underlying PD-MCI is important for the development of new therapeutic targets, including the reduction of insoluble amyloid-β accumulation or the inhibition of oligomeric α-synuclein accumulation. Finally, non-pharmacological interventions such as cognitive intervention programmes (Jean *et al.*, 2010; Naismith *et al.*, 2013) or physical exercise should be investigated further (Hindle *et al.*, 2013).

1.8 Study summary, objectives and hypotheses

In summary, PD is a complex neurodegenerative disorder with impairments of movement, gait, mood and cognition. The incidence of dementia is six times higher than that expected in controls, with a cumulative prevalence approaching 80% in community studies. Moreover, up to a quarter of people with Parkinson's disease will have subtle cognitive deficits (mild cognitive impairment) at diagnosis. The cognitive profile of PDD differs from that of AD, with deficits in attention being one of the strongest predictors of PDD. Overall, executive function and visuospatial deficits are more prominent in PDD, but it is not known which pattern of deficit is associated with a more rapid rate of cognitive decline. Although the pathophysiology underlying these deficits is likely to be varied, deposition of amyloid within the cortex and cholinergic loss throughout the frontal cortex are likely to contribute. CSF may be a potential biomarker for cognitive decline in PD, with previous studies indicating that low levels of amyloid-β 1-42 are associated with memory impairment. A surrogate marker of cortical cholinergic status is short latency afferent inhibition, a simple neurophysiological technique that has been shown to be abnormal in AD and DLB, where cholinergic loss is a fundamental neurophysiological component.

Aims and objectives:

The overarching aim of this thesis was therefore to define and characterise cognitive subtypes early Parkinson's and to determine the interplay between putative markers of protein deposition and neurochemical dysfunction.

This aim was achieved through the following objectives:

1) To investigate cognition in early Parkinson's disease.
2) To investigate the relationship between amyloid-β and cognition (including specific cognitive domains) in early Parkinson's disease.
3) To investigate the relationship between short latency afferent inhibition (SAI) and cognition in early Parkinson's disease.

Hypotheses:

1. Cognitive deficits will be frequently observed, even in a very early cohort of Parkinson's disease patients.
2. Low CSF amyloid-β in early PD will be associated with cognitive decline.
3. Low CSF amyloid-β in early PD will be associated with impaired mnemonic function, that is, a more "posterior" mediated function.
4. Abnormal short latency afferent inhibition (SAI) will be associated with low baseline CSF amyloid-β levels, reflecting greater underlying cholinergic dysfunction and Alzheimer-related pathology.
5. Abnormal short latency afferent inhibition will correlate with cognitive impairment.
6. Abnormal SAI will be associated with more posteriorly mediated cognitive functions.
7. Executive dysfunction at baseline will not be associated with CSF or SAI abnormalities.

Chapter 2 Cognition in early Parkinson's disease – the ICICLE-PD study

2.1 General ICICLE-PD Methodology

The Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation-PD (ICICLE-PD) study is a twin centre longitudinal observational study, the aim of which is to better understand the anatomical, biochemical and genotypic mechanisms underlying the evolution of PDD from disease onset. Through centres in Newcastle and Cambridge, participants are followed up every 18 months, where the diagnosis of PD is reassessed and further clinical, neuropsychological, imaging and laboratory measures are performed.

2.2 Baseline Assessment

Between June 2009 and December 2011, we attempted to identify all newly diagnosed PD patients from outpatient clinics in Newcastle-upon-Tyne/Gateshead and Cambridgeshire, UK. A total of 166 primary care practices were identified and encouraged to refer patients with suspected parkinsonism. We also informed colleagues in secondary care and invited them to refer all patients with suspected parkinsonism. This group included neurologists (n=48), geriatricians (n=17), and Parkinson's disease nurse specialists (n=14). PD was diagnosed by a movement disorder specialist according to the UK Brain Bank criteria for idiopathic PD (Hughes *et al.*, 1992). Exclusion criteria comprised: Parkinsonism diagnosed prior to the onset of the study; insufficient working knowledge of English (defined as insufficient to perform the neuropsychological assessments or questionnaires in the opinion of the assessor); significant memory impairment at presentation (defined as MMSE score < 24), or meeting DSM IV (Association, 2000) or the MDS criteria for dementia (Emre *et al.*, 2007); subjects who did not have the capacity to give informed consent (as assessed by criteria laid out in the MHA code of practice, section 4-3); history consistent with DLB (McKeith *et al.*, 2005), atypical parkinsonian syndromes (including multiple system atrophy or progressive supranuclear palsy, diagnosed according to accepted criteria (Litvan *et al.*, 2003)) repeated strokes or stepwise progression of symptoms, leading to a diagnosis of 'vascular parkinsonism'; and, exposure to dopamine receptor blocking agents at the onset of symptoms.

To control for the effects of normal ageing and to generate normative values for cognitive tests, unrelated controls of similar age and sex to patients were recruited through local advertising (including primary care settings), word of mouth and community groups. Carers and spouses of patients with PD were not used as controls to limit bias; for example, sleep disruption or mood disorder in a patient could influence carer response. None of the controls had a history of major psychiatric disorders, cognitive impairment, stroke or a movement disorder, but were not pre-screened for memory problems to ensure that this was a representative sample. All control subjects underwent clinical and neuropsychological testing, and were given the option of participating in laboratory and MRI studies. Global cognitive scores in control participants were comparable to published age- and educationally-matched normative data for the MMSE (Crum *et al.*, 1993) and Montreal Cognitive Assessment (MoCA) (Dalrymple-Alford *et al.*, 2010).

The study was approved by the Newcastle and North Tyneside Research Ethics Committee and performed according to the Declaration of Helsinki, with all subjects providing written informed consent.

2.3 Clinical Assessment

Clinical and demographic data collected comprised a detailed history of disease onset; disease duration; comorbidities; family history of neuropsychiatric disease; level of education and medication use. Clinical assessments were performed by trained examiners and included a standardised neurological assessment, the MDS-revised Unified Parkinson's Disease Rating Scale (MDS UPDRS) (Goetz *et al.*, 2008b) and Hoehn and Yahr stage (Hoehn and Yahr, 1967). Depressive symptoms were assessed using the Geriatric Depression Scale-15 (GDS-15) score (Yesavage *et al.*, 1983) and functional status and well-being were measured by the Parkinson's disease Quality of Life Questionnaire (PDQ-39) (Peto *et al.*, 1995). Motor phenotype was calculated using the MDS-UPDRS revision (Goetz *et al.*, 2008b) of the method described by Jankovic (Stebbins *et al.*, 2013) whereby patients are categorised into tremor dominant (TD), postural instability with gait difficulty (PIGD), or indeterminate (ID) motor subtypes based on the ratio between mean tremor score versus and mean postural instability gait difficulty score. Ratios of ≥ 1.15 were classified as TD and ≤ 0.90 as PIGD (Stebbins

et al., 2013). Levodopa equivalent daily dose (LEDD) was calculated for all dopaminergic medications (Tomlinson *et al.*, 2010). Participants underwent baseline assessments, with planned evaluation every 18 months until the end point of dementia (diagnosed according to the Movement Disorder Society criteria (Emre *et al.*, 2007)) or death.

2.4 Neuropsychological Assessment

Global cognitive function was assessed with the MMSE (Folstein *et al.*, 1975) and Montreal Cognitive Assessment (MoCA) (Nasreddine *et al.*, 2005; Zadikoff *et al.*, 2008). Although traditionally used as a screening test for cognitive impairment in the wider population, the MMSE has not been validated in PD and problems such as accuracy, sensitivity (especially in early cognitive impairment) and "ceiling effect" have called into question its use (Hobson and Meara, 1999; Athey *et al.*, 2005; Zadikoff *et al.*, 2008; Nazem *et al.*, 2009). One study demonstrated that over half of those who were classified as "normal" cognition by MMSE were impaired on the MoCA (Nazem *et al.*, 2009). In addition, executive and visuospatial domains are poorly represented by the MMSE. Originally developed as a brief screening tool to detect mild cognitive impairment (MCI) as a precursor to AD with a score of less than 26/30 being predictive of MCI (Nasreddine *et al.*, 2005), the MoCA has been shown to be valuable in screening for both dementia and MCI in PD (Hoops *et al.*, 2009; Dalrymple-Alford *et al.*, 2010). At a score of <21/30, the MoCA has a 81% sensitivity, 95% specificity, negative predictive value of 92% and a positive predictive value of 87% for the diagnosis of PDD, with the corresponding figures for MCI at a score of <26/30 of 90, 75, 61 and 95%, respectively (Dalrymple-Alford *et al.*, 2010). Although other scales of global cognition, such as the Addenbrooke's Cognitive Examination-Revised (ACE-R) are available and have shown to be useful screening tools for PD-MCI (McColgan *et al.*, 2012), the MoCA can be completed in 10 minutes and is one of the recommended global screening tools by the Movement Disorder Society Task Force on MCI (Litvan *et al.*, 2012).

Premorbid IQ was estimated with the National Adult Reading Test (NART) (Nelson and O'Connell, 1978). Where relevant, subjects were assessed 'on' dopaminergic medication, although due to the short disease duration, motor and cognitive fluctuations were not problematic in this study. Participants were asked to refrain

from drinking caffeine or smoking for one hour prior to appointments, to reduce the risk of confounding due to stimulant effects and to standardise neuropsychological testing.

Five cognitive domains were assessed as part of the neuropsychological battery. Attention and working memory were measured using the Cognitive Drug Research (CDR) computerised battery (Ballard *et al.*, 2002; Wesnes *et al.*, 2002). Scores of simple reaction time, choice reaction time and digit vigilance mean time (all in milliseconds (msec)) were summed to produce a Power of Attention (PoA) score, with a higher score indicating more severe impairment (Wesnes *et al.*, 2002) (Table 2-1). PoA has been used as both a primary and secondary outcome of attentional function in acetylcholinesterase inhibitor (AChE-I) studies in PDD and DLB (Wesnes *et al.*, 2002; Emre *et al.*, 2004; Wesnes *et al.*, 2005; Rowan *et al.*, 2007), and as a measure of attention when correlated with clinical characteristics (Taylor *et al.*, 2008; Allcock *et al.*, 2009). Digit vigilance accuracy was also evaluated as part of this domain, which was chosen due to its large effect size when compared with controls. Memory was assessed with Pattern Recognition Memory (PRM), Spatial Recognition Memory (SRM) [sensitive to impairment of temporal and frontal lobe function, respectively (Owen *et al.*, 1995b)] and Paired Associates Learning (PAL) from the computerised Cambridge Neuropsychological Test Automated Battery (CANTAB) (Sahakian *et al.*, 1988; Robbins *et al.*, 1994; Owen *et al.*, 1995a) (Table 2-1). As this battery requires touch screen testing, participants were initially screened for visual, motor and comprehensive deficits using a motor screening test (MOT). Paired associates learning is a visuospatial test of learning and memory that is sensitive to both temporal and frontal damage and has been shown to predict Alzheimer's disease with a high degree of accuracy (Swainson *et al.*, 2001; Blackwell *et al.*, 2004). Executive function was determined using the modified ('one touch') version (OTS) of the Tower Of London task from the CANTAB battery, a test of planning requiring working memory (Owen *et al.*, 1995a) that has been shown to activate fronto-parietal-caudate circuitry (Baker *et al.*, 1996; Cheesman *et al.*, 2005); phonemic fluency (words beginning with F, A and S in one minute) (Benton, 1968) and semantic fluency (animals in 90 seconds) (Goodglass, 1972), tests sensitive to fronto-striatal and fronto-parietal dysfunction (Miller, 1985). For phonemic fluency, Cambridge participants (n=61) only underwent testing of words

beginning with F, and this data was therefore combined with the Newcastle data to produce an aggregate score. In line with previous studies in PD assessing cognition using the CANTAB battery (Sahakian *et al.*, 1988; Lewis *et al.*, 2003; Foltynie *et al.*, 2004; Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a), scores of total number correct for PRM (maximum 24) and SRM (maximum 20) were used, with OTS assessed using the total number of problems solved on first attempt (maximum 20). For PAL, the parameters measured were stages completed (score 0-8), total number errors, total number of trials and mean trials to success. Previous studies using PAL have used a combination of these parameters. After discussion with the CANTAB team in Cambridge and after inspecting the distributions of the data, mean trials to success was included, with a higher score indicating a greater degree of impairment. The pentagon copying item within the MMSE was graded using a modified 0-2 rating scale (Ala *et al.*, 2001; Williams-Gray *et al.*, 2007a) as a measure of visuospatial function. Language domain was assessed using the naming (0-3) and sentence (0-2) subsets of the MoCA test.

Table 2-1 Computerised battery tests

Cognitive test	Description	Measure
CDR		
Simple reaction time (SRT)	Subject instructed to press 'YES' button as quickly as possible every time the word 'yes' appeared on computer screen. 30 stimuli delivered at varying inter-stimulus intervals.	Response time (ms); response time variability (%)
Choice reaction time (CRT)	Either the word 'no' or the word 'yes' randomly appeared on screen, and the patient instructed to press the corresponding button as quickly as possible. 30 stimuli delivered at varying intervals.	Response time (ms); response time variability (%); % of accurate responses
Digit vigilance	A target digit was randomly selected and constantly displayed to the right of the screen. A series of digits was then presented in the centre of the screen at the rate of 150 per minute and the subject told to press the 'YES' button as quickly as possible every time the digit in the series matched the target digit.	Response time (ms); response time variability (%); % of accurate responses; number of errors
CANTAB		
Pattern recognition memory (PRM)	Subjects were initially shown a series of coloured patterns inside a box in the centre of the screen. In the next stage, pairs of patterns were shown in the centre, & the subject was asked to touch the pattern they had already seen during the initial phase.	Number correct (max 24); % correct; latency to correct (ms)
Spatial recognition memory (SRM)	In the 1st phase, unfilled white squares were shown at different locations on the screen. During the 2nd phase, 2 squares appeared simultaneously on the screen and the subject had to select the correct location had been used before in the 1st phase.	Number correct (max 20); % correct; latency to correct (ms)
Paired associates learning (PAL)	Initially 6 boxes were placed in different locations on the screen and were 'opened' one at a time, with 1 displaying a coloured pattern. The pattern then appeared in the screen centre, & the subject was asked to touch which box had contained the pattern. This was repeated with 2, 3 & up to 6 patterns. Finally the number of boxes was increased to 8 with 8 patterns to identify.	Stages completed (max 8); total errors; total trials; mean trials to success
One touch stockings of Cambridge (OTS)	2 sets of 3 stockings were displayed on the touch screen, with the subject asked to rearrange the balls in the bottom display to match the position in the top of the screen.	Problems solved on 1st choice (max 20); mean choices to correct; latency to correct (ms)

MoCA was not performed on the first 24 participants as it was introduced slightly later in the study. A small number of subjects did not undergo all 11 neuropsychological

tests and were coded as missing data. For example, CDR could not be completed in two PD patients due to technical issues (Table 2-2).

Mild cognitive impairment was determined using the recently published Movement Disorder Society criteria (Litvan *et al.*, 2012). The ICICLE neuropsychological battery was devised prior to the publication of the MDS PD-MCI guidelines. Therefore, the visuospatial domain was not as well covered as we would have anticipated. Other domains were, however, well covered. Subjects were classified as level 1 MCI (abbreviated assessment, possible MCI) if they scored <26 on the MoCA. Level 2 criteria (comprehensive assessment) necessitated impairment on at least two neuropsychological tests, represented by either two impaired tests in one cognitive domain or one impaired test in two different domains. A test score was considered 'impaired' if it was 1, 1.5 or 2 standard deviations (SDs) below the mean score of the control subjects which was approximately normally distributed. For non-normally distributed data, even after transformation (pentagon, naming, language and digit vigilance scores), cut-offs were used that gave approximately the correct percentage of people impaired (according to the normal distribution) for that corresponding SD. For example, the mean plus 1 SD cut-off for naming was 2 or less as 14% of controls scored 0, 1 or 2. Subjects were further classified as single- or multiple-domain amnestic or nonamnestic according to the criteria.

Table 2-2 Missing cognitive data

	MoCA	MMSE	CDR	CANTAB	Verbal fluency	Pentagon	Language
Control no. missing	2	0	5	5	1	0	2
Reason for missing data	Missing data		Equipment failure (n=3), missing data (n=2)	Visual impairment (n=4), missing data (n=1)	Missing data		Missing data
PD no. missing	24	0	2	12	3	0	24
Reason	Introduced later in study		Equipment failure	Visual impairment (n=3), missing data (n=9)	Missing data		Introduced later in study

2.5 Statistical Analysis

Statistical analyses were performed with SPSS 19.0 (SPSS, Chicago, IL). Data were examined for normality with visual histograms and Kolmogorov-Smirnov test. Means and SDs for general characteristics and cognitive test scores were calculated. The primary statistical analysis consisted of descriptive statistics comparing those classified as MCI level 1 or level 2 with the PD-cognitively normal (CN) groups, and within MCI level 2, whether they met the criteria at 1, 1.5 or 2 SD below normative values. Means were compared using Student t-tests or ANOVA for normally distributed and Mann-Whitney or Kruskal-Wallis test for data that were non-normally distributed. Pearson correlation (parametric distributions) or Spearman's rank correlation (non-parametric) coefficients were calculated to assess the bivariate association between cognitive and clinical or biochemical parameters. A linear regression model was used to control for covariates including age and education; logistic regression was used for binary dependent variables. Collinearity diagnostics were inspected to test for multi-collinearity, with an average variance inflation factor (VIF) greater than one considered problematic. The Durbin–Watson statistic was used to identify autocorrelation (values less than one or greater than three were a cause for concern). In all regression models, standardised residuals were inspected to ensure they were approximately normally distributed and between ±3.0. Cook's distances were used to check for cases exerting undue influence in any model. Mixed linear modelling was used with age as a covariate to compare neuropsychological tests between groups (controls versus PD participants). The effect size of each cognitive test was calculated using Glass's delta, by calculating the mean difference (mean cognitive test score of controls – mean cognitive test score of PD participants) divided by the SD. *A priori* correction for multiple comparisons was not made due to the exploratory nature of the investigation; if a parameter was significant, even if by chance, it was felt it merited further analysis (Rothman, 1990; Perneger, 1998; Feise, 2002). However, if multiple corrections were required for defining clinical significance, a Bonferroni correction was applied for multiple comparisons. Pearson Chi-square tests were used to compare between-group distribution of proportions, with Fisher's exact test used if the expected frequency in any group was < 5. For parametric tests, all p values reported are two-tailed. A p value of < 0.05 was deemed as significant.

2.6 Results – cognition in early Parkinson's disease

2.6.1 Baseline characteristics of study participants

682 patients with parkinsonism were approached and, of these, 226 with idiopathic PD consented (Figure 2-1). Seven were subsequently excluded (one due to a diagnosis of dementia; one due to insufficient command of the English language; one due to vascular parkinsonism and four due to normal Dopamine Transporter scans (FP-CIT SPECT)). Those that declined to take part (n=312) were older than those who participated (71.5 vs. 65.9 years, $p<0.001$, unpaired t-test). 101 age- and sex-matched controls were also recruited, with two excluded (one due to a diagnosis of essential tremor and one because of a glioblastoma multiforme incidentally found on MRI). Therefore, 219 PD subjects and 99 controls participated. With the exception of a statistically significant difference in disease duration (6.0 *vs.* 4.3 months) which was not clinically significant, PD participants from Newcastle and Cambridge were well-matched (Table 2-3). Baseline characteristics are shown in Table 2-4. In keeping with early PD, mean disease duration was less than six months (mean 5.5 months) with relatively mild motor disease (mean MDS UPDRS score 27.1) and 83% of participants were Hoehn and Yahr Stage 1 or 2. PD participants scored significantly lower on the MoCA and MMSE and higher on the GDS-15 than the controls, but there were no differences in the years of education. Control participants tended to be older, and although this did not reach statistical significance, age was included as a covariate in all analyses. Mean LEDD was 178 mg/day in PD subjects, with only 16.4% of participants being treatment naive. The most commonly medication prescribed was a dopamine agonist, and there was no significant difference in the number of PD participants versus controls who were taking antidepressants.

Figure 2-1 Flow diagram of PD participants and assessments

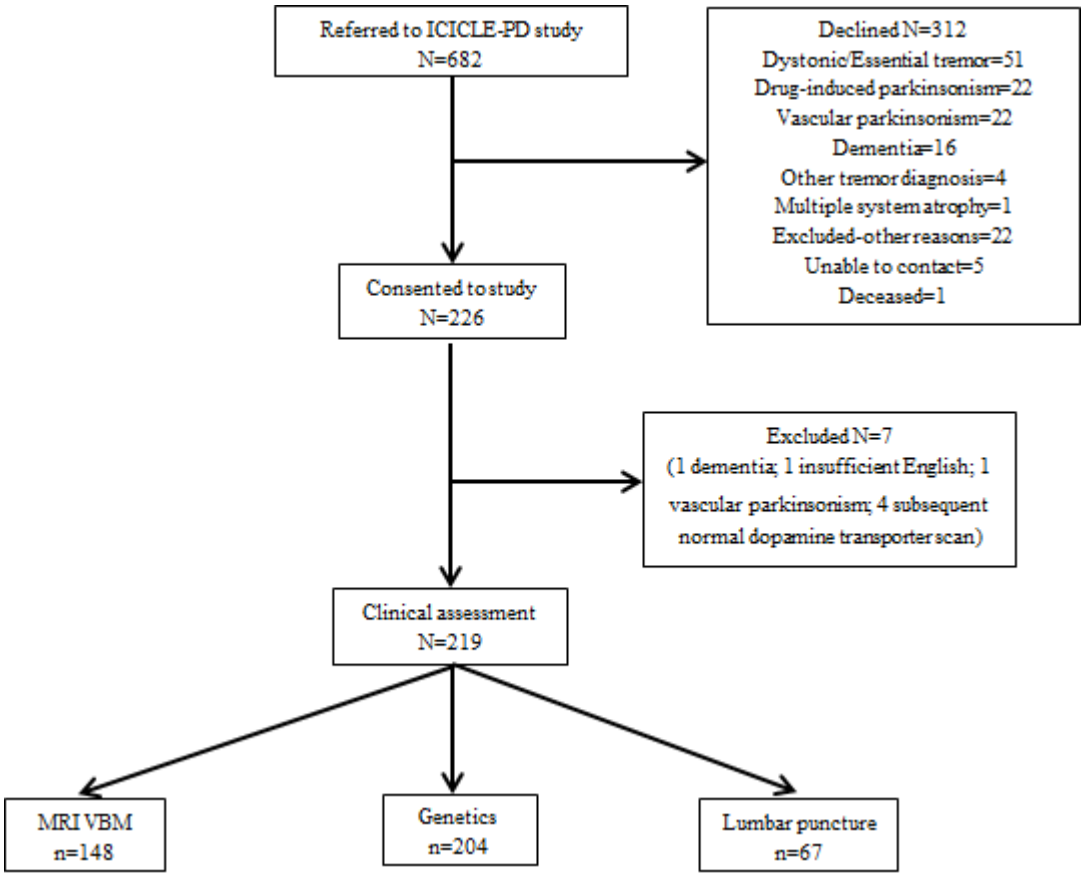


Table 2-3 Comparison of Newcastle and Cambridge PD participant demographics

Characteristic	**Newcastle (n=158)**	**Cambridge (n=61)**	**P value**
Age	66.5 (10.3)	64.4 (7.8)	0.103[a]
Male gender	104 (65.8)	36 (59.0)	0.347[b]
Disease duration (mo)	6.0 (4.5)	4.3 (6.0)	**<0.001**
MDS UPDRS III	27.1 (12.1)	28.8 (11.2)	0.373
LEDD (mg/d)	177.8 (146.9)	178.3 (171.3)	0.849
Education (years)	12.8 (3.9)	12.8 (3.0)	0.450
NART	114.6 (10.8)	113.4 (9.1)	0.143
GDS-15	2.9 (2.6)	3.0 (2.9)	0.888
MoCA[c]	25.1 (3.6)	25.8 (2.7)	0.499
MMSE	28.6 (1.4)	29.0 (1.0)	0.120

Table 2-4 Demographics and clinical characteristics of PD and control participants (unadjusted)

Characteristic	Controls (n=99)	PD (n=219)	p value
Age (years)	67.9 (8.2, 48.0 – 88.2)	65.9 (9.7, 35.0 – 87.3)	0.057[a]
Male gender	54 (54.5%)	140 (63.9%)	0.112[b]
Education (years)	13.1 (3.4)	12.8 (3.6)	0.356
Disease duration (months)	-	5.5 (5.0)	-
Hoehn & Yahr stage I	-	57 (26.0%)	-
Hoehn & Yahr stage II	-	125 (57.1%)	-
Hoehn & Yahr stage III	-	36 (16.4%)	-
Hoehn &Yahr stage IV	-	1 (0.5%)	-
MDS UPDRS part 3	-	27.6 (11.9)	-
Levodopa equivalent dose (mg/d)	-	178.0 (153.7)	-
Treatment naive	-	36 (16.4%)	-
Levodopa use	-	64 (29.2%)	-
Dopamine agonist use	-	83 (37.9%)	-
Monoamine oxidase inhibitor	-	78 (35.6%)	-
Amantadine	-	3 (1.4%)	-
Antidepressant use	10 (10.1%)	35 (16.0%)	0.164[b]
NART	116 (8.7)	114 (10.3)	0.366
MoCA[c]	27.0 (2.5)	25.3 (3.4)	<0.001
MMSE	29.0 (1.2)	28.7 (1.3)	0.010
GDS 15	1.0 (1.5)	2.9 (2.7)	<0.001

Data are mean (SD) or number (%), and range for age. MDS UPDRS=Movement Disorder Society Unified Parkinson's Disease Rating Scale; NART=National Adult Reading Test; MoCA=Montreal Cognitive Assessment; MMSE=Mini Mental State Examination; GDS 15=Geriatric Depression Scale-15; [a]Unpaired t-test; [b] Pearson Chi-square test; the remainder used Mann-Whitney test; [c]n=292 (controls=97;PD=195).

2.6.3 Cognitive profile of early PD

The cognitive profile of control and PD participants are shown in Table 2-5. Cognitive test scores on all domains, apart from language, were significantly lower in PD than control participants, even after controlling for age using ANCOVA. The greatest effect sizes for cognitive tests between PD and control participants were seen in tests of attention and OTS, a test of executive function.

Table 2-5 Cognitive profiles of all controls versus PD participants. Comparisons performed using ANCOVA with age as a covariate; values are adjusted means (SD)

Characteristic	Controls (n=99)	PD all (n=219)	p value	Effect size of cognitive test
MoCA	27.1 (2.5)	25.2 (3.4)	<0.001	0.76
MMSE	29.1 (1.2)	28.7 (1.3)	0.006	0.35
Power of attention (msec)	1272.2 (136.0)	1381.8 (233.7)	<0.001	0.81
Digit vigilance accuracy (%)	96.3 (5.8)	92.0 (12.8)	0.002	0.75
Pattern recognition memory	20.8 (2.5)	19.6 (3.1)	0.001	0.48
Spatial recognition memory	16.2 (1.8)	15.3 (2.2)	<0.001	0.51
Paired associates learning	1.8 (0.5)	2.0 (0.8)	0.009	0.42
One touch stockings	16.6 (2.5)	14.4 (4.2)	<0.001	0.84
Phonemic fluency	13.1 (4.7)	11.8 (4.7)	0.024	0.28
Semantic fluency	24.2 (6.1)	21.0 (6.6)	<0.001	0.52
Pentagon copying	2.0 (0.2)	1.8 (0.4)	0.009	0.51
Naming	2.8 (0.4)	2.9 (0.3)	0.365	0.09
Sentence	1.7 (0.6)	1.6 (0.6)	0.104	0.22

81 out of 195 PD participants who completed the MoCA (41.5%) scored less than 26 and therefore met the criteria for level 1 MCI, compared with 22.7% in the control group. Those who met the criteria for level 1 MCI were older, had a longer disease duration, fewer years of education, higher depression scores and were on higher doses of dopaminergic medications than those with PD who were cognitively normal (PD-CN) (Table 2-6). After adjusting for age and education in an ANCOVA, cognitive scores were worse in all tests apart from PoA in those with MCI. Level 2 criteria were met by 144 (65.8%) of PD participants at 1 SD below normative values, 93 (42.5%) at 1.5 SD and 49 (22.4%) at 2 SD. The corresponding figures for controls were 43 (43.4%) at 1 SD, 21 (21.2%) at 1.5 SD and 9 (9.1%) at 2 SD. PD participants who met level 2 criteria were older, completed fewer years of education, had greater motor disability and scored higher on the GDS than those who were cognitively normal (Table 2-7). Cognitive scores were poorer in all tests in those with level 2 MCI, even after adjusting for age and education in an ANCOVA. 54.5% of those classified as level 2 MCI at 1 SD also met criteria for level 1 MCI, with the corresponding figures for 1.5 and 2 SD 65.5 and 73.3%, respectively. Conversely, 90.1% of those with a MoCA score of less than 26 were impaired at level 2 criteria at 1 SD, 70.4% at 1.5 SD and 40.7% at 2 SD.

When PD participants were dichotomised by motor phenotype into those who were tremor dominant (TD) and non-TD, there was no difference in the proportions who were represented in the MCI groups either at level 1 (Table 2-6) or level 2 (Table 2-7) compared with PD-CN. Those who were non-TD scored significantly worse on semantic fluency than TD participants (adjusted mean score 19.6 *vs.* 22.5, p=0.002 after Bonferroni correction); but otherwise there were no significant differences between the groups.

Table 2-6 Cognitive profile of PD participants according to level 1 MCI criteria

Characteristic	Level 1 PD-CN (n=114)	Level 1 MCI (n=81)	p value	Adjusted p value[a]
Age (years)	64·1	69	<0.001	-
Male gender (n)	72	53	0.764	-
Education (years)	13.8	11.5	<0.001	-
Disease duration (mo)	4.7	6.4	0.022	-
MDS UPDRS part 3	26.4	29.7	0.54	-
Proportion non-TD phenotype (%)	45.6	51.9	0.390	-
LEDD (mg/d)	157.1	203.5	0.039	-
MoCA	27.7	21.9	<0.001	<0.001
MMSE	29.1	28.2	<0.001	<0.001
GDS 15	2.6	3.3	0.023	0.027[b]
Power of attention (msec)	1361.5	1417.2	0.02	0.311
Digit vigilance accuracy (%)	94.2	88.3	<0.001	0.018
Pattern recognition memory	20.6	18.1	<0.001	<0.001
Spatial recognition memory	15.7	14.8	0.006	0.043
Paired associates learning	1.8	2.3	<0.001	<0.001
One touch stockings	15.8	12.4	<0.001	<0.001
Phonemic fluency	13.2	10	<0.001	<0.001
Semantic fluency	22.6	18.7	<0.001	0.004
Pentagon copying	1.9	1.7	<0.001	0.007
Naming	3	2.8	<0.001	<0.001
Sentence	1.8	1.4	<0.001	0.001

Values are mean (SD); [a]Cognitive scores adjusted for age and years of education; [b]GDS adjusted for age; non-TD=non tremor dominant motor phenotype; LEDD=levodopa equivalent dose

Table 2-7 Cognitive profiles of PD participants according to level 2 MCI criteria

Characteristic	Level 2 PD-CN (n=75)	Level 2 MCI 1 SD (n=51; cumulative n=144)	Level 2 MCI 1.5 SD (n=44; cumulative n=93)	Level 2 MCI 2 SD (n=49; cumulative n=49)	p value	Adjusted p value[a]
Age (years)	61.2	67.3	68.9	69	<0.001	-
Male gender (n)	42	35	29	34	0.356	-
Education (years)	14.4	13.1	11.2	11.4	<0.001	-
Disease duration (mo)	5.3	6.2	5.8	4.8	0.43	-
MDS UPDRS part 3	22.8	27.2	31.7	31.6	<0.001	-
Proportion non-TD phenotype (%)	53.3	39.2	54.5	49.0	0.384	-
LEDD (mg/d)	177.5	149.2	202.4	186.8	0.362	-
MoCA	27.4	26.1	24.5	22.3	<0.001	<0.001
MMSE	29.3	28.8	28.4	27.9	<0.001	<0.001
GDS 15	2.3	2.8	2.9	4	0.016	<0.001[b]
Power of attention (msec)	1266	1310	1403.9	1601.9	<0.001	<0.001
Digit vigilance accuracy (%)	97.5	96.7	92.2	78.9	<0.001	<0.001
Pattern recognition memory	21.8	19.9	19.2	16.9	<0.001	<0.001
Spatial recognition memory	16.9	15.3	14.9	13.4	<0.001	<0.001
Paired associates learning	1.6	1.8	2.2	2.6	<0.001	<0.001
One touch stockings	16.9	14.8	14.1	11.1	<0.001	<0.001
Phonemic fluency	14.3	11.8	9.7	9.8	<0.001	<0.001

Semantic fluency	25	21.5	19.1	16.7	<0.001	<0.001
Pentagon copying	2	1.9	1.8	1.6	<0.001	<0.001
Naming	3	2.9	2.8	2.8	0.009	0.021
Sentence	1.9	1.6	1.5	1.4	0.001	0.003

[a]Cognitive scores adjusted for age and years of education; [b]GDS adjusted for age; non-TD=non tremor dominant motor phenotype; LEDD=levodopa equivalent daily dose

2.6.4 MCI subtypes

Among the five cognitive domains, memory impairment was the most common domain affected in PD participants at 1, 1.5 and 2 SD below normative values (24.2, 15.1 and 5.5%, respectively). This was followed by executive dysfunction in 24.7, 11.0 and 4.1% of participants and attention/working memory impairment in 20.1, 12.3 and 5.5%, respectively (Figure 2-2). Visuospatial impairment occurred in 13.2, 13.2 and 1.8%. Only 5.0% of participants were impaired in the language domain at 1 SD below the control mean. In control participants, the memory domain was also the most common domain affected at 1, 1.5 and 2 SD below normative values (12.1, 8.1 and 2.0%, respectively). This was followed by executive dysfunction in 9.1, 3.0 and 1.0% and attention in 7.1, 3.0 and 1.0%, respectively. Visuospatial dysfunction was seen in 5.1% at 1 and 1.5 SD only, with 6.1, 1.0 and 1.0% impaired in language domain at 1, 1.5 and 2 SD, respectively (Figure 2-2). When level 2 MCI criteria were applied at 1.5 SD, 12.8% of those with PD were classified as nonamnestic single-domain MCI (naMCI-sd), 7.7% had amnestic (aMCI-sd), 5.0% were nonamnestic multiple-domain (naMCI-md) and 8.2% had aMCI-md (Figure 2-3). For controls, the corresponding figures were 8.1% for naMCI-sd, 5.1% for aMCI-sd, 0% for naMCI-md and 3.0% for aMCI-md (Figure 2-3). The remainder of those classified as MCI had mixed deficits and did not fall within one classification. This included subjects who were impaired in just two tests but in different domains, meaning that there was no “pure” domain impairment.

Figure 2-2 Graphical representation of percentage of PD and control participants impaired according to single cognitive domain

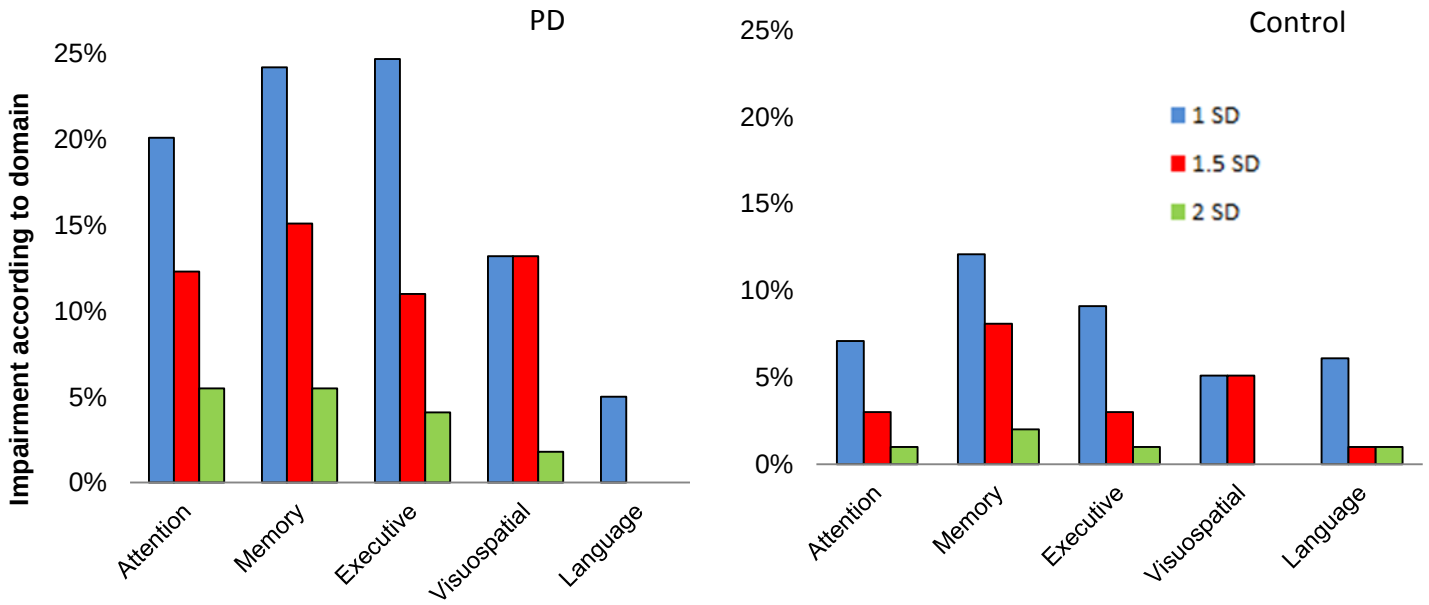
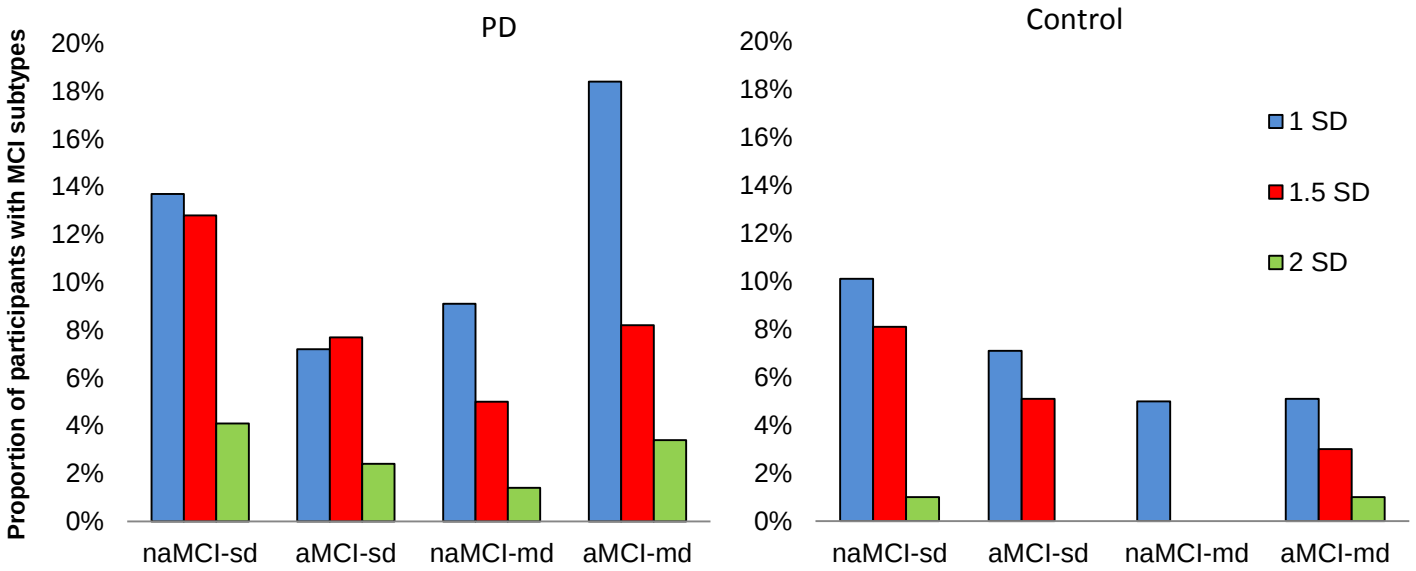


Figure 2-3 Graphical representation of PD and control participants impaired according to subtypes of MCI



na = non-amnestic; a = amnestic; sd = single-domain; md = multiple-domain

2.7 Discussion – cognition in early PD

This study has shown that in a large community-acquired cohort of early PD participants, PD-MCI is common, with 42.5% meeting level 2 MDS criteria at 1.5 SD below normative means. The clinical profile of these subjects differed from those with normal cognition, being significantly older, with greater motor impairment and depression. This is the first study to apply the new MDS PD-MCI criteria to a cohort of early PD.

Previous studies of patients with early or untreated PD have shown frequencies of PD-MCI between 14.8 and 36% (Foltynie *et al.*, 2004; Muslimovic *et al.*, 2005; Aarsland *et al.*, 2009b; Poletti *et al.*, 2012). A critical review of PD-MCI with a comprehensive literature review found a mean frequency of 26.7% (Litvan *et al.*, 2011), and an earlier large meta-analysis 25.8% (Aarsland *et al.*, 2010). However, very recent work using the MDS PD-MCI criteria in a prevalent PD cohort found a similar rate of 41%, when level 2 criteria were used at 1.5 SDs below normative values and the authors did not include cognitive complaint as part of the criteria (Marras *et al.*, 2013). The higher prevalence of PD-MCI in this study may be explained by our adoption of the new MDS PD-MCI criteria, which are generally considered to be less conservative than other definitions of MCI. In addition, by using two computerised test systems we may have increased the precision of measuring cognition. Further work is required to validate the new MDS MCI criteria in longitudinal assessments, and to determine the optimum type and number of cognitive tests that will best predict future cognitive decline. In addition, future studies should also determine the prognostic utility of MCI in PD and whether this diagnosis does predict future cognitive decline. A recent longitudinal study suggests that a diagnosis of MCI at baseline assessment is associated with a relative risk of dementia during follow-up of 39, although it should be noted that a significant proportion (21.6%) of those diagnosed with MCI reverted to normal cognition over three years (Pedersen *et al.*, 2013). Furthermore, the sensitivity and specificity of the screening tests used to determine level 1 PD-MCI should be further examined, as 27% of PD participants classified here as level 2 MCI at 2 SDs below normative values had normal MoCA scores. In keeping with our work, the aforementioned study found similar performance of the MoCA, with a specificity of only 44% in those who scored 26 or less (Marras *et al.*, 2013).

In common with a number of studies , we found that nonamnestic single-domain was the most common PD-MCI subtype (Janvin *et al.*, 2006; Caviness *et al.*, 2007; Aarsland *et al.*, 2009b; Mamikonyan *et al.*, 2009; Aarsland *et al.*, 2010; Sollinger *et al.*, 2010; Litvan *et al.*, 2011; Goldman *et al.*, 2012a). This is in contrast to the general population, where amnestic MCI is the most common subtype. MCI in the general population is generally less common than in PD, with prevalence rates of between 14 to 18% in those aged 70 and over; indeed, the annual progression to dementia in these patients may only be between 6-10% (Petersen *et al.*, 2009). The sub-optimal sensitivity of the MMSE in detecting cognitive impairment in PD is consistent with earlier studies (Nasreddine *et al.*, 2005; Zadikoff *et al.*, 2008).

The largest effect sizes seen for individual cognitive tests in PD participants were OTS and PoA from the frontal-executive and attention domains, respectively. These are likely to represent differing underlying pathophysiology, with PoA dependent on cortical cholinergic function and OTS with dopamine-dependent fronto-parietal-caudate function.

Motor phenotype did not come up as a determinant of MCI, which is somewhat surprising in view of its well-established association with dementia (Alves *et al.*, 2006; Burn *et al.*, 2006; Williams-Gray *et al.*, 2007a; Taylor *et al.*, 2008). It is recognised that motor phenotype does change over the course of the disease, with the majority of the transition seen from TD to PIGD (Alves *et al.*, 2006); it is therefore possible that our participants were at such an early disease stage that the true influence of motor phenotype has not yet emerged. Also, motor phenotype was calculated using the new MDS-UPDRS scale (Stebbins *et al.*, 2013), which does require further validation in larger, longitudinally assessed cohorts of PD subjects. Participants with non-TD disease did score worse on semantic fluency that those with TD disease, however, and this test has been previously identified as a predictor of future dementia (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a).

The principal strengths of this hypothesis-driven prospective study are its multimodal design and recruitment of a large community cohort with early disease. Through future longitudinal evaluation, we will determine whether those participants currently classified as PD-MCI at baseline are at increased risk of PDD, and the subtypes that ultimately predict cognitive decline. Previous cross-sectional studies of MCI have been

limited by small sample sizes and disease heterogeneity. Our sample is further strengthened by a matched control group who underwent detailed assessments.

Limitations include the fact that not all of the participants were treatment-naïve, which may limit the generalizability to all early stage PD patients, but arguably this makes the findings more relevant to clinicians and researchers. A large number of patients declined to take part in the study, which may limit how we translate the findings to the wider PD population. Participants who declined to take part in the study were older, were often caring for frail relatives, may have had a greater number of co-morbidities and thus are potentially more likely to develop dementia. These participants may have reflected the inclusion of geriatric medicine clinics as sources of referral which we believe enhanced our ability to include patients who may have otherwise not contributed. Apathy and depression may also have played a role in the refusal to participate, as both have been shown to be common in early disease (Khoo *et al.*, 2013). The population was largely recruited from the community, which may have introduced bias towards an increase in those with cognitive impairment. Certainly we noted that a significant proportion of our control participants also met the criteria for MCI, which is likely to represent the fact that we did not pre-screen for cognition. Again we would argue that this suggests that these controls were more representative of the wider global community. In terms of the cognitive measures used, domains of attention, memory and executive function were well covered, but testing of visuospatial function and language was limited, which may be one explanation for the low frequency of impairment observed in these domains and limit to absolute applicability of the MDS criteria. However, inability to copy intersecting pentagons has been shown to be a significant predictor of dementia risk (Williams-Gray *et al.*, 2009a), and there is evidence from other studies that language function is less likely to be affected in early cognitive dysfunction in PD (Caviness *et al.*, 2007).

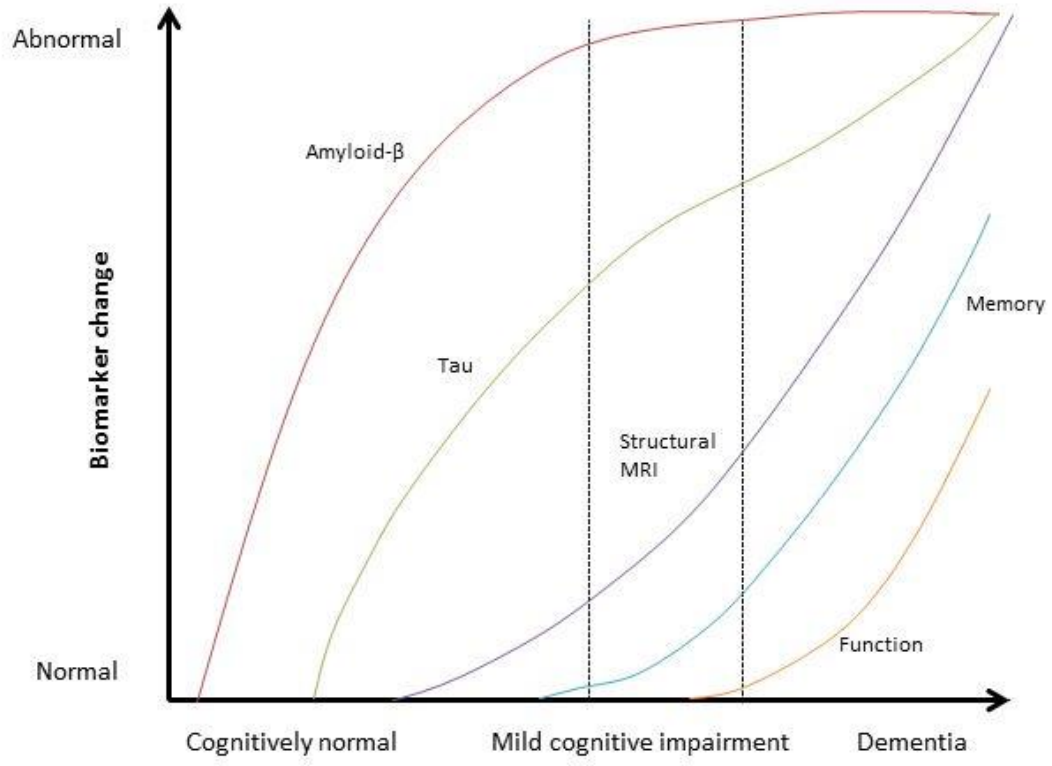
In summary, the ICICLE-PD study has shown that mild cognitive impairment is more common than previously reported in patients with newly diagnosed PD. Future longitudinal assessment will determine whether those participants are at increased risk of PDD, allowing for identification of disease modification strategies and targeted treatment options.

Chapter 3 Cerebrospinal fluid as a biomarker for cognitive decline in Parkinson's disease

3.1 Rationale for cerebrospinal fluid as a biomarker in neurodegenerative disease

Although the diagnosis of different neurodegenerative diseases may be based on clinical findings and imaging markers, often the earliest pathological alterations may be undetected before clinical findings become apparent. There is evidence that in Alzheimer's disease (AD), the earliest changes seen are in amyloid deposition, followed by neurodegeneration and cell loss. These precede detectable cognitive changes by many years (Jack *et al.*, 2010)(Figure 3-1).

Figure 3-1 Changes in biomarkers in AD (adapted from Jack et al, 2010)



Earlier recognition of these patients with neurodegenerative disorders and biochemical abnormalities using biomarkers is important because it allows prognostication with early and accurate diagnosis, allows identification of those patients who may be selected for neuroprotective or disease-modifying therapies and it offers insight into the underlying pathophysiology of the disease. Furthermore,

sensitive and specific biomarkers that permit the detection of preclinical disease could also offer objective measures of disease progression and outcome in clinical trials. Potential biomarkers in such diseases include structural or functional imaging, genetic markers, blood or cerebrospinal fluid (CSF). As shown in Figure 3-1, imaging changes may not be seen until late in the disease process; therefore, CSF may be a more suitable alternative.

CSF bathes the central nervous system (CNS) and may reflect brain pathology and brain metabolic systems more accurately than plasma or serum, although CSF proteins are derived from both blood and brain tissue (Pan *et al.*, 2007; van Dijk *et al.*, 2010). Proteins derived from blood enter the CSF via the choroid plexus, and follow the flow of CSF to the ventricles and subarachnoid space, thus creating an increasing protein gradient from ventricular to lumbar CSF (van Dijk *et al.*, 2010). This has implications for the site of CSF withdrawal, as does a traumatic tap with subsequent blood contamination (Teunissen *et al.*, 2009). Some CSF proteins are also influenced by circadian rhythm. In view of these variations, a standardised protocol for the collection of CSF has been produced (Teunissen *et al.*, 2009).

The most widely studied peptides in neurodegenerative diseases and dementia are amyloid-β and tau. More recently, α-synuclein has been investigated as a potential biomarker for synucleinopathies. These proteins are discussed in further detail below.

3.1.1 Amyloid-β

Amyloid-β (Aβ) proteins are a key component of extracellular amyloid plaques and cerebrovascular amyloid that form part of the pathological hallmark of AD (Glenner and Wong, 1984; Selkoe, 1996; Verbeek *et al.*, 2003). Aβ is derived from the proteolytic cleavage of Aβ precursor protein (APP), with the subsequent peptide production dependent on the length of amino acid chain produced by the enzymes β- then γ-secretases at the C-terminus. The peptides produced from this cleavage vary in their ability to aggregate, depending on their length and the degree of post-translational oxidation (Mollenhauer and Trenkwalder, 2009). The longer 42-amino acid form, Aβ42, is a major constituent of neuritic amyloid plaques, and is highly prone to oligomerization and aggregation into fibrils (Jarrett *et al.*, 1993; Selkoe, 1996; Verbeek *et al.*, 2003; Irvine *et al.*, 2008). It is this conformation of oligomers and fibrils that precipitate neurotoxicity. Aβ40, containing 40 residues, is more abundantly

produced than Aβ42 and is also found within neuritic amyloid plaques, although there is some evidence that Aβ42 is the more toxic of the species (Jarrett *et al.*, 1993). Aβ40 is not found within diffuse plaques, which are not fibrillar and contain almost exclusively Aβ42 proteins; these are thought to represent precursors of mature, neuritic plaques (Irvine *et al.*, 2008). Aβ is found within the CSF (Seubert *et al.*, 1992; Shoji *et al.*, 1992), with a robust inverse correlation found between CSF Aβ and ante- and post-mortem amyloid plaque pathology in AD (Clark *et al.*, 2003; Strozyk *et al.*, 2003; Fagan *et al.*, 2006; Grimmer *et al.*, 2009; Jack *et al.*, 2010). AD, DLB and PDD participants with positive PiB PET imaging consistently demonstrate low CSF Aβ42 levels (Fagan *et al.*, 2006; Grimmer *et al.*, 2009; Maetzler *et al.*, 2009), with neuropathological amyloid plaque numbers in the neocortex and hippocampus strongly associated with lower CSF Aβ42 levels (Strozyk *et al.*, 2003). The reason for reduced Aβ in CSF is thought to be due to sequestration within the parenchyma of senile plaques, with subsequently lower circulating levels available to diffuse into the CSF, although other reasons include the non-detection of Aβ oligomers and its binding to other proteins (Irvine *et al.*, 2008). Reduced circulating CSF Aβ42 levels have been found in other conditions, including Creutzfeldt-Jakob disease, where plaque pathology may be part of prion disease but does not form a major extent of the disease burden (Mollenhauer *et al.*, 2011a). Hence it has been postulated that Aβ42 may be a more non-specific measure of neurodegeneration. Although CSF Aβ42 and Aβ40 have demonstrated considerable variation in hour-to-hour sampling over a 36-hour period in non-demented control participants (Bateman *et al.*, 2007), this was not replicated in a small number of mildly impaired older adults sampled every 6 hours over the same time period (Moghekar *et al.*, 2012). Overall, however, low CSF Aβ is likely to represent a suitable biomarker of cortical Aβ plaque load.

3.1.2 Tau

Tau operates as a microtubule-associated protein, stabilising microtubules that are required for the axonal transport of cytoplasmic organelles within the neuron (Verbeek *et al.*, 2003). Tau is usually only mildly phosphorylated in health, but in AD becomes hyperphosphorylated, precipitating tau aggregation, accumulation of insoluble paired helical filaments and tangle formation, ultimately leading to neuronal cell death and leakage of tau into the CSF (Verbeek *et al.*, 2003). Hence, although total tau is

measured, phosphorylated tau levels in addition may be a more specific marker of tangle formation. In AD, intracellular neurofibrillary tangles are found in close proximity to Aβ plaques, lending support to the concept of Aβ induced neurotoxicity (Irvine *et al.*, 2008), although tau pathology is also found in other neurodegenerative disorders without Aβ pathology, including progressive supranuclear palsy, frontotemporal dementia and corticobasal degeneration. Tau is phosphorylated at a number of sites, including threonine 181, 205 and 231. Assays that detect tau phosphorylated at threonine 181 (P181-tau) are commonly used in clinical practice as a measure of phosphorylated tau. Neither total- or P181-tau were found to significantly vary in repeated measures over a 36-hour time period (Moghekar *et al.*, 2012), and these markers of tau plus Aβ42 remained stable over a 6 month assessment period in subjects with AD (Blennow *et al.*, 2007). In AD, increased CSF levels of total and phosphorylated tau correlated with neuropathological neurofibrillary tangle scores (Tapiola *et al.*, 1997; Buerger *et al.*, 2006; Tapiola *et al.*, 2009), suggesting that CSF tau measurements may be an appropriate antemortem measure of tangle deposition. However, tau levels are greatly elevated in Creutzfeldt-Jakob disease (CJD) (Otto *et al.*, 2002) and increased in acute stroke (Hesse *et al.*, 2001), suggesting that tau may also be a marker of general neuronal loss.

3.1.3 α-synuclein

As discussed in Chapter 1, pre-synaptic α-synuclein is the major constituent of Lewy bodies and Lewy neurites, and is found in PD and other synucleinopathies. Like amyloid, α-synuclein proteins first coalesce as soluble oligomers, then aggregate as protofibrils before forming insoluble fibrils (El-Agnaf *et al.*, 2003). There is evidence that these intermediate soluble oligomers and protofibrils may be the pathogenic species causing neuronal death and neurodegeneration (El-Agnaf *et al.*, 2003). α-synuclein can be detected in plasma, CSF and within blood cells (Henchcliffe *et al.*, 2011). To date, most studies have demonstrated lower total CSF α-synuclein in PD patients compared to controls (Tokuda *et al.*, 2006; Mollenhauer *et al.*, 2008; Hong *et al.*, 2010; Mollenhauer *et al.*, 2011b; Shi *et al.*, 2011) and compared to AD subjects (Mollenhauer *et al.*, 2011b; Tateno *et al.*, 2012), although others have shown no difference (Borghi *et al.*, 2000; Ohrfelt *et al.*, 2009; Reesink *et al.*, 2010). Reduced α-synuclein has been detected even in *de novo* PD patients (Mollenhauer *et al.*, 2013).

Synucleinopathies have generally been shown to have reduced CSF α-synuclein compared with tauopathies (Ballard *et al.*, 2010; Mollenhauer *et al.*, 2011b; Shi *et al.*, 2011), and in the largest study to date on CSF biomarkers in neurodegenerative disease, α-synuclein levels of less than 1.6 pg/μL had a positive predictive value of 90.7 % for a synucleinopathy (Mollenhauer *et al.*, 2011b). The explanation for the decrease in CSF α-synuclein could be due to intracellular aggregation, resulting in reduced extracellular release, alterations in gene transcription or a greater clearance of the protein from CSF (Mollenhauer and Trenkwalder, 2009). There is some evidence that α-synuclein increases with age (Hong *et al.*, 2010) and decreases with PD severity (Tokuda *et al.*, 2006), although this has not been consistently shown. α-synuclein is affected by blood contamination of the CSF and therefore red cell count should be controlled for (Hong *et al.*, 2010). As stated above, it has been postulated that oligomeric not total α-synuclein may be the toxic species in PD, and more recent studies that measured CSF oligomeric α-synuclein have found increased levels in PD (Tokuda *et al.*, 2010) and DLB (Paleologou *et al.*, 2009). To date there has been little investigation of α-synuclein and cognition, although one small study in DLB found that lower α-synuclein levels correlated with lower scores on MMSE and verbal fluency (Reesink *et al.*, 2010). Therefore, in view of conflicting results to date, definitive conclusions cannot be drawn on whether CSF measurement of α-synuclein in PD is a useful potential cognitive marker.

3.1.4 Other CSF proteins

Other CSF proteins that have been explored in neurodegenerative diseases include markers of inflammation such as interleukins, neuropeptides, neurofilaments and oxidative stress markers (Hu *et al.*, 2010; van Dijk *et al.*, 2010; Henchcliffe *et al.*, 2011). A further protein product indicative of oxidative stress and mitochondrial dysfunction is DJ-1, which has shown promise as a possible biomarker of PD (Hong *et al.*, 2010). In a large well conducted study, decreased DJ-1 levels in the CSF were found in PD compared with AD and control participants, with a sensitivity and specificity for patients with PD versus controls of 90 and 70%, respectively. Further work is required to validate these findings in wider neurodegenerative populations.

3.2 Cerebrospinal fluid in Alzheimer's disease and other dementias

The typical pattern of CSF findings in AD is well established (Verbeek *et al.*, 2003), and more recently, this pattern of decreased Aβ42 with increased total-tau (T-tau) and P181-tau has been recommended as supportive criteria for the diagnosis of AD (Dubois *et al.*, 2010). General patterns of CSF biomarkers in other forms of cognitive impairment are shown in Table 3-1. Changes seen in mild cognitive impairment (MCI) are similar to those seen in AD and predict the development of future AD (Mattsson *et al.*, 2009; Visser *et al.*, 2009), although it is likely that longitudinal cognitive decline only occurs in those with increased P181-tau levels (Desikan *et al.*, 2012). In DLB, the pattern of CSF markers is similar to that found in AD and has been hypothesised to be due to the burden of AD pathology (Parnetti *et al.*, 2008). However, DLB may be differentiated from AD by increased Aβ40 and reduced α-synuclein levels, thought to be due to disease-specific mechanisms of amyloid deposition driven by interactions with α-synuclein pathology (Bibl *et al.*, 2006). Changes seen in frontotemporal dementia include raised tau levels that are greater than in PD and controls, but less than changes seen in AD (Parnetti *et al.*, 2011). In addition, a modest reduction in Aβ42 and increase in Aβ40 has been found, with a more specific and marked reduction in Aβ38; this may be due to a disease specific phenomenon, masking of epitopes to antibodies or an upregulation of other Aβ peptides (Bibl *et al.*, 2007). Lastly, Creutzfeldt-Jakob disease is characterised by a massive increase in tau levels due to neuronal death, with reduced Aβ42 despite the relative absence of plaque pathology. It has been hypothesised that this may be due to the influence of prion proteins impeding Aβ metabolism, or a possible common chaperone complex that shows a high affinity binding and epitope masking of Aβ42 (Mollenhauer *et al.*, 2011a).

Table 3-1 CSF markers in cognitive impairment

Diagnosis	T-tau	P-Tau	Aβ42	Aβ40	Aβ42: Aβ40	Other comments	References
AD	↑↑	↑↑	↓↓	↔↓	↓	ApoE4 ↓ Aβ42 levels	(Verbeek *et al.*, 2003; Vemuri *et al.*, 2009)
MCI-AD	↑	↑	↓	-	↓		(Shaw *et al.*, 2009; Vemuri *et al.*, 2009; Koyama *et al.*, 2012)
DLB	↑	↑	↓	↑	-	↓ αsyn	(Mollenhauer *et al.*, 2005; Bibl *et al.*, 2006; Bibl *et al.*, 2010; Andersson *et al.*, 2011; Mollenhauer *et al.*, 2011b)
FTD	↑	↑	↔↓	↑	-	↓↓ Aβ38	(Bibl *et al.*, 2007; Parnetti *et al.*, 2011)
CJD	↑↑↑	↑↑↑	↓↓	↔	-	↑ αsyn	(Otto *et al.*, 2002; Mollenhauer *et al.*, 2008; Mollenhauer *et al.*, 2011a)

AD = Alzheimer's disease; MCI = mild cognitive impairment; DLB = dementia with Lewy bodies; FTD = frontotemporal dementia; CJD = Creutzfeldt-Jakob disease

3.3 CSF amyloid and tau as biomarkers of cognition in Parkinson's disease

In recent years, there has been an exponential increase in the number of studies assessing potential CSF biomarkers that may be predictive of AD, DLB, PD, PDD and atypical parkinsonian disorders. Differences in definition of the neurodegenerative disorder, differing CSF protein measurement systems (for example, enzyme linked immunosorbent assay (ELISA) versus bead-based approach in the Luminex platform) and diverse reading techniques (namely spectrometry versus luminescence) result in difficulties drawing comparisons in specific protein levels across studies. The majority of this chapter will discuss studies that have specifically looked at cognition and/or phenotypes in Parkinson's disease (highlighted in yellow in Table 3-2). In non-demented PD (PDND) participants, most studies have not demonstrated a significant difference in total tau levels between PDND and controls (Molina *et al.*, 1997; Steur *et al.*, 1998; Kanemaru *et al.*, 2000; Sjogren *et al.*, 2000; Lins *et al.*, 2004; Parnetti *et al.*, 2008; Compta *et al.*, 2009; Alves *et al.*, 2010; Parnetti *et al.*, 2011). The results are

similar for P181-tau, although only later studies specifically measured this protein (Parnetti *et al.*, 2008; Compta *et al.*, 2009; Alves *et al.*, 2010; Parnetti *et al.*, 2011). One recent small study did demonstrate increased total tau, total tau/Aβ42 ratio and clusterin (a glycoprotein released under cytotoxic conditions) levels in PD participants with a short disease duration compared to controls; the authors postulated that the findings may be due to high levels of neurodegeneration in early disease (Vranova *et al.*, 2010). In contrast, two large biomarker studies found that both total and P181-tau were lower in PD participants compared with controls (Shi *et al.*, 2011; Hall *et al.*, 2012), although it should be noted that both of these studies were designed to assess the accuracy of a number of CSF biomarkers across a number of neurodegenerative diseases, and not specifically to compare PD with other groups. In terms of amyloid markers and PDND participants, early studies plus one recent study did not demonstrate differences in Aβ42 levels compared to controls (Kanemaru *et al.*, 2000; Sjogren *et al.*, 2000; Holmberg *et al.*, 2003; Lins *et al.*, 2004; Parnetti *et al.*, 2011), whereas later studies have found consistently decreased Aβ42 levels in PDND subjects (Parnetti *et al.*, 2008; Compta *et al.*, 2009; Alves *et al.*, 2010; Shi *et al.*, 2011). Aβ40 and Aβ38 are also lower in these patients (Alves *et al.*, 2010). These changes may be present even before clinical PD manifests. In a recent study of asymptomatic and symptomatic *LRRK2* carriers, there was a trend towards lower CSF analytes in those with clinical disease compared to those with asymptomatic mutations, although this did not reach statistical significance (Aasly *et al.*, 2012). In the group overall, reduced CSF Aβ42 and tau levels correlated with lower striatal dopaminergic function as determined by PET tracers, with a significant association between Aβ42 and ^{18}F-6-fluoro-L-dopa uptake.

Table 3-2 Summary of studies assessing amyloid and tau CSF biomarkers in PD

Reference	Patients	Biomarkers	Results	Other notes
(Molina *et al.*, 1997)	C=25 (62.8 yrs); PDND=26 (66 yrs)	Total tau (ELISA Innogenetics)	Mean PD=132.8 *vs* C=159.9 pg/ml (not significant)	Tau not correlated with age, age onset PD, disease duration, UPDRS or H&Y stage.
(Steur *et al.*, 1998)	C=16 (58 yrs); PDND=67; PDD=48 (all PD mean age 62 yrs)	Total tau (ELISA Innogenetics), AST	Tau=155, 199, 204 ng/L (C, PDND, PDD) (not significant)	Tau & AST not correlated with MMSE, disease duration, dementia duration, age or sex. PDD defined MMSE<26.
(Kanemaru *et al.*, 2000)	C=19 (74.7 yrs); PDND=15 (72.9 yrs); DLB=11 (75.1 yrs); AD=24 (75.8 yrs)	Aβ42, total tau (ELISA Innotest)	Aβ42=713.6, 617.1, 349.2, 284.1 pg/ml (C, PD, DLB, AD) Tau=115.1, 116.6, 137.5, 460.1 pg/ml	Aβ42 signif ↓ in DLB & AD; tau signif ↑ in AD.
(Sjogren *et al.*, 2000)	C=32 (71.5 yrs); PDND=23 (70.7 yrs); AD=60 (66 yrs); FTD=17 (62.4 yrs)	Aβ42, total tau (ELISA Innogenetics), GAP-43	Aβ42=772, 610, 381, 553 pg/mL (C, PD, AD, FTD) Tau=307, 313, 743, 366 pg/mL	Aβ42 & tau no different in PD & controls but ↑ & ↓ cf AD, respectively.
(Holmberg *et al.*, 2003)	C=32 (65.9); PD=48 (62.3); MSA=36 (63.7); PSP=15 (68.5)	Aβ42 (ELISA Innotest)	Aβ42=800, 812, 552, 729 pg/mL (C, PD, MSA, PSP)	Signif ↓ Aβ42 in MSA only cf controls
(Lins *et al.*, 2004)	C=12 (62.8 yrs); PDND=12 (69.3 yrs); NPH=12 (75 yrs); AD=12 (71.8 yrs); VD=12 (76.4 yrs)	Aβ42, total tau (ELISA Innogenetics)	Aβ42=648, 598, 403, 454, 580 pg/mL (C, PD, NPH, AD, VD) Tau=224, 227, 267, 729, 280 pg/mL	Aβ42 & tau no different cf controls or VD.
(Bibl *et al.*, 2006)	C=23 (68.5 yrs); PDD=21 (72.4 yrs); DLB=21 (71.5 yrs); AD=23 (69.5 yrs)	Aβ37, 38, 39, 40 & 42 (Aβ-SDS–PAGE/immunoblot analysis)	Signif ↓ Aβ42 in AD & DLB cf controls; PDD not signif	DLB ↑ $A\beta40^{ox}$; Aβ42/37 accurately discriminated between dementias & controls. PDD diagnosed using DSM IV criteria for dementia
(Mollenhauer *et al.*, 2006b)	C=41 (70yrs); PDND=23 (72 yrs); PDD=73 (72 yrs)	Aβ42, total tau (ELISA Innogenetics), ApoE genotyping	Aβ42=641, 559, 466 pg/ml (C, PD, PDD); signif ↓PDD *vs* PD & C Tau=148, 216, 214 pg/ml; signif	Aβ42 & tau not correlated with age, disease duration, UPDRS, H&Y or MMSE. Changes marked in those with ApoE3/3.

			PDD *vs* C only	PDD diagnosed MMSE<25
(Parnetti *et al.*, 2008)	C=20 (60 yrs); PD=20 (62 yrs); PDD=18 (65 yrs); DLB=19 (70 yrs); AD=23 (70 yrs)	Aβ42, total tau, P tau (ELISA Innogenetics) MMSE, MODA, NPI	Aβ42=1014, 788, 647, 373, 544 pg/mL (C, PD, PDD, DLB, AD) Total tau=177, 160, 286, 508, 960 pg/mL P tau=42, 37, 52, 55, 119 pg/ml	Aβ42 signif ↓ in all groups cf controls; no difference between PD & PDD. No signif difference in total tau in controls, PD or PDD (↑DLB & AD). P tau ↑ in AD only. DLB only, trend towards ↓ Aβ42 & ↑ disease duration & ↑ total tau & ↓MMSE/MODA scores
***(Compta *et al.*, 2009)**	C=30 (71.1 yrs); PDND=20 (69.1 yrs); PDD=20 (72.5 yrs)	Aβ42, total tau, P tau (ELISA Innogenetics) Neuropsychological Assessment	Aβ42=638, 539, 410 pg/mL (C, PDND, PDD) Total tau=220, 247, 463 pg/mL P tau=31, 29, 46 pg/ml Signif ↑ T & P tau in PDD *vs* PDND & controls. Signif linearity in Aβ42 between 3 groups. No correlation with UPDRS or H&Y. MANOVA- age signif ass with total tau & P tau.	All PD- T & P tau signif associated with recall, recognition, naming & visuoperceptive deficits. Aβ42 signif ↓ in those with phonemic & semantic deficits & positive correlation with semantic fluency. PDND- Aβ42 signif ↓ in px with impaired phonemic fluency & positively correlated with this variable. PDD- T & P tau signif associated with impaired recognition & naming.
(Montine *et al.*, 2010)	C=115 (68 yrs); aMCI=24 (68 yrs); AD=49 (68 yrs); PD=41 (64 yrs); PD-CIND=58 (66 yrs); PDD=11 (71 yrs)	Aβ42, total tau, P181 tau (AlzBio3 Luminex kits from Innogenetics)	Aβ42=378, 322, 313, 220 pg/mL (C, PD, PD-CIND, PDD) Total tau=60, 54, 53, 33 pg/mL P tau=25, 20, 20, 17 pg/ml	PD-CIND defined as clinical dementia rating of 0.5 but without dementia. Aβ42 signif ↓ in PD-CIND & PDD cf controls. T tau levels unchanged in 3 PD groups. P tau signif ↓ in PD & PD-CIND cf controls. 15% PD, 29% PD-CIND & 45% PDD abnormal ↑ P tau/ Aβ42, cf >90% aMCI & AD.
(Bibl *et al.*, 2010)	C=40 (64.5 yrs); PDD=21 (73.2); DLB=15 (71.4); AD=45 (70.9)	Aβ42, total tau (ELISA Innotest); Aβ peptide patterns Aβ-SDS-PAGE/immunoblot	Aβ42=0.79, 0.51, 0.37, 0.41 ng/mL (C, PDD, DLB, AD) Total tau=0.23, 0.31, 0.37, 0.62 ng/mL	PDD signif ↓ Aβ42 & Aβ42% & ↑ Aβ40% cf controls. ↑ Aβ40% & ↓ Aβ42 in DLB vs PDD. Low Aβ42/tau sensitive marker for AD.

			Aβ42%=11.7, 7.5, 7.1, 4.4 Aβ40%=0.8, 1.1, 1.8, 0.9	↑ Aβ40% potential marker of DLB.
(Vranova *et al.*, 2010)	C=30 (58.8 yrs); PD=32 (59.9)	Aβ42, total tau (ELISA Biosource, Innogenetics);cystatin C, clusterin ELISA, Biovendor)	Total tau=194 vs 225 ng/L (C vs PD) Clusterin=9004 vs 4668 IU/L	Signif ↑ tau PD vs C. Signif ↑ tau, tau/ Aβ42 & clusterin in PD with symptoms <2 yrs cf > 2 yrs. May be due to high levels of neurodegeneration.
(Alves *et al.*, 2010)	C=36 (66.4 yrs); PD=109 (67.2); AD=20 (68.5)	Aβ38, 40 & 42 (Meso Scale Discovery kit by electrochemiluminiscence); Total & P181 tau (ELISA, Innogenetics); Full neuropsychological testing	Aβ42=445, 362, 208 pg/mL (C, PD, AD) Aβ40=7070, 5973, 6104 pg/mL Aβ38=629, 484, 514 pg/mL Total tau=228, 224, 435 pg/mL P tau=51, 58, 89 pg/ml	Drug-naïve PD patients. Signif ↓ Aβ42, Aβ40 & Aβ38 in PD cf controls. No difference in tau (ANCOVA with age, sex & education as covariates). Signif ass between Aβ42, Aβ40 & Aβ38 and memory in multiple sequential regression (not attentional-executive or visuospatial domains). Tau did not correlate with cognitive scores.
(Siderowf *et al.*, 2010)	PDND=45 (73 yrs)	Aβ42, total tau, P181 tau (Luminex platform with Innogenetics); Longitudinal f/u and DRS-2	Baseline Aβ42= 224 pg/mL Total tau=52 pg/mL P tau=18 pg/mL All patients had 1 year f/u, 20 had 2 year f/u and 3 evaluated at 3 years.	At baseline, no association between CSF & cognition. ↓ Aβ42 strongly associated with cognitive ↓ over time. Aβ42 ≤ 192 had ↓in DRS score that was 6.1 points greater than those > 192. Largest effect size for attention, then conceptualization then memory subscore. ↓ Aβ42 in ApoE4 carriers.
(Mulugeta *et al.*, 2011b)	C=12 (73.5 yrs); PDD=20 (73); DLB=23 (74); AD=30 (75.5)	Aβ38, 40 & 42 (Meso Scale Discovery kit by electrochemiluminiscence); Total & P181 tau (ELISA, Innogenetics)	Aβ42=337, 287, 223, 192 pg/mL (C, PDD, DLB, AD) Aβ40=8286, 5036, 5507, 5461 pg/mL Aβ38=635, 404, 385, 440 pg/mL Total tau=250, 303, 303, 382 pg/mL P tau=58, 57, 60, 86 pg/ml	No difference between PDD & DLB. Aβ42/Aβ38 strongest marker for differentiation of AD & DLB. Age & MMSE score correlated with total tau, Aβ38 & Aβ40 in all subjects. Aβ38 correlated with disease duration. Aβ42 correlated with UPDRS.
(Shi *et al.*, 2011)	C=137 (58.9 yrs); PD=126 (63.8); AD=50 (68.1); MSA=32 (60.3)	Aβ42, total tau, P tau (ELISA Innogenetics); Flt3 ligand,	Aβ42=404, 333, 209, 312 pg/mL (C, PD, AD, MSA)	T & P tau tended to ↑ with age. Aβ42 & T/P tau ↓ in PD & MSA cf

		fractalkine, DJ-1, α-syn	Total tau=62, 55, 95, 47 pg/mL P tau=29, 21, 57, 22 pg/ml	controls. Fractalkine/Aβ42 ↑ with UPDRS score. ↑Flt3 differentiated PD from MSA.
(Mulugeta *et al.*, 2011a)	C=12 (74.1 yrs); PDD=21 (73.6); DLB=24 (74 yrs); AD=50 (74.4)	Aβ38, 40 & 42 (Meso Scale Discovery kit by electrochemiluminiscence); Total & P181 tau (ELISA, Innogenetics); sAPPα & sAPPβ	Aβ42=479, 264, 262, 275 pg/mL (C, PDD, DLB, AD) Aβ40=7835, ?, 5833, 5297 pg/mL Aβ38=785, 366, 384, 814 pg/mL Total tau=269, 308, 297, 600 pg/mL P tau=58, 66, 63, 89 pg/ml	No signif differences in APP between groups, but APP correlated with MMSE. In LBD, signif correlations between sAPP*α* and sAPP*β* with all A*β* species & T-Tau, but not P-tau.
(Andersson *et al.*, 2011)	PDD=17 (73 yrs); DLB=47 (76); AD=150 (75)	Aβ42, total tau, P tau (ELISA Innogenetics)	Aβ42=566, 402, 409 (PDD, DLB, AD) ng/L T tau=314, 434, 625 ng/L P tau=51, 56, 78 ng/L	DLB ↑ T tau & ↓ Aβ42 cf PDD. In DLB, not PDD, CSF proteins correlated with MMSE memory scores.
***(Compta *et al.*, 2011a)**	C=9 (70 yrs); PDND=19 (69); PDD=19 (73)	Aβ42, total tau, P tau (ELISA Innogenetics); tau genotypes	Aβ42=667, 570, 389 pg/mL (c, PDND, PDD) T tau=248, 217, 358 pg/mL P tau=34, 28, 35 pg/mL	rs242557 tau gene polymorphism associated with ↑ T & P tau but only in those with Aβ42 < 500pg/mL. H1/H1 carriers ↑ P tau in PDD only. No signif difference in Aβ42 levels in ApoE4 carriers and non-carriers.
(Parnetti *et al.*, 2011)	C= 32 (61.9 yrs); PD=38 (69.3); DLB=32 (71.4); AD=48 (68.7); FTD=31 (64.3)	Aβ42, total tau, P tau (ELISA Innogenetics); αsyn (SuperSignal ELISA)	Aβ42=69, 43, 18, 35, 15 (C, PD, DLB, AD, FTD) ng/mL T tau=190, 232, 341, 734, 424 pg/mL P tau=35, 42, 49, 98, 59 pg/mL αsyn=69, 43, 18, 35, 15 mg/mL	αsyn levels signif ↓ in pathological groups cf controls. Inverse correlation between αsyn & T tau. In PD, Aβ42, T & P tau levels not different to controls. T tau/αsyn & P tau/αsyn ↓ showed best discrimination of PD.
(Mollenhauer *et al.*, 2011b)	C=23 (73 yrs); PD=273 (72); DLB=66 (72); MSA=15 (70); PSP=8 (72)	Aβ42, total tau (ELISA Innogenetics); αsyn (ELISA system mSA1/Syn1-BB)	Aβ42=499, 474, 378, 460, 529 (C, PD, DLB, MSA, PSP) pg/mL T tau=267, 180, 192, 146, 154 pg/mL αsyn=2.2, 1.3, 1.3, 1.1, 1.8 µg/µL	αsyn signif different across groups. The only signif predictor for αsyn was clinical diagnosis. αsyn < 1.6 µg/µL predicted synucleinopathies with 90.7% accuracy.
(Leverenz *et al.*, 2011)	PDND=22 (68.7 yrs)	Aβ42, total tau (Luminex-based MAP); BDNF;	Aβ42=544 pg/mL T tau=137 pg/mL	Correlation between processing speed & Aβ42, Aβ42/t-tau & BDNF.

		Neuropsychological testing	BDNF=285 pg/mL	Signif association between semantic fluency (vegetable) & Aβ42/t-tau. Effects attenuated by age.
(Hall *et al.*, 2012)	C=107 (70 yrs); PD=90 (63); PDD=33 (76); DLB=70 (74); AD=48 (78); PSP=45 (70); MSA=48 (64); CBD=12 (71)	Aβ42, T-tau, P tau & αsyn (Luminex MAP); NFL	Aβ42=630, 612, 559, 447, 362, 576, 589, 595 ng/mL (C, PD, PDD, DLB, AD, PSP, MSA, CBD) T tau= 473, 371, 365, 413, 840, 429, 528, 611 ng/mL P tau=49, 46, 52, 51, 103, 43, 42, 46 ng/mL αsyn= 67, 55, 59, 59, 94, 70, 56, 56 ng/mL	NFL associated with disease severity. Age correlated with αsyn in PD, controls & MSA. PDD ↑ P tau & NFL cf PD. PD & PDD ↓ T tau cf controls; PD ↓P tau. PD, PDD, DLB & MSA signif ↓ αsyn cf controls; AD signif ↑ cf other groups. NFL ↑ PSP, MSA & CBD cf others.
***(Compta *et al.*, 2012)**	C=12 (71.5 yrs); PDND=18 (69); PDD=15 (73)	Aβ42, total tau, P tau (ELISA Innogenetics); MRI	Aβ42=659, 579, 348 pg/mL (C, PDND, PDD) Total tau=237, 218, 395 pg/mL P tau=33, 28, 35 pg/ml	Signif negative association between grey matter volume & T/P tau; signif positive association for grey matter volume & Aβ42 in whole PD group for mostly frontal & temporal structures.
(Aasly *et al.*, 2012)	Asymptomatic LRRK2 carriers=18 (51 yrs); LRRK2 + PD=8 (67)	Aβ42, total tau, P tau (ELISA Innogenetics); Dopaminergic PETs	Aβ42=601 vs 504 (carriers vs LRRK2+PD) pg/mL T tau=36 vs 24 pg/mL P tau=21 vs 17 pg/mL	↓ Aβ42 & tau correlated with ↓ striatal DA function on PET. Signif association between Aβ42 & 18F-6-fluoro-L-dopa uptake.
(Jellinger, 2012)	C=17 (68.7 yrs); PD-NT=6 ; PD-TD=6 (mean age all PD 63.2); AD=27 (68.7)	Aβ42, total tau (ELISA Innogenetics)	Aβ42=658, 394, 147, 376 pg/mL (C, PD-NT, PD-TD, AD) T tau=223, 147, 193, 760 pg/mL Tau/ Aβ42=29, 45, 19.3, 49.5	Cortex tau/Aβ42 signif ↑ in PD-NT & AD cf controls and TD-PD. Note- difficult to see how this calculated, & also Aβ42 ↓ in PD-TD (lower than AD & PD-NT).
(Vranova *et al.*, 2012)	C=19 (56.2 yrs); PD-EDO=17 (48.9); PD-NT=16 (65.6); PD-TD=15 (67.3); AD=18 (68.4)	Aβ42, total tau (ELISA Innogenetics); tau/ Aβ42	Aβ42=830, 809, 631, 791, 423 ng/L (C, PD-EDO, PD-NT, PD-TD, AD) T tau=209, 235, 435, 263, 554 ng/L Tau/ Aβ42=0.28, 0.29, 0.55, 0.34, 1.5	PD-NT signif ↑ tau & tau/ Aβ42 cf controls, PD-EDO & PD-TD. In PD-NT, tau correlated with H&Y stage. No signif difference between AD & PD-NT in terms of tau or tau/ Aβ42.
(Alves *et al.*, 2013)	PD-PIGD=39 (67 yrs); PD-TD=60 (67.5)	Aβ38, 40 & 42 (Meso Scale Discovery kit by electrochemiluminiscence)	Aβ42=307 vs 408 pg/mL (PIGD vs TD) Aβ40=5664 vs 6453 pg/mL Aβ38=390 vs 578 pg/mL	PIGD signif ↓ Aβ42 & Aβ38 cf TD. PIGD signif ↓ all Aβ peptides cf controls. PIGD severity inversely associated with Aβ42 & Aβ38.

	Aβ42 & Aβ38 associated with lower limb bradykinesia.

C= Controls; PDND= PD non-demented; PDD= PD dementia; PD-CIND= PD cognitively impaired non-demented; PD-NT= PD non tremor-dominant; PD-TD= PD tremor dominant; PD-EDO= early disease onset; PIGD= postural instability gait difficulty; DLB= dementia with Lewy bodies; AD= Alzheimer's disease; MSA= multiple system atrophy; PSP= progressive supranuclear palsy; FTD= frontotemporal dementia; NPH= normal pressure hydrocephalus; VD= vascular dementia; aMCI= amnestic mild cognitive impairment; UPDRS= Unified PD Rating Scale; H&Y= Hoehn & Yahr stage; MMSE= mini mental state examination; MODA= Milan Overall Dementia Assessment; NPI= Neuropsychiatric Inventory; DRS-2= Dementia Rating Scale; AST= aspartate aminotransferase; ApoE= apolipoprotein; sAPP= soluble isoforms of amyloid precursor protein; NFL= neurofilament light chain; DA= dopamine; *= studies with patients from same cohort; cf=compared with.

With respect to cognitive impairment in PD, there is consistent evidence that there are differences in CSF markers in those with PDD compared to controls or PDND subjects, although one small early study that only measured total tau and aspartate aminotransferase (AST) did not find differences between these groups (Steur *et al.*, 1998). In DLB and PDD, CSF findings in DLB were similar to that in AD, with higher T-tau and lower Aβ42 in DLB than PDD, lending support to the hypothesis that these patients may have a larger AD burden (Andersson *et al.*, 2011). In addition, Aβ42 and T-tau levels may to be related to dementia severity and duration in DLB but not PDD, with one study reporting a negative association between CSF Aβ42 and disease duration and T-tau and cognitive scores (Parnetti *et al.*, 2008).

The first study to accurately characterise Aβ42 and T-tau in a cohort with PDD was in 2006, where Aβ42 was significantly lower and tau levels were higher compared to PDND and control subjects and to controls, respectively (Mollenhauer *et al.*, 2006b). This pattern was most marked in the subgroup of PDD patients who were carriers of the ApoE3 genotype; this is in contrast with findings in the general population, where ApoE4 genotype predicts lower Aβ42 levels (Morris *et al.*, 2010), and may have been due to the small numbers studied. Of note, however, PDD was diagnosed only with a MMSE score of less than 25 (Mollenhauer *et al.*, 2006b). In the next study that compared Aβ42, total and P181-tau in controls, PDND, PDD (n=18), DLB and AD subjects, Aβ42 was decreased in all groups compared with controls, but no difference was seen between PDND and PDD. Total and P181-tau were not significantly different between the control, PD or PDD groups, with no correlations between measures of global cognitive function and CSF markers (Parnetti *et al.*, 2008). In contrast, a further study found associations between neuropsychological deficits and CSF markers (Compta *et al.*, 2009). In addition to a graduation in Aβ42 levels from high (controls) to intermediate (PDND) to low (PDD) and greater total plus P181-tau in PDD compared to PDND subjects, increased tau levels were associated with memory, naming and visuospatial deficits, with lower Aβ42 found in those with impairments of verbal fluency (Compta *et al.*, 2009). Aβ42 correlated with phonetic fluency in PD patients with and without dementia (PDND), leading the authors to postulate that underlying Alzheimer-type pathology in PD was associated with cortical cognitive function, and that low CSF Aβ42 with impaired phonetic fluency in PDND could represent an early

marker of cognitive dysfunction. In the same group of patients, certain tau gene polymorphisms defined raised total and P181-tau levels, but only in the presence of low CSF Aβ42 values (Compta *et al.*, 2011a), with greater tau levels negatively correlated with frontal and temporal grey matter volume on MRI (Compta *et al.*, 2012). These changes are consistent with findings from the AD literature (Buongiorno *et al.*, 2011). In a community cohort of 109 *de novo* PDND patients, CSF Aβ42, Aβ40 and Aβ38 levels were reduced compared to controls, although no difference in tau was found (Alves *et al.*, 2010). In contrast to the results from Compta *et al*, these early stage PD subjects did not display correlations between tau and neuropsychological assessments, but Aβ peptides were significantly associated with impairments on the memory domain in multiple sequential regression, implying that Aβ metabolism is altered early in the disease course and may influence the heterogeneity of the pattern and course of cognitive decline associated with PD (Alves *et al.*, 2010). In comparison, a more recent study with 22 non-demented PD patients showed no correlations between Aβ42 or total tau and memory, processing speed or executive function after controlling for age, but did demonstrate a significant association between category fluency (naming vegetables) and Aβ42/T-tau ratio (Leverenz *et al.*, 2011). These results should be interpreted with caution due to the small sample size and the possible inconsistencies in the results, with category fluency (naming animals) revealing no relationship with any CSF parameters. A much larger cross-sectional study of 345 individuals compared CSF Aβ42, T-tau and P-tau levels in controls, amnestic MCI, AD, PD, PD cognitively impaired not demented (PD-CIND) and PDD patients (Montine *et al.*, 2010). Progressively lower Aβ42 levels were found in PD, PD-CIND and PDD patients, whereas average T-tau was unchanged and P181-tau levels were decreased compared to controls. One-third of PD-CIND and a half of PDD patients had the biomarker "signature" of AD (with an increased P181-tau/Aβ42 ratio) compared to over 90% of those with amnestic MCI or AD, leading the authors to propose that abnormal Aβ42 metabolism may be a common mechanism for PD-CIND and PDD.

The only published longitudinal study to date to look at the relationship between CSF biomarkers and cognitive decline in PD showed a strong association between reduced Aβ42 and cognitive decline over one year follow-up (Siderowf *et al.*, 2010). No

association was seen between T-tau and P-tau and cognitive decline. Although at baseline assessment there was no association between biomarkers and cognition, subjects with baseline CSF Aβ42 levels of ≤ 192 pg/mL (measured using Luminex platform) were at greatest risk of deterioration in cognitive scores over time, exhibiting a decline of an average of 5.8 more points per year on the Mattis Dementia Rating Scale (DRS-2) compared to subjects above this cut-off, after adjusting for age, disease duration and baseline cognitive status. Low CSF Aβ42 was associated with decline in multiple subscales of the DRS-2, with the largest effect size seen for attention, then conceptualization, then memory subscores. An accompanying editorial highlighted the need for longitudinal studies and better defined more homogeneous cohorts to validate these findings (Aarsland and Ravina, 2010).

Lastly, very recent studies in PD have examined the relationship of different phenotypes and CSF parameters (Alves *et al.*, 2010; Jellinger, 2012; Vranova *et al.*, 2012; Alves *et al.*, 2013). PD participants with a non-tremor dominant phenotype seem to have a pattern of CSF markers similar to changes seen in AD, with significantly higher T-tau levels and T-tau/Aβ42 ratio compared to tremor-dominant (TD) disease, early-onset disease or control participants (Vranova *et al.*, 2012). In non-tremor dominant participants, T-tau correlated with disease severity, as measured by Hoehn and Yahr scale (Vranova *et al.*, 2012). Although there was a trend towards reduced Aβ42 in this phenotype, this did not reach statistical significance. In comparison, newly diagnosed unmedicated PD participants with the postural instability gait difficulty (PIGD) phenotype at diagnosis had lower Aβ42 and Aβ38 levels compared with a TD presentation (Alves *et al.*, 2013). These Aβ peptides were associated with PIGD subscore severity and lower limb bradykinesia, suggesting that Aβ metabolism may influence motor function in addition to cognitive consequences even in early stage disease (Alves *et al.*, 2013). Tau levels were not reported in this study.

In conclusion, studies of CSF in PD have been largely cross-sectional in nature (with the exception of Siderowf's study), and frequently limited by small sample size and heterogeneous study groups. Overall, low CSF Aβ42 levels have consistently been associated with cognitive impairments in non-demented PD subjects, possibly related to AD and temporal lobe pathology. Results for tau and cognition have been conflicting, with some studies suggesting an increase, others no change and some

suggesting lower tau levels in those with PD and cognitive impairment. Alzheimer-type changes in the CSF seem to correlate with neuropsychological assessments of posteriorly mediated cortical impairments, although further work is required to clarify these findings with longitudinal evaluation over time.

Therefore, as part of the ICICLE-PD study, we aimed to determine whether certain CSF parameters may predict cognition in a homogeneous, well-defined cohort of early PD patients. We hypothesised that low CSF amyloid-β would be associated with cognitive decline, and that low CSF amyloid-β would be associated with more posteriorly-mediated cognitive dysfunction.

3.4 Specific CSF methodology

Lumbar puncture (LP) was performed on a subset of consenting participants as part of the ICICLE-PD study, within a four month period after the initial assessment. To standardise collections, all LPs were performed between 8 and 10am after an overnight fast and whilst withholding Parkinson's mediations, as some biomarkers may be altered by circadian rhythms and by medication use (Teunissen *et al.*, 2009). LPs were performed in the left lateral or sitting position, depending on the flexibility of the patient and presence of tremor. After appropriate local anaesthesia with lignocaine, a 20 gauge needle in L3-L5 was used to collect up to 10ml of CSF. Samples that were free of visual contamination by blood were centrifuged within 15 minutes of collection at 2000g at 4^{o}C for 10 minutes, with the supernatant then divided into aliquots of 0.25mL and frozen at -80^{o}C in labelled polypropylene cryovials. These cryovials have low protein binding potential and should not influence biomarker outcome (Teunissen *et al.*, 2009). Matched serum samples were taken simultaneously and centrifuged at 4000g at 19^{o}C for 10 minutes. CSF analysis was kindly provided by Professor Brit Mollenhauer and her team in Gottingen, Germany. Frozen samples were transported on dry ice without thawing, and immediately analysed for Aβ42, Aβ40, T-tau, tau phosphorylated at amino acid 181 (P181-tau) and total α-synuclein (αsyn). Samples were analysed using commercially available assays (tau protein: INNOTEST hTAU Antigen; p-tau: INNOTESTTM PHOSPHO-TAU(181P); Aß42: INNOTEST TH ß-AMYLOID (1-42) all Fujirebio Inc/Innogenetics, Gent, Belgien; p-tau and Aß40: hAmyloid ß40 Elisa ABETA GmbH, Heidelberg, Germany) (Mollenhauer *et al.*, 2006a). CSF αsyn values were determined as published previously with slight modifications (Mollenhauer *et al.*,

2008; Kruse *et al.*, 2012). Capture antibody MJF-1 clone 12.1 (kindly provided by Liyu Wu, Epitomics, Burlingame, CA, USA) was used at 3µg/ml. Detection was performed using Anti-α-Synuclein clone 42/α-Synuclein (BD Biosciences, Heidelberg, Germany) at 1µg/ml. An electrochemiluminescence-based detection system as developed by Meso Scale Discovery™ was used instead of classical ELISAs, due to its higher sensitivity and smaller amount of CSF required (Kruse *et al.*, 2012). αsyn standards were used in the range of 6 - 25000 pg/ml, with lower limits of detection was less than 10pg/ml. Intra-assay coefficients of variation were usually less the 20%, in most cases even less than 10%.

3.5 Statistical analysis

Statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL). Data were examined for normality with visual histograms and the Kolmogorov-Smirnov test. Means were compared using unpaired t-tests if normally distributed or the Mann-Whitney test for non-normally distributed data. Pearson Chi-square tests were used to compare between-group distribution of proportions. Pearson correlation (for normally distributed data) or Spearman's rank correlation coefficients (non-normal) were calculated to assess bivariate associations between neuropsychological tests and CSF parameters. If these were significant or reached near-significance, correlations were examined using scatter plots to determine whether the relationship was linear. A linear regression model (forced entry) was then used to control for age and education, as these co-variates are known to influence cognition. Due to exploratory nature of the study, we did not adjust for multiple comparisons; if a parameter was significant, even if by chance, it was felt that it merited further analysis (Rothman, 1990; Perneger, 1998; Feise, 2002).

In the second step of analysis, regression models were used to examine the contribution of CSF markers (independent variables) to cognition (dependent variable) and to determine whether CSF markers were an independent predictor of cognition. MoCA (global cognitive function), One Touch Stockings (OTS) of Cambridge (executive function), semantic fluency (executive domain), Power of Attention (PoA, attention domain), pattern recognition memory (PRM), paired associates learning (PAL) (both memory domains), pentagon score (visuospatial function) and total language score were analysed separately. Generally, one test from each domain was examined,

although semantic fluency was investigated in addition to OTS as there is evidence that impairment in this test predicts subsequent PDD (Williams-Gray *et al.*, 2007a). Both PAL plus PRM were assessed as part of the memory domain, as PRM was associated with Aβ42 using bivariate correlations and has been shown to be sensitive to temporal lobe damage, thus more 'posteriorly' mediated (Owen *et al.*, 1995b). PAL was also explored as part of the memory domain as this test is sensitive to both temporal and frontal damage and has been shown to predict Alzheimer's disease with a high degree of accuracy (Swainson *et al.*, 2001; Blackwell *et al.*, 2004). Age (Hughes *et al.*, 2000; Aarsland *et al.*, 2001; Hobson and Meara, 2004; Aarsland *et al.*, 2007b; Williams-Gray *et al.*, 2007a; Uc *et al.*, 2009; Williams-Gray *et al.*, 2009a) and education (Green *et al.*, 2002) influence cognition in PD and in the general population. Other potential contributors to cognition include motor phenotype (Aarsland *et al.*, 2003a; Alves *et al.*, 2006; Burn *et al.*, 2006; Uc *et al.*, 2009), disease severity (Hughes *et al.*, 2000; Aarsland *et al.*, 2001; Green *et al.*, 2002; Uc *et al.*, 2009), dopaminergic medication (Kehagia *et al.*, 2010) and presence of depression (Starkstein *et al.*, 1992). Therefore, age and education were controlled for in each model, and the associations of each cognitive test with motor phenotype score, UPDRS III score, LEDD and GDS were examined using bivariate correlation and controlled for if there was a significant or near significant association ($p<0.1$). These clinical predictors were entered in the first level of the model using forced entry, then Aβ42, Aβ40, P-tau and α-synuclein ('mechanistic' predictors) were entered in the second level using a stepwise method, to determine which, if any of the CSF parameters made a significant independent contribution to cognition. Collinearity diagnostics were inspected to test for multi-collinearity, with an average variance inflation factor (VIF) greater than one considered problematic. The Durbin–Watson statistic was used to identify autocorrelation (values less than one or greater than three were a cause for concern). In all regression models, standardised residuals were inspected to ensure they were approximately normally distributed and between ±3.0. There was no evidence of cases exerting undue influence in any model, as evidenced by Cook's distances ($D<1$).

In the final stage of analysis, the cases who were defined as level 2 mild cognitive impairment (PD-MCI) at 1.5 standard deviations (SDs) as per the methods outlined in Chapter 2 were compared to those who were cognitively normal (PD-CN) with respect

to the CSF values. The mean values of Aβ42, Aβ40, T-tau, P-tau and α-synuclein in those with PD-MCI versus PD-CN were initially compared using t-test or Mann-Whitney test, depending on the distribution of the parameters. A binary logistic regression model (forced entry) was then used to control for age and education. For the model to be a significant fit of the data, the -2 x log-likelihood statistic and its associated chi-square statistic were < 0.05. Residuals were examined to ensure model fit, and standardised residuals > 3 were inspected. There was no evidence of cases exerting undue influence in any model, as evidenced by Cook's distances (D<1). Model fit was reported using Cox & Snell's R^2 and Nagelkerke's R^2.

3.6 Results

3.6.1 General and clinical characteristics

67 PD participants consented to lumbar puncture, 56 from Newcastle and 11 from Cambridge. Comparison with those who did not consent is shown in Table 3-3. The sample was representative with the exception of years of education and depression scores. Those who did not consent scored slightly worse on digit vigilance, paired associates learning and pattern recognition memory cognitive tests. No samples were discarded due to blood contamination.

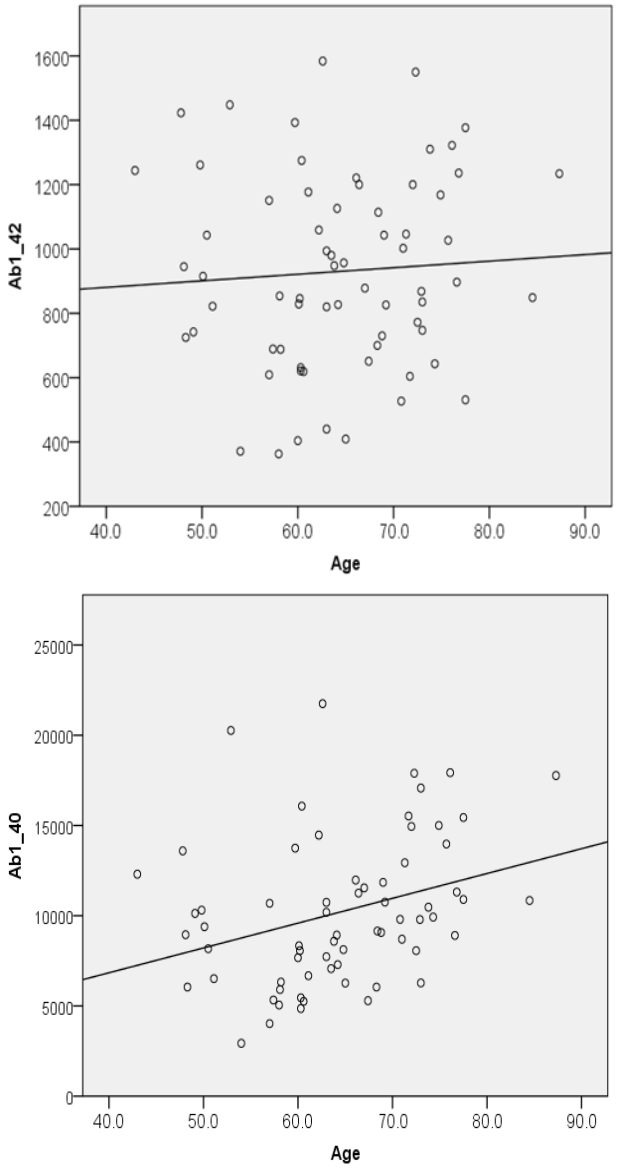
Table 3-3 Characteristics of participants who did and did not consent to lumbar puncture

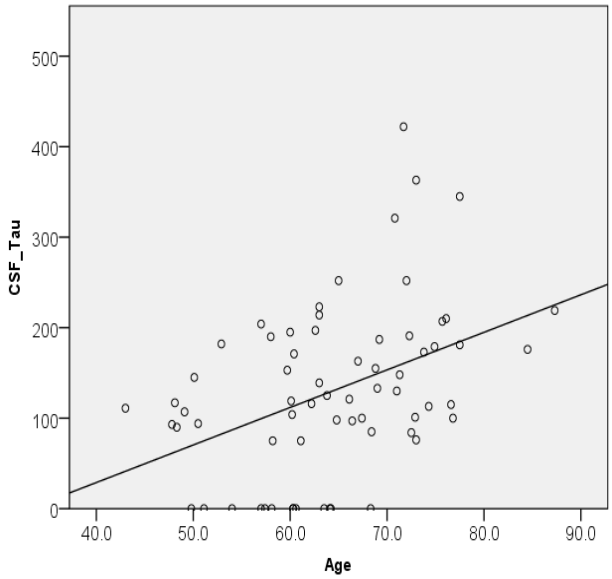
Characteristic	CSF +ve (n=67)	CSF –ve (n=152)	P value
Age (years)[a]	64.5 (9.4)	66.6 (9.8)	0.141
Male gender[b]	39 (58.2%)	101 (66.4%)	0.242
Disease duration (mo)	5.3 (5.6)	5.6 (4.7)	0.249
MDS UPDRS III	26.8 (11.8)	27.9 (11.9)	0.513
LEDD (mg/d)	140.3 (98.1)	194.6 (170.3)	0.071
Education (years)	13.5 (3.9)	12.5 (3.5)	**0.035**
GDS-15	2.4 (2.5)	3.1 (2.7)	**0.025**
MoCA[c]	25.2 (3.3)	25.5 (3.7)	0.314
MMSE	28.8 (1.1)	28.6 (1.3)	0.439
Phonemic fluency	11.3 (4.6)	12.0 (4.8)	0.386
Semantic fluency	22.0 (5.8)	20.7 (7.0)	0.062
PoA	1341.7 (163.0)	1396.1 (257.9)	0.261
Digit vigilance	94.4 (11.1)	91.0 (13.3)	**0.003**
PAL	1.82 (0.7)	2.1 (0.9)	**0.004**
PRM	20.7 (2.6)	19.2 (3.3)	**0.001**
SRM	15.6 (2.1)	15.2 (2.3)	0.331
OTS	14.5 (3.8)	14.5 (4.3)	0.798
Pentagon	1.9 (0.4)	1.8 (0.4)	0.219
Language	4.5 (0.7)	4.5 (0.7)	0.507

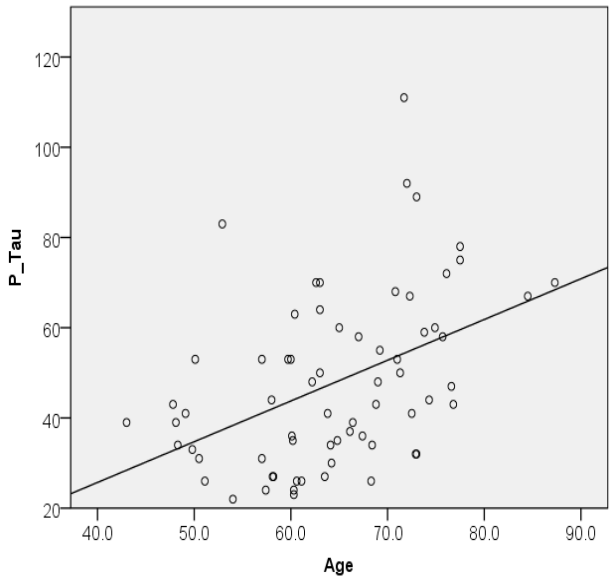
Data shown are mean (SD) or number (%). [a]Unpaired t-test, [b]Pearson Chi-Square; the remainder used Mann-Whitney test. [c]MoCA completed in 195 PD participants. PoA= power of attention; PAL= paired associates learning; PRM= pattern recognition memory; SRM= spatial recognition memory; OTS= one touch stockings of Cambridge.

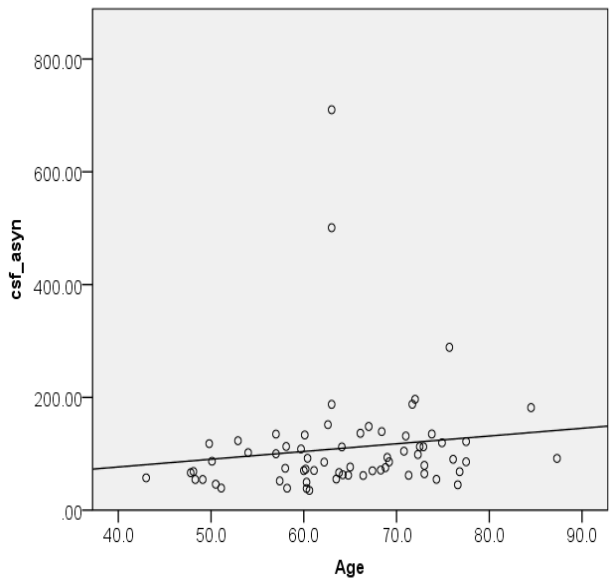
Scatter plots of the CSF analytes with age are shown in Figure 3-2. It was noted that there were two outliers in the α-syn data. One of these was noted to be a 'reddish sample' at analysis, and therefore analysis was performed with and without this to determine whether this unduly influenced the statistics. The findings below are with the outlier included, and any differences in the significance levels without it are reported in addition. The lower limit of detection for T-tau was <75 pg/ml, therefore these samples were coded as 0 pg/ml.

Figure 3-2 Scatter plots demonstrating the relationship between age and CSF markers




CSF_Tau
500
400
300
200
100
0
40.0
50.0
60.0
70.0
80.0
90.0
Age


P_Tau
120
100
80
60
40
20
40.0
50.0
60.0
70.0
80.0
90.0
Age


csf_asyn
800.00
600.00
400.00
200.00
.00
40.0
50.0
60.0
70.0
80.0
90.0
Age

As expected, there were significant positive correlations between Aβ42 and Aβ40 (r=0.698, p<0.001, Pearson's correlation coefficient) and T- and P181-tau (0.902, p<0.001, Spearman's rho). α-syn also correlated with Aβ42 (r=0.264, p=0.031), Aβ40 (r=0.455, p<0.001), T-tau (r=0.530, P<0.001) and P181-tau (r=0.571, p<0.001) (all Spearman's rho). In keeping with previous studies, there were significant correlations between age and T-tau and P181-tau (r=0.412 and r=0.466, respectively, p<0.001, Spearman's rho), age and Aβ40 (r=0.315, p=0.009, Pearson's correlation coefficient) and age and αsyn (r=0.278, p=0.023, Spearman's rho) (Parnetti *et al.*, 2011; Shi *et al.*, 2011; Hall *et al.*, 2012). None of the CSF markers correlated with measures of disease duration or severity, the latter as measured by the MDS-UPDRS. 15 subjects (22.4%) had pathological P181-tau levels above normal (>60 pg/ml), and five (7.5%) had Aβ42 <500 pg/ml. Raw CSF values are displayed in Table 3-4, including all PD participants and separate values for males and females. With the exception of α-syn, which showed a slightly higher mean value in females compared to males, there were no significant differences between each gender.

Table 3-4 Raw CSF marker values in all PD participants, and males vs females

	PD all (n=67)	Male (n=39)	Female (n=28)	P value (M vs F)
Age (years)	64.5 (9.4)	65.7 (10.5)	62.7 (7.3)	0.181[a]
Aβ42 (pg/ml)	930.5 (299.7)	935.2 (285.0)	923.8 (324.3)	0.879[a]
Aβ40 (pg/ml)	10203 (4092)	10102 (3833)	10342 (4497)	0.815[a]
T-tau (pg/ml)	130.4 (93.2)	127.4 (95.9)	134.6 (90.8)	0.358
P-tau (pg/ml)	47.8 (19.1)	47.0 (19.7)	48.9 (18.6)	0.620
αsyn (pg/ml)	110.2 (100.2)	100.3 (83.7)	124.0 (119.8)	**0.033**

Values are mean (SD). [a]Unpaired t-test, the remainder used Mann-Whitney test.

3.6.2 CSF and cognition

Associations between CSF analytes and cognitive variables were then examined using simple bivariate correlations, and are shown in Table 3-5. If a significant or near significant value was found, scatter plots were examined and a linear regression model

was used to control for age and education to determine if this significance remained (Table 3-6).

There was a significant association between Aβ42 and PRM (r=0.285, p=0.021, Spearman's rho), and there was a trend towards an association between Aβ42 and MoCA and Aβ42 and phonemic fluency (r=0.226, p=0.075, Spearman's rho; r=-0.220, p=0.073, Pearson's correlation coefficient, respectively). Further trends were found between Aβ40 and total language score (r=0.218, p=0.085, Spearman's rho) and P-tau and semantic fluency (r=-0.211, p=0.086, Spearman's rho). Scatter plots of these associations are shown in Figure 3-3.

Table 3-5 Bivariate analysis for CSF and cognitive variables

	CSF									
	Aβ42 r[a]	*p*	**Aβ40** r[a]	*p*	**T-tau** r[b]	*p*	**P-tau** r[b]	*p*	**αsyn** r[b]	*p*
MoCA[b]	.226	***.075***	.107	*.405*	-.196	*.124*	-.132	*.303*	.033	*.799*
PoA[a]	-.110	*.374*	-.020	*.871*	.124	*.316*	.126	*.311*	.097	*.435*
Digit vigilance[b]	.029	*.814*	-.044	*.723*	-.072	*.565*	-.108	*.386*	-.107	*.387*
Phonemic[a]	-.220	***.073***	-.110	*.374*	.090	*.471*	.030	*.809*	.001	*.996*
Semantic[a]	.131	*.292*	-.072	*.561*	-.169	*.171*	-.211	***.086***	.017	*.891*
OTS[b]	.102	*.418*	.114	*.367*	.007	*.955*	.009	*.942*	.060	*.636*
PRM[b]	.285	***.021***	.188	*.134*	-.054	*.671*	-.023	*.855*	-.058	*.648*
SRM[a]	.182	*.146*	.126	*.317*	-.036	*.778*	-.061	*.631*	-.149	*.235*
Pentagons[b]	.061	*.624*	.015	*.901*	-.087	*.483*	-.111	*.372*	-.026	*.832*
Language[b]	.188	*.141*	.218	***.085***	-.024	*.854*	.038	*.765*	.172	*.177*

[a]Normally distributed data; [b]non-normally distributed; r value using Pearson's correlation coefficient if both variables are normally distributed and Spearman's rho if not. MoCA= Montreal Cognitive Assessment; PoA= power of attention; phonemic= phonemic fluency; semantic= semantic fluency; OTS= one touch stockings of Cambridge; PRM= pattern recognition memory; SRM= spatial recognition memory; language= total language score.

Figure 3-3 Scatter plot demonstrating relationships between CSF markers and cognitive assessments

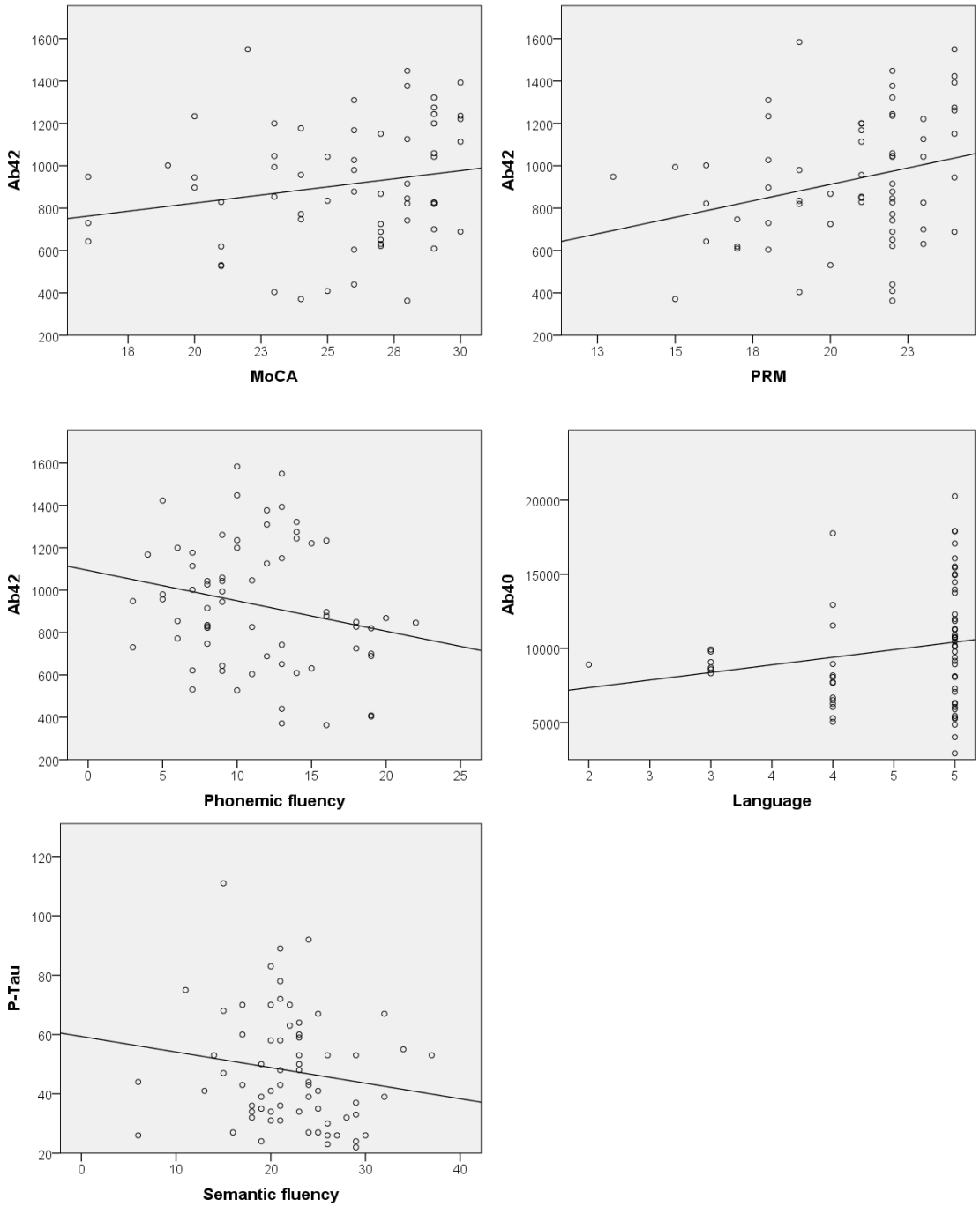


Table 3-6 Predictors of association between cognition and CSF biomarkers

Regression	B	SE	β	p
MoCA				
Linear regression model (df=3, n=62), p = 0.002, R^2 = .215				
Age	-.105	.049	-.253	0.037
Education	.277	.107	.302	0.012
Aβ42	.003	.002	.245	**0.041***
PRM				
Linear regression model (df=3, n=64), p = 0.003, R^2 = .207				
Age	-.060	.032	-.217	0.064
Education	.191	.080	.273	0.021
Aβ42	.003	.001	.316	**0.008***
Phonemic fluency				
Linear regression model (df=3, n=66), p <0.001, R^2 = .259				
Age	.030	.054	.061	0.580
Education	.541	.128	.461	<0.001
Aβ42	-.003	.002	-.205	0.064
Language				
Linear regression model (df=3, n=62), p = 0.056, R^2 = .119*				
Age	-.018	.011	-.215	0.115
Education	.034	.023	.187	0.137
Aβ40	.000055	.000	.296	**0.031***
Semantic fluency				
Linear regression model (df=3, n=66), p = 0.035, R^2 = .127				
Age	-.170	.081	-.275	0.040
Education	.296	.178	.200	0.101
P-tau	.001	.041	.003	0.982

In a linear regression model that allowed the co-variates of age and education to be controlled for, Aβ42 was associated with global cognition, as measured by MoCA (β

standardised coefficient=0.245, p=0.041). Together, age, education and Aβ42 explained 21.5% of the variance in MoCA score (p=0.002). Similarly, Aβ42 remained associated with PRM, a test of temporal lobe function (β standardised coefficient=0.316, p=0.008), with this CSF marker plus age and education explaining 20.7% of the variance in PRM score (p=0.003). By contrast, the significant associations between Aβ42 and phonemic fluency and P-tau and semantic fluency were lost after controlling for the aforementioned variables in the model (β standardised coefficient=-0.205, p=0.064 and β=0.003, p=0.982, respectively). Although the overall model did not explain the variance in language (R^2=0.119, p=0.056), reduced Aβ40 was correlated with poorer language score (β=0.296, p=0.031). However, it can be seen from the scatter plots in Figure 3-3 that the relationship between language and Aβ40 was not linear, and therefore firm conclusions cannot be drawn from these statistics.

To further explain cognition using CSF parameters, tests from each cognitive domain were entered into a linear regression model as the dependent variable and are shown below:

1. Global cognition (Montreal Cognitive Assessment)

Table 3-7 Regression model statistics and coefficients of variables for MoCA score in PD (n=63)

Independent	β	P-value
Model 1		
Age	-.220	0.070
Education	.250	0.051
UPDRS 3	-.178	0.156
R^2 = .185; P-value of change = 0.007		
Model 2		
Age	-.261	**0.030***
Education	.250	**0.045***
UPDRS 3	-.165	0.176
Aβ42	.237	**0.046***
R^2 = .239 ; P-value of change = 0.046		

In a bivariate analysis, MoCA score demonstrated a negative association with motor severity score which reached near significance (r=-.0.240, p=0.058, Spearman's rho); therefore UPDRS plus age and education were entered into the regression model. A greater level of Aβ42 was an independent determinant of MoCA score and explained 5.4% of variability (Table 3-7). In the final model, age, education and Aβ42 were independent determinants, explaining 24% of variability in MoCA. Motor severity was not significant, and the remainder of the CSF parameters were excluded from the model as they were also not significant.

2. Executive function (One Touch Stockings of Cambridge)

Table 3-8 Regression model statistics and coefficients of variables for OTS score (n=61)

Independent	β	P-value
Model 1		
Age	-.280	0.024
Education	.160	0.207
UPDRS 3	-.246	0.053
Motor phenotype score	.096	0.431
R^2 = .197; P-value of change = 0.014		
Model 2		
Age	-.261	**0.006***
Education	.250	0.123
UPDRS 3	-.165	0.093
Motor phenotype score	.075	0.528
Aβ40	.237	**0.047***
R^2 = .253 ; P-value of change = 0.047		

In addition to age and education, OTS displayed a significant negative association with UPDRS 3 score (r=-0.268, p=0.031), with a trend towards a positive correlation with motor phenotype score (a greater score indicating more tremor-dominant features, r=0.213, p=0.099). A larger Aβ40 value was an independent determinant of OTS score, explaining 5.6% of variability (Table 3-8). The final model explained 25% of variability in OTS, with age and Aβ40 independent determinants. As there was only a trend

towards significance between OTS and motor phenotype score and the latter value was not significant in either model, the analysis was repeated without motor phenotype being entered into the initial step. This changed the model, with the final model explaining 24% of variability in OTS (P-value of change=0.030), and age (β=-0.391, p=0.003) plus P-tau (β=0.284, p=0.003) independent determinants.

3. Executive function (semantic fluency)

Table 3-9 Regression model statistics and coefficients of variables for semantic fluency (n=67)

Independent	β	P-value
Model 1		
Age	-.285	**0.015***
Education	.211	0.069
LEDD	-.253	**0.029***
R^2 = .191; P-value of change = 0.004		

In a bivariate analysis for explanatory variables for semantic fluency, levodopa equivalent daily dose (LEDD, mg/day) negatively correlated with test score (r=-0.253, p=0.039). LEDD plus age and education explained 19% of variance in semantic fluency, with age and LEDD significant independent predictors in the final model. None of the CSF parameters added to the final model and were therefore not included, although Aβ42 showed a trend towards significance (β=.197, p=0.086).

4. Attention (Power of Attention)

Table 3-10 Regression model statistics and coefficients of variables for PoA (n=66)

Independent	β	P-value
Model 1		
Age	.203	0.073
Education	-.177	0.116
GDS	.427	**<0.001***
R^2 = .251; P-value of change < 0.001		

PoA score is a sum of reaction times, with a higher score indicating more impairment, and showed a positive correlation with GDS score (r=0.341, p=0.005) in bivariate analysis. Again there were no CSF markers that added to the model with age, education and GDS explaining 25% of variance in PoA. Only GDS was an independent determinant (β=0.427, p<0.001).

5. Memory (pattern recognition memory)

Table 3-11 Regression model statistics and coefficients of variables for PRM (n=65)

Independent	β	P-value
Model 1		
Age	-.202	0.073
Education	.148	0.200
UPDRS 3	-.404	0.001
R^2 = .261; P-value of change < 0.001		
Model 2		
Age	-.225	**0.035***
Education	.173	0.113
UPDRS 3	-.401	**<0.001***
Aβ42	.312	**0.004***
R^2 = .357; P-value of change = 0.004		

PRM was negatively associated with UPDRS (r=-0.371, p=0.002). Aβ42 was an important determinant of PRM score, explaining nearly 10% variability in the final model (Table 3-11). Age, education, motor severity score and Aβ42 together accounted for 36% variability in PRM score, with age, UPDRS and Aβ42 independent predictors.

6. Memory (paired associates learning)

Table 3-12 Regression model statistics and coefficient variables for PAL (n=65)

Independent	β	P-value
Model 1		
Age	.372	0.002
Education	-.239	0.039
R^2 = .212; P-value of change = 0.001		
Model 2		
Age	.521	**<0.001***
Education	-.292	**0.009***
P-tau	-.354	**0.005***
R^2 = .310; P-value of change = 0.005		

Paired associates learning (PAL) 'mean trials to success' was used as the score within this test (with a greater score indicating worse performance), as this was used in Chapter 2 as part of the definition of mild cognitive impairment. No other clinical variables correlated with PAL score apart from age and education, and in the final model, these plus P-tau explained 31% of variance in PAL score. All variables were independent determinants, with increasing P-tau levels predicting a better test score. However, it was noted that the model did not meet one of the assumptions required for generalisation, with a maximum standardised residual value of > ±3.0 (one value was 4.4). Therefore, the model was repeated with this case excluded. This did not change the overall model (R^2=31%, p=0.005), with age (β=.533, p<0.001), education (β=-.279, p=0.014) and P-tau (β=-.359, p=0.005) remaining independent contributors to PAL score. The model of PAL was also repeated using PAL 'total errors' (again a higher score indicating more impairment) and the same variables, as total error score has been shown to was very sensitive to the testing of an MCI (non-PD) group and also the prediction of AD (Swainson *et al.*, 2001; Blackwell *et al.*, 2004). In the final model, only age and education were included and both were significant contributors, explaining 24% of PAL total error score, but P-tau was no longer significant (p=0.082). The standardised residuals were almost within ± 3.0 (one residual was 3.3).

7. Visuospatial function (pentagon score)

Table 3-13 Regression model statistics and coefficient variables for pentagon score (n=67)

Independent	β	P-value
Model 1		
Age	-.173	0.160
Education	.119	0.356
UPDRS	-.130	0.312
R^2 = .275; P-value of change = 0.172		

Pentagon score demonstrated a trend towards an association with UPDRS on bivariate analysis (r=-0.211, p=0.086). The final model, although explaining 28% of variance in pentagon score, was not significantly better at predicting pentagon score than using the mean pentagon score, with no clinical or CSF characteristics independent determinants of visuospatial function. In addition, the assumptions for generalisation of the model were not met, with the standardised residual scores ranging from -5.2 to 0.927.

8. Language

Table 3-14 Regression model statistics and coefficient variables for language (n=62)

Independent	β	P-value
Model 1		
Age	-.135	0.274
Education	.153	0.237
UPDRS 3	-.065	0.628
GDS	-.169	0.206
LEDD	-.283	0.027
R^2 = .189; P-value of change = 0.034		
Model 2		
Age	-.271	**0.037***
Education	.197	0.113
UPDRS 3	.012	0.929
GDS	-.133	0.298
LEDD	-.364	**0.004***
Aβ40	.356	**0.010***
R^2 = .283; P-value of change = 0.010		

Language score was significantly inversely associated with both LEDD (r=-0.327, p=0.009) and GDS (r=-0.291, p=0.022), with a trend towards correlation with UPDRS (r=-0.210, p=0.098). These plus age and education were therefore entered into the first step of the model, with these clinical variables plus Aβ40 explaining 28% of variability in language score. In the final model, younger age, lower LEDD and increased Aβ40 were independent determinants of language score, with Aβ40 explaining 9% of the variance. Aβ42 was also a significant predictor (partial correlation=.268, p=0.044), but was forced out by Aβ40 and hence not included in the final model. As motor severity showed only a trend towards significance in association with language score and did not independently contribute to either model, the model was repeated with this variable removed. This did not alter the final model, which explained 28% variability with age, LEDD and Aβ40 being independent predictors of

language score. Below is a summary table of the contributors to cognition discussed in this section, using clinical and CSF parameters in regression models.

Table 3-15 Summary table of independent contributors to cognitive assessments

	MoCA	OTS	Semantic fluency	PoA	PRM	PAL	Pentagon	Language
Age	X	X	X		X	X		X
Education	X					X		
UPDRS 3					X			
LEDD			X					X
GDS				X				
Aβ42	X				X			
Aβ40		X*						X
P-tau		X*				X		

X=significant determinant of cognition in the final regression model; *Differing CSF parameters included, depending on whether motor phenotype included in model

3.6.3 CSF and MCI

To further examine CSF and its relationship with MCI, participants were dichotomised into those who met the criteria for level 2 MCI at 1.5 standard deviations (SDs) and those who were cognitively normal (see Chapter 2 for description of methods as to how MCI was defined). CSF values according to cognitive status are shown in Table 3-16. PD participants who met criteria for level 2 MCI (PD-MCI, n=21) had significantly fewer years of education than those who were cognitively normal (PD-CN, n=46) (11.2 *vs.* 14.5 years, p<0.001, Mann-Whitney test). The PD-MCI cohort had significantly lower mean Aβ40 levels compared with PD-CN (8781 *vs.* 10,852 pg/ml, p=0.029, t-test), and there was a trend towards reduced Aβ42 in the MCI group (840.2 *vs.* 971.6 pg/ml, p=0.096, t-test). Initially there was no significant differences in T-tau or P-tau in either group (p=0.456 and p=0.425, respectively, Mann-Whitney test).

A binary logistic regression model was used to control for age and education, as increased age is associated with risk of MCI (Foltynie *et al.*, 2004; Muslimovic *et al.*, 2005; Williams-Gray *et al.*, 2007a; Kim *et al.*, 2009; Mamikonyan *et al.*, 2009; Aarsland *et al.*, 2010; McColgan *et al.*, 2012) and there was a significant difference between the number of years of education between the two groups. After controlling for these

variables, significant differences in all CSF parameters remained between the two cognitive groups, with the exception of α-synuclein. T-tau, P-tau, Aβ42 and Aβ40 values were all significantly lower in the PD-MCI groups (p=0.032, p=0.039, p=0.041 and p=0.009, respectively) (Figure 3-4 and Table 3-17 to Table 3-20). Inspection of residuals revealed that two cases (case 23 and 43, both from the PD-MCI group) had standardised residuals > 3. The analysis was therefore repeated with these cases excluded, which did not change the significance of the models or CSF parameters (p=0.020, p=0.035, p=0.017 and p=0.005 for T-tau, P-tau, Aβ42 and Aβ40, respectively).

Of the five participants with Aβ42 levels less than 500 pg/ml, only one was classified as PD-MCI, with a corresponding figure of three out of 15 subjects with P-tau levels greater than 60 pg/ml. Three of the low Aβ42 and six of the high P-tau group scored less than 26 on the MoCA, thus meeting the criteria for level 1 MCI.

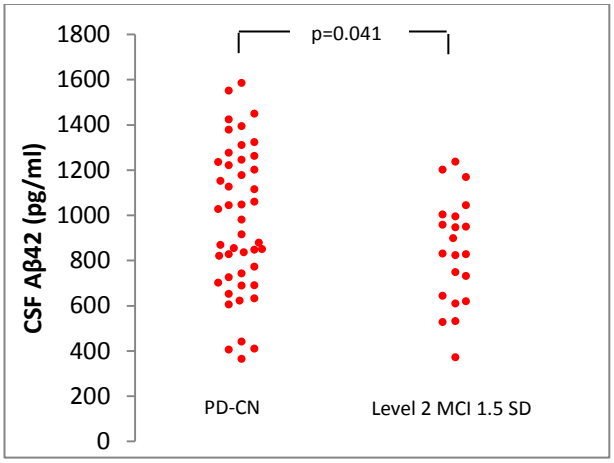
Table 3-16 CSF profile according to cognitive status

	Level 2 PD-CN	**Level 2 MCI 1.5 SD**	**Unadjust-ed p**	**adjusted p[b]**
No. patients	46	21		
Age (yrs)	63.6 (9.7)	66.3 (8.7)	0.291	-
Education (yrs)	14.5 (4.0)	11.2 (2.8)	**<0.001[a]**	-
CSF T-tau (pg/ml)	135.2 (92.1)	119.9 (97.1)	0.456[a]	**0.032**
CSF P-tau (pg/ml)	49.2 (19.8)	44.8 (17.4)	0.425[a]	**0.039**
CSF Aβ42 (pg/ml)	971.6 (319)	840.2 (234.6)	0.096	**0.041**
CSF Aβ40 (pg/ml)	10852 (4369)	8781 (3035)	**0.029**	**0.009**
αsyn (pg/ml)	119.3 (116.6)	90.3 (43.9)	0.358[a]	0.182

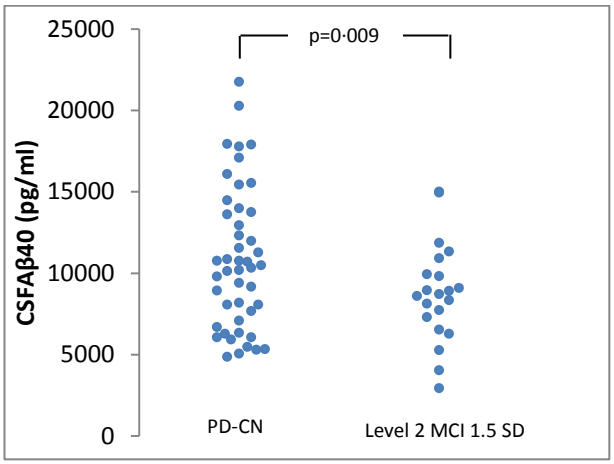
Values are mean (SD); [a]Mann-Whitney test; remainder unpaired t-test;

[b]Adjusted for age and education in a binary logistic regression model.

Figure 3-4 Scatter plot of (A) CSF Aβ42 and (B) CSF Aβ40 levels (pg/ml) in PD-CN and PD-MCI at 1.5 SD below normative values



(A)



(B)

Table 3-17 Binary logistic regression statistics for MCI and T-tau

	B (SE)	P-value	Exp (B)	95% CI for Exp (B)	
				Lower	Upper
Age	.069 (.041)	0.088	1.072	.990	1.160
Education	-.401 (.128)	0.002*	.670	.521	.860
T-tau	-.009 (.004)	0.032*	.991	.983	.999

R^2 = .249 (Cox & Snell), .350 (Nagelkerke); Model Chi-squared (3) = 19.17, p < 0.001

Table 3-18 Binary logistic regression statistics for MCI and P-Tau

	B (SE)	P-value	Exp (B)	95% CI for Exp (B)	
				Lower	Upper
Age	.069 (.041)	0.094	1.071	.988	1.161
Education	-.377 (.124)	0.002*	.686	.537	.875
P-tau	-.042 (.020)	0.039*	.959	.922	.998

R^2 = .247 (Cox & Snell), .347 (Nagelkerke); Model Chi-squared (3) = 19.02, p < 0.001

Table 3-19 Binary logistic regression statistics for MCI and Aβ42

	B (SE)	P-value	Exp (B)	95% CI for Exp (B)	
				Lower	Upper
Age	.035 (.036)	0.326	1.036	.966	1.111
Education	-.376 (.131)	0.004*	.687	.531	.887
Aβ42	-.002 (.001)	0.041*	.998	.996	1.000

R^2 = .241 (Cox & Snell), .339 (Nagelkerke); Model Chi-squared (3) = 18.50, p < 0.001

Table 3-20 Binary logistic regression statistics for MCI and Aβ40

	B (SE)	P-value	Exp (B)	95% CI for Exp (B)	
				Lower	Upper
Age	.087 (.046)	0.058	1.090	.997	1.192
Education	-.394 (.131)	0.003*	.675	.522	.871
Aβ40	-3.10×10^{-4} (1.18×10^{-4})	0.009*	.9997	.9995	.9999

R^2 = .301 (Cox & Snell), .422 (Nagelkerke); Model Chi-squared (3) = 23.96, p < 0.001

Lastly, the CSF parameters of those with level 2 PD-MCI (n=21) were then examined further in an exploratory analysis. Due to the small numbers impaired in each cognitive domain, participants were instead dichotomised into those with single- or multiple-domain MCI and those with amnestic *vs.* nonamnestic impairments. No significant differences between mean CSF levels were seen in single- (n=9) *vs*. multiple- (n=12) domain MCI (Aβ42: 792.3 *vs.* 876.2, p=0.400; Aβ40: 8207.3 *vs*. 9210.9, p=0.468; T-tau: 134.8 *vs*. 108.7, p=0.556; P-tau: 44.3 *vs*. 45.2, p=0.917; asyn: 87.2 *vs*. 92.6, p=0.788, respectively). Interestingly, PD subjects with amnestic MCI (n=11) had significantly lower T-tau levels compared with nonamnestic MCI (n=10; 77.8 *vs.* 166.1, respectively, p=0.034). P-tau levels showed a trend towards significance (amnestic 38.7 *vs.* nonamnestic 51.5, p=0.093). The amnestic and nonamnestic participants did not differ significantly in mean Aβ42 (837.1 vs. 843.7, respectively, p=0.951), Aβ40 (8214.1 vs. 9404.2, p=0.383) or asyn levels (79.5 vs. 102.2, p=0.248). When these values were entered into a binary logistic regression model, however, with amnestic/nonamnestic as the dependent variable, and age, education plus T-tau or P-tau as co-variates, neither T-tau nor P-tau were significant determinants. Furthermore, the model chi-square was not significant, indicating that the addition of the variables to the model did not significantly affect its predictive power (for T-tau model chi-square statistic (3) = 7.54, p>0.05; *B*=.019, p=0.052. For P-tau, model chi-square statistic (3) = 5.64, p>0.05; *B*=.086, p=0.108).

3.7 Discussion: CSF as a biomarker for cognition in PD

In line with previous studies, CSF Aβ42 and Aβ40 levels were found to be significantly lower in those with impaired cognition (Mollenhauer *et al.*, 2006a; Compta *et al.*, 2009; Montine *et al.*, 2010), with no differences in αsyn levels. It should be noted, however, that only total αsyn and not oligomeric αsyn was measured; the latter has been postulated to be the pathogenic species that may precipitate neuronal degeneration (Paleologou *et al.*, 2009). Aβ42 correlated with both global cognition and visual PRM, the latter being sensitive to deficits of temporal lobe function. Aβ42 and Aβ40 are major fibrillar components of amyloid plaques, one of the pathological hallmarks of AD (Selkoe, 1996; Caughey and Lansbury, 2003). Although the pathophysiology of PD-MCI is poorly understood and likely to be heterogeneous, it is most likely the result of a complex and varying interaction between neurotransmitter dysfunction, synaptic

pathology, protein aggregation and neuronal damage. To date, only one neuropathological study in well-characterised PD-MCI in which participants were followed prospectively has been reported (Adler *et al.*, 2010). The neuropathology was heterogeneous, with some cases exhibiting limbic or neocortical Lewy body (LB) pathology and the remainder predominantly consisting of brainstem LB pathology. Diffuse amyloid plaques were seen in the majority of cases, with two of the amnestic MCI subgroup meeting the neuropathological criteria for AD. Reduced Aβ42 in our participants with PD-MCI therefore suggests that these patients may have a higher burden of distinct pathology based within medial temporal lobe of an Alzheimer's-type, a finding strengthened by the correlation with PRM on neuropsychological testing. PRM is insensitive to levodopa therapy (Lange *et al.*, 1992) and has been associated with scores on a dementia screen in a large group of PD patients (Owen *et al.*, 1993). It is therefore possible that even with disease duration of less than six months, alterations in Aβ metabolism are present in a subset of patients, leading to Aβ fibrillization, aggregation and deposition of amyloid plaques, with subsequent increased risk of future dementia. Indeed there is evidence that Aβ alterations occur early and may affect motor circuits in addition to cognition, with reduced Aβ in *de novo* PD patients (Alves *et al.*, 2013; Halliday, 2013). By contrast, a trend towards decreased T-tau and P181-tau in subjects with PD-MCI was found, consistent with some (Alves *et al.*, 2010; Montine *et al.*, 2010) but not all (Compta *et al.*, 2009) previous work. Indeed, there is evidence that tau levels are higher in AD and DLB than PDD (Parnetti *et al.*, 2008; Andersson *et al.*, 2011). These findings are consistent with the temporal ordering of biomarker abnormalities seen in AD, which may have some translational application in the development of cognitive decline in PD: Here markers of Aβ deposition occur early and are only followed by markers of neuronal death (such as increased tau) much later in the disease trajectory. The findings are also consistent with the autopsy study from Adler and colleagues in PD-MCI which demonstrated little neurofibrillary tangle deposition (Adler *et al.*, 2010). This is the first work on CSF findings in PD-MCI classified by the MDS criteria, and the small differences in tau levels seen between the groups are of uncertain significance, with further work required including longitudinal data to clarify the findings.

Some of the most interesting results from this work are derived from the regression models using CSF parameters to explain cognition. Aβ42 was an independent contributor to global cognition, as measured by MoCA score, and explained 5% of variability. As discussed above, Aβ42 was also an independent predictor of PRM, sensitive to deficits of temporal lobe function (Owen *et al.*, 1995b) and hence a more 'posteriorly' mediated cognitive test. Semantic fluency has also been postulated to have a more posterior cortical than fronto-striatal basis and has been shown to predict cognitive decline in one incident cohort of PD (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a), but amyloid pathology did not seem to contribute in this study. Lesion studies have demonstrated that frontal lobe injuries produce similar deficits on phonemic and semantic fluency, whilst temporal lobe damage produces a greater impairment in semantic compared to phonemic fluency (Henry and Crawford, 2004a). In addition, PD patients are significantly more impaired on semantic relative to phonemic fluency, which is likely to reflect the dependence on semantic memory in the former test (Henry and Crawford, 2004b). The neural networks of semantic memory, which require activation of long-term memory, are thought to originate in the temporal lobe, specifically in the medial temporal lobe (Pihlajamaki *et al.*, 2000). It is therefore surprising that Aβ did not contribute to variability in semantic fluency and may indicate a differing underlying pathophysiological process, such as cholinergic loss, dopaminergic denervation or cortical Lewy body deposition. There was, however, a trend towards significance, and alternatively the study could have been underpowered to detect such differences. Most previous studies of CSF in PD have not demonstrated an overall association between semantic fluency and Aβ (Alves *et al.*, 2010; Leverenz *et al.*, 2011), and although one study did report that CSF Aβ was significantly lower in those with impaired semantic and phonemic fluencies with a significant positive correlation with semantic fluency (Compta *et al.*, 2009), the disease duration of the participants was much greater than in the present study (mean 10 years).

Executive function as measured by OTS, a test of planning requiring working memory (Owen *et al.*, 1995a) that has been shown to activate fronto-parietal-caudate circuitry (Baker *et al.*, 1996; Cheesman *et al.*, 2005), was more difficult to interpret in the context of CSF biomarkers. In the initial regression model, Aβ40 contributed to OTS score, but after removing motor phenotype, it was P-tau that was a significant

contributor. In addition, the latter relationship was positive, indicating that greater P-tau levels predict a better score on OTS. OTS is thought to be primarily mediated by cortical dopamine levels (Williams-Gray *et al.*, 2009a). It has been hypothesised that in PD, the reason for reduced tau and Aβ levels seen in the CSF is due to deposition of soluble tau and Aβ oligomers, which instead of forming insoluble tangles and plaques precipitate the aggregation of α-synuclein into soluble oligomers then the insoluble fibrils found in Lewy bodies (Shi and Zhang, 2011). If this is true, it may explain in part why there is a positive relationship between P-tau and better scores on both OTS and PAL, with greater tau levels indicating reduced Lewy body burden. Indeed, the finding that tau contributed to PAL 'mean trials to success' score but not 'total errors' score strengthens this assumption, as 'total error' score has been shown to predict AD (Swainson *et al.*, 2001; Blackwell *et al.*, 2004). These findings also correspond with those indicating reduced tau levels in those with amnestic versus nonamnestic MCI. Although both T-tau and P-tau demonstrated a strong positive correlation with α-synuclein, this does not disprove the above hypothesis, and may be because total and not soluble α-synuclein levels were measured; the latter is thought to be the more toxic species (Paleologou *et al.*, 2009). Hence this would support the thinking that cognitive impairment in PD has a somewhat different pathology to AD.

Neither attention nor visuospatial function had CSF parameters in their final model. Attention is likely to be cholinergically mediated, at least in part (Yarnall *et al.*, 2011), and therefore it is predictable that amyloid and tau pathology did not contribute to the model. Although one study in PD did demonstrate an association between attention subscores and low CSF Aβ42, the effect was only seen after longitudinal assessment (Siderowf *et al.*, 2010). It is likely that the associations and independent contributions of CSF parameters will change over time in our ICICLE-PD study. The lack of contribution of biomarkers to visuospatial function is perhaps more unexpected, given the previous work from Cambridge indicating that impaired pentagon copying early in disease is predictive of subsequent cognitive decline (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). Again it is possible that this could be due to pathology that is not detected by CSF assays, such as the presence of cortical Lewy bodies. Also, it should be noted that in this study pentagon copying, with possible scores of 0, 1 or 2,

was the sole measure of visuospatial function and therefore may not have the sensitivity required to delineate independent contributors.

Lastly, the finding that Aβ40 contributed to variability in language function is intriguing. As discussed above, Aβ40 is less prone to aggregate as compared to Aβ42, and it has been suggested that Aβ40 is more likely to reflect Aβ peptide production from amyloid precursor protein rather than amyloid deposition per se (Andreasson *et al.*, 2007; Alves *et al.*, 2010). The language tests that were used as part of the study were relatively crude and form part of the MoCA, but it is well established that localisation of language function occurs in the temporal lobe and hence is a more posteriorly mediated cognitive domain (Spitsyna *et al.*, 2006). Although language function may be less affected than other cognitive domains in PD (Troster, 2008; Barone *et al.*, 2011), the finding that Aβ pathology significantly contributes to language variability verifies the results for PRM above, and suggests that lower Aβ40 and Aβ42 increases the risk of subsequent cognitive decline due to temporal lobe AD-type pathology.

The strengths of this project are its accurate characterisation of a homogeneous cohort of early PD participants and relatively large numbers of patients for a CSF study. The analysis of CSF biomarkers was performed by an internationally-renowned team with multiple previous publications on the subject. However, it should be noted that our mean values for CSF parameters were somewhat higher than those seen in other studies using an electroluminescence platform (Mollenhauer *et al.*, 2011b). The lack of comparators in the form of either control or AD participants who underwent CSF sampling could also be viewed as a weakness. It can be seen from Figure 3-4 that there is considerable overlap in individual Aβ42 and Aβ40 levels between PD-MCI and PD-CN, and hence it is unlikely that CSF alone can be used as a biomarker for cognitive decline. However, in the future, hopefully lower levels of these analytes could be used with other risk markers to predict subsequent risk of cognitive impairment. As discussed in chapter 2, some of the neuropsychological domains may be simplistic in terms of localisation of separate functions. Lastly, without neuropathological data, it is difficult to exclude concomitant AD pathology as a cause for our findings.

In conclusion, significantly lower Aβ42 and Aβ40 levels were found in PD-MCI, and reduced Aβ42 correlated with temporal lobe impairments. Lower CSF Aβ42 and Aβ40 levels could therefore be used in a set of risk markers to determine those at a greater

risk of cognitive decline, with these subjects targeted for future early therapeutic intervention and disease modification.

Chapter 4 Short latency afferent inhibition as a biomarker for cognitive decline in Parkinson's disease

4.1 Rationale for short latency afferent inhibition as a biomarker in Parkinson's disease

The deposition of cortical and subcortical Lewy bodies is implicated in the underlying pathophysiology of dementia in PD (Aarsland *et al.*, 2005a), with recent clinicopathological studies demonstrating a synergistic role for Alzheimer-type pathology in PDD (Lashley *et al.*, 2008; Kempster *et al.*, 2010; Compta *et al.*, 2011b). Additionally, imaging (Bohnen *et al.*, 2003) and post-mortem (Tiraboschi *et al.*, 2000) studies support a cholinergic basis for cognitive impairment, although other neurotransmitters are likely to be involved. Cholinergic dysfunction and its role in PDD are discussed more extensively in Chapter 1 (section 1.6.5). The pathophysiology of mild cognitive impairment in PD patients (PD-MCI) is less well characterised, with some evidence suggesting that acetylcholine deficiency may contribute (Meyer *et al.*, 2009).

A biomarker that could predict those at risk of cognitive decline would be invaluable in allowing appropriate targeting of therapeutic interventions. Short latency afferent inhibition (SAI) is one possibility and provides an *in vivo* non-invasive measurement of cholinergic function, substantiated by studies demonstrating decreased SAI in Alzheimer's disease (AD) (Di Lazzaro *et al.*, 2002; Nardone *et al.*, 2008a), DLB (Di Lazzaro *et al.*, 2007b) and after administration of the muscarinic antagonist scopolamine in normal subjects (Di Lazzaro *et al.*, 2000). Most recently, SAI was found to be significantly impaired in patients with PDD (Celebi *et al.*, 2012). To date, there have been no studies exploring the cholinergic basis of PD-MCI using SAI, or indeed examining SAI in early PD. The aim of this part of the study was therefore to determine whether subjects with early PD had evidence of cholinergic dysfunction, and to establish whether SAI contributed to cognitive dysfunction. We hypothesised that PD participants would have reduced SAI compared to controls, and that those with PD-MCI would have decreased SAI compared to cognitively normal PD patients (PD-CN). An additional hypothesis was that more posteriorly-mediated cognitive tests would show greater association with SAI, being putatively more cholinergically mediated.

4.2 What is short latency afferent inhibition?

SAI can be described as the inhibition of the motor cortex that occurs approximately 20 milliseconds (ms) after median nerve stimulation at the wrist (Tokimura *et al.*, 2000; Chen *et al.*, 2008; Chen, 2013). It is seen as suppression of a motor evoked potential (MEP) in hand muscles, produced by transcranial magnetic stimulation (TMS) of the contralateral motor cortex, after a conditioning afferent stimulation of the median nerve, and is measured by averaging conditioned/unconditioned MEPs (%) with a greater value indicating less inhibition. For example, a value of 45% would indicate that the mean MEP responses were reduced to 45% of the test unconditioned response. SAI is thought to be cortical in origin, as an early study demonstrated through recordings of reduced descending corticospinal volleys induced by TMS following median nerve stimulation in conscious patients with implanted electrodes in the cervical epidural space (Tokimura *et al.*, 2000). The same study also established that SAI arises around 1ms after latency of the N20 component (a negativity that usually peaks around 20ms after the stimulus) of the somatosensory evoked potential achieved after median nerve stimulation, and lasts for around 8ms (Tokimura *et al.*, 2000; Chen *et al.*, 2008). Although the exact underlying pathway of SAI is unknown, it is likely to involve a fast pathway from the periphery to the motor cortex, via thalamocortical projection to either the motor cortex directly or the sensory then motor cortex (Chen, 2013). As discussed above, SAI is reduced in cholinergically-mediated dementias ((Di Lazzaro *et al.*, 2002; Di Lazzaro *et al.*, 2007b; Nardone *et al.*, 2008a) and after administration of the muscarinic antagonist scopolamine (Di Lazzaro *et al.*, 2000); it is also increased after a single dose of a cholinesterase inhibitor (Di Lazzaro *et al.*, 2002) and SAI response predicts long term response to this drug (Di Lazzaro *et al.*, 2005a). Therefore acetylcholine (ACh) is likely to be involved in mediating SAI.

Other neurotransmitters that may influence SAI in normal or diseased states include γ-aminobutyric acid (GABA) and dopamine. Previous studies have shown that diazepam increases (or has no effect) (Di Lazzaro *et al.*, 2007a) whilst lorazepam and zolpidem decrease SAI (Di Lazzaro *et al.*, 2005c; Di Lazzaro *et al.*, 2007a). Benzodiazepines act on $GABA_A$ receptors, with differing drugs having different affinity for receptor subtypes. It is possible that the reason for these distinct actions on SAI could be due to actions on

separate receptor subtypes, as diazepam is relatively non-selective, the affinity of lorazepam for various GABA receptor subtypes is unknown, and zolpidem has a high affinity for α1-subunits bearing $GABA_A$ receptors (Di Lazzaro *et al.*, 2005c). The interaction between cortical ACh and GABA is complex, with human pathological studies demonstrating ACh-induced activation of GABA neurones with subsequent triggering of GABAergic postsynaptic currents (Alkondon *et al.*, 2000), and animal models indicating decreased ACh release following administration of GABA receptor agonists (Imperato *et al.*, 1993) and enhanced release with $GABA_A$ antagonists (Giorgetti *et al.*, 2000; Diez-Ariza *et al.*, 2002). Whilst these studies using different benzodiazepines indicate that GABA receptors are involved in the circuitry underlying SAI, it is difficult to be certain entirely what the effects of changes in the synaptic/extrasynaptic concentration of endogenous and non-specific GABA would be. GABA receptors are likely to contribute to other cortical circuits including short-interval intracortical inhibition (SICI) and long interval cortical inhibition (LICI), both of which interact with SAI (Alle *et al.*, 2009; Udupa *et al.*, 2009). Other neurotransmitters are unlikely to be involved in SAI in healthy subjects as quetiapine, a serotoninergic, dopaminergic, histaminergic and α1&2 adrenergic antagonist, has no significant effect on SAI or SICI (Di Lazzaro *et al.*, 2005b).

There is also evidence that dopamine may modulate cortical cholinergic function and hence modify SAI in some diseases. Administration of levodopa (Martorana *et al.*, 2009) and dopamine agonists (Martorana *et al.*, 2013) restores SAI in AD but has no effect in healthy controls. In PD, SAI was reported to be normal in patients off dopaminergic medications but reduced on the affected side after administration of dopaminergic medication (Sailer *et al.*, 2003). However, other researchers argue that SAI is actually pathologically increased in PD, and that treatment with levodopa restores this (Di Lazzaro *et al.*, 2004a).

In summary, SAI is an inhibitory cortical circuit that is likely to reflect ACh function, although other neurotransmitters may also be involved. Quantification of SAI provides a mechanism to investigate neuronal circuits and alterations in different disease states, allowing increased understanding of the disease pathogenesis.

4.2.1 Other inhibitory and facilitatory cortical circuits

TMS allows the non-invasive investigation of excitatory and inhibitory cortical circuits and their underlying neurobiology. It involves stimulation of corticospinal neurones via a magnetic field produced by an electrical current passed through shielded wire coils (Cantello *et al.*, 2002; Rogasch and Fitzgerald, 2013). TMS applied to the primary motor cortex (M1) with sufficient intensity produces a motor evoked potential (MEP) in the target muscle. In addition to SAI, a number of other inhibitory and facilitatory circuits can be investigated using TMS, and these plus their interactions are shown in Table 4-1 (Chen, 2004; Muller-Dahlhaus *et al.*, 2008; Paulus *et al.*, 2008; Alle *et al.*, 2009; Freitas *et al.*, 2011; Ni *et al.*, 2011; Tsutsumi *et al.*, 2012a; Tsutsumi *et al.*, 2012b).

Table 4-1 Summary of cortical circuits investigated with TMS

Circuit	Method of measurement of individual circuits	Neuro-transmission	Interaction with other circuits
SAI	TMS of M1 preceded by median nerve stimulus at ISI of ~20ms	ACh (+/- $GABA_A$)	Reduces SIHI and LICI
SICI	Subthreshold conditioning stimulus applied ipsilateral to a suprathreshold test stimulus ('paired pulse TMS') over M1 at an ISI of 1-5ms	Largely via inhibitory $GABA_A$-R	Reduces SAI
LICI	Paired pulse TMS with 2 suprathreshold stimuli applied over M1 at an ISI of 50-200ms	Inhibitory $GABA_B$-R	Reduces SIHI
SIHI	Inhibition seen after conditioning stimulus delivered to *contralateral* M1, followed by test stimulus at 6-15ms	Transcallosal pathways ?via $GABA_B$-R	Reduces SICI and SAI
LAI	As for SAI but with ISI of ~200ms	Unknown	Inhibits LICI
ICF	As for SICI but with longer ISIs of 7-20ms	?Excitatory glutamate via NMDA-R (+/- inhibitory $GABA_A$)	Increased by LAI

SICI=short-interval intracortical inhibition; LICI=long-interval intracortical inhibition; SIHI=short-latency interhemispheric inhibition; LAI=long latency afferent inhibition; ICF=intracortical facilitation; M1=primary motor cortex; ISI=interstimulus interval; NMDA-R=N-Methyl-D-aspartic acid receptor.

4.2.2 Short latency afferent inhibition in neurodegenerative diseases

As discussed above, it has been established that SAI is reduced in AD (Di Lazzaro *et al.*, 2002; Di Lazzaro *et al.*, 2004b; Di Lazzaro *et al.*, 2005a; Nardone *et al.*, 2008a). Acetylcholinesterase inhibitors (AChE-I) can restore this loss (Di Lazzaro *et al.*, 2002; Di Lazzaro *et al.*, 2004b), with rivastigmine producing an increase in SAI from a mean baseline of 83.2% to 59.4% inhibition after a single dose in one study (Di Lazzaro *et al.*, 2002), and from 86.2% to 70.1% in a larger cohort (Di Lazzaro *et al.*, 2004b). There was no significant change in SAI in the controls given the drug (Di Lazzaro *et al.*, 2002). Both baseline SAI and increase in SAI after treatment with rivastigmine predict long-term response to the drug, as measured by the Global Deterioration Scale (Di Lazzaro *et al.*, 2005a). In this latter study, change in SAI following administration of an AChE-I correlated with changes in assessments of long-term verbal memory and verbal fluency (Di Lazzaro *et al.*, 2005a). Moreover, specific behavioural disorders are associated with cholinergic dysfunction, with hallucinations correlating with SAI in DLB and with affective symptoms in AD (Marra *et al.*, 2011).

In mild cognitive impairment (MCI), the data on SAI is less clear. Three studies have examined SAI in MCI in non-parkinsonian patients; an earlier study in amnestic MCI did not demonstrate an impairment in SAI compared to controls (Sakuma *et al.*, 2007), but more recent findings in those with more advanced cognitive impairments who displayed abnormalities on functional neuroimaging support the hypothesis that degeneration of cholinergic interneurons is an early process in MCI, with reduced SAI in those with cognitive deficits (Tsutsumi *et al.*, 2012a). Participants with MCI displayed a mean SAI of 85% compared to 50% in the control group; interestingly there was no difference in those who were and were not taking donepezil (Tsutsumi *et al.*, 2012a). In a further study examining MCI subtypes, only those with amnestic MCI-multiple domain had reduced SAI compared with other subtypes and controls (Nardone *et al.*, 2012b). Administration of donepezil in a sample of these participants significantly increased SAI, and in all participants, SAI significantly correlated with tests of verbal memory, attention and executive function (with the exception of verbal fluency).

In contrast to AD and DLB, where there is a profound loss of cholinergic transmission, SAI is normal in frontotemporal dementia (Di Lazzaro *et al.*, 2006) and in some subjects

with vascular dementia (Di Lazzaro *et al.*, 2008), thus supporting the role of SAI as a non-invasive putative measure of cholinergic loss. However, other studies have demonstrated that cholinergic loss may be a feature of vascular dementia, with reduced SAI in patients compared to controls (Nardone *et al.*, 2008b), although it should be noted that individual responses varied widely. In addition, emerging work in the hereditary small vessel disease Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), where white matter lesions can affect cholinergic projections, has shown a loss of SAI (Manganelli *et al.*, 2008; Palomar *et al.*, 2013). One small study included eight patients with progressive supranuclear palsy (PSP), where degeneration of cholinergic neurones in the pedunculopontine nucleus (PPN) and nucleus basalis of Meynert (nbM) is part of the pathology (Nardone *et al.*, 2005). Perhaps surprisingly, there was no difference in SAI between PSP patients and controls; the authors postulated that the cholinergic system is affected differently in PD and PSP, with loss of presynaptic cholinergic neurones and upregulation of muscarinic ACh receptors in PD but not PSP (Nardone *et al.*, 2005).

Recent work has examined SAI in subjects with and without idiopathic REM sleep behaviour disorder (iRBD) (Nardone *et al.*, 2012c). Over half the participants with iRBD also had MCI, with mean SAI significantly smaller in those with iRBD than controls (72.2% SAI in iRBD vs. 43.1% in controls). SAI correlated with iRBD disease duration, global cognition and measures of episodic memory and executive functions (Nardone *et al.*, 2012c).

4.2.3 Short latency afferent inhibition and ageing

Because of evidence indicating that sensorimotor integration is altered during ageing with a decline in cholinergic function, a number of studies have examined the effect of age on SAI and other cortical circuits. Earlier studies did not demonstrate differences in SAI between young and 'elderly' subjects (Oliviero *et al.*, 2006; Degardin *et al.*, 2011), although mean ages of older participants were only 71 and 62, respectively. In more recent work with larger numbers and increased age (31 participants, mean age 70.3 years, range 65-82), SAI was significantly reduced in the older compared to younger age group, with approximately half of the senior group exhibiting low levels of inhibition, and a number exhibiting facilitation (average conditioned MEP ≥ 100%) (Young-Bernier *et al.*, 2012a). Within the older age group, SAI did not correlate with

increasing age *per se*, but did predict motor performance. The same authors published a further study in the same groups of patients and found that SAI explained approximately 10% of the variance in memory scores (which was significant), but did not predict executive function (Young-Bernier *et al.*, 2012b).

4.2.4 Short latency afferent inhibition in Lewy body diseases

Studies that have explored SAI in Lewy body diseases are shown in Table 4-2. The first study in PD assessed 10 young prevalent PD participants on and off dopaminergic medications (mean age 58.2 years) and 10 age-matched controls. In PD subjects on the more affected side, SAI was normal in the 'off' state (~50% of conditioned MEP response) but was decreased after dopaminergic medication (~100%) (Sailer *et al.*, 2003). SAI did not correlate with scores of motor severity or disease duration. The authors of a consequent study of just three PD subjects and 12 age-matched controls argued that SAI was enhanced in PD in the 'off' state, and that this is restored by L-dopa administration (Di Lazzaro *et al.*, 2004a). In the affected side of these hemiparkinsonian participants, SAI was 22.1% versus 54.1% on the unaffected side. The findings of increased SAI in PD was confirmed by a further study, whose authors reasoned that this could be due to upregulation of postsynaptic muscarinic ACh receptors due to loss of presynaptic cholinergic projective neurones (Nardone *et al.*, 2005). Here SAI was 24.2% in PD versus 44% in PSP and 46.2% in controls, with no association found between SAI and neuropsychological data.

Three studies have evaluated SAI in DLB. Although an initial study did not demonstrate differences in SAI between DLB and control participants (Nardone *et al.*, 2006), two further studies challenged these findings, with significant reductions in SAI (Di Lazzaro *et al.*, 2007b; Marra *et al.*, 2011). The initial study by Nardone *et al* was criticised for the use of outdated diagnostic criteria for DLB and for the lack of randomisation when performing SAI under different conditions. In keeping with outcomes in AD, administration of an AChE-I restored SAI in three out of four DLB patients (Di Lazzaro *et al.*, 2007b) and correlated with hallucination scores (Marra *et al.*, 2011). Recently SAI was also found to be impaired in Parkinson's disease dementia (PDD), with the degree of SAI correlating with tests of global plus specific cognitive functions (Celebi *et al.*, 2012).

Table 4-2 Studies to date on SAI in Lewy body diseases

Reference	Group, n	Age (mean), years	Disease duration	Diagnosis	Medications	Muscle used; ISIs tested	Main SAI findings	Other comments
(Sailer *et al.*, 2003)	PD = 10; C = 10	PD= 58.2; C= 59.5	PD = 7.4 years	Not discussed	Performed 'on' and 'off' DA meds	R + L FDI; ISI 20ms	SAI normal off med (~50% vs. controls ~65%), ↓ in 'on' state (~100%)	DA med ↓SAI on more affected side but not on less affected side (~60%). All patients right-handed
(Di Lazzaro *et al.*, 2004a)	PD = 3; C = 12	PD= 67.3; C= 73.1	'Newly diagnosed'	Not discussed	No DA meds	R + L FDI; ISIs N20 + 2-8ms	Enhanced SAI on affected side in PD (22.1%)	No difference between unaffected side of PD (54.1%) & controls (45.3%)
(Nardone *et al.*, 2005)	PD = 10 (6 with dementia); PSP = 8 (4 with dementia); C = 15	PD= 66.5; PSP= 68.2; C= 73.1	PD= 22.4; PSP= 19.1 months	PD-Ward and Gibb criteria PSP- not discussed Dementia- DSM-III-R criteria	No anticholinergic drugs or drugs affecting motor cortex excitability; 'off' DA meds	R or L FDI, depending on side affected by PD; ISIs N20 + 2-8ms	Enhanced SAI in PD (24.2%); no difference between PSP (44%) and controls (46.2%)	No separate data given on those with cognitive impairment. No correlation of SAI with cognitive scores
(Nardone *et al.*, 2006)	DLB = 10; AD = 13; C = 15	DLB= 71.2; AD= 69.6; C= 67.5	DLB= 30.6; AD= 32.2 months	DLB- McKeith 1996 consensus	No anticholinergics, DA, or meds	FDI (dominant hand); ISIs	SAI reduced in AD (84.2%) but not DLB (56.4%)	SAI performed without randomisation of

				criteria AD- NINCDS-ADRDA criteria	affecting motor cortex excitability	N20 + 2-8ms	compared with controls (46.8%)	different conditions, and 1996 criteria (not revised) used for DLB diagnosis. No correlation of SAI with MMSE or DRS
(Di Lazzaro *et al.*, 2007b)	DLB = 10; AD = 10; C = 10	DLB= 73.1; AD= 72.1; C= 72	DLB= 25.5; AD= 32.0 months	DLB- Revised consortium criteria 2005 AD- NINCDS-ADRDA criteria	No AChE-I or drugs affecting CNS	L FDI; ISIs N20 + 2-8ms	SAI reduced in DLB (93.2%) and AD (90.8%) cf with controls (42.8%)	4 DLB patients also given AChE-I & SAI repeated- 3 showed large ↑ in SAI following drug
(Sailer *et al.*, 2007)	PD + bilateral STN DBS = 7; C = 7	PD = 56.1; C = 56.0	PD = 14 years	Not discussed	'On' & 'off' DA meds	R or L FDI; ISI N20 + 3ms	SAI ↓ in MED-ON/STIM-OFF (~84%) state but normal in MED-ON/ STIM-ON (~54%)	Normal SAI in MED-OFF/STIM-OFF (~60%) state vs. MED-OFF/ STIM ON (~62%) controls (~56%)
(Manganelli *et al.*, 2009)	PD+VH= 10; PD–VH= 12; C = 11	PD+VH = 70.4; PD-VH = 65.5; C = 62.4	PD+VH = 8.7; PD-VH = 9.0 years	Not discussed; Not demented	No anticholinergic or anti-depressant meds; 'on' medication	R or L FDI (according to most affected side); ISIs N20 + 2, 4 & 8ms	SAI reduced in PD+VH (89.4%) cf PD-VH (46.5%) & controls (46.4%). All PD - 66% SAI	MCI present in 16/22 PDs. PD+VH more impaired on tests of attention/ frontal & visuospatial functions
(Marra *et al.*, 2011)	DLB = 18; AD = 18; C =	DLB = 73.0; AD = 71.8; C = 71.8	DLB = 28.8; AD = 34.0 months	DLB- Revised consortium	No AChE-I, antidepressant or	L FDI; ISIs N20 + 2-	SAI reduced in DLB (73.8%) and	SAI correlated with hallucinations in

	10			criteria 2005 AD- NINCDS-ADRDA criteria	antipsychotics	8ms	AD (85.3%) cf controls (42.8%)	DLB and euphoria in AD
(Celebi *et al.*, 2012)	PD = 10; PDD = 10; AD = 10; C = 10	PD = 72.0; PDD = 75.0; AD = 76.1; C = 72.1	PD = 2.3; PDD = 8.4; AD = 2.8 years	PD- UK Brain Bank criteria PDD- MDS consensus criteria AD- NINCDS-ADRDA criteria	No anticholinergic or AChE-I; 'off' medication	R or L FDI; ISIs N20 + 1-8ms	SAI reduced in PDD (91.4%) & AD (94.7%) patients, but normal in PD (61.4%) & controls (55.5%)	Negative correlation between SAI & MMSE, attention, executive, memory & visuospatial function
(Nardone *et al.*, 2012a)	PD+RBD = 10; PD-RBD = 13; C = 15	PD+RBD = 65.9; PD-RBD = 63.7; C = 66.4	PD+RBD = 5.0; PD-RBD = 6.0 years	PD- not discussed; RBD- American Academy of Sleep Medicine criteria	'On' DA medication; no anticholinergics or drugs affecting motor cortex excitability	R or L FDI, depending on handed-ness; ISIs N20 + 2-8ms	SAI ↓ in PD+RBD (77.4%) vs. PD-RBD (49%) & controls (45.4%)	SAI values correlated with episodic verbal memory, executive and visuospatial function in PD
(Rochester *et al.*, 2012)	PD = 22; C = 22	PD = 70.2; C = 67.4	PD = 19.8 months	QSBB criteria	'On' DA meds; off meds affecting cortical excitability	R or L FDI, depending on side affected; ISIs N20 – N20 + 4ms	SAI ↓ in PD (77%) vs. 54% in controls	↓ SAI independent determinant of gait speed, explaining 37% variability
(Yarnall *et al.*,	PD-MCI = 11; PD-CN =	PD-MCI = 73.3; PD-CN = 66.9;	PD-MCI = 20; PD-CN = 20.5	PD- QSBB	'On' DA meds; off meds affecting	R or L FDI, depending	SAI ↓ in PD-MCI (88.4%) vs. PD-	SAI negatively correlated with

2013)	11; C = 22	C = 67.9	months	criteria PD-MCI-MoCA <26	cortical excitability	on side affected; ISIs N20 – N20 + 4ms	CN (62.8%) & controls (55.7%)	MoCA in PD but not controls

PD= Parkinson's disease; AD= Alzheimer's disease; DLB= dementia with Lewy bodies; C= healthy controls; PSP= progressive supranuclear palsy; DA= dopaminergic; AChE-I= acetylcholinesterase inhibitor; FDI= first dorsal interosseous; STN DBS= subthalamic nucleus deep brain stimulation; VH= visual hallucinations; RBD= REM sleep behaviour disorder; MMSE= mini mental state examination; DRS= Mattis Dementia Rating Scale; QSBB= Queen Square Brain Bank criteria; MCI= mild cognitive impairment; CN= cognitively normal; MoCA= Montreal Cognitive Assessment; cf=compared with.

A small study showed that in PD participants with subthalamic nucleus (STN) deep brain stimulation (DBS), SAI is normal in the 'off' state regardless of whether the stimulator is on (MED-OFF, STIM-ON or STIM-OFF, respectively), reduced in the MED-ON/STIM-OFF state and restored to normal in the MED-ON/STIM-ON condition (Sailer *et al.*, 2007). Possible mechanisms for the STN DBS normalisation hypothesised by the authors included alterations in neuronal firing patterns, increased motor cortex excitability or a direct effect on cortical structures (Sailer *et al.*, 2007).

A study in patients with PD and visual hallucinations, believed to reflect functional involvement of cortical cholinergic circuits, showed reduced SAI compared to those without hallucinations (Manganelli *et al.*, 2009). In this study, 16 of the 22 PD participants were also found to have MCI, with more severe impairments in tests probing attention/frontal lobe and visuospatial functions in those with hallucinations. Similarly, in PD plus RBD, where cholinergic degeneration is recognised (Kotagal *et al.*, 2012a) and which is a risk factor for dementia (Postuma *et al.*, 2012), SAI was reduced compared to PD participants without RBD and to controls (Nardone *et al.*, 2012a).

We examined SAI in our cohort of early PD patients (Rochester *et al.*, 2012; Yarnall *et al.*, 2013). SAI was reduced in PD compared to control subjects and was an independent predictor of gait speed in those with PD (Rochester *et al.*, 2012). Furthermore, SAI is decreased in PD-MCI, as defined by the Movement Disorder Society level 1 criteria (MoCA score < 26/30) (Yarnall *et al.*, 2013).

4.2.5 Limitations of SAI studies in PD

To date, studies in PD have been small and contradictory, with some studies demonstrating enhanced (Di Lazzaro *et al.*, 2004a; Nardone *et al.*, 2005), reduced (Sailer *et al.*, 2003; Sailer *et al.*, 2007; Rochester *et al.*, 2012) or normal SAI (Celebi *et al.*, 2012). The amount of inhibition seems to correlate with global cognitive function and tests of attention/executive, memory and possibly visuospatial function. Work thus far has been limited by small sample sizes, disease heterogeneity and additional pathologies. No studies have examined SAI in PD-MCI, where cholinergic dysfunction may contribute to the cognitive deficits (Meyer *et al.*, 2009). We therefore sought to determine the relationship between PD-MCI, cognition and SAI in a large cohort of well-characterised early PD participants.

4.3 Specific project methodology

4.3.1 Participants

PD and control participants were recruited as part of the ICICLE-PD study in Newcastle (see Chapter 2). Due to timings of this specific project, the majority of PD subjects were tested as part of their 18 month assessments as a convenience sample (see Results for details), whilst most of the controls underwent SAI at baseline. Neuropsychological and clinical assessments were performed as discussed in Chapter 2. Exclusion criteria were those participants with contraindications to magnetic stimulation (including metallic heart valve, cranial aneurysm clips, previous seizures), co-morbid diseases that could potentially affect somatosensory evoked potentials (such as a peripheral neuropathy or myelopathy) and a tremor score of >2 on the MDS-UPDRS (Goetz *et al.*, 2008b), as this adversely affects electromyogram (EMG) readings. The SAI protocol used in these experiments was based on the method described by Tokimura and colleagues (Tokimura *et al.*, 2000) and followed international consensus criteria (Chipchase *et al.*, 2012). SAI was performed off medications affecting the central nervous system where possible (including benzodiazepines and antidepressants), and 'on' dopaminergic medications to reduce patient discomfort and improve acceptability of the protocol. Anticholinergic drugs were stopped for at least five days prior to testing, and no participants were taking cholinesterase inhibitors. The technician performing TMS was blinded to the cognitive status of participants.

4.3.2 Recordings

Surface EMGs were recorded from abductor pollicis brevis (APB) and first dorsal interosseous (FDI) of the most symptomatic (patients) or the dominant upper limb (controls) with adhesive Ag-AgCI gel electrodes (Biosense Medical Ltd). The active electrode was placed over the muscle belly and the reference electrode over the proximal metacarpophalangeal joint. Somatosensory evoked potentials (SEPs) were recorded via adhesive electrodes (Neuroline 720, Ambu, Denmark) applied to the scalp (contralateral to median nerve stimulation; see below) after appropriate skin preparation, using a bipolar montage with the non-inverting electrode 2cm anterior to C3/C4 (according to the 10-20 system), the inverting electrode 2cm posterior to C3/C4

(depending on the side of stimulation) and the reference electrode placed on the forehead.

Signals were amplified (EMG gain 1000-2000; EEG gain 50k) and bandpass filtered (EMG 30 Hz-2kHz; EEG 3Hz-2kHz), using a Digitimer D360 system (Letchworth Garden City, Herts, UK), before being digitised at 5kHz by a Power1401 interface (Cambridge Electronic Design Ltd, Cambridge, UK) connected to a computer running Spike2 software (Cambridge Electronic Design Ltd).

4.3.3 Nerve stimulation

SEPs were obtained by stimulating the median nerve in the more symptomatic arm in patients and on the dominant side in controls. Stimuli (single pulses; pulse width 200μs; range 4 to 25 mA) were delivered to the median nerve at the wrist using a constant current stimulator (Digitimer DS7A) via adhesive electrodes (cathode proximal; Biosense Medical Ltd). The intensity of the stimulus was adjusted to just above motor threshold, as determined by a visible twitch in the APB muscle.

4.3.4 Magnetic stimulation

Transcranial magnetic stimulation (TMS) of the motor cortex was performed using a high power Magstim 200 (Magstim Co. Whitland, Dyfed, Wales) and circular TMS coil (130mm diameter). The vertex (Cz) was measured using standard procedures and marked with indelible ink. The circular coil was then placed with the vertex marker positioned in the centre of the coil and the handle of the coil (held by the experimenter) posterior. An anticlockwise coil current was used to stimulate the left hemisphere (right hand) and a clockwise coil-current for the right hemisphere (left hand). The direction of the coil current used (and therefore the hemisphere stimulated) varied between patients (and controls). In PD patients TMS was delivered to the hemisphere opposite the most affected side, whereas in controls the dominant hemisphere was targeted. Motor evoked potentials (MEPs) were recorded from the contralateral FDI muscle. Resting motor threshold (RMT) was determined as the percentage of maximum stimulator output which elicited a liminal MEP (approximately 50μV in 5 out of 10 trials) at rest. MEPs were digitized (see above) and stored for later analysis, when they were analysed blind to the diagnostic category.

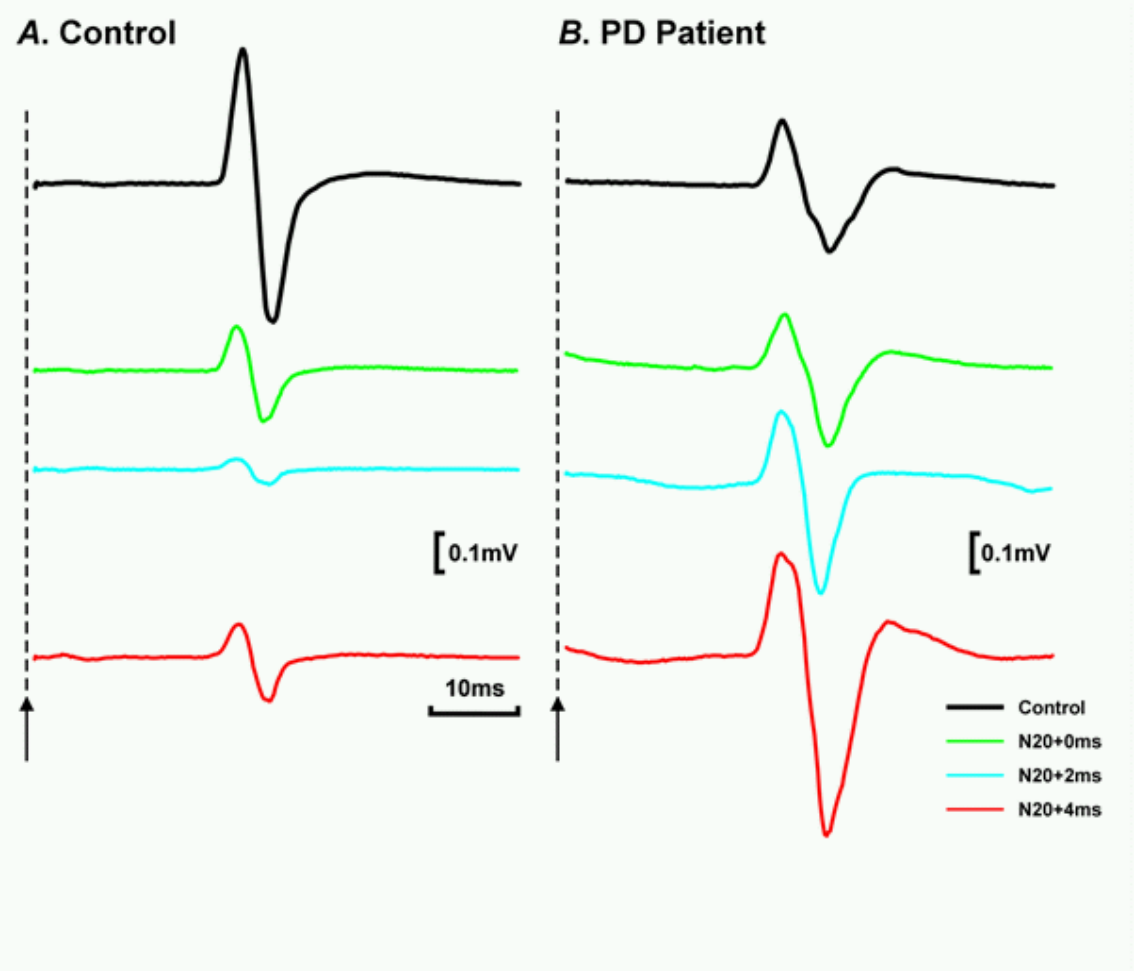
4.3.5 Short latency afferent inhibition

MEPs were conditioned by median nerve stimulation. Conditioning stimuli delivered to the median nerve preceded cortical TMS by varying interstimulus intervals (ISIs). These were determined relative to the latency of the N20 component of the average SEP to median nerve stimulation (averages of 2000 raw sweeps). SAI was randomly tested at five different ISIs with 10 trials at each ISI (from N20 in 1ms increments until N20+4ms), with 20 unconditioned (test) stimuli also delivered randomly (Figure 4-1). The peak to peak amplitude of the conditioned MEPs at each ISI were averaged and expressed as a percentage of the averaged unconditioned MEP (basal MEP). To reduce variability, the conditioned responses were combined across all ISIs and expressed as the percentage of the unconditioned MEP to provide a grand mean of SAI, as described by others (Di Lazzaro *et al.*, 2000; Nardone *et al.*, 2008a). Participants were given visual feedback to maximise complete relaxation, and any EMGs contaminated by interference were excluded.

4.3.6 Statistics

Statistics were carried out as for Chapter 2 and 3. In addition, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were also performed. The assumption of homogeneity of variance was not violated (Levene Statistic $p>0.05$). Our *a priori* hypotheses were that PD participants would have decreased SAI compared to controls, and that those with PD-MCI would have a further decrement compared to the PD-CN group. Therefore, in the ANOVA analysis, *contrast 1* compared PD *vs.* controls, and *contrast 2* PD-MCI *vs*. PD-CN participants. Polynomial linear trends were established, with the groups coded as control=1, PD-CN=2 and PD-MCI=3 and weighted as -2, +1 and +1, respectively for *contrast 1* and 0, +1 and -1, respectively for *contrast 2*. Significance values are reported as one-tailed with a p value of <0.05 taken as significant. Hochberg's GT2 post hoc pairwise comparisons (as the samples sizes were different) reported for the comparisons between PD-MCI *vs.* controls and PD-CN *vs.* control subjects. Further analyses were performed using ANCOVA to control for influences on SAI. Simple planned contrasts were performed and adjusted means calculated.

Figure 4-1 Short latency afferent inhibition. Conditioned and unconditioned motor evoked potentials (MEPs) recorded from: ***A.*** A control subject (aged 66); ***B.*** A patient with Parkinson's disease (aged 83). Baseline (unconditioned) MEPs are plotted in black and conditioned MEPs are plotted in colour (time intervals between the conditioning median nerve stimulation and the TMS test pulse are shown in parentheses): green (N20); blue (N20+2ms); and red (N20+4ms). Average MEPs (n=20) have been plotted.



4.4 Results

4.4.1 General and clinical characteristics

Recruitment of participants for this study is outlined in Figure 4-2. Five PD subjects were recruited at baseline, and the remainder at their 18 month assessments, due to the timing of an ethical amendment. The majority of controls were approached at baseline, but to increase numbers, additional participants who had not previously undergone TMS were consented when reviewed at 18 months. Five PD participants declined to take part. A number were unable to undergo SAI (PD n=13, controls n=15); reasons included previous seizures (n=3), metallic prostheses (n=15), use of anticholinergic and other medications that affect SAI that could not be stopped prior to testing (n=3) and significant co-morbid disease including sensory axonal neuropathy and cervical myelopathy (n=7). Six subjects could not tolerate either the median nerve stimulation or TMS required to produce meaningful MEPs. Other reasons for not obtaining SAI data included technical issues (for example, no clear N20 obtained), tremor score and participants not approached due to the timings of their assessments. Lastly, following SAI evaluation, nine were subsequently excluded due to poor quality EMG recordings, drug effects and one due to re-diagnosis from PD to progressive supranuclear palsy (PSP).

Comparisons of participants who were available for SAI (PD n=87 (1 participant excluded due to PSP), control n=86) who did and did not undergo SAI are shown in Table 4-3. PD participants who underwent SAI had less severe motor disease (MDS UPDRS 3 score 30.9 vs. 36.7, p=0.033) and had better MMSE scores (28.8 vs. 28.0, p=0.017) than those who did not, but were otherwise well-matched. Controls undergoing SAI scored higher (i.e. better function) on MoCA, MMSE and spatial recognition memory (SRM) scores than those who did not, but otherwise the group was representative. General and clinical comparisons of those PD and control participants who had SAI performed are shown in Table 4-4. There were no significant differences in age, gender, handedness or years of education between the groups. PD subjects scored higher on depression scores and lower on the majority of cognitive tests than controls, with the exception of digit vigilance, verbal fluency, pentagon score and language. Memory sub-scores were unavailable for one PD participant

(refused computerised testing); with attention scores unavailable for a different PD subject (missing data).

Figure 4-2 Flow diagram of recruitment of SAI

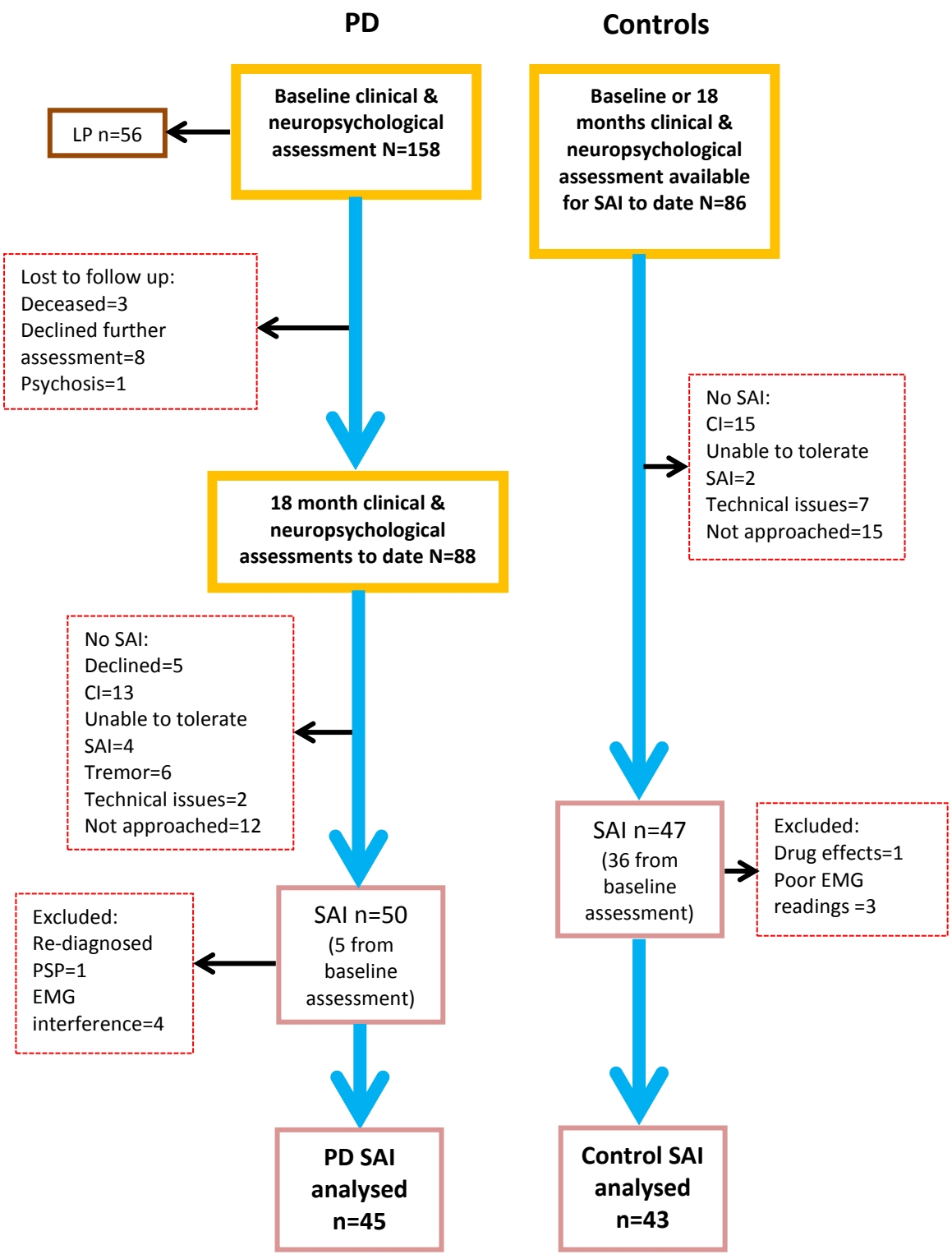


Table 4-3 Comparisons of participants who did and did not undergo SAI

Characteristic	Control			PD		
	SAI- (n=43)	SAI+ (n=43)	*p*	SAI- (n=42)	SAI+ (n=45)	*p*
Age (yrs)[a]	67.0	69.2	0.240	67.5	69.3	0.396
Male gender (%)[b]	24 (56)	26 (61)	0.662	27 (64)	28 (62)	0.842
Education (yrs)	13.0	13.4	0.481	13.8	12.7	0.241
Disease duration (mo)	-	-	-	22.7	21.4	0.882
UPDRS 3[a]	-	-	-	36.7	30.9	**0.033***
LEDD (mg/d)[a]	-	-	-	374.7	331.5	0.285
PIGD score	-	-	-	0.87	0.71	0.233
Non-TD phenotype (n,%)[b]	-	-	-	25 (60)	30 (67)	0.490
GDS	1.0	0.8	0.341	2.9	2.6	0.190
MoCA	26.8	28.0	**0.034***	25.3	25.9	0.517
MMSE	28.8	29.4	**0.013***	28.0	28.8	**0.017***
PoA (msec)	1279	1271	0.705	1410	1358	0.311
Digit Vigilance (%)	95.9	97.0	0.273	92.1	92.2	0.561
PRM	20.7	21.2	0.189	20.4	19.7	0.274
SRM	15.8	16.8	**0.021***	15.0	15.1	0.710
PAL	2.0	1.8	0.072	2.3	2.1	0.665
OTS	16.0	17.1	0.194	13.3	15.1	0.079
Phonemic fluency[a]	39.0	41.5	0.338	37.7	37.0	0.824
Semantic fluency[a]	23.9	24.9	0.438	21.3	22.4	0.466
Pentagon	1.9	2.0	0.309	1.8	1.8	0.514
Language	4.5	4.7	0.219	4.4	4.6	0.153

[a]Unpaired t-test; [b]Chi-squared test; remainder used Mann-Whitney; Values are mean (%); PoA=power of attention; PRM=pattern recognition memory; SRM=spatial recognition memory; PAL=paired associates learning; OTS=one touch stockings of Cambridge.

Table 4-4 Demographic and descriptive data for PD and control participants who underwent SAI

Characteristic	Control (n=43)	PD (n=45)	p value
Age[a]	69.2 (8.4, 50.6-89.7)	69.3 (10.3, 45.0-88.8)	0.939
Male gender[b]	26 (60.5%)	28 (62.2%)	0.866
Handedness (R/L)[c]	40/3	40/5	0.714
Education	13.4 (3.5)	12.7 (3.5)	0.347
GDS	0.8 (1.4)	2.6 (3.3)	**0.001***
MoCA	28.0 (1.9)	25.9 (4.1)	**0.018***
MMSE	29.4 (0.8)	28.8 (1.4)	**0.018***
PoA	1271 (145)	1358 (186)	**0.025***
Digit vigilance	97.0 (4.9)	92.2 (14.4)	0.095
PRM	21.2 (2.4)	19.7 (3.0)	**0.012***
SRM	16.8 (1.7)	15.1 (3.0)	**0.009***
PAL	1.8 (0.7)	2.1 (0.7)	**0.028***
OTS	17.1 (1.8)	15.1 (4.1)	**0.008***
Phonemic fluency[a]	41.5 (12.2)	37.0 (13.5)	0.108
Semantic fluency[a]	24.9 (5.7)	22.4 (8.0)	0.098
Pentagon	2.0 (0.2)	1.8 (0.5)	0.056
Language	4.7 (0.6)	4.6 (0.8)	0.295

[a]Unpaired t-test; [b]Chi-squared test; [c]Fisher's exact test; remainder used Mann-Whitney; Values are mean (SD, range for age).

4.4.2 General neurophysiology results

Recordings were made from the dominant hand in control subjects (40 right-handed and 3 left-handed) and in the side of onset or most symptomatic arm in participants with PD (27 right-dominant disease, of whom 25 were right-handed, and 18 left-dominant disease, of whom 3 were left-handed). Neurophysiological parameters for both groups are shown in Table 4-5. There were no significant differences in N20, median nerve motor threshold or resting motor threshold between PD and controls. Mean SAI was significantly reduced in PD compared to control participants (77.4% of MEP test response *vs.* 59.0%, p=0.001, unpaired t-test), although individual responses varied widely (Figure 4-4). The amount of inhibition showed a trend towards a

reduction in PD participants at the N20 interval (p=0.102), but was significantly less at N20+1 to N20+4ms (p=0.031, p=0.012, p=0.001 and p<0.001, respectively). Figure 4-4 demonstrates that there was an obvious outlier in both the control and PD groups (control SAI=128.6%, PD SAI=181.6%). These figures were checked, with no obvious reason for the lack of inhibition (for example, no medications implicated); therefore they were included in the final analyses. Removing these and re-analysing the difference in SAI did not alter the overall significance (PD mean SAI 57.3 ± 19.7%, control mean SAI 75.1 ± 24.7%, p<0.001). Figure 4-3 shows the difference in SAI at each interstimulus interval in both groups, with a clear separation at each interstimulus interval.

Table 4-5 Neurophysiological parameters of PD and control participants

	Control	PD	P value
N20 (ms)	22.0 (2.7)	22.2 (1.7)	0.698
MNMT (mA)	11.6 (4.4)	11.4 (4.8)	0.806
RMT (% max stim)	40.0 (5.8)	39.3 (6.6)	0.655
SAI	59.0 (22.3)	77.4 (29.2)	**0.001***

Data are mean (SD); MNMT=median nerve motor threshold; RMT=resting motor threshold of maximum stimulator output; p values obtained using unpaired t-test.

Figure 4-3 Short latency afferent inhibition for PD and control participants at each interstimulus interval ; *p<0.05

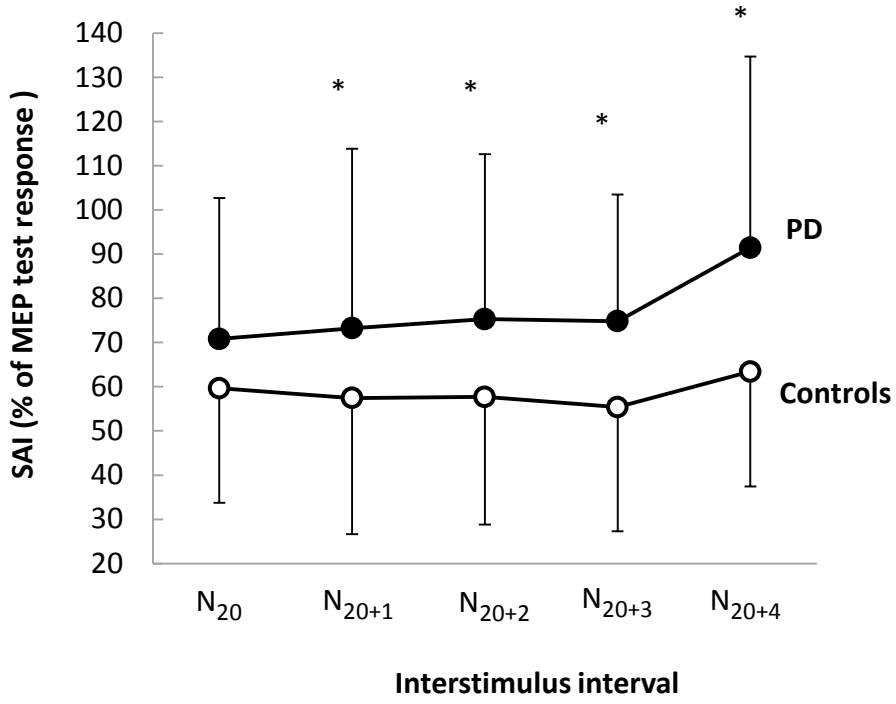
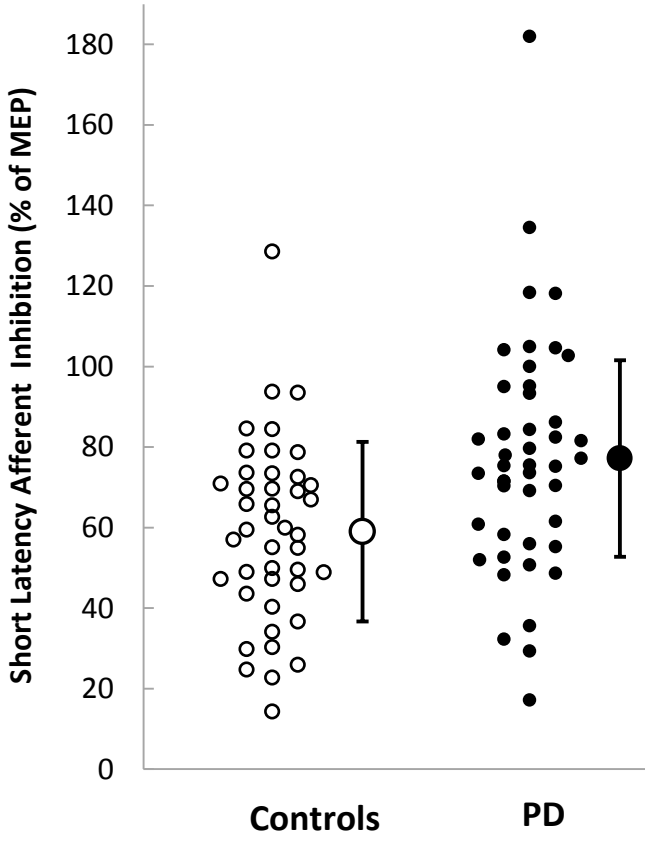


Figure 4-4 Scatterplot of percentage inhibition of SAI in PD and control subjects ; mean and SD are also displayed



4.4.3 Short latency afferent inhibition analysis

The relationship between SAI and clinical characteristics for all participants are shown in Figure 4-5. There was a significant association between age and SAI, with the amount of inhibition reduced as age increased (r=0.325, p=0.002, Pearson's *r*). A negative correlation was found between SAI and years of education and global cognitive score as measured by MoCA (r=-0.317, p=0.003 and r=-0.238, p=0.023, respectively, Spearman's rho). There was no correlation between SAI and depression score (r=0.124, p=0.249, Spearman's rho). These findings were largely influenced by the PD group, as when analysed separately, SAI correlated with age only in the control group (r=0.303, p=0.048), with no correlation seen between SAI and years of education or MoCA (r=-0.129, p=0.410 and r=.148, p=0.345, respectively). In all participants there was no significant difference in SAI between males and females (70.5% *vs.* 65.1%, respectively, p=0.373, unpaired t-test). PD subjects demonstrated a positive correlation with SAI and age (r=0.366, p=0.013), and a negative correlation between SAI and years of education completed (r=-0.445, p=0.002), MoCA score (r=-0.410, p=0.005) and MMSE (r=-0.336, p=0.024, Spearman's rho). There was no association between the amount of inhibition and GDS, disease duration, disease severity (as measured by MDS UPDRS 3), PIGD score or LEDD (r=0.097, r=-0.106, r=0.236, r=0.016 and r=-0.031, respectively, Spearman's rho).

4.4.4 Short latency afferent inhibition and cognition

SAI was examined in relation to cognition in PD and control participants. Initially participants were classified by control group, cognitively normal PD (PD-CN) and PD with mild cognitive impairment (PD-MCI) according to the MDS level 1 criteria (a MoCA score of less than 26). General characteristics of each group are shown in Table 4-6. There were significant group differences for age, years in education, GDS, disease severity, MoCA score and SAI (for SAI, $F(2, 85) = 11.96$, $p<0.001$). The planned contrasts revealed a significant difference between PD and controls ($t(85) = 4.06$, $p<0.001$ (one-tailed)), and that the PD-MCI group had significantly less inhibition than the PD-CN (93.4 ± 30.6% *vs.* 67.7 ± 24.0%) group ($t(85) = -3.40$, $p<0.001$ (one-tailed)). Post hoc tests (Hochberg's GT2) revealed a significant difference between PD-MCI *vs.* controls ($p<0.001$), but no difference between PD-CN and control participants (67.7 ± 24.0% *vs.* 59.0 ± 22.3%, p=0.376). There was, however, a large degree of overlap

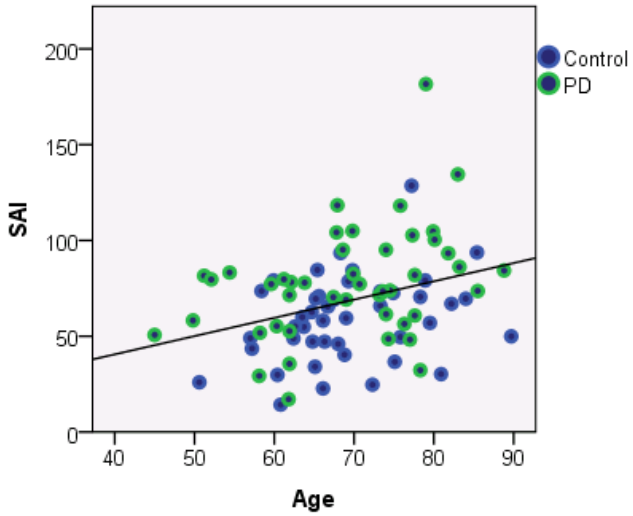
between the groups (Figure 4-6). Excluding the two SAI outliers as discussed above did not affect the overall results.

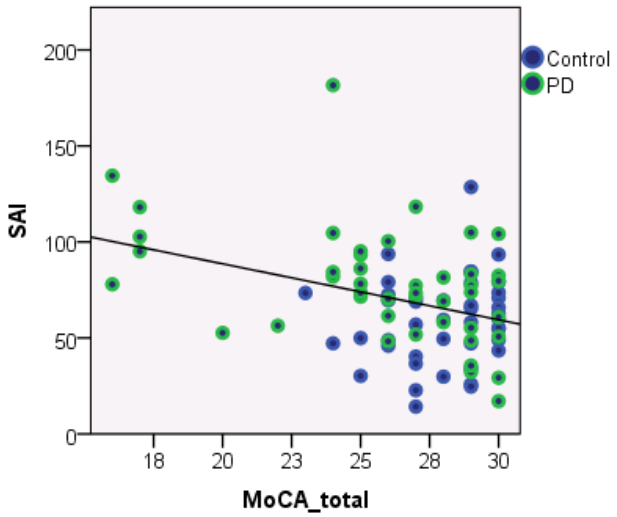
Table 4-6 Characteristics of controls, PD cognitively normal (PD-CN) and PD mild cognitive impairment (PD-MCI), according to MDS level 1 MCI criteria

	Control (n=43)	**PD-CN (n=28)**	**PD-MCI (n=17)**	**p**
Age (yrs)[a]	69.2	65.3	76.0	**0.001***
Male gender (n, %)[b]	26 (61)	16 (57)	12 (71)	0.659
Education (yrs)	13.4	13.9	10.9	**0.001***
Disease duration (mo)	-	21.7	20.8	0.682
MDS UPDRS[a]	-	27.3	36.8	**0.003***
LEDD (mg/d)[a]	-	341.3	315.5	0.597
GDS	0.8	2.3	3.3	**0.003***
MoCA	28.0	28.4	21.8	**<0.001***
SAI (%, SD)[a]	59.0 (22.3)	67.7 (24.0)	93.4 (30.6)	**<0.001***

Data are mean (SD for SAI); [a]ANOVA; [b]Pearson Chi-Squared; remainder used Kruskal-Wallis test.

Figure 4-5 Scatter plots demonstrating the relationship between SAI and clinical characteristics in PD and control subjects; A) SAI and age; B) SAI and MoCA score; C) SAI and years of education





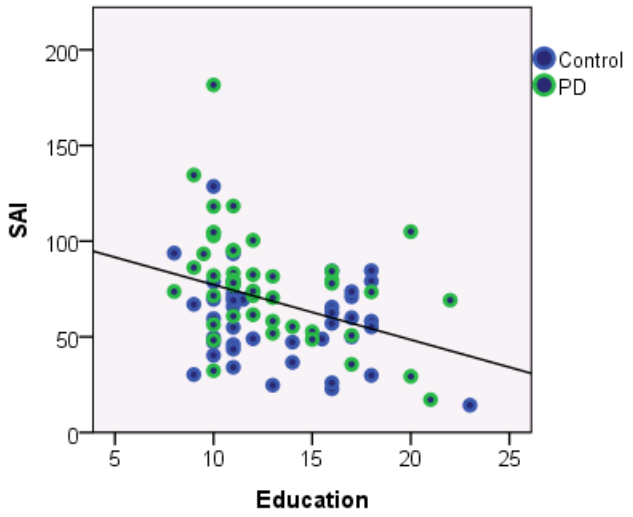
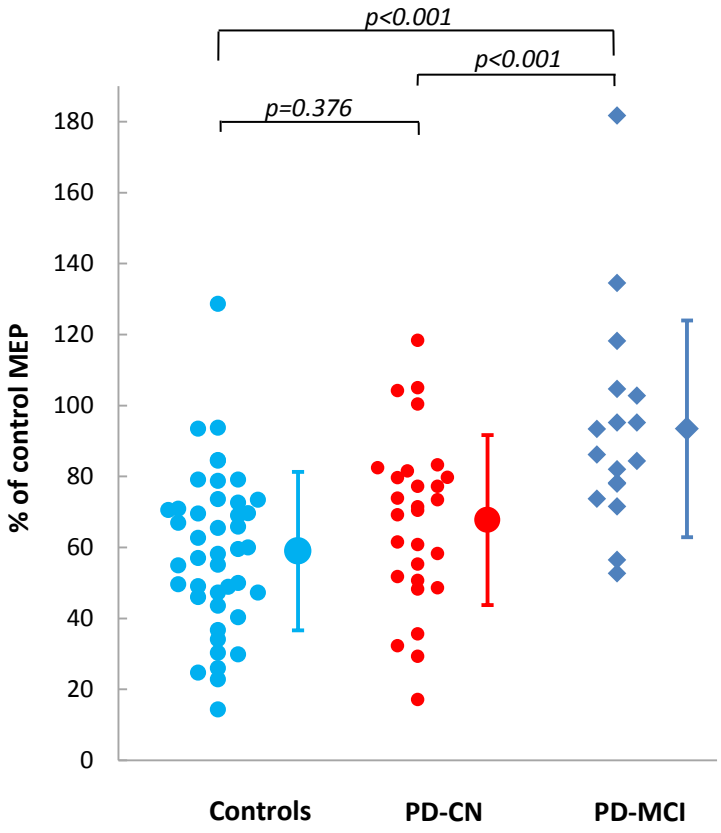


Figure 4-6 Scatter plot showing mean values of SAI for controls, PD cognitively normal (PD-CN) and PD mild cognitive impairment (PD-MCI) according to level 1 criteria ; error bars are SDs



The group differences in SAI remained after controlling for age, education and GDS in an ANCOVA ($F(2, 82) = 6.97$, $p<0.01$), although simple planned contrasts revealed that only the PD-MCI showed significantly less inhibition compared to the control group (adjusted mean SAI 86.8 *vs.* 59.4%; $t(82) = -3.64$, $p<0.001$) but not compared to PD-CN (adjusted mean SAI 86.8 *vs.* 71.2%; $t(82) = -1.93$, $p>0.05$). The difference between PD-CN and control subjects was also not significant (adjusted mean 71.2 *vs.* 59.4%, p=0.057). Disease severity using MDS UPDRS was not controlled for, due to a lack of association between SAI (see above) or cognition (see Chapter 2).

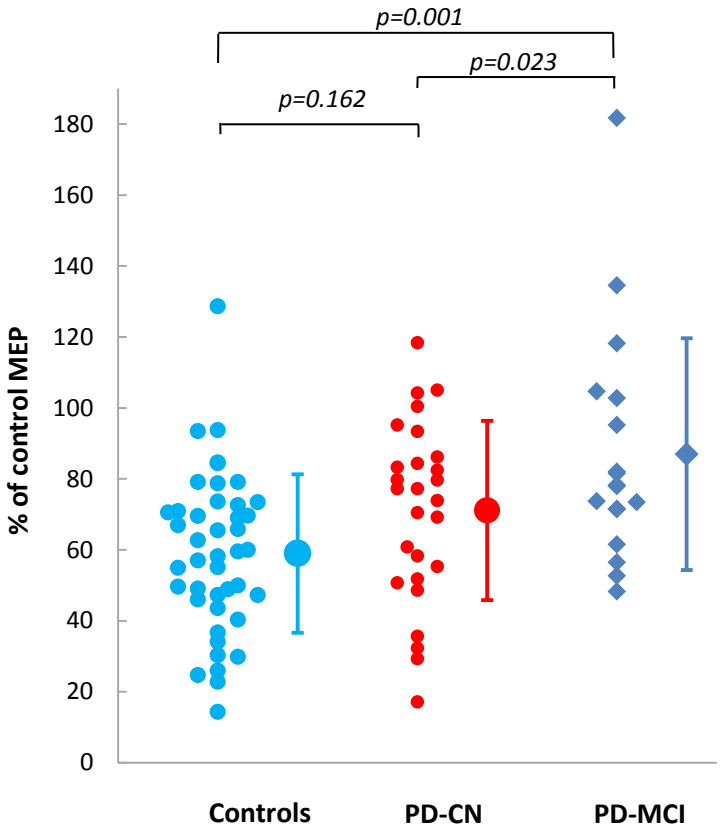
When PD participants were classified as PD-CN (n=27) or PD-MCI (n=18) using level 2 criteria at 1.5 SDs below normative values (see Chapter 2 for details of methods), there was again a significant difference in the amount of inhibition across the PD-MCI, PD-CN and control groups (87.0 ± 32.7% *vs.* 71.1 ± 25.2% *vs.* 59.0 ± 22.3%, respectively, $F(2, 85) = 7.80$, $p<0.001$) (Table 4-7), but with considerable overlap between participants

(Figure 4-7). Planned contrasts revealed that there was a significant difference between PD vs. controls ($t(85) = 3.64$, $p<0.001$ (one-tailed)), and that the PD-MCI group had significantly less inhibition than PD-CN subjects ($t(85) = -2.04$, $p<0.05$ (one-tailed)). In post hoc tests (Hochberg's GT2), there was a significant difference in inhibition between the PD-MCI and control group (p=0.001) but not PD-CN and control subjects (p=0.162).

Table 4-7 Characteristics of control, PD-CN and PD-MCI groups according to MDS level 2 criteria

	Control (n=43)	**PD-CN (n=27)**	**PD-MCI (n=18)**	**p**
Age (yrs)[a]	69.2	67.1	72.6	0.157
Male gender (n, %)[b]	26 (61)	16 (59)	12 (67)	0.870
Education (yrs)	13.4	13.7	11.4	**0.039***
Disease duration (mo)	-	22.2	20.2	0.531
MDS UPDRS[a]	-	28.0	35.1	**0.030***
LEDD (mg/d)[a]	-	326.9	338.5	0.809
GDS	0.8	2.5	2.9	**0.003***
MoCA	28.0	28.2	22.6	**<0.001***
SAI (%, SD)[a]	59.0 (22.3)	71.1 (25.2)	87.0 (32.7)	**<0.001***

Figure 4-7 Scatter plot of mean SAI values for controls, PD-CN and PD-MCI according to MDS level 2 criteria



After controlling for age, education and GDS in an ANCOVA, these group differences remained significant ($F(2, 82) = 5.56$, $p<0.01$). In planned simple contrasts, PD-MCI participants had significantly reduced inhibition compared to the control group (adjusted mean SAI 81.9 *vs.* 59.5%; $t(82) = -3.11$, $p<0.01$) but not PD-CN subjects (adjusted mean 73.7%; $t(82) = -1.09$, $p>0.1$). The control group demonstrated a greater degree of SAI compared to PD-CN ($p=0.026$). The analysis was repeated excluding the two outliers (one from the control and one from PD-MCI group), which did not alter the results.

Finally, those PD subjects with level 2 MCI at 1.5 SDs below normative values (n=18) were then examined. Mean SAI values were compared in those with single- (n=10) and multiple- (n=8) domain MCI. No significant difference was seen between the groups (single 79.1 ± 26.0% *vs.* multiple 96.8 ± 39.0%, $p=0.268$). In addition, when dichotomised into amnestic (n=9) *vs.* nonamnestic (n=9) MCI, there was no difference in inhibition (SAI 80.9 ± 22.4% *vs.* 93.0 ± 41.1%, respectively, $p=0.450$). When

amnestic/nonamnestic was entered into a binary logistic regression model as the dependent variable, with age, education and SAI as co-variates, the model chi-square was non-significant, plus SAI was not a significant determinant (chi-square(3) = 3.50, p>0.05; SAI *B*=.007, p=0.678). Results were similar for single/multiple-domain (model chi-square(3) = 1.78, p>0.05; SAI *B*=.018, p=0.326). The small numbers impaired may mean that these analyses are underpowered to detect differences between the groups.

4.4.5 Cognitive domains and short latency afferent inhibition

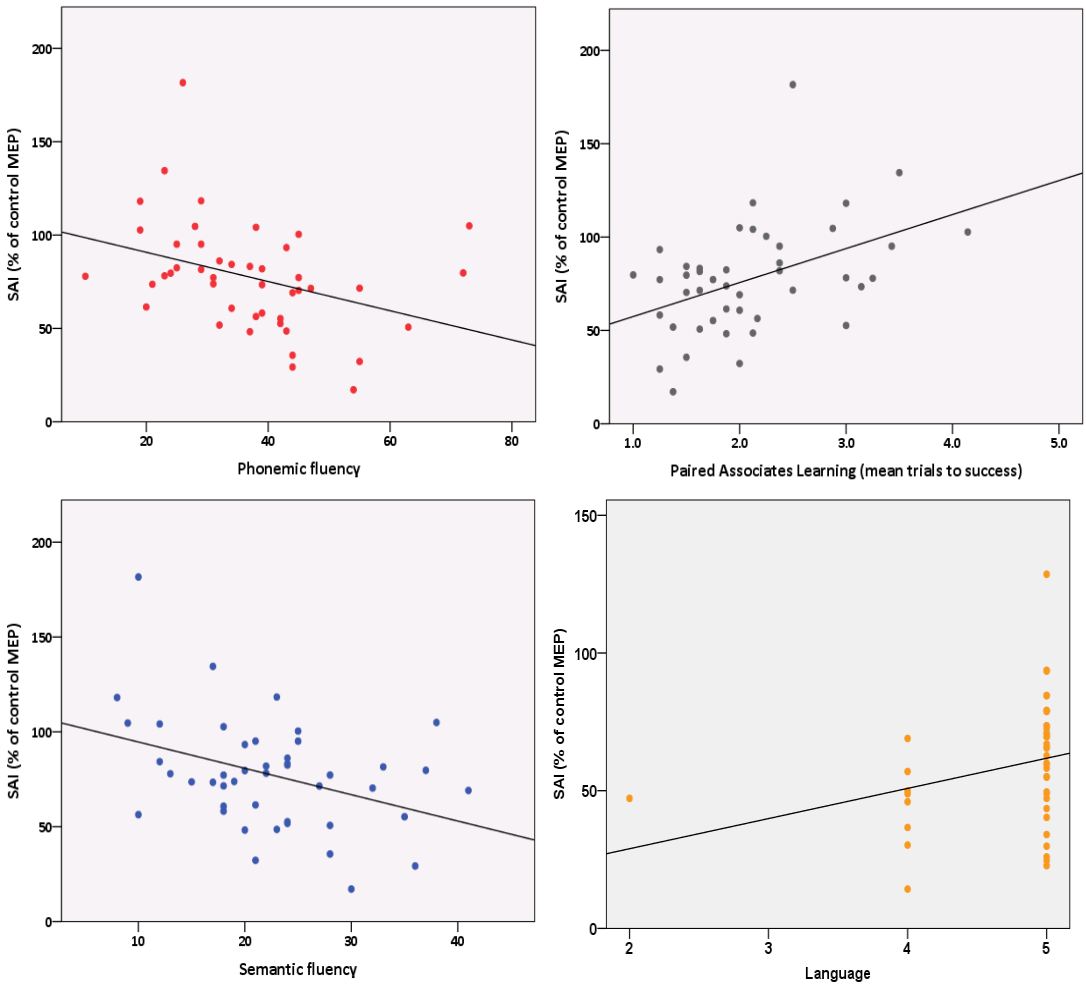
Associations between SAI and cognitive variables in the PD and control subjects were examined using simple bivariate correlations, shown in Table 4-8. There were negative correlations between SAI and both phonemic and semantic fluency in PD but not control participants (r=-0.362, p=0.015; r=-0.380, p=0.010, respectively, Pearson's *r*), with higher scores on verbal fluency correlating with greater inhibition of MEP response. In addition, a significant positive correlation was found between paired associates learning (PAL) and SAI in PD subjects (r=0.439, p=0.003, Spearman's rho). PAL (mean trials to success) is a visuospatial test of learning and memory, with a higher score indicating a greater degree of impairment; therefore here a higher score correlated with reduced inhibition. Scatter plots of these correlations are shown in Figure 4-8. Although a significant correlation was found between language score and SAI in controls, it can be seen from Figure 4-8 that the relationship was non-linear and therefore violated one of the assumptions for correlation.

Table 4-8 Bivariate analysis of explanatory cognitive variables for SAI in PD and control subjects

	Control (n=43)	PD (n=45)
Power of Attention (PoA)		
r	0.226	0.095
p value	0.145	0.541
Digit vigilance		
r	0.108	-0.210
p value	0.490	0.170
Pattern recognition memory (PRM)		
r	-0.196	-0.238
P value	0.208	0.120
Spatial recognition memory (SRM)		
r	-0.062	0.026
p value	0.692	0.866
Paired associates learning (PAL)		
r	-0.064	**0.439**
p value	0.684	**0.003***
One touch stockings (OTS)		
r	-0.180	-0.185
p value	0.249	0.228
Phonemic fluency[a]		
r	0.058	**-0.362**
p value	0.712	**0.015***
Semantic fluency[a]		
r	0.029	**-0.380**
p value	0.855	**0.010***
Pentagon copying		
r	0.112	-0.239
p value	0.475	0.114
Language		
r	**0.368**	-0.167
p value	**0.015***	0.273

[a]Pearson's *r*; remainder used Spearman's rho

Figure 4-8 Scatter plots demonstrating relationships between SAI and phonemic fluency, PAL and semantic fluency in PD and SAI and language in control subjects



To further evaluate the relationship between cognition in PD and SAI, tests from each cognitive domain were entered into a linear regression model as the dependent variable and are shown below:

1. Global cognition (Montreal Cognitive Assessment)

Table 4-9 Regression model statistics and coefficients of variables for MoCA score in PD (n=44)

Independent	β	p value
Model 1		
Age	-.220	0.177
Education	.112	0.500
UPDRS III	-.358	0.053
Motor phenotype score	.155	0.284
GDS	.064	0.692
R^2 = .315; P-value of change= 0.011		
Model 2		
Age	-.130	0.404
Education	.005	0.975
UPDRS III	-.353	**0.042***
Motor phenotype score	.259	0.070
GDS	.097	0.523
SAI	-.388	**0.014***
R^2 = .419; P-value of change= 0.014		

In a bivariate analysis, MoCA score demonstrated a significant negative correlation with motor severity score (r=-0.497, p=0.001, Spearman's rho) and near significant correlation with GDS (r=-0.269, p=0.074) and motor phenotype score (a greater score indicating more tremor-dominant features; r=0.291, p=0.056). Therefore, these factors plus age and education were entered into the regression model. A greater degree of inhibition (that is, a lower SAI percentage) was an independent determinant of MoCA score and independently explained 10% of variability (Table 4-9). In the final model, motor severity score and SAI were independent determinants, explaining 42% of variability. The regression model was repeated for control participants, with age, education then SAI entered into the model (Model 1 R^2=.267, P-value of change=0.002; Model 2 R^2=.365, P-value of change=0.018). The final model explained 37% of variability on MoCA score, with age (β=-.605; p<0.001) and SAI (β=.335, p=0.018) independent predictors of MoCA score. However, the relationship between SAI and MoCA score was positive, indicating that reduced inhibition (a greater SAI percentage)

predicted better function, which is not consistent with other results or indeed our knowledge of SAI.

2. Executive function (One Touch Stockings of Cambridge)

Table 4-10 Regression model statistics and coefficients of variables for OTS score (n=44)

Independent	β	p value
Model 1		
Age	.079	0.607
Education	.112	0.465
UPDRS III	-.524	**0.002***
R^2 = .303; P-value of change= 0.002		
Model 2		
Age	.118	0.458
Education	.058	0.720
UPDRS III	-.534	**0.002***
SAI	-.143	0.357
R^2 = .318; P-value of change= 0.357		

In addition to age and education, OTS displayed a significant negative association with UPDRS 3 score (r=-0.432, p=0.003), and only this motor severity score was significant in the final model, which explained 32% variability in OTS.

3. Executive function (phonemic and semantic fluency)

Table 4-11 Regression model statistics and coefficients of variables for phonemic fluency (n=45)

Independent	β	p value
Model 1		
Age	.093	0.521
Education	.333	**0.026***
UPDRS III	-.418	**0.007***
R^2 = .359; P-value of change < 0.001		
Model 2		
Age	.142	0.338
Education	.264	0.087
UPDRS III	-.423	**0.006***
SAI	-.201	0.160
R^2 = .390; P-value of change= 0.160		

As both tests of verbal fluency correlated with SAI, these were also included as part of executive function to further explore cognition. In a bivariate analysis of explanatory variables for both phonemic and semantic fluency, UPDRS 3 negatively correlated with test score (r=-0.525, p<0.001 for both, Pearson's *r*). Age, years of education, motor severity and SAI explained 39% of the variance in phonemic fluency (Table 4-11), although only UPDRS 3 score was an independent predictor in the final model. Similarly, for semantic fluency, the aforementioned variables explained a high degree of variance (48%), but SAI was not an independent predictor of semantic fluency in the final model (Table 4-12).

Interestingly, when PD participants were dichotomised into whether they were or were not impaired on semantic fluency at 1 SD below normative values (normal, n=35, mean SAI=70.7 ± 23.1% *vs.* impaired, n=10, mean SAI 100.9 ± 36.9%, p=0.003); 1.5 SD below normal (n=38, SAI=72.6 ± 24.4% *vs.* impaired n=7, SAI=103.9 ± 39.9%, p=0.007); and 2 SD (normal, n=41, mean SAI=73.8 ± 24.1% *vs.* impaired n=4, SAI=115.2 ± 51.6%, p=0.005), significant differences were seen between normal and impaired groups. These differences remained robust after controlling for age, education and UPDRS 3 in

an ANCOVA. Differences were not seen in controls, or in PD participants who were subsequently dichotomised by phonemic fluency or PAL scores.

Table 4-12 Regression model statistics and coefficients of variables for semantic fluency (n=45)

Independent	β	p value
Model 1		
Age	-.170	0.202
Education	.425	**0.002***
UPDRS III	-.261	0.058
R^2 = .472; P-value of change < 0.001		
Model 2		
Age	-.146	0.285
Education	.392	**0.007***
UPDRS III	-.263	0.058
SAI	-.096	0.466
R^2 = .479; P-value of change= 0.466		

4. Attention (Power of Attention)

Table 4-13 Regression model statistics and coefficients of variables for PoA (n=44)

Independent	β	p value
Model 1		
Age	.296	0.075
Education	-.026	0.873
R^2 = .094; P-value of change= 0.131		
Model 2		
Age	.255	0.134
Education	.031	0.858
SAI	.172	0.316
R^2 = .117; P-value of change= 0.316		

PoA, a sum of reaction time scores, showed no correlations with other clinical or demographic characteristics in a bivariate analysis. Therefore only age and education were entered in to a model with SAI. These three variables together were a poor fit of

a model for PoA, explaining only 12% of variance, with non-significant P-values of change and no independent predictors in the final model.

5. Memory (paired associates learning)

Table 4-14 Regression model statistics and coefficient variables for PAL (n=44)

Independent	β	p value
Model 1		
Age	.314	0.058
Education	-.083	0.606
UPDRS III	.174	0.308
R^2 = .219; P-value of change= 0.018		
Model 2		
Age	.223	0.171
Education	.038	0.818
UPDRS III	.196	0.235
SAI	.326	**0.042***
R^2 = .298; P-value of change= 0.042		

As part of the memory domain, paired associates learning (PAL) was assessed, as a significant correlation had been demonstrated between this variable and SAI. 'Mean trials to success' was used as the score within this test (with a greater score indicating worse performance), as this was used in Chapter 2 as part of the definition of mild cognitive impairment. PAL correlated with UPDRS 3 score in a bivariate association (r=0.323, p=0.033, Spearman's rho), therefore this plus age, education and SAI were entered into the regression model. The final model explained 30% of variance in PAL score, with only SAI an independent determinant (β=.328, p=0.042) explaining 8% of variability. Reduced inhibition (that is, a greater percentage SAI value) predicted a worse test score. The same model in control participants was not significant (Model 1 R^2=.105, P-value of change=0.108; Model 2 R^2=.124, P-value of change=0.367), and SAI was not an independent predictor (β=-.146, p=0.367).

6. Visuospatial function (pentagon score)

Table 4-15 Regression model statistics and coefficient variables for pentagon score (n=45)

Independent	β	p value
Model 1		
Age	-.164	0.308
Education	.228	0.158
R^2 = .108; P-value of change= 0.091		
Model 2		
Age	-.144	0.386
Education	.200	0.245
SAI	-.082	0.626
R^2 = .113; P-value of change= 0.626		

Pentagon score was not associated with other clinical variables on bivariate analysis. The final model explained only 11% of variance in pentagon score, and was not significantly better at predicting pentagon score than using the mean pentagon score, with no clinical characteristics independent determinants of visuospatial function. In addition, the assumptions for generalisation of the model were not met, with the standardised residual scores ranging from -3.5 to 0.974, and these were non-normally distributed.

7. Language

Table 4-16 Regression model statistics and coefficient variables for language (n=45)

Independent	β	p value
Model 1		
Age	-.001	0.998
Education	-.013	0.935
UPDRS III	-.421	**0.016***
R^2 = .175; P-value of change= 0.049		
Model 2		
Age	.045	0.790
Education	-.078	0.652
UPDRS III	-.426	**0.015***
SAI	-.187	0.252
R^2 = .200; P-value of change= 0.252		

Language scores demonstrated a trend towards a negative correlation with motor severity score (r=-0.275, p=0.067), therefore UPDRS 3 plus age and education were entered into the initial model, with these plus SAI explaining 20% of variability in language score. In the final model, only UPDRS was a significant determinant of language function, with increasing motor severity associated with poorer language score (β=-.426, p=0.015). Below is a summary table of the contributors to cognition discussed in this section, using clinical and SAI parameters in regression models.

Table 4-17 Summary table of independent contributors to cognitive assessments

	MoCA	OTS	Phonemic fluency	Semantic fluency	PoA	PAL	Pentagon	Language
Age								
Education				X				
UPDRS 3	X	X	X					X
LEDD								
GDS								
SAI	X					X		

X=significant determinant of cognition in the final regression model

4.4.6 Hemispheric effects of short latency afferent inhibition

To be certain that hemispheric effects had no impact on the results (Helmich *et al.*, 2005), PD patients in whom the dominant arm was not the most affected arm were excluded and the analysis limited to those in whom the most affected limb was the dominant limb (PD all = 28). These were compared to the control group in whom the dominant arm was tested (40 right-handed; 3 left-handed). Mean SAI was 84.3 ± 31.9% for PD participants and 59.0 ± 22.3% for controls ($p<0.001$, unpaired t-test). ANOVA confirmed that SAI was significantly different across the groups when PD subjects were classified according to level 1 MCI criteria ($F(1, 69) = 15.50$, $p<0.001$), with PD-MCI (n=10) showing significantly less inhibition that both PD-CN (mean SAI 106.6% *vs.* 71.9%, p=0.002, Hochberg's GT2 post hoc test) and controls ($p<0.001$), but no difference between controls and PD-CN (p=0.179). These differences were robust when controlling for age, education and depression score. Equally, differences between the groups were maintained when the PD participants were classified according to level 2 MCI criteria ($F(2, 68) = 13.40$, $p<0.001$, ANOVA). Moreover, after excluding left-hand dominant patients and controls, the results remained significant.

4.5 Discussion

These results demonstrate that cholinergic dysfunction occurs early in both PD and in PD-MCI, as evidenced by the fact that SAI is abnormal in these patients, but not in those with PD and normal cognition or in control participants. SAI may therefore serve as a biomarker of cholinergic dysfunction in a sub-group of people with PD who are at increased risk of dementia. This concept is strengthened by the finding that in PD but not control participants, greater SAI was an independent determinant of MoCA score, a sensitive screening tool for determining MCI in PD and PDD.

This is the first study to explore SAI in PD-MCI patients, and the largest study to investigate SAI in PD. Much of the previous work on SAI and cognitive impairment has involved those with Alzheimer's disease (AD), where cholinergic dysfunction is well established as a pathophysiological basis for dementia. Although an earlier study in non-parkinsonian patients with amnestic MCI did not demonstrate an impairment in SAI (Sakuma *et al.*, 2007), more recent findings in those with more advanced disease support the concept of cholinergic loss in MCI, with reduced SAI in those with cognitive deficits (Tsutsumi *et al.*, 2012a) or with amnestic multiple-domain MCI (Nardone *et al.*,

2012b). The cholinergic deficit in the mid-frontal cortex is more severe in Lewy body dementia (LBD) compared with AD (Tiraboschi *et al.*, 2000; Bohnen *et al.*, 2003), and it is therefore possible that SAI may be more sensitive in detecting MCI in PD than in early AD.

The pathophysiology of cognitive impairment in Lewy body diseases is not limited to cholinergic dysfunction, and is likely to involve both other neurotransmitters (serotonin, GABA, glutamate), plus α-synuclein aggregation, Lewy body deposition, neurofibrillary tangles and amyloid plaque burden (Francis, 2009). These mechanisms are unlikely to operate discretely and may have synergistic effects on cognition. SAI provides a relatively simple, non-invasive and inexpensive neurophysiological method to evaluate cholinergic dysfunction, and we propose that it could serve as a biomarker with other measures to determine those at increased risk of dementia, thereby providing an opportunity for early clinical and pharmacological interventions. Other inhibitory neurotransmitters that may also influence cholinergic neurotransmission include GABA, as diazepam increases (or has no effect) (Di Lazzaro *et al.*, 2007a) whilst lorazepam decreases SAI (Di Lazzaro *et al.*, 2005c). Whilst these studies using different benzodiazepines indicate that GABA receptors are involved in the circuitry underlying SAI, as discussed earlier in this chapter it is difficult to be certain entirely what the effects of changes of endogenous and non-specific GABA would be. Therefore, GABA could potentially influence the results, and further work is required to establish the effects on SAI. GABA receptors are likely to contribute to other cortical circuits including short-interval intracortical inhibition (SICI) and long interval cortical inhibition (LICI), both of which interact with SAI (Alle *et al.*, 2009; Udupa *et al.*, 2009). It is possible that changes in SAI could be related to changes in these other circuits; however, whilst it is established that cholinergic loss occurs in the basal forebrain in PD (Tiraboschi *et al.*, 2000), loss of GABAergic neurones is not a feature of PD (Kish *et al.*, 1986; Halliday *et al.*, 2005; Emir *et al.*, 2012). We therefore suggest that the reduction in SAI seen here in PD-MCI may be related to loss of cholinergic neurones. Moreover, we could be certain that the effects of hemispheric dominance (Helmich *et al.*, 2005) had no impact, because our results remained robust after limiting the analysis to right-handed controls and patients in whom the right arm was both the dominant and most affected.

Age correlated with SAI in both control and PD participants, strengthening recent work suggesting that there is an age-related reduction in cholinergic modulation of inhibitory cortical circuits (Young-Bernier *et al.*, 2012a). This is the first study that has suggested a link between SAI and education, and raises the possibility that in addition to delaying or protecting against AD and vascular dementia in the general population (Hall *et al.*, 2007; Hall *et al.*, 2009; Meng and D'Arcy, 2012), greater cognitive reserve in PD may protect against future decline, as measured by SAI.

In PD participants but not controls, SAI was found to correlate with global cognition, verbal fluency and memory subscores. SAI was an independent determinant of MoCA and PAL in regression analyses. PAL is a visuospatial test of learning and memory that is sensitive to both temporal and frontal damage and has been shown to predict Alzheimer's disease with a high degree of accuracy (Swainson *et al.*, 2001; Blackwell *et al.*, 2004); it is therefore possible that poor scores on PAL plus reduced SAI could predict cholinergic deficit and future cognitive decline in PD. Additionally, PD participants who were impaired in semantic fluency tests showed significantly less inhibition than those who had normal scores. This supports previous work suggesting that poor performance on this posteriorly mediated cognitive test also predicts cognitive decline (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). It was surprising that SAI did not predict tests of attention, which are thought to be cholinergically mediated, at least in part (Bohnen *et al.*, 2006b; Amici and Boxer, 2007; Yarnall *et al.*, 2011). Possible reasons for the lack of association between these variables include the short disease duration or the fact that SAI is more a measure of cholinergic loss from the nbM rather than the PPN, where degeneration in PD is well-recognised (Jellinger, 1988; Zweig *et al.*, 1989; Rinne *et al.*, 2008). Neuronal loss from the nbM would lead to loss of ascending cholinergic projections to the cortex and be reflected in abnormal SAI, whereas cholinergic neuronal loss in PPN would be postulated to lead to thalamic dysfunction primarily.

Strengths of this study are that a homogenous group of well-characterised participants with early Parkinson's disease were evaluated, with age- and sex-matched controls. The extent of SAI in the controls was largely similar to that of previously published studies, with a comparable reduction in inhibition in our subjects with PD-MCI and

previous AD or DLB participants (Di Lazzaro *et al.*, 2002; Di Lazzaro *et al.*, 2004b; Di Lazzaro *et al.*, 2005a; Di Lazzaro *et al.*, 2007b).

One weakness of this study is that we do not have evidence of test-retest reliability. Older age has been associated with increased variability in SAI, with TMS procedures displaying a degree of variability with time (Young-Bernier *et al.*, 2012a). No 'normal' values for SAI exist in older adults; therefore we cannot be certain that the variations seen are within normal limits for this age group. Future work should include test-retest reliability in PD and control participants. It should be noted, however, that clear differences were seen between the groups studied, which supports the validity of the findings.

Other weaknesses include the fact that other inhibitory circuits (such as SICI and LICI) were not measured, and thus we cannot be absolutely sure of GABAergic contribution to cognitive impairment. Further differences in methodologies in this work compared with established publishers (Di Lazzaro *et al.*, 2000; Chen *et al.*, 2008; Di Lazzaro *et al.*, 2008; Nardone *et al.*, 2008a) include that only ISIs of 1 to 4ms were utilised and averaged (other studies used a grand mean of ISIs at 2 to 8 ms), and we used a circular TMS coil (other studies used a figure-of-eight coil). Whilst paired-pulse TMS paradigms were all initially described using figure-of-eight coils (outside diameter 90mm for each circle) placed over the motor 'hotspot' of the muscle being studied, it has become increasingly clear that equally reliable results can be obtained using a circular coil (130mm outside diameter) placed over the vertex (Trompetto *et al.*, 1999; Zoghi *et al.*, 2003; Badawy *et al.*, 2011). With a hand held circular coil there is very little movement-related MEP variability, and it can be accurately and reproducibly repositioned during a study, particularly during longitudinal studies, as the vertex is easily marked. However, as this was a multi-modal longitudinal study, the risk of patient fatigue is significant; therefore practical steps to ensure comfort are important. In addition, it should be noted that a large number of both PD and control subjects were unable to participate for both clinical and non-clinical reasons. Therefore, whether these study findings are applicable to a wider patient group could be questioned. In particular, as PD participants with a tremor score of greater than 2 on the MDS UPDRS III were excluded, these findings may not be valid for those with tremor-dominant disease.

Although the largest SAI study reported in this patient group to date, this sample size is still relatively modest, and the findings require validation in larger cohorts. Participants were studied on dopaminergic medications in contrast to earlier studies (Sailer *et al.*, 2003; Di Lazzaro *et al.*, 2004a; Nardone *et al.*, 2005), and therefore whether SAI is abnormal in the off state cannot be determined. However, the levodopa equivalent doses used in this study were small (mean 331.5 mg/day). Previous studies have demonstrated reduced SAI in PD patients on dopaminergic medications (Sailer *et al.*, 2003; Sailer *et al.*, 2007; Shukla *et al.*, 2013); possible reasons for the differences in this study include shorter disease duration, lower doses of dopaminergic medications and less heterogeneity amongst our patient group. We acknowledge that examining PD participants on and off treatment would strengthen the assumption that the effect on SAI seen in our subjects was not an artefact of dopaminergic medications on SAI, or that PD patients with MCI are more sensitive to dopaminergic medications, and would provide further useful information. Furthermore, since this study was cross-sectional, the assertion that reduced SAI may act as a biomarker for cognitive decline in PD-MCI clearly requires confirmation in the context of a longitudinal trial. Due to the heterogeneity of the disease, it is unlikely that a single biomarker will predict dementia in PD, but SAI in combination with other biomarkers may prove useful.

In conclusion, short latency afferent inhibition is impaired in PD-MCI and correlated with global cognition, verbal fluency and memory assessments. SAI may represent a biomarker for early cholinergic dysfunction in these subjects in conjunction with other clinical measures to predict future cognitive decline.

Chapter 5 Predicting cognitive impairment in Parkinson's disease: a multimodal approach

5.1 Combining cerebrospinal fluid and short latency afferent inhibition to predict cognition

As discussed in previous chapters, PD is a heterogeneous disorder, and it is unlikely that a single biomarker will have the required sensitivity or specificity to accurately predict cognitive decline in individual patients. Therefore, a multimodal approach using a combination of biomarkers may permit more precise prognostication. Earlier chapters have established that Aβ protein levels (namely CSF Aβ40 and Aβ42) and cholinergic dysfunction (measured using SAI) may identify high-risk individuals, and hence it would seem reasonable to combine these biomarkers to determine whether these could work synergistically. This theory has biological plausibility, with increasing evidence in animal models of an interaction between the cholinergic system and Aβ in both disease states and normal ageing (Kar *et al.*, 2004; Schliebs and Arendt, 2006).

There is growing work suggesting that Aβ has neuromodulatory effects in addition to its known neurotoxicity (Mura *et al.*, 2010a; Mura *et al.*, 2012). High concentrations of this protein have been shown to inhibit the cholinergic control of dopamine release (via both muscarinic and nicotinic receptors) plus GABA and glutamate in the rat nucleus accumbens, caudate putamen and hippocampus (Preda *et al.*, 2008; Grilli *et al.*, 2010; Mura *et al.*, 2010b; Mura *et al.*, 2012). However, lower Aβ concentrations potentiated cholinergic release of glutamate (Mura *et al.*, 2012). Additionally, picomolar-nanomolar concentrations of Aβ peptides are found with normal brain cells, with evidence that they decrease ACh concentrations via mechanisms including reduction of choline uptake at presynaptic hippocampal neurones, reducing choline acetyltransferase activity and reduced ACh release from synaptic vesicles (Kar *et al.*, 2004). Of note, these changes occur without apparent neurotoxicity. Although the above studies demonstrating a relationship between Aβ and cholinergic function have been in animal models, the work is supported in post-mortem subjects with AD, where an association between cholinergic activity and Aβ concentration has been demonstrated (Beach *et al.*, 2000).

Aβ is derived from the proteolytic cleavage of Aβ precursor protein (APP), with the subsequent peptide production dependent on the length of amino acid chain produced by sequential action of β- then γ-secretases. If instead APP is cleaved by α-secretase, full length Aβ peptide production is prevented, with soluble APPα formed in its place (Selkoe, 2003; Schliebs and Arendt, 2006). ACh influences the production of Aβ, with both muscarinic agonists and AChE inhibitors increasing the production of soluble APPα and reducing total Aβ concentration (Fisher *et al.*, 2003; Schliebs and Arendt, 2006). Conversely, scopolamine, a potent muscarinic antagonist, increased fibrillar Aβ40 and Aβ42 levels in transgenic mice (Liskowsky and Schliebs, 2006), whilst PD subjects treated with antimuscarinic medications for greater than two years demonstrated greater amyloid plaque densities than those who had not been exposed to these medications (Perry *et al.*, 2003).

In summary, there is evidence that Aβ peptide production and deposition adversely affects ACh synthesis and release, and that ACh in turn may alter APP processing. Therefore, an analysis was performed on a subset of ICICLE participants who had undergone both CSF sampling and SAI, to determine whether any relationship existed between cholinergic dysfunction and amyloid pathology.

5.2 Specific methods

Participants who had consented to both lumbar puncture at baseline visit and subsequent neurophysiology measurement (SAI) as part of the ICICLE study have already been described in Chapters 2-4. As only PD patients had CSF sampling, control data are not included here. Data of these participants were inspected visually and with scatter plots, and any correlation assessed using Pearson's *r* or Spearman's rho, depending on the distribution of the data. Scatter plots were constructed, with reference lines demarcating participants as above or below median values.

5.3 Results – general characteristics

Of the 45 PD participants who had undergone SAI, 24 also had lumbar puncture at their baseline visit. 22 of these had SAI at their 18 month visit, with two having SAI at baseline. Characteristics of those who had SAI with or without CSF sampling are shown in Table 5-1. Those who had both SAI and CSF showed a trend towards younger age, lower dopaminergic medications, better MoCA score and superior memory score

(measured using paired associates learning, PAL) and had a shorter disease duration. However, there were no differences in neurophysiological measurements between the two groups.

Table 5-1 Characteristics of PD participants with SAI plus/minus CSF

	SAI – CSF (n=21)	**SAI + CSF (n=24)**	**P value**
Age (years)[a]	72.1 (10.2)	66.9 (10.0)	**0.088**
Male gender (n, %)[b]	12 (57.1)	16 (66.7)	0.511
PD duration (months)	22.8 (9.8)	20.2 (5.5)	**0.007***
UPDRS 3[a]	31.4 (9.6)	30.4 (12.0)	0.749
LEDD (mg/d)[a]	373.7 (132.0)	294.6 (168.6)	**0.090**
Education	12.1 (3.3)	13.3 (3.6)	0.105
GDS	3.5 (4.1)	1.9 (2.3)	0.296
MoCA	24.7 (4.3)	26.9 (3.7)	**0.050**
PoA (ms)	1393 (172)	1329 (195)	0.150
OTS	14.8 (3.1)	15.5 (4.9)	0.195
PAL	2.3 (0.8)	1.9 (0.6)	**0.054**
Pentagon	1.8 (0.5)	1.8 (0.5)	0.862
Language	4.5 (0.8)	4.6 (0.8)	0.669
SAI (%)[a]	77.5 (25.7)	77.4 (32.4)	0.984
N20[a]	21.8 (2.9)	22.7 (25.8)	0.319
MNMT (mA)[a]	10.9 (4.5)	11.8 (5.2)	0.526
TMS MSO (%)[a]	38.0 (5.9)	40.4 (7.1)	0.216

Data are mean (SD) or number (%) for gender; [a]Unpaired t-test; [b]Pearson Chi-Square; the remainder used Mann-Whitney test; MNMT=median nerve motor threshold; TMS MSO=transcranial magnetic stimulation maximum stimulator output

5.4 Combining CSF and SAI

Results from regression modelling (chapters 3 and 4) were visually examined initially to determine whether a relationship existed between CSF data, SAI and cognition (Table 5-2). Reduced inhibition (that is, a greater value for SAI) and lower CSF Aβ42 levels both predicted poorer scores on global cognition, as assessed using MoCA. Neither CSF parameters nor SAI predicted attention or visuospatial function. Greater Aβ42 values predicted improved temporal function, as measured using pattern recognition memory (PRM), and both Aβ42 and Aβ40 were significant predictors of language function, although Aβ40 was the stronger predictor. Aβ40 or P-tau values were significant contributors to executive function, depending on whether motor phenotype score was or was not included in the final model, respectively. Lastly, both reduced inhibition and greater P-tau levels were significant contributors to temporal/frontal lobe function, as measured using paired associates learning (PAL).

Table 5-2 Predictors of cognition using regression modelling

Domain	**SAI**	**CSF**		
		Aβ42	*Aβ40*	*P-Tau*
Global cognition	X	X	O	O
Attention	O	O	O	O
Executive	O	O	X*	X*
Memory				
Temporal	O	X	O	O
Temporal/frontal	X	O	O	X
Visuospatial	O	O	O	O
Language	O	X	X	O
Single vs. multiple	O	O	O	O
Amnestic vs. nonamnestic	O	O	O	O

*Significant predictors varied according to whether motor phenotype was included in the final model; x=significant determinant of cognition in final regression model; o=non-significant determinant of cognition

Bivariate associations were then explored between SAI and CSF parameters. No significant correlation was seen between SAI and Aβ42 (r=-0.056, p=0.794, Pearson's *r*), SAI and Aβ40 (r=0.185, p=0.387, Pearson's *r*), SAI and P-tau (r=0.254, p=0.232, Spearman's rho) or SAI and α-synuclein (r=0.135, p=0.529, Pearson's *r*). There was a trend towards increased SAI and T-tau (r=0.349, p=0.095, Pearson's *r*). The lack of association may be due to the fact that this part of the analysis could have been underpowered to detect differences. Controlling for age in a partial correlation did not alter these significance levels. Scatter plots were then constructed, with the x- and y-reference lines dichotomised around the median values for the amount of inhibition and the CSF parameter for this sample to indicate the area of "high-risk" for both biomarkers.

Figure 5-1 demonstrates the first scatter plot for SAI versus Aβ42 levels, with the hatched area representing those participants with both reduced inhibition and reduced Aβ42. Of the four participants within this section, three scored less than 26 on the MoCA (therefore meeting level 1 MCI criteria) and also met level 2 MCI criteria at 1.5 SDs below normative values (shown in red and labelled as 'a', respectively) at their baseline ICICLE assessment. Interestingly, these same three participants also scored well below 2 SDs on semantic fluency, and named less than 12 animals in 90 seconds (11, 6 and 6 animals for the three subjects). One participant ('b') has subsequently been diagnosed with PDD prior to his 36 month assessment. The fourth participant ('c' and blue dot on plot) was cognitively normal, but also had Aβ40, T-tau and P-tau levels below median values. All four participants were aged over 60 (mean age 70), and the three who were cognitively impaired were also male. Motor phenotype was mixed, with one subject tremor-dominant, one indeterminate and two PIGD at baseline assessment. The three other participants in the whole group who scored less than 26 on the MoCA (shown as red dots) were near the median values for SAI and /or Aβ42; these same three subjects also met the criteria for level 2 MCI at 1.5 SDs below normal.

Figure 5-1 Scatter plot of SAI versus Aβ42 levels ; reference lines are across the median values for both parameters; hatched area represents those at highest risk according to previous results; red dots represent those with a baseline MoCA score < 26; a=participant with baseline level 2 MCI at 1.5 SDs below normative values; b=participant subsequently diagnosed PDD; c= Aβ42, Aβ40, T-tau and P-tau values all below median values

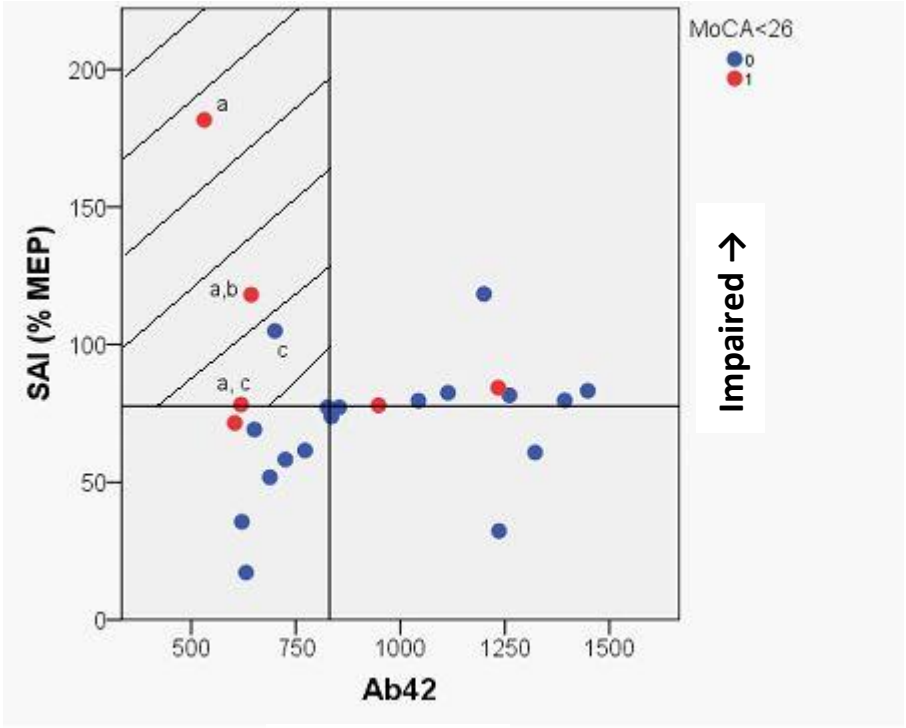
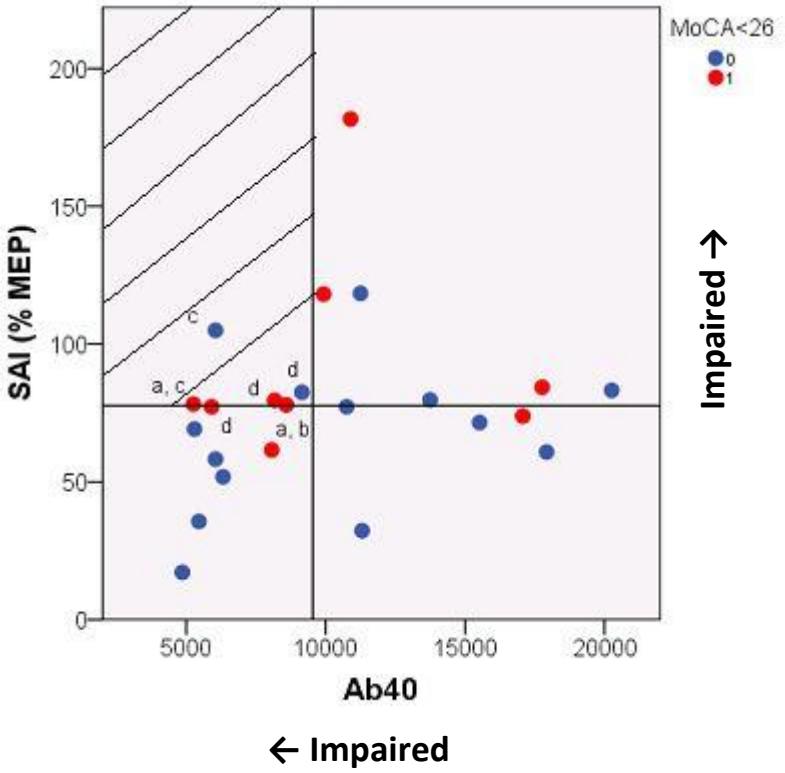


Figure 5-2 shows the scatter plot of SAI versus Aβ40 levels. Six participants were within the hatched "high-risk" area; two of these ('c') have already been discussed above as they also had Aβ42, T-tau and P-tau levels below the median. Four of the six participants met level 1 criteria for MCI at baseline visit (labelled as red); two of these also met level 2 MCI criteria ('a'). One subject ('b'; additional to that seen in Figure 5-1) has subsequently been diagnosed as PDD. Mean age was lower than the SAI/Aβ42 group (62 years), with only two of the six subjects scoring poorly in tests of semantic fluency. Two of the participants within the hatched area were female, and four were assessed as PIGD at baseline.

Figure 5-2 Scatter plot of SAI versus Aβ40 levels ; reference lines are across the median values for both parameters; hatched area represents those at highest risk according to previous results; red dots represent those with a baseline MoCA score < 26; a=participant with baseline level 2 MCI at 1.5 SDs below normative values; b=participant subsequently diagnosed PDD; c= Aβ42, Aβ40, T-tau and P-tau values all below median values; d= Aβ40, T-tau and P-tau values all below median values



Participants identified as "high-risk" using T-tau and P-tau levels did not seem to be as discriminatory (Figure 5-3 and Figure 5-4). Five of the six participants also had lower Aβ42 and/or Aβ40 levels and have already been discussed above ('c' or 'd'). The remaining participant (unlabelled, blue dot within hatched area) scored normally on cognitive testing, although perhaps notably he did have a history of RBD, which has been postulated as a predictor of dementia risk (Postuma *et al.*, 2012). Half of the six participants scored less than 26 on the MoCA, with one of these also meeting criteria for level 2 MCI ('a, c'). This latter participant was the only one to score below normative values on semantic fluency. Again two of the six subjects were female, and mean age within these six was 62 years. Generally, the scatter plots show that SAI tends to be more variable at lower levels of T- and P-tau.

Figure 5-3 Scatter plot of SAI versus T-tau levels ; reference lines are across the median values for both parameters; hatched area represents those at highest risk according to previous results; red dots represent those with a baseline MoCA score < 26; c= Aβ42, Aβ40, T-tau and P-tau values all below median values; d= Aβ40, T-tau and P-tau values all below median values

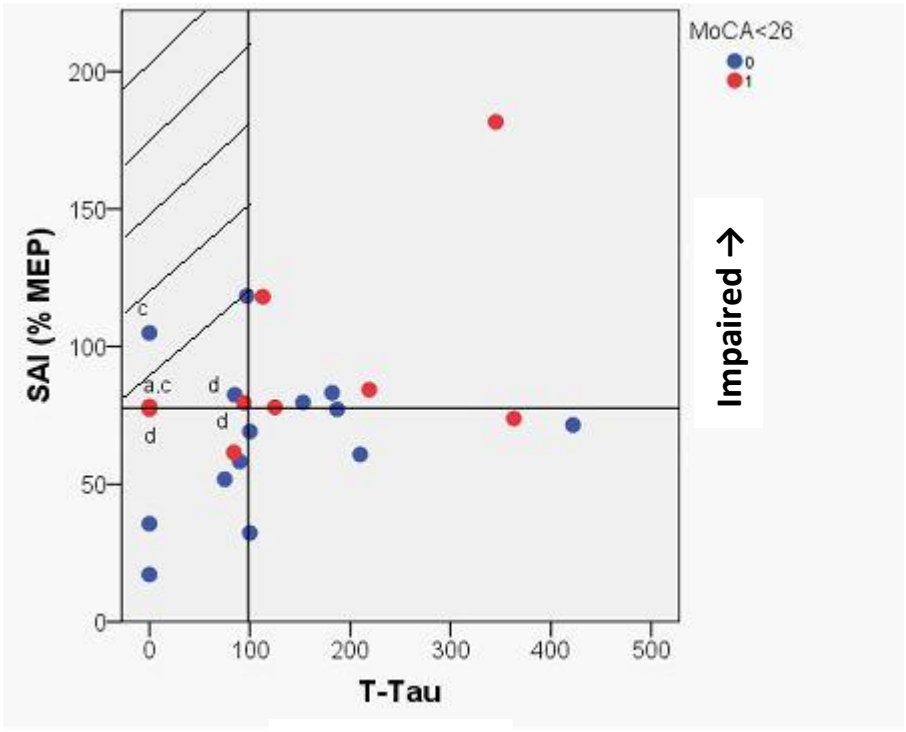
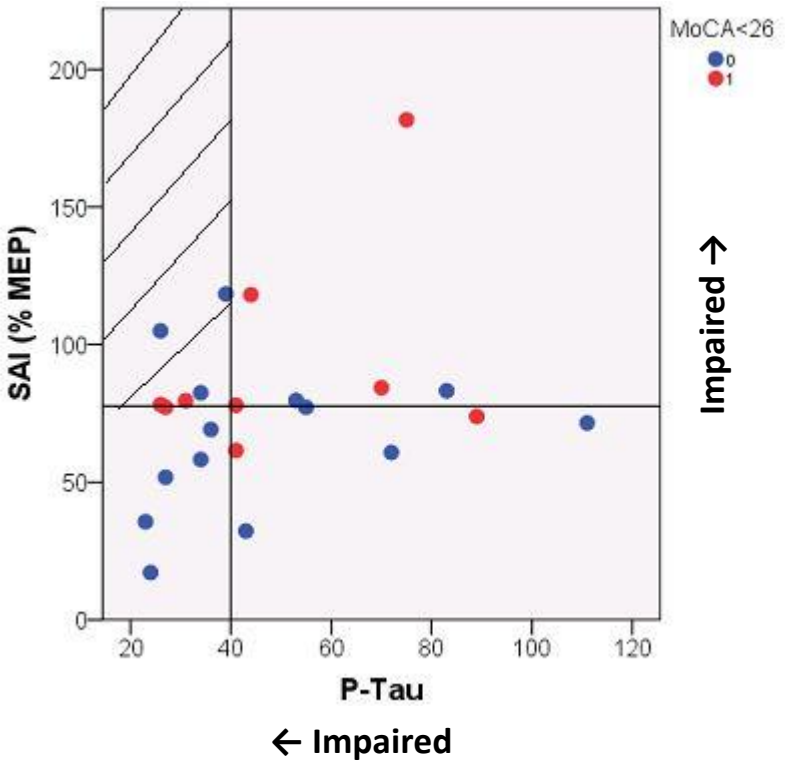


Figure 5-4 Scatter plot of SAI versus P-tau levels ; participants in hatched area as for T-Tau



5.5 Discussion – combining CSF and SAI biomarkers

These data provide some weak evidence that combining CSF parameters and SAI may be useful in determining PD patients at risk of future cognitive decline. In particular, both lower baseline CSF Aβ42 and reduced inhibition (greater SAI absolute value) predicted poorer global cognitive scores, and decreased baseline P-tau levels plus decreased SAI predicted poorer memory function (temporal/frontal impairment, as measured using PAL) (Table 5-2). It was clear that these were independent but not held concurrently in a model, although combining the two biomarkers together did identify more MCI than each alone. Therefore, it is conceivable that participants with a combination of these abnormalities may be at risk of dementia, allowing targeted treatment and earlier therapeutic manipulation in studies aiming to halt progression of cognitive decline. The potential interaction of amyloid deposition and the cholinergic system has been discussed in the introduction. There is also biological plausibility for a link between tau and acetylcholine, with an *in vitro* study demonstrating a decrease in tau phosphorylation following administration of muscarinic agonists (Sadot *et al.*, 1996). In AD models, Aβ accumulation with associated cholinergic dysfunction and impaired G-protein coupling leads to neurodegeneration, tau phosphorylation and

neuronal loss (Thathiah and De Strooper, 2009). However, an increase in tau protein levels following stimulation of nicotinic acetylcholine receptors (Hellstrom-Lindahl *et al.*, 2000; Kar *et al.*, 2004) may offer an explanation for the findings here, with cholinergic loss precipitating a relative reduction in tau levels in those with, or at risk of, cognitive impairment.

No significant correlations were found between any CSF parameter and SAI. This is in contrast to a study in AD (n=19), where SAI was inversely correlated with Aβ42 levels and positively correlated with P-tau (Martorana *et al.*, 2012). Possible reasons for the differences seen here include differing pathologies and disease processes, in addition to the early disease status of our participants. However, when scatter plots were used to dichotomise participants into "high-risk" categories, more interesting patterns emerged. The most robust link seemed to be in those with Aβ42 levels less than median values and reduced SAI, where 75% of the participants also showed evidence of MCI using both level 1 and level 2 criteria. One of these has subsequently been diagnosed as PDD, and these same three participants all performed poorly on a test of semantic fluency. These findings support those from a comparable cohort of early PD participants in Cambridgeshire, where inability to name more than 20 animals in 90 seconds was a predictor of subsequent dementia diagnosis at three and five years (Williams-Gray *et al.*, 2007a; Williams-Gray *et al.*, 2009a). Our participants within this quadrant may therefore be at increased risk of future cognitive decline due to their more posteriorly-mediated cortical impairments due to cholinergic dysfunction and Aβ deposition. Just two of the participants within the quadrant that contained reduced Aβ40 levels combined with reduced SAI had evidence of level 2 MCI, with four scoring less than 26 on the MoCA. One of these participants has since been diagnosed with PDD. Decreased T-tau and P-tau plus impaired SAI was a less discriminative grouping, with half the participants also having a concomitant diagnosis of level 1 MCI but only one also meeting criteria for level 2.

A potential weakness of this analysis is that these assessments were performed at different time points, with all CSF samples collected at the baseline visit, whilst the majority of neurophysiological assessments were performed at 18 months. Comparison between the two time points may therefore be potentially misleading. However, no difference was found in cholinergic dysfunction as measured by cortical

AChE activity between PD participants with early (less than three years) or advanced disease (Shimada *et al.*, 2009); therefore it is possible that between baseline and 18 months there is no discernible acetylcholine loss and hence the extent of SAI may not change. To date, there have been no studies examining longitudinal measurement of SAI, and future studies should address this in PD. In addition, the small numbers of participants who underwent both CSF and SAI assessment may have resulted in a type 1 error. Lastly, lack of control data for this exploratory analysis could be perceived as a weakness. Obtaining CSF from neurologically normal controls is notoriously difficult from various perspectives, however, not least participant acceptability and ethical considerations.

In conclusion, there is evidence that a multimodal approach of combinations of biomarkers using CSF and SAI may increase the accuracy of prediction of future cognitive decline. In particular, reduced Aβ42 and impaired SAI, surrogate markers of Aβ deposition and cholinergic loss, respectively, may be the most accurate combination to determine early PD participants at highest risk of dementia.

Chapter 6 Conclusion and future directions

The prevalence of Parkinson's disease and thus the burden of associated non-motor symptoms will increase in future years due to secular trends in the age-structure of populations, both UK and worldwide. Rising age in the general population often equates to an increase in co-morbidities and associated polypharmacy. Therefore, the detection and if possible, prevention, of cognitive impairment associated with Parkinson's disease is of upmost importance for future generations in terms of healthcare costs, social care and prevention of morbidity and mortality. Cognition is also intricately linked with other non-motor aspects of the disorder, including mood disturbance, gait alterations and falls. A treatment that improved cognition may also have an impact on these other important aspects of PD. A biomarker that could identify those who will develop cognitive impairment early in the disease process would permit more effective use of putative disease-modifying therapies and potentially contribute towards the identification of novel, tractable drug targets. Possible biomarkers include laboratory measures of blood and cerebrospinal fluid (CSF), neurophysiological procedures, imaging techniques such as magnetic resonance imaging and parameters of motor function comprising measures of gait and balance.

The ICICLE-PD study is therefore timely, with its overarching aims being to determine the mechanisms underlying the evolution of PDD from early disease, to establish clinical risk factors associated with high dementia risk and to determine possible biomarkers predictive of future cognitive decline. The latter two objectives were the subject of this thesis, the principal aim of which was to define cognitive subtypes in early Parkinson's disease and to determine the interplay between putative markers of protein deposition and neurochemical dysfunction.

PD-MCI, which may represent a pre-dementia state, was common in a large cohort of early PD participants. 42.5% of PD participants met new MDS criteria for level 2 MCI at 1.5 SDs below normative values, compared to 21.1% of controls; these values are greater than previously reported for early PD. These PD participants were significantly older, with greater motor disease severity, lower educational levels and had higher depression scores compared to the PD-CN group. The most common single cognitive domain to be affected was memory, although a majority of participants were classified

as nonamnestic single-domain MCI according to subtype. This is in contrast to a non-PD older population, where amnestic MCI is the most common subtype. Of the tests used to assess cognition, the largest effect sizes were seen in those probing frontal-executive and attention domains, which may reflect differing underlying pathophysiological processes.

Analyses in the subgroup of PD participants who underwent CSF sampling allowed further investigation of the underlying mechanisms and pathophysiology of cognition in early PD. Reduced amyloid-β levels were associated with inferior global cognitive score and pattern recognition memory, the latter of which is sensitive to temporal lobe dysfunction. Mean Aβ40 and Aβ42 levels were also significantly lower in our participants with PD-MCI compared to those with normal cognition. These results indicate that cognition in early PD may be mediated by amyloid deposition, possibly within the temporal lobe. Correlation between Aβ42 levels and pattern recognition memory was in keeping with one of the *a priori* hypotheses of this thesis: that reduced CSF amyloid-β would be associated with more posteriorly-mediated cognitive dysfunction. The finding that Aβ40 was a significant contributor to language function in a regression model would also be in keeping with this hypothesis. Somewhat unexpectedly, and contradictory to outcomes from the AD literature, we found that lower CSF tau levels were seen in those with MCI compared to the PD-CN group. In accordance with this, P-tau was a significant predictor of paired associates learning, with poorer scores associated with reduced tau. It is possible that these reduced tau levels may be due to tau deposition, with subsequent aggregation of α-synuclein and formation of cortical Lewy bodies, although this remains speculative at present and requires corroboration in post-mortem study.

Further understanding of the pathophysiology of mild cognitive impairment was illustrated by the SAI data, which indicated that cholinergic dysfunction is likely to be involved. In all participants, increased age was associated with a decrease in SAI, confirming that an age-related cholinergic loss occurs, regardless of disease state. Cognitive reserve also seemed to protect against cholinergic loss to some degree, with improved inhibition seen with greater number of years spent in education. PD participants demonstrated significantly reduced mean SAI compared to control participants, confirming imaging studies that have shown that cholinergic loss is

present even in early disease. In addition, cholinergic dysfunction is likely to be more severe in those with mild cognitive impairment, as SAI was abnormal in these participants, but not in those with PD and normal cognition. SAI correlated with scores on global cognition, verbal fluency and memory tests, indicating that there may also be a cholinergic basis to impairments within these domains.

The heterogeneity of PD means that is it unlikely a single biomarker will predict dementia risk, and hence SAI and CSF were examined together to determine if a multimodal approach could improve accuracy. One of our *a priori* hypotheses was that abnormal SAI at 18 months would be associated with reduced Aβ42 levels, and although there was no direct correlation between these two parameters, both were significant predictors of poorer scores of global cognition. In addition, participants who had both reduced Aβ42 and SAI values greater than the median were more cognitively impaired, with a majority meeting criteria for MCI. Therefore, combining these biomarkers may enhance accuracy of cognitive classification.

Future studies should involve the longitudinal assessment of participants, to determine whether PD-MCI is a pre-dementia state, and whether those with abnormalities on CSF and SAI parameters are associated with more rapid cognitive decline and/or dementia. It would also be useful to correlate cognition with falls and gait disturbance, as there is evidence these are associated both in PD and in the general population. A treatment that would encompass these important non-motor characteristics would be enormously beneficial in reducing the morbidity and mortality related to the disease, with a net gain of improvement in both patient and carer quality of life. One of the strengths of the ICICLE-PD study is that both controls and PD participants are to be assessed every 18 months; hence progression to defined end-points will increase in the coming years. This, coupled with planned future brain tissue donation, will allow us to establish a robust set of biomarkers for the evolution of PDD. Future work should also concentrate on assessing the validity of SAI as a biomarker. We have started to assess test-retest variability in controls, and we aim to increase the numbers and perform repeat SAI on PD participants.

In conclusion, mild cognitive impairment is common in patients with early PD. Abnormal SAI and significantly lower Aβ42 and Aβ40 levels were found in PD-MCI, and this correlated with memory function. We hypothesise that these subjects are at a

greater risk of cognitive decline and suggest that these participants should be targeted for future early therapeutic intervention and disease modification. Longitudinal assessment will help determine which clinical, laboratory and neurophysiological measures best predict those who will ultimately develop PDD.

Appendix A – Publications, awards and presentations arising from this thesis

Awards

Movement Disorder Society Junior Award for outstanding and innovative research for '*Characterising Mild Cognitive Impairment in Incident Parkinson's Disease: The ICICLE-PD Study.'* Oral presentation at Plenary Session of the 17th International Congress of Parkinson's Disease and Movement Disorders in Sydney, Australia, June 19 2013.

Publications

1. Mild cognitive impairment in Parkinson's disease. Yarnall AJ, Rochester L, Burn DJ. Accepted June 2013, *Age and Ageing*.

2. The Incidence of Parkinson's Disease in the North East of England. Duncan GW, Khoo TK, Yarnall AJ, O'Brien JT, Brayne CE, Coleman SY, Brooks DJ, Barker RA, Burn DJ. Accepted June 2013, *Age and Ageing*.

3.Characterising Mild Cognitive Impairment in Incident Parkinson's Disease: The ICICLE-PD Study. Yarnall AJ, Duncan GW, Breen D, Khoo TK, Brooks DJ, Coleman S, O'Brien JB, Barker RA, Burn DJ. Under review, *Neurology*.

4.Frequency of primary sleep disorder in patients with newly diagnosed Parkinson's Disease. Prudon B, Duncan GW, Khoo TK, Yarnall AJ, Burn DJ and Anderson KN. Under review, *Movement Disorders.*

5.Health-related quality of life in early Parkinson's disease: the impact of non-motor symptoms. Duncan GW, Khoo TK, Yarnall AJ, O'Brien JT, Coleman SY, Brooks DJ, Barker RA, Burn DJ. Under review, *Movement Disorders*.

5.Falls in Patients with Dementia (book chapter). Rochester L, Lord S, Yarnall AJ, Burn DJ in *Movement disorders in dementia*. Submitted June 2013.

6.Dementia in Parkinson's Disease (book chapter)**.** Burn DJ and Yarnall AJ, in *Non-Motor Symptoms of Parkinson's disease*, Second Edition, Oxford University Press, 2013.

7.Bone Health and Neurological Disease. Dobson R, Yarnall AJ, Noyce A, Giovannoni G. *Practical Neurology* 2013; 13: 70–79.

8.Short latency afferent inhibition: a biomarker for mild cognitive impairment in Parkinson's disease? Yarnall AJ, Rochester L, Baker M, David R, Khoo TK, Duncan GW, Galna B, Burn DJ. *Movement Disorders* 2013: DOI: 10.1002/mds.25360.

9.Rapid Eye Movement Sleep Behaviour Disorder in Parkinson's Disease: Magnetic Resonance Imaging Study. Ford AH, Duncan GW, Firbank MJ, Yarnall AJ, Khoo TK, Burn DJ, and O'Brien JT. *Movement Disorders* 2013: DOI: 10.1002/mds.25367.

10.The Spectrum of Non-Motor Symptoms in Early Parkinson's Disease. Khoo TK, Yarnall AJ, Duncan GW, Coleman S, O'Brien JT, Brooks DJ, Barker RA, Burn DJ. *Neurology* 2013: 80; 1–6.

11.New Horizons in the pathogenesis, assessment and management of movement disorders. Duncan GW, Yarnall AJ, Marrinan S, Burn DJ. *Age and Ageing* 2013; 42: 2–10.

12.Hot Topic: Further Evidence That Amyloid-β Oligomer and Cellular Prion Protein Interaction Produces Deleterious Consequences in Alzheimer's Disease. Yarnall AJ. *Movement Disorders* 2012; 27: 1612

13.Cholinergic dysfunction contributes selectively to gait disturbance in early PD: an in vivo evaluation with short latency afferent inhibition. Rochester L, Yarnall AJ, David R, Baker MR, Lord S, Galna B, Burn DJ. *Brain* 2012: 135; 2779–2788.

14.Parkinson's disease. Yarnall AJ, Archibald N, Burn DJ. *Medicine* 2012 40(10).

15.Amantadine-induced myoclonus in a patient with progressive supranuclear palsy. Yarnall AJ, Burn DJ. *Age and Ageing* 2012: 41(5); 695-696.

16.Falling short: Underestimation of fracture risk in atypical parkinsonian syndromes. Yarnall AJ, Duncan GW, Khoo TK, Burn DJ. *Parkinsonism and Related Disorders* 2012:18;692-693

17.The interplay of cholinergic function, cognition and falls in Parkinson's disease. Yarnall AJ, Rochester L, Burn DJ. *Movement Disorders* 2011:26; 2496-2503.

18. Hot Topic: Low CSF a-Synuclein Levels May Facilitate the Prediction of Synucleinopathies. Yarnall AJ. *Movement Disorders* 2011: 26 (7); 1194

International presentations

1. Characterising Mild Cognitive Impairment in Incident Parkinson's Disease: The ICICLE-PD Study

Yarnall AJ, Breen DP, Duncan GW, Khoo TK, Coleman SY, Evans JR, Rowe J, O'Brien JT, Wesnes K, Robbins TW, Brooks DJ, Barker RA, Burn DJ.

Oral Plenary Session and poster presentation at the *17th International Congress of Parkinson's Disease and Movement Disorders, Sydney June 2013.*

2. Apomorphine: a potential modifier of amyloid deposition in Parkinson's disease?

Yarnall AJ, Lashley T, Ling H, O'Sullivan SS, Lees A, Revesz T, Burn DJ.

Poster presentation at the *17th International Congress of Parkinson's Disease and Movement Disorders, Sydney June 2013.*

3. Short latency afferent inhibition: a biomarker for mild cognitive impairment in Parkinson's disease?

Yarnall AJ, Rochester L, David R, Khoo TK, Duncan GW, Galna B, Baker MR, Burn DJ.

Poster presentation at the *American Neurological Association meeting, Boston October 2012*

4. Cholinergic dysfunction in Parkinson's disease with mild cognitive impairment: a short latency afferent inhibition study.

Yarnall AJ, Rochester L, David R, Duncan GW, Khoo TK, Baker MR, Burn DJ.

Poster presentation at the *16th International Congress of Parkinson's Disease and Movement Disorders, Dublin June 2012*

5. Anticholinergic load: is there a cognitive cost in early Parkinson's disease?

Yarnall AJ, Khoo TK, Duncan GW, Rochester L, Barker RA, Burn DJ.

Poster presentation at the *16th International Congress of Parkinson's Disease and Movement Disorders, Dublin June 2012*

6. Atypical parkinsonian syndromes and fracture risk- are patients adequately managed?

Yarnall AJ, Duncan GW, Khoo TK, Burn DJ.

Poster presentation at the *16th International Congress of Parkinson's Disease and Movement Disorders, Dublin June 2012*

Selected also as an oral presentation for the Guided Poster Tours

7. Mild cognitive impairment in PD: does cholinergic dysfunction contribute?

Yarnall AJ, Rochester L, David R, Baker M, Lord S, Burn DJ

Poster presentation at the *8th International Congress on mental Dysfunction and Other Non-Motor Symptoms in Parkinson's disease and Related Disorders, Berlin, May 2012*

Appendix B - Questionnaire and Scales used in this thesis

MDS-UPDRS

The Movement Disorder Society (MDS)-sponsored new version of the UDPRS is founded on the critique that was formulated by the Task Force for Rating Scales in Parkinson's disease (*Mov Disord* 2003;18:738-750). Thereafter, the MDS recruited a Chairperson to organize a program to provide the Movement Disorder community with a new version of the UDPRS that would maintain the overall format of the original UPDRS, but address issues identified in the critique as weaknesses and ambiguities. The Chairperson identified subcommittees with chairs and members. Each part was written by the appropriate subcommittee members and then reviewed and ratified by the entire group. These members are listed below.

The MDS UPDRS has four parts: Part I (non-motor experiences of daily living), Part II (motor experiences of daily living, Part III (motor examination) and Part IV (motor complications). Part I has two components: IA concerning a number of behaviors that are assessed by the investigator with all pertinent information from patients and caregivers and IB that is completed by the patient with or without the aid of the caregiver, but independently of the investigator. It can, however, be reviewed by the rater to ensure that all questions are answered clearly and the rater can help explain any perceived ambiguities. Part II is designed to be a self-administered questionnaire like Part IB, but can be reviewed by the investigator to ensure completeness and clarity. Of note, the official versions of Part1A, Part1B and Part2 of the MDS-UPDRS do not have separate on or off ratings. However, for individual programs or protocols the same questions can be used separately for on and off. Part III has instructions for the rater to give or demonstrate to the patient; it is completed by the rater. Part IV has instructions for the rater and also instructions to be read to the patient. This part integrates patient-derived information with the rater's clinical observations and judgments and is completed by the rater.

The authors of this new version are:

Chairperson: Christopher G. Goetz
Part I: Werner Poewe (chair), Bruno Dubois, Anette Schrag
Part II: Matthew B. Stern (chair), Anthony E. Lang, Peter A. LeWitt
Part III: Stanley Fahn (chair), Joseph Jankovic, C. Warren Olanow
Part IV: Pablo Martinez-Martin (chair), Andrew Lees, Olivier Rascol, Bob van Hilten
Development Standards: Glenn T. Stebbins (chair), Robert Holloway, David Nyenhuis
Appendices: Cristina Sampaio (chair), Richard Dodel, Jaime Kulisevsky
Statistical Testing: Barbara Tilley (chair), Sue Leurgans, Jean Teresi,
Consultant: Stephanie Shaftman, Nancy LaPelle

Contact person: Christopher G. Goetz, MD
Rush University Medical Center
1725 W. Harrison Street, Suite 755
Chicago, IL USA 60612

Telephone 312-942-8016
Email: cgoetz@rush.edu

July 1, 2008

Part II: Motor Aspects of Experiences of Daily Living (M-EDL)

2.1 SPEECH

Over the past week, have you had problems with your speech?

0: Normal:	Not at all (no problems).	
1: Slight:	My speech is soft, slurred or uneven, but it does not cause others to ask me to repeat myself.	
2: Mild:	My speech causes people to ask me to occasionally repeat myself, but not everyday.	☐
3: Moderate:	My speech is unclear enough that others ask me to repeat myself every day even though most of my speech is understood.	
4: Severe:	Most or all of my speech cannot be understood.	

	SCORE
2.2 SALIVA & DROOLING Over the past week, have you usually had too much saliva during when you are awake or when you sleep? 0: Normal: Not at all (no problems). 1: Slight: I have too much saliva, but do not drool. 2: Mild: I have some drooling during sleep, but none when I am awake. 3: Moderate: I have some drooling when I am awake, but I usually do not need tissues or a handkerchief. 4: Severe: I have so much drooling that I regularly need to use tissues or a handkerchief to protect my clothes.	☐
2.3 CHEWING AND SWALLOWING Over the past week, have you usually had problems swallowing pills or eating meals? Do you need your pills cut or crushed or your meals to be made soft, chopped or blended to avoid choking? 0: Normal: No problems. 1: Slight: I am aware of slowness in my chewing or increased effort at swallowing, but I do not choke or need to have my food specially prepared. 2: Mild: I need to have my pills cut or my food specially prepared because of chewing or swallowing problems, but I have not choked over the past week. 3: Moderate. I choked at least once in the past week. 4: Severe: Because of chewing and swallowing problems, I need a feeding tube.	☐

	SCORE
2.4 EATING TASKS Over the past week, have you usually had troubles handling your food and using eating utensils? For example, do you have trouble handling finger foods or using forks, knifes, spoons, chopsticks? 0: Normal: Not at all (No problems). 1: Slight: I am slow, but I do not need any help handling my food and have not had food spills while eating. 2: Mild: I am slow with my eating and have occasional food spills. I may need help with a few tasks such as cutting meat. 3: Moderate: I need help with many eating tasks but can manage some alone. 4: Severe: I need help for most or all eating tasks.	☐
2.5 DRESSING Over the past week, have you usually had problems dressing? For example, are you slow or do you need help with buttoning, using zippers, putting on or taking off your clothes or jewelry? 0: Normal: Not at all (no problems). 1: Slight: I am slow but I do not need help. 2: Mild: I am slow and need help for a few dressing tasks (buttons, bracelets). 3: Moderate: I need help for many dressing tasks. 4: Severe: I need help for most or all dressing tasks.	☐

	SCORE
2.6 HYGIENE Over the past week, have you usually been slow or do you need help with washing, bathing, shaving, brushing teeth, combing your hair or with other personal hygiene? 0: Normal: Not at all (no problems). 1: Slight: I am slow but I do not need any help. 2: Mild: I need someone else to help me with some hygiene tasks. 3: Moderate: I need help for many hygiene tasks. 4: Severe: I need help for most or all of my hygiene tasks.	☐
2.7 HANDWRITING Over the past week, have people usually had trouble reading your handwriting? 0: Normal: Not at all (no problems). 1: Slight: My writing is slow, clumsy or uneven, but all words are clear. 2: Mild: Some words are unclear and difficult to read. 3: Moderate: Many words are unclear and difficult to read. 4: Severe: Most or all words cannot be read.	☐
2.8 DOING HOBBIES AND OTHER ACTIVITIES Over the past week, have you usually had trouble doing your hobbies or other things that you like to do? 0: Normal: Not at all (no problems). 1: Slight: I am a bit slow but do these activities easily. 2: Mild: I have some difficulty doing these activities. 3: Moderate: I have major problems doing these activities, but still do most. 4: Severe: I am unable to do most or all of these activities.	☐

	SCORE
2.9 TURNING IN BED Over the past week, do you usually have trouble turning over in bed? 0: Normal: Not at all (no problems). 1: Slight: I have a bit of trouble turning, but I do not need any help. 2: Mild I have a lot of trouble turning and need occasional help from someone else. 3: Moderate: To turn over I often need help from someone else. 4: Severe: I am unable to turn over without help from someone else.	☐
2.10 TREMOR Over the past week, have you usually had shaking or tremor? 0: Normal: Not at all. I have no shaking or tremor. 1: Slight: Shaking or tremor occurs but does not cause problems with any activities. 2: Mild: Shaking or tremor causes problems with only a few activities. 3: Moderate: Shaking or tremor causes problems with many of my daily activities. 4: Severe: Shaking or tremor causes problems with most or all activities.	☐
2.11 GETTING OUT OF BED, A CAR, OR A DEEP CHAIR Over the past week, have you usually had trouble getting out of bed, a car seat, or a deep chair? 0: Normal: Not at all (no problems). 1: Slight: I am slow or awkward, but I usually can do it on my first try. 2: Mild: I need more than one try to get up or need occasional help. 3: Moderate: I sometimes need help to get up, but most times I can still do it on my own. 4: Severe: I need help most or all of the time.	☐

	SCORE
2.12 WALKING AND BALANCE Over the past week, have you usually had problems with balance and walking? 0: Normal: Not at all (no problems). 1: Slight: I am slightly slow or may drag a leg. I never use a walking aid. 2: Mild: I occasionally use a walking aid, but I do not need any help from another person. 3: Moderate: I usually use a walking aid (cane, walker) to walk safely without falling. However, I do not usually need the support of another person. 4: Severe: I usually use the support of another persons to walk safely without falling.	☐
2.13 FREEZING Over the past week, on your usual day when walking, do you suddenly stop or freeze as if your feet are stuck to the floor. 0: Normal: Not at all (no problems). 1: Slight: I briefly freeze but I can easily start walking again. I do not need help from someone else or a walking aid (cane or walker) because of freezing. 2: Mild: I freeze and have trouble starting to walk again, but I do not need someone's help or a walking aid (cane or walker) because of freezing. 3: Moderate: When I freeze I have a lot of trouble starting to walk again and, because of freezing, I sometimes need to use a walking aid or need someone else's help. 4: Severe: Because of freezing, most or all of the time, I need to use a walking aid or someone's help.	☐

This completes the questionnaire. We may have asked about problems you do not even have, and may have mentioned problems that you may never develop at all. Not all patients develop all these problems, but because they can occur, it is important to ask all the questions to every patient. Thank you for your time and attention in completing this questionnaire.

Part III: Motor Examination

Overview: This portion of the scale assesses the motor signs of PD. In administering Part III of the MDS-UPDRS the examiner should comply with the following guidelines:

At the top of the form, mark whether the patient is on medication for treating the symptoms of Parkinson's disease and, if on levodopa, the time since the last dose.

Also, if the patient is receiving medication for treating the symptoms of Parkinson's Disease, mark the patient's clinical state using the following definitions:
ON is the typical functional state when patients are receiving medication and have a good response.
OFF is the typical functional state when patients have a poor response in spite of taking medications.

The investigator should "rate what you see". Admittedly, concurrent medical problems such as stroke, paralysis, arthritis, contracture, and orthopedic problems such as hip or knee replacement and scoliosis may interfere with individual items in the motor examination. In situations where it is absolutely impossible to test (e.g., amputations, plegia, limb in a cast), use the notation "**UR**" for Unable to Rate. Otherwise, rate the performance of each task as the patient performs in the context of co-morbidities.

All items must have an integer rating (no half points, no missing ratings).

Specific instructions are provided for the testing of each item. These should be followed in all instances. The investigator demonstrates while describing tasks the patient is to perform and rates function immediately thereafter. For Global Spontaneous Movement and Rest Tremor items (3.14 and 3.17), these items have been placed purposefully at the end of the scale because clinical information pertinent to the score will be obtained throughout the entire examination.

At the end of the rating, indicate if dyskinesia (chorea or dystonia) was present at the time of the examination, and if so, whether these movements interfered with the motor examination.

3a Is the patient on medication for treating the symptoms of Parkinson's Disease? ☐ No ☐ Yes

3b If the patient is receiving medication for treating the symptoms of Parkinson's Disease, mark the patient's clinical state using the following definitions:

☐ ON: On is the typical functional state when patients are receiving medication and have a good response.

☐ OFF: Off is the typical functional state when patients have a poor response in spite of taking medications.

3c Is the patient on Levodopa ? ☐ No ☐ Yes

3.C1 If yes, minutes since last levodopa dose: _____

	SCORE
3.1 SPEECH Instructions to examiner: Listen to the patient's free-flowing speech and engage in conversation if necessary. Suggested topics: ask about the patient's work, hobbies, exercise, or how he got to the doctor's office. Evaluate volume, modulation (prosody) and clarity, including slurring, palilalia (repetition of syllables) and tachyphemia (rapid speech, running syllables together). 0: Normal: No speech problems. 1: Slight: Loss of modulation, diction or volume, but still all words easy to understand. 2: Mild: Loss of modulation, diction, or volume, with a few words unclear, but the overall sentences easy to follow. 3: Moderate: Speech is difficult to understand to the point that some, but not most, sentences are poorly understood. 4: Severe: Most speech is difficult to understand or unintelligible.	☐
3.2 FACIAL EXPRESSION Instructions to examiner: Observe the patient sitting at rest for 10 seconds, without talking and also while talking. Observe eye-blink frequency, masked facies or loss of facial expression, spontaneous smiling and parting of lips. 0: Normal: Normal facial expression. 1: Slight: Minimal masked facies manifested only by decreased frequency of blinking. 2: Mild: In addition to decreased eye-blink frequency, Masked facies present in the lower face as well, namely fewer movements around the mouth, such as less spontaneous smiling, but lips not parted. 3: Moderate: Masked facies with lips parted some of the time when the mouth is at rest. 4: Severe: Masked facies with lips parted most of the time when the mouth is at rest.	☐

	SCORE
3.3 RIGIDITY Instructions to examiner: Rigidity is judged on slow passive movement of major joints with the patient in a relaxed position and the examiner manipulating the limbs and neck. First, test without an activation maneuver. Test and rate neck and each limb separately. For arms, test the wrist and elbow joints simultaneously. For legs, test the hip and knee joints simultaneously. If no rigidity is detected, use an activation maneuver such as tapping fingers, fist opening/closing, or heel tapping in a limb not being tested. Explain to the patient to go as limp as possible as you test for rigidity. 0: Normal: No rigidity. 1: Slight: Rigidity only detected with activation maneuver. 2: Mild: Rigidity detected without the activation maneuver, but full range of motion is easily achieved. 3: Moderate: Rigidity detected without the activation maneuver; full range of motion is achieved with effort. 4: Severe: Rigidity detected without the activation maneuver and full range of motion not achieved.	☐ Neck ☐ RUE ☐ LUE ☐ RLE ☐ LLE
3.4 FINGER TAPPING Instructions to examiner: Each hand is tested separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to tap the index finger on the thumb 10 times as quickly AND as big as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problems. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) the amplitude decrements near the end of the 10 taps. 2: Mild: Any of the following: a) 3 to 5 interruptions during tapping; b) mild slowing; c) the amplitude decrements midway in the 10-tap sequence. 3: Moderate: Any of the following: a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st tap. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L

	SCORE
3.5 HAND MOVEMENTS Instructions to examiner: Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to make a tight fist with the arm bent at the elbow so that the palm faces the examiner. Have the patient open the hand 10 times as fully AND as quickly as possible. If the patient fails to make a tight fist or to open the hand fully, remind him/her to do so. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problem. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the task. 2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the task. 3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st open-and-close sequence. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L
3.6 PRONATION-SUPINATION MOVEMENTS OF HANDS Instructions to examiner: Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to extend the arm out in front of his/her body with the palms down; then to turn the palm up and down alternately 10 times as fast and as fully as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problems. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the sequence. 2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the sequence. 3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing c) the amplitude decrements starting after the 1st supination-pronation sequence. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L

	SCORE
3.7 TOE TAPPING Instructions to examiner: Have the patient sit in a straight-backed chair with arms, both feet on the floor. Test each foot separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the heel on the ground in a comfortable position and then tap the toes 10 times as big and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problem. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) amplitude decrements near the end of the ten taps. 2: Mild: Any of the following: a) 3 to 5 interruptions during the tapping movements; b) mild slowing; c) amplitude decrements midway in the task. 3: Moderate: Any of the following: a) more than 5 interruptions during the tapping movements or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) amplitude decrements after the first tap. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L
3.8 LEG AGILITY Instructions to examiner: Have the patient sit in a straight-backed chair with arms. The patient should have both feet comfortably on the floor. Test each leg separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the foot on the ground in a comfortable position and then raise and stomp the foot on the ground 10 times as high and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problems. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) amplitude decrements near the end of the task. 2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowness; c) amplitude decrements midway in the task. 3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing in speed; c) amplitude decrements after the first tap. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L

	SCORE
3.9 ARISING FROM CHAIR Instructions to examiner: Have the patient sit in a straight-backed chair with arms, with both feet on the floor and sitting back in the chair (if the patient is not too short). Ask the patient to cross his/her arms across the chest and then to stand up. If the patient is not successful, repeat this attempt a maximum up to two more times. If still unsuccessful, allow the patient to move forward in the chair to arise with arms folded across the chest. Allow only one attempt in this situation. If unsuccessful, allow the patient to push off using his/her hands on the arms of the chair. Allow a maximum of three trials of pushing off. If still not successful, assist the patient to arise. After the patient stands up, observe the posture for item 3.13 0: Normal: No problems. Able to arise quickly without hesitation. 1: Slight: Arising is slower than normal; or may need more than one attempt; or may need to move forward in the chair to arise. No need to use the arms of the chair. 2: Mild: Pushes self up from arms of chair without difficulty. 3: Moderate: Needs to push off, but tends to fall back; or may have to try more than one time using arms of chair, but can get up without help. 4: Severe: Unable to arise without help.	☐
3.10 GAIT Instructions to examiner: Testing gait is best performed by having the patient walking away from and towards the examiner so that both right and left sides of the body can be easily observed simultaneously. The patient should walk at least 10 meters (30 feet), then turn around and return to the examiner. This item measures multiple behaviors: stride amplitude, stride speed, height of foot lift, heel strike during walking, turning, and arm swing, but not freezing. Assess also for "freezing of gait" (next item 3.11) while patient is walking. Observe posture for item 3.13 0: Normal: No problems. 1: Slight: Independent walking with minor gait impairment. 2: Mild: Independent walking but with substantial gait impairment. 3: Moderate: Requires an assistance device for safe walking (walking stick, walker) but not a person. 4: Severe: Cannot walk at all or only with another person's assistance.	☐

	SCORE
3.11 FREEZING OF GAIT Instructions to examiner: While assessing gait, also assess for the presence of any gait freezing episodes. Observe for start hesitation and stuttering movements especially when turning and reaching the end of the task. To the extent that safety permits, patients may NOT use sensory tricks during the assessment. 0: Normal: No freezing. 1: Slight: Freezes on starting, turning or walking through doorway with a single halt during any of these events, but then continues smoothly without freezing during straight walking. 2: Mild: Freezes on starting, turning or walking through doorway with more than one halt during any of these activities, but continues smoothly without freezing during straight walking. 3: Moderate: Freezes once during straight walking. 4: Severe: Freezes multiple times during straight walking.	☐
3.12 POSTURAL STABILITY Instructions to examiner: The test examines the response to sudden body displacement produced by a quick, forceful pull on the shoulders while the patient is standing erect with eyes open and feet comfortably apart and parallel to each other. Test retropulsion. Stand behind the patient and instruct the patient on what is about to happen. Explain that s/he is allowed to take a step backwards to avoid falling. There should be a solid wall behind the examiner, at least 1-2 meters away to allow for the observation of the number of retropulsive steps. The first pull is an instructional demonstration and is purposely milder and not rated. The second time the shoulders are pulled briskly and forcefully towards the examiner with enough force to displace the center of gravity so that patient MUST take a step backwards. The examiner needs to be ready to catch the patient, but must stand sufficiently back so as to allow enough room for the patient to take several steps to recover independently. Do not allow the patient to flex the body abnormally forward in anticipation of the pull. Observe for the number of steps backwards or falling. Up to and including two steps for recovery is considered normal, so abnormal ratings begin with three steps. If the patient fails to understand the test, the examiner can repeat the test so that the rating is based on an assessment that the examiner feels reflects the patient's limitations rather than misunderstanding or lack of preparedness. Observe standing posture for item 3.13 0: Normal: No problems: Recovers with one or two steps. 1: Slight: 3-5 steps, but subject recovers unaided. 2: Mild: More than 5 steps, but subject recovers unaided. 3: Moderate: Stands safely, but with absence of postural response; falls if not caught by examiner. 4: Severe: Very unstable, tends to lose balance spontaneously or with just a gentle pull on the shoulders.	☐

	SCORE
3.13 POSTURE Instructions to examiner: Posture is assessed with the patient standing erect after arising from a chair, during walking , and while being tested for postural reflexes. If you notice poor posture, tell the patient to stand up straight and see if the posture improves (see option 2 below). Rate the worst posture seen in these three observation points. Observe for flexion and side-to-side leaning. 0: Normal: No problems. 1: Slight: Not quite erect, but posture could be normal for older person. 2: Mild: Definite flexion, scoliosis or leaning to one side, but patient can correct posture to normal posture when asked to do so. 3: Moderate: Stooped posture, scoliosis or leaning to one side that cannot be corrected volitionally to a normal posture by the patient. 4: Severe: Flexion, scoliosis or leaning with extreme abnormality of posture.	☐
3.14 GLOBAL SPONTANEITY OF MOVEMENT (BODY BRADYKINESIA) Instructions to examiner: This global rating combines all observations on slowness, hesitancy, and small amplitude and poverty of movement in general, including a reduction of gesturing and of crossing the legs. This assessment is based on the examiner's global impression after observing for spontaneous gestures while sitting, and the nature of arising and walking. 0: Normal: No problems. 1: Slight: Slight global slowness and poverty of spontaneous movements. 2: Mild: Mild global slowness and poverty of spontaneous movements. 3: Moderate: Moderate global slowness and poverty of spontaneous movements. 4: Severe: Severe global slowness and poverty of spontaneous movements.	☐
3.15 POSTURAL TREMOR OF THE HANDS Instructions to examiner: All tremor, including re-emergent rest tremor, that is present in this posture is to be included in this rating. Rate each hand separately. Rate the highest amplitude seen. Instruct the patient to stretch the arms out in front of the body with palms down. The wrist should be straight and the fingers comfortably separated so that they do not touch each other. Observe this posture for 10 seconds. 0: Normal: No tremor. 1: Slight: Tremor is present but less than 1 cm in amplitude. 2: Mild: Tremor is at least 1 but less than 3 cm in amplitude. 3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude. 4: Severe: Tremor is at least 10 cm in amplitude.	☐ R ☐ L

SCORE

3.16 KINETIC TREMOR OF THE HANDS

Instructions to examiner: This is tested by the finger-to-nose maneuver. With the arm starting from the outstretched position, have the patient perform at least three finger-to-nose maneuvers with each hand reaching as far as possible to touch the examiner's finger. The finger-to-nose maneuver should be performed slowly enough not to hide any tremor that could occur with very fast arm movements. Repeat with the other hand, rating each hand separately. The tremor can be present throughout the movement or as the tremor reaches either target (nose or finger). Rate the highest amplitude seen.

0: Normal:	No tremor.
1: Slight:	Tremor is present but less than 1 cm in amplitude.
2: Mild:	Tremor is at least 1 but less than 3 cm in amplitude.
3: Moderate:	Tremor is at least 3 but less than 10 cm in amplitude.
4: Severe:	Tremor is at least 10 cm in amplitude.

☐ R

☐ L

3.17 REST TREMOR AMPLITUDE

Instructions to examiner: This and the next item have been placed purposefully at the end of the examination to allow the rater to gather observations on rest tremor that may appear at any time during the exam, including when quietly sitting, during walking and during activities when some body parts are moving but others are at rest. Score the maximum amplitude that is seen at any time as the final score. Rate only the amplitude and not the persistence or the intermittency of the tremor.
As part of this rating, the patient should sit quietly in a chair with the hands placed on the arms of the chair (not in the lap) and the feet comfortably supported on the floor for 10 seconds with no other directives. Rest tremor is assessed separately for all four limbs and also for the lip/jaw. Rate only the maximum amplitude that is seen at any time as the final rating.

Extremity ratings

0: Normal:	No tremor.
1: Slight.:	< 1 cm in maximal amplitude.
2: Mild:	> 1 cm but < 3 cm in maximal amplitude.
3: Moderate:	3 - 10 cm in maximal amplitude.
4: Severe:	> 10 cm in maximal amplitude.

☐ RUE

☐ LUE

☐ RLE

☐ LLE

Lip/Jaw ratings

0: Normal:	No tremor.
1: Slight:	< 1 cm in maximal amplitude.
2: Mild:	> 1 cm but < 2 cm in maximal amplitude.
3: Moderate:	> 2 cm but < 3 cm in maximal amplitude.
4: Severe:	> 3 cm in maximal amplitude.

☐ Lip/Jaw

3.18 CONSTANCY OF REST TREMOR

SCORE

Instructions to examiner: This item receives one rating for all rest tremor and focuses on the constancy of rest tremor during the examination period when different body parts are variously at rest. It is rated purposefully at the end of the examination so that several minutes of information can be coalesced into the rating.

0:	Normal:	No tremor.
1:	Slight:	Tremor at rest is present < 25% of the entire examination period.
2:	Mild:	Tremor at rest is present 26-50% of the entire examination period.
3:	Moderate:	Tremor at rest is present 51-75% of the entire examination period.
4:	Severe:	Tremor at rest is present > 75% of the entire examination period.

☐

DYSKINESIA IMPACT ON PART III RATINGS

A. Were dyskinesias (chorea or dystonia) present during examination? ☐ No ☐ Yes

B. If yes, did these movements interfere with your ratings? ☐ No ☐ Yes

HOEHN AND YAHR STAGE

0: Asymptomatic.

1: Unilateral involvement only.

2: Bilateral involvement without impairment of balance.

3: Mile to moderate involvement; some postural instability but physically independent; needs assistance to recover from pull test.

4: Severe disability; still able to walk or stand unassisted.

5: Wheelchair bound or bedridden unless aided.

☐

Scripted MMSE and C.D.T.

Subject and test details

Name		
NHS Number		
Date of birth - Age	/ /	yrs
Assessed by		
Reason assessed		
Date assessed - Time	/ /	:

Introduce yourself and put the patient at ease, for example –

"Do you mind if we do a short memory test? I do one with everyone I see. Some of the questions are easy, some are harder. Everyone makes mistakes so don't worry if you can't answer some of them"

Score 1 for a correct response and 0 for an incorrect response.

1 Orientation

"What year are we in?" *(exact only)*

"What season is this?"

"What month are we in?"

"What is today's date?" *(allow error of one day)*

"What day of the week is it today?" *(exact only)*

"What country are we in?"

"What county are we in?"
(Accept Newcastle, Northumberland or Tyne & Wear)

"What city / town / village are we in?"

"What is this address / the name of this place?"

"Name 2 streets nearby" OR " What floor/ ward is this?"
(Ask the former if at home or the latter if in hospital)

2 Registration *(Allow 3 trials – score first only)*

"I'm going to give you the names of three objects. When I've finished I'd like you to repeat them and then remember them."

Apple (Ball)

Table (Car)

Penny (Man)

3 Attention – concentration

"I would like you to take 7 away from 100."
"Now keep taking 7 away until I tell you to stop."
Record each answer below, awarding one point for each answer which is 7 less than the previous.
(An alternative question for people who have never been able to calculate is - "Can you spell WORLD backwards?")

\- - - -

4 Recall

"What were the names of the three objects I asked you to repeat and remember a little while ago?"

Apple (Ball)

Table (Car)

Penny (Man)

5 Naming

"What is this called?" *(show pen or pencil)*

"What is this called?" *(show watch)*

6 Comprehension

"Can you repeat this phrase 'No ifs, ands or buts'?"

"Can you read and do this?" *(show close your eyes)*

7 Praxis

"Can you copy this drawing?" *(show pentagon)*

"Can you think up & write a complete sentence?"

"Take this paper in your right/left hand, fold it in half with both hands and put it down on your lap."

Takes paper in right /left hand

Folds paper

Lays paper on lap

MMSE Score

8 Clock Drawing Test *(not part of MMSE)*

"Imagine this circle is the face of a clock. Put in all the numbers." "Now set the hands to ten past eleven"

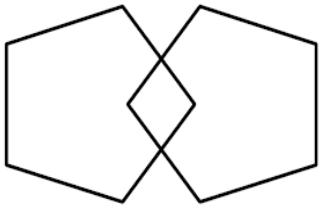
All numbers present

Numbers placed correctly

Hands placed correctly

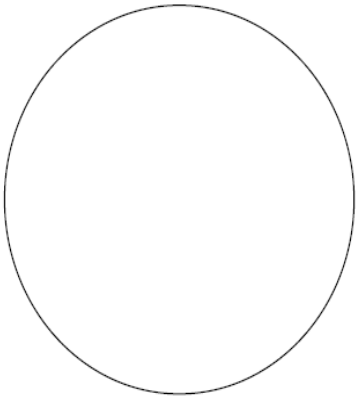
Comment on any factors which could have influenced performance e.g. sensory deficits or behaviour / mental state / physical state at time of testing.

Close your eyes



Sentence

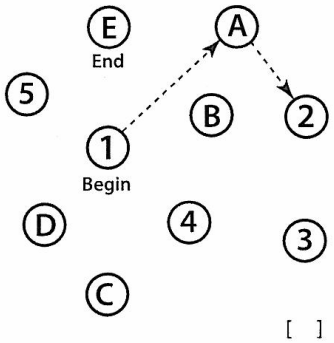
Clock Face

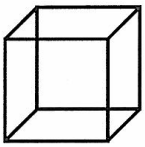


MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE





Copy cube

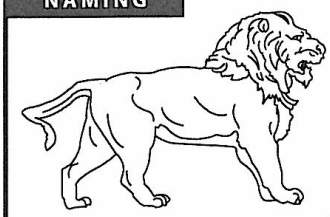
[ ] [ ]

Draw CLOCK (Ten past eleven) (3 points)

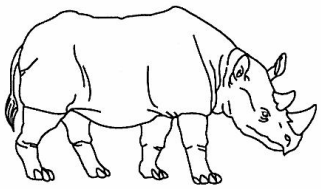
[ ] Contour [ ] Numbers [ ] Hands

POINTS __/5

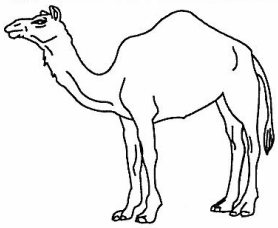
NAMING



[ ]



[ ]



[ ]

__/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

__/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

__/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

__/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

__/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

__/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

__/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

__/6

 Version 7.1 **www.mocatest.org** Normal ≥ 26 / 30

TOTAL __/30

Add 1 point if ≤ 12 yr edu

Administered by: _____

Geriatric Depression Scale GDS-15

Subject ID: _____ Date: _____

Choose the best answer for the way you have felt over the last week:

Please circle:

1.	Are you basically satisfied with your life?	YES	**NO**
2.	Have you dropped many of your interests and activities?	**YES**	NO
3.	Do you feel that your life is empty?	**YES**	NO
4.	Do you often get bored?	**YES**	NO
5.	Are you in good spirits most of the time?	YES	**NO**
6.	Are you afraid that something bad is going to happen to you?	**YES**	NO
7.	Do you feel happy most of the time?	YES	**NO**
8.	Do you often feel helpless?	**YES**	NO
9.	Do you prefer to stay at home, rather than going out and doing new things?	**YES**	NO
10.	Do you feel that you have more problems with your memory than most?	**YES**	NO
11.	Do you think that it is wonderful to be alive now?	YES	**NO**
12.	Do you feel pretty worthless the way you are now?	**YES**	NO
13.	Do you feel full of energy?	YES	**NO**
14.	Do you feel that your situation is hopeless?	**YES**	NO
15.	Do you feel that most people are better off than you are?	**YES**	NO

Total score:

CARU Operating Procedure

Short Latency Afferent Inhibition

General Preparation

1) For PD participants, check MDS UPDRS 3.4, 3.5 and 3.6 bilaterally pre- and post-procedure
2) Ensure no contraindications:
    - Pregnancy
    - Seizures
    - Cardiac pacemaker/metallic heart valve/aneurysm clips
    - Metalwork in body
3) Ensure no mobile phones in pockets of patient and tester, remove credit cards from pockets and take badge off
4) Ensure stimulators unarmed (median nerve stimulator on 0)
5) Switch on equipment
6) Ensure patient relaxed, with arms on pillow placed over arms of chair
7) Clean dominant arm and contralateral scalp with alcohol wipes (for PD use most affected arm)

EMG Preparation

Channel 1 – not working

Channel 2 – APB (abductor pollicis brevis)

Channel 3 – FDI (first dorsal interossei)

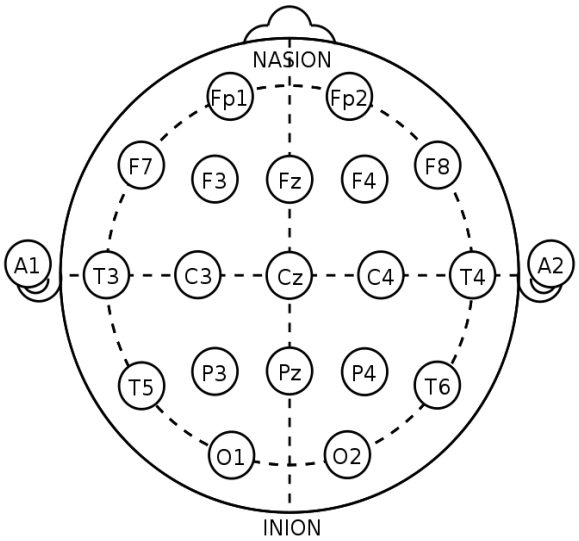
Channel 4 - EEG

Channel 5 – EDC (extensor digitorum communis)

1) Place small adhesive EMG electrodes and attach wires over following sites:
    - Thenar eminence with reference electrode over 1st MCP joint
    - FDI with reference over 2nd MCP joint
    - EDC (landmark= move 3rd and 4th fingers) with reference over tendon
    - Common reference over radial styloid process
2) Place median nerve stimulator electrodes over median nerve at wrist crease (ulna side of Palmaris longus tendon – press thumb and 3rd finger together to elicit this) with reference 3cm distal/lateral to tendon (cathode=purple=proximal; anode=white=distal)
3) Secure wires with micropore tape to arm and pillow

EEG Preparation

1) Measure electrode positions on scalp in accordance with 10-20 international EEG system and record distances:
    - Nasion – inion
    - Tragus – tragus
    - Intersecting line = vertex (Cz)
    - Measure and mark 3cm lateral, then 2cm anterior and 2cm posterior (F3 and P3, respectively)



Cz to C3 = 3cm

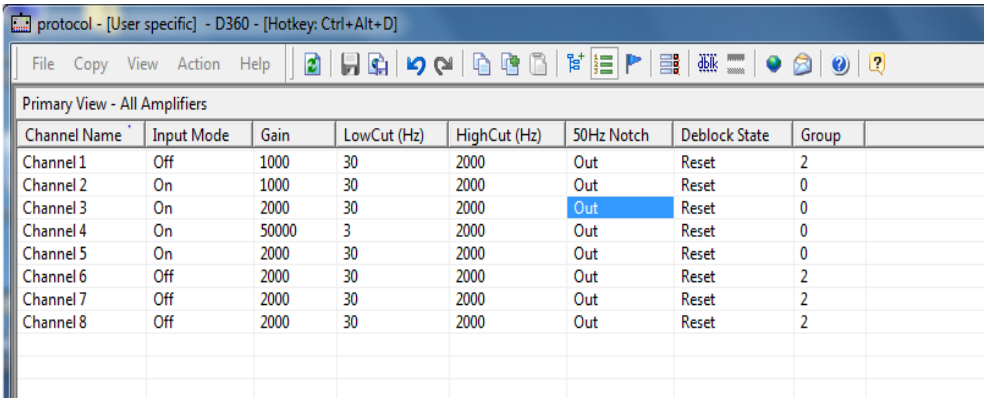
C3 to F3 = 2cm

C3 to P3 = 2cm

2) Apply conducting gel over forehead, F3 and P3 and apply EEG electrodes:
    - Grey = reference = forehead
    - Anode = yellow = F3
    - Cathode = purple = P3
3) Place gauze over F3 and P3 electrodes then Coban over head to secure
4) Secure wires by taping to shoulder

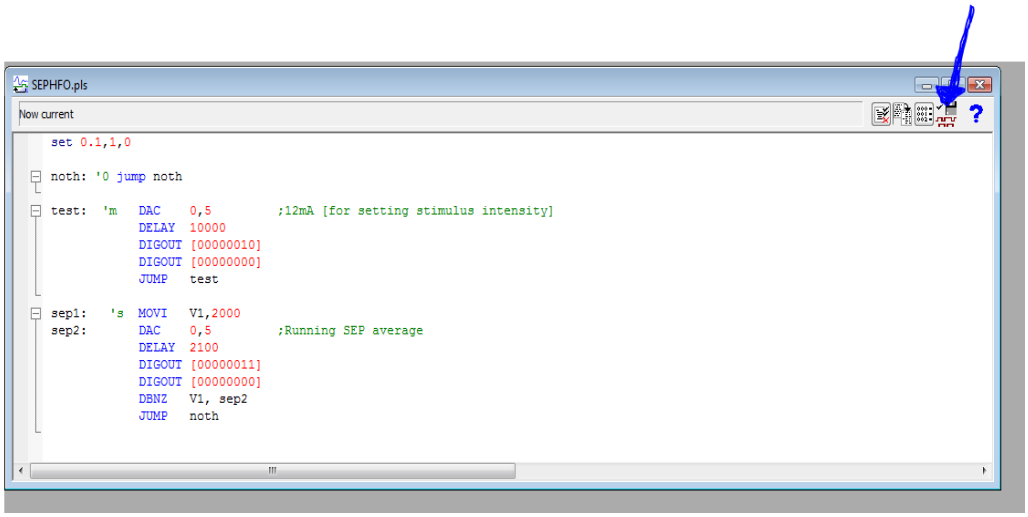
Determining N20

1) Check Digitimer gains as below:

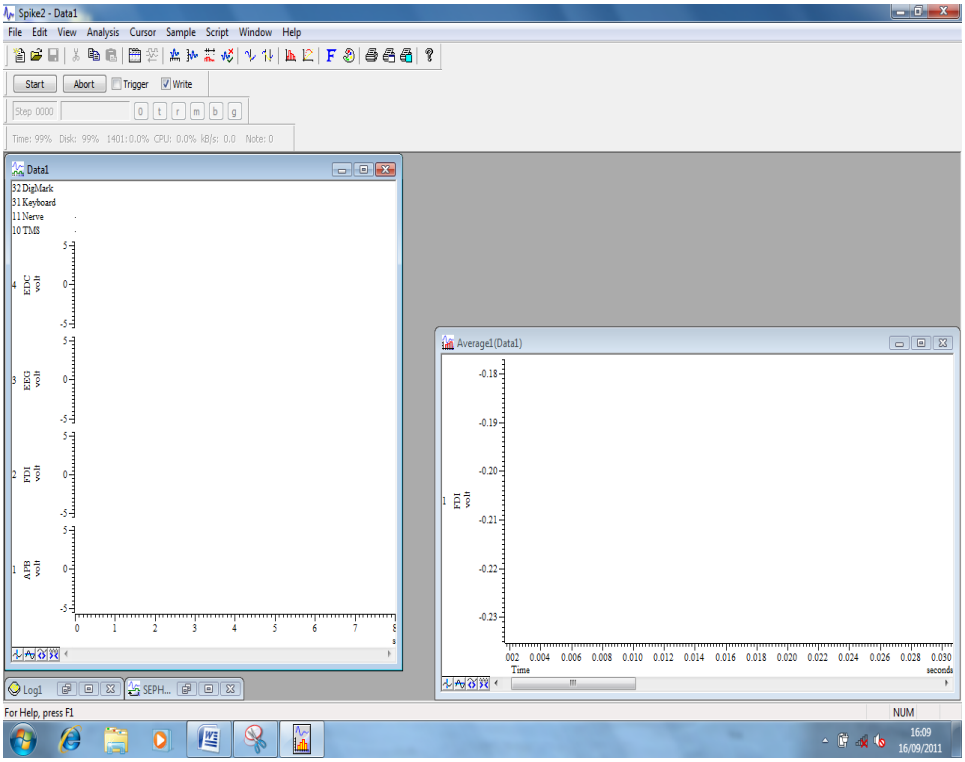


Channel Name	Input Mode	Gain	LowCut (Hz)	HighCut (Hz)	50Hz Notch	Deblock State	Group
Channel 1	Off	1000	30	2000	Out	Reset	2
Channel 2	On	1000	30	2000	Out	Reset	0
Channel 3	On	2000	30	2000	Out	Reset	0
Channel 4	On	50000	3	2000	Out	Reset	0
Channel 5	On	2000	30	2000	Out	Reset	0
Channel 6	Off	2000	30	2000	Out	Reset	2
Channel 7	Off	2000	30	2000	Out	Reset	2
Channel 8	Off	2000	30	2000	Out	Reset	2

2) Open Spike 2 sampling configuration:
Local disc C / spike 2 / work / SAI / .pls files / SEPFHO / run now /
'sample now' (see picture of button) /'start' button:



Ensure on the SEPHFO screen that the screen is current by pressing button below blue arrow

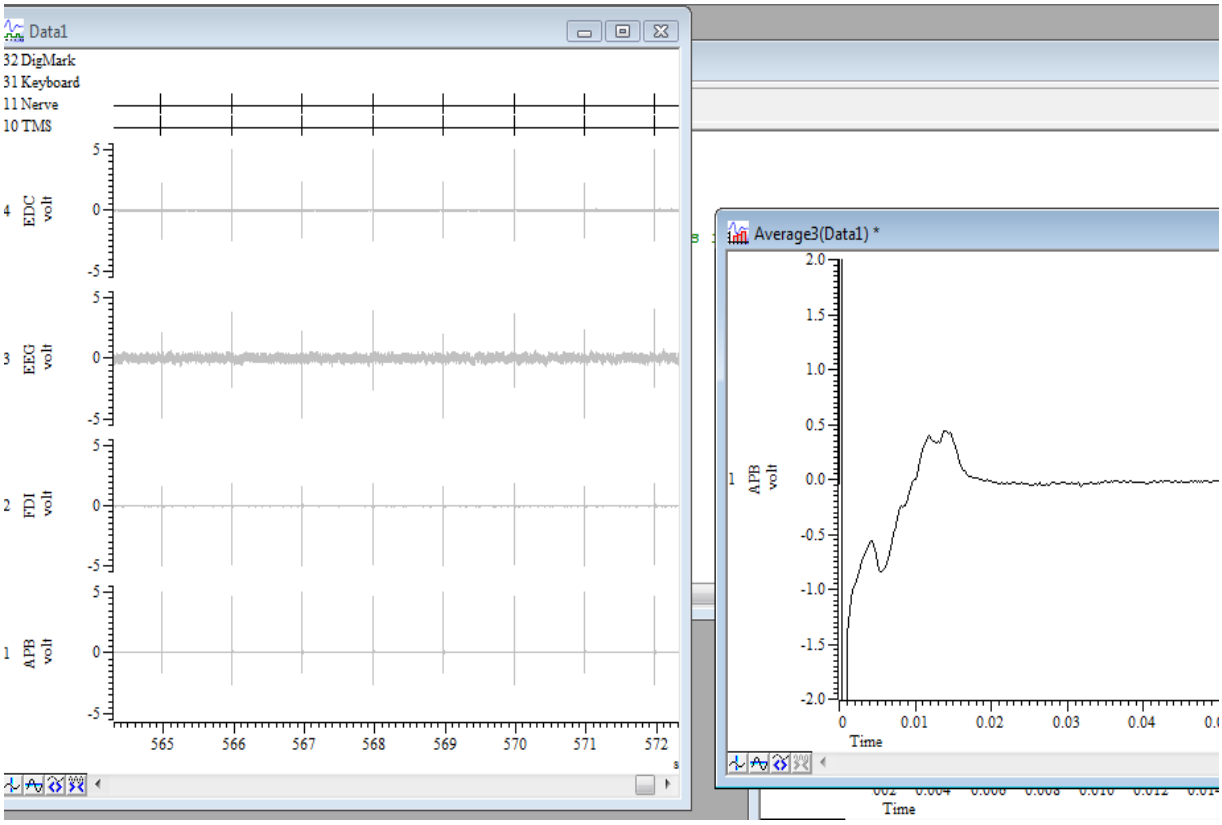


3) Check EEG working- ask patient to close eyes and clench teeth
4) Check EMG working- move thumb/1st finger/3rd and 4th finger against resistance
5) May need to reduce gain if these EMG readings fill -5 to +5 to avoid clipping
6) Set up average:

   Analysis / new result view / waveform average / APB / trigger nerve / unclick mean / new / all data / ok / last data / 2 seconds

   Do not 'Optimise display'

7) Median nerve stimulation – warn patient it may feel like pins and needles:
   - Check pulse width 200 uV
   - Turn on clear button
   - Turn on output (button up)
   - Click on 'm' button
   - Increase voltage until you find luminal threshold (see M wave on screen = direct activation of muscle)

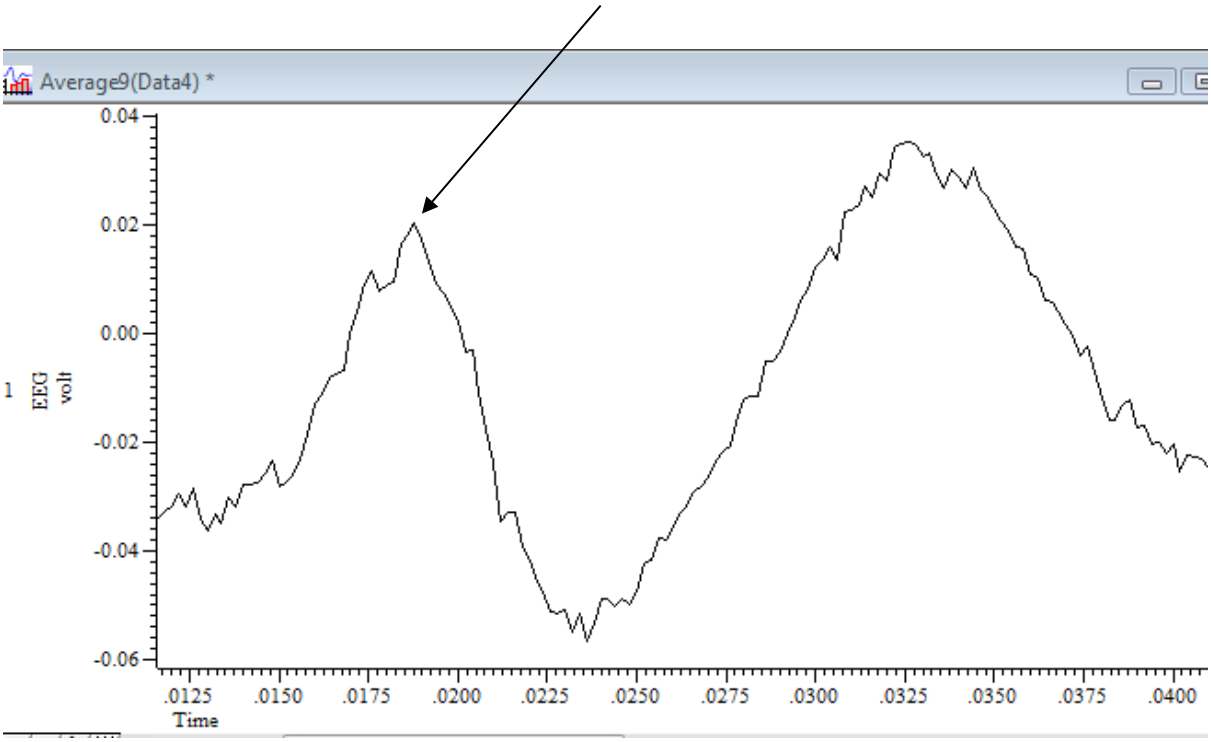


- Note threshold
- Add 20% to find SEP and note this
- NB –need to optimise Y axis- right click on Y axis, optimise Y axis, change gain from 2 to -2

8) Click on abort
9) Click on 'sample now' button on top left of screen
10) Ensure SEPFHO configuration open and current, then Analysis / new result view / waveform average / EEG /display mean / all data / optimise data
11) Click 'write' then 'start' then 's' (=SEPs)
12) Check on average screen. 2000 sweeps takes around 8 minutes, may need to enlarge EEG screen
13) When sweeps have finished, click on data screen (not average screen):
File / save / local disc C / data storage / save as e.g.20110824INC061MRB00

(New directory)

14) On average 2 screen, check N20:
Click on 'cursor' / label mode / position



15) Note peak at N20 (e.g.19.6 = 0.0196) and negative peak at p15 (e.g. 15.8 = 0.0158)
16) Do not save average screen
17) Close data 2
18) Remove EEG electrodes
19) Turn median nerve output off on digitimer box (isolate switch)

TMS

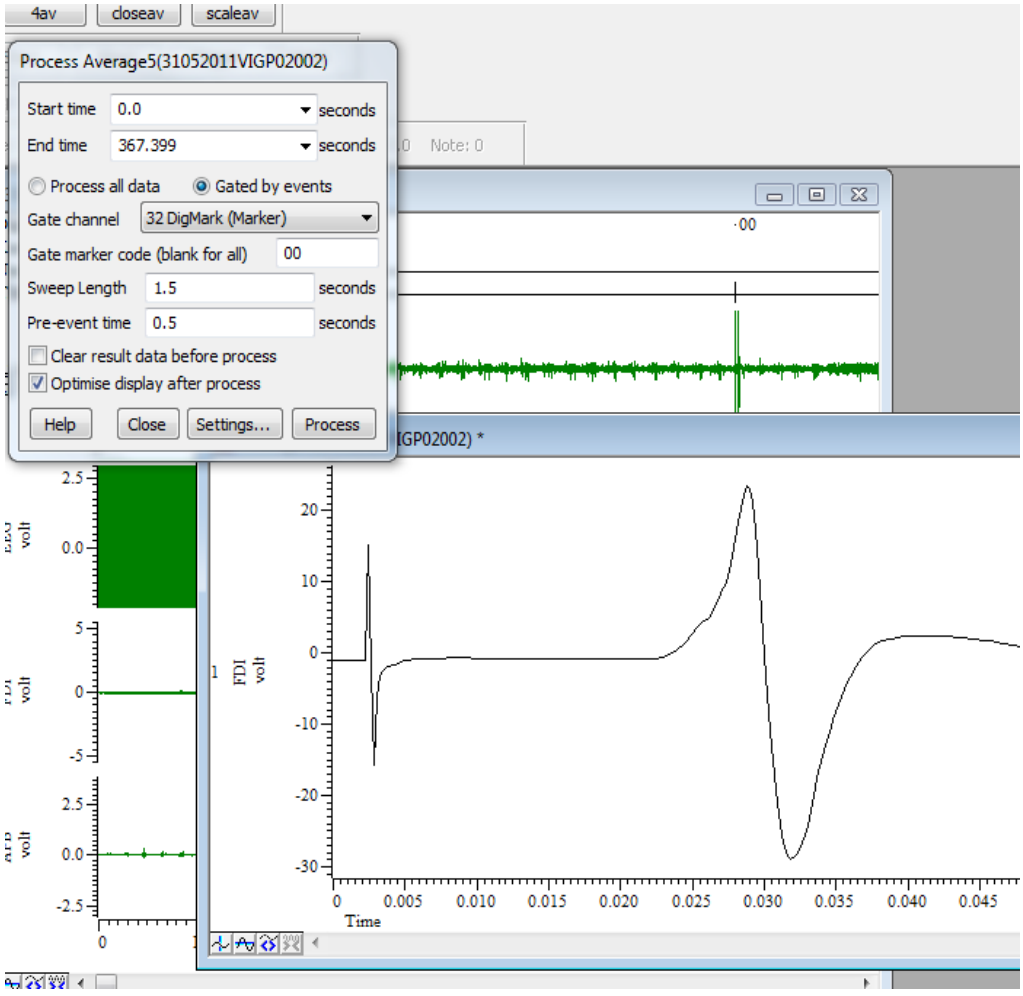
1) Open file / open / SAI (ensure 'files of type' are sequencer files.pls) / SAI.diffint
2) In proc 2-6, insert N20 in proc 2 then add 1ms each time e.g 206, 216 etc and save – Ctrl 'S'
   Do not input to proc1 or 'both' – only change proc 2 and continue to proc 6
3) Analysis / new result view / waveform average / FDI channel / trigger – TMS / unclick mean / new / last 5 seconds / take 'off' optimise display / apply / ok
4) Place Magstim with centre of coil over vertex. If using R arm, A side up; L arm = B side up
5) Magstim power on and armed
6) Green button arms
7) Take 'write' off
8) Start
9) Click on 't' (=TMS)
10) Start at 30% maximum stimulator output (MSO) and increase until MEP (~5 µV in size) is elicited in APB in 50% of 10 trials, to nearest 1% MSO. This is the resting motor threshold (RMT). Note this value

11) Increase MSO to 20% above this for SAI testing and note this, where 20 TMS only trials will be randomly alternated with 50 conditioned trials at N20, N20 +1ms, N20+2ms, N20 +3ms and N20 +4ms.
12) Turn on median nerve output
13) Click on 'write' and then 'g' (=go)
14) Click on data screen:
File / save / local disc C / date storage e.g. 20110824INC061MRB01

Analysis

1) Open the data, then Analysis / new result view / waveform average / FDI / trigger = 32DigMark (Marker) / display mean / new / Gated by events / insert gate marker code separately for each value (i.e. 00 for TMS alone, 01 for TMS plus N20 etc)

2) Measure peaks on average screen



References

Aarsland, D., Andersen, K., Larsen, J.P., Lolk, A. and Kragh-Sorensen, P. (2003a) 'Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study', *Archives of Neurology*, 60(3), pp. 387-92.

Aarsland, D., Andersen, K., Larsen, J.P., Lolk, A., Nielsen, H. and Kragh-Sorensen, P. (2001) 'Risk of dementia in Parkinson's disease - A community-based, prospective study', *Neurology*, 56(6), pp. 730-736.

Aarsland, D., Andersen, K., Larsen, J.P., Perry, R., Wentzel-Larsen, T., Lolk, A. and Kragh-Sorensen, P. (2004) 'The rate of cognitive decline in Parkinson disease', *Archives of Neurology*, 61(12), pp. 1906-1911.

Aarsland, D., Ballard, C., Walker, Z., Bostrom, F., Alves, G., Kossakowski, K., Leroi, I., Pozo-Rodriguez, F., Minthon, L. and Londos, E. (2009a) 'Memantine in patients with Parkinson's disease dementia or dementia with Lewy bodies: a double-blind, placebo-controlled, multicentre trial', *Lancet Neurology*, 8(7), pp. 613-618.

Aarsland, D., Bronnick, K., Ehrt, U., De Deyn, P.P., Tekin, S., Emre, M. and Cummings, J.L. (2007a) 'Neuropsychiatric symptoms in patients with Parkinson's disease and dementia: frequency, profile and associated care giver stress', *Journal of Neurology, Neurosurgery & Psychiatry*, 78(1), pp. 36-42.

Aarsland, D., Bronnick, K. and Fladby, T. (2011) 'Mild Cognitive Impairment in Parkinson's Disease', *Current Neurology and Neuroscience Reports*, 11(4), pp. 371-378.

Aarsland, D., Bronnick, K., Larsen, J.P., Tysnes, O.B., Alves, G. and Norwegian ParkWest Study, G. (2009b) 'Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest study', *Neurology*, 72(13), pp. 1121-6.

Aarsland, D., Bronnick, K., Williams-Gray, C., Weintraub, D., Marder, K., Kulisevsky, J., Burn, D., Barone, P., Pagonabarraga, J., Allcock, L., Santangelo, G., Foltynie, T., Janvin, C., Larsen, J.P., Barker, R.A. and Emre, M. (2010) 'Mild cognitive impairment in Parkinson disease: a multicenter pooled analysis', *Neurology*, 75(12), pp. 1062-9.

Aarsland, D., Kvaloy, J.T., Andersen, K., Larsen, J.P., Tang, M.X., Lolk, A., Kragh-Sorensen, P. and Marder, K. (2007b) 'The effect of age of onset of PD on risk of dementia', *Journal of Neurology*, 254(1), pp. 38-45.

Aarsland, D., Larsen, J.P., Tandberg, E. and Laake, K. (2000) 'Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study', *Journal of the American Geriatrics Society*, 48(8), pp. 938-42.

Aarsland, D., Litvan, I., Salmon, D., Galasko, D., Wentzel-Larsen, T. and Larsen, J.P. (2003b) 'Performance on the dementia rating scale in Parkinson's disease with dementia and dementia with Lewy bodies: comparison with progressive supranuclear palsy and Alzheimer's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 74(9), pp. 1215-1220.

Aarsland, D., Pahlhagen, S., Ballard, C.G., Ehrt, U. and Svenningsson, P. (2012) 'Depression in Parkinson disease-epidemiology, mechanisms and management', *Nature Reviews Neurology*, 8(1), pp. 35-47.

Aarsland, D., Perry, R., Brown, A., Larsen, J.P. and Ballard, C. (2005a) 'Neuropathology of dementia in Parkinson's disease: a prospective, community-based study', *Annals of Neurology*, 58(5), pp. 773-6.

Aarsland, D., Zaccai, J. and Brayne, C. (2005b) 'A systematic review of prevalence studies of dementia in Parkinson's disease', *Movement Disorders*, 20(10), pp. 1255-1263.

Aasly, J.O., Shi, M., Sossi, V., Stewart, T., Johansen, K.K., Wszolek, Z.K., Uitti, R.J., Hasegawa, K., Yokoyama, T., Zabetian, C.P., Kim, H.M., Leverenz, J.B., Ginghina, C., Armaly, J., Edwards, K.L., Snapinn, K.W., Stoessl, A.J. and Zhang, J. (2012) 'Cerebrospinal fluid amyloid beta and tau in LRRK2 mutation carriers', *Neurology*, 78(1), pp. 55-61.

Abdo, W.F., van de Warrenburg, B.P.C., Burn, D.J., Quinn, N.P. and Bloem, B.R. (2010) 'The clinical approach to movement disorders', *Nature Reviews Neurology*, 6(1), pp. 29-37.

Adler, C.H. and Beach, T.G. (2010) 'Variability of diffuse plaques and amyloid angiopathy in Parkinson's disease with mild cognitive impairment', *Acta Neuropathologica*, 120(6), pp. 831-831.

Adler, C.H., Caviness, J.N., Sabbagh, M.N., Shill, H.A., Connor, D.J., Sue, L., Evidente, V.G.H., Driver-Dunckley, E. and Beach, T.G. (2010) 'Heterogeneous neuropathological findings in Parkinson's disease with mild cognitive impairment', *Acta Neuropathologica*, 120(6), pp. 827-8.

Ala, T.A., Hughes, L.F., Kyrouac, G.A., Ghobrial, M.W. and Elble, R.J. (2001) 'Pentagon copying is more impaired in dementia with Lewy bodies than in Alzheimer's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 70(4), pp. 483-8.

Alcalay, R.N., Caccappolo, E., Mejia-Santana, H., Tang, M.X., Rosado, L., Reilly, M.O., Ruiz, D., Ross, B., Verbitsky, M., Kisselev, S., Louis, E., Comella, C., Colcher, A., Jennings, D., Nance, M., Bressman, S., Scott, W.K., Tanner, C., Mickel, S., Andrews, H., Waters, C., Fahn, S., Cote, L., Frucht, S., Ford, B., Rezak, M., Novak, K., Friedman, J.H., Pfeiffer, R., Marsh, L., Hiner, B., Siderowf, A., Payami, H., Molho, E., Factor, S., Ottman, R., Clark, L.N. and Marder, K. (2012) 'Cognitive performance of GBA mutation carriers with early-onset PD The CORE-PD study', *Neurology*, 78(18), pp. 1434-1440.

Alkondon, M., Pereira, E.F.R., Eisenberg, H.M. and Albuquerque, E.X. (2000) 'Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks', *Journal of Neuroscience*, 20(1), pp. 66-75.

Allam, M.F., Campbell, M.J., Hofman, A., Del Castillo, A.S. and Fernandez-Crehuet Navajas, R. (2004) 'Smoking and Parkinson's disease: systematic review of prospective studies', *Movement Disorders*, 19(6), pp. 614-21.

Allan, L., McKeith, I., Ballard, C. and Kenny, R.A. (2006) 'The prevalence of autonomic symptoms in dementia and their association with physical activity, activities of daily living and quality of life', *Dementia and Geriatric Cognitive Disorders*, 22(3), pp. 230-237.

Allan, L.M., Ballard, C.G., Allen, J., Murray, A., Davidson, A.W., McKeith, I.G. and Kenny, R.A. (2007) 'Autonomic dysfunction in dementia', *Journal of Neurology Neurosurgery and Psychiatry*, 78(7), pp. 671-677.

Allcock, L.M., Kenny, R.A., Mosimann, U.P., Tordoff, S., Wesnes, K.A., Hildreth, A.J. and Burn, D.J. (2006) 'Orthostatic hypotension in Parkinson's disease: association with cognitive decline?', *International Journal of Geriatric Psychiatry*, 21(8), pp. 778-83.

Allcock, L.M., Rowan, E.N., Steen, I.N., Wesnes, K., Kenny, R.A. and Burn, D.J. (2009) 'Impaired attention predicts falling in Parkinson's disease', *Parkinsonism & related disorders*, 15(2), pp. 110-115.

Alle, H., Heidegger, T., Krivanekova, L. and Ziemann, U. (2009) 'Interactions between short-interval intracortical inhibition and short-latency afferent inhibition in human motor cortex', *Journal of Physiology*, 587(21), pp. 5163-5176.

Alves, G., Bronnick, K., Aarsland, D., Blennow, K., Zetterberg, H., Ballard, C., Kurz, M.W., Andreasson, U., Tysnes, O.B., Larsen, J.P. and Mulugeta, E. (2010) 'CSF amyloid-beta and tau proteins, and cognitive performance, in early and untreated Parkinson's Disease: the Norwegian ParkWest study', *Journal of Neurology Neurosurgery and Psychiatry*, 81(10), pp. 1080-1086.

Alves, G., Forsaa, E.B., Pedersen, K.F., Dreetz Gjerstad, M. and Larsen, J.P. (2008) 'Epidemiology of Parkinson's disease', *Journal of Neurology*, 255 Suppl 5, pp. 18-32.

Alves, G., Larsen, J.P., Emre, M., Wentzel-Larsen, T. and Aarsland, D. (2006) 'Changes in motor subtype and risk for incident dementia in Parkinson's disease', *Movement Disorders*, 21(8), pp. 1123-30.

Alves, G., Pedersen, K.F., Bloem, B.R., Blennow, K., Zetterberg, H., Borm, G.F., Dalaker, T.O., Beyer, M.K., Aarsland, D., Andreasson, U., Lange, J., Tysnes, O.B., Zivadinov, R. and Larsen, J.P. (2013) 'Cerebrospinal fluid amyloid-beta and phenotypic heterogeneity in de novo Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 84(5), pp. 537-543.

Amici, S. and Boxer, A. (2007) 'Oiling the Gears of the Mind: Roles for Acetycholine in the Modulation of Attention', in Miller, B.L., Cummings, Jeffrey L. (ed.) *The Human Frontal Lobes: Functions and Disorders*. Second Edition edn. New York: Guilford Press, pp. 135-145.

Andersson, M., Zetterberg, H., Minthon, L., Blennow, K. and Londos, E. (2011) 'The cognitive profile and CSF biomarkers in dementia with Lewy bodies and Parkinson's disease dementia', *International Journal of Geriatric Psychiatry*, 26(1), pp. 100-105.

Andreasson, U., Portelius, E., Andersson, M.E., Blennow, K. and Zetterberg, H. (2007) 'Aspects of beta-amyloid as a biomarker for Alzheimer's disease', *Biomarkers in Medicine*, 1(1), pp. 59-78.

Apaydin, H., Ahlskog, J.E., Parisi, J.E., Boeve, B.F. and Dickson, D.W. (2002) 'Parkinson disease neuropathology: later-developing dementia and loss of the levodopa response', *Archives of Neurology*, 59(1), pp. 102-12.

Arendt, T., Bigl, V., Arendt, A. and Tennstedt, A. (1983) 'Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's Disease', *Acta Neuropathologica*, 61(2), pp. 101-8.

Association, A.P. (2000) *Diagnostic and Statistical Manual of Mental Disorders (IV-TR)*. 4th Edition (Text Revised) edn. Washington DC, US: American Psychiatric Publishing.

Athey, R.J., Porter, R.W. and Walker, R.W. (2005) 'Cognitive assessment of a representative community population with Parkinson's disease (PD) using the Cambridge Cognitive Assessment-Revised (CAMCOG-R)', *Age & Ageing*, 34(3), pp. 268-73.

Baba, T., Kikuchi, A., Hirayama, K., Nishio, Y., Hosokai, Y., Kanno, S., Hasegawa, T., Sugeno, N., Konno, M., Suzuki, K., Takahashi, S., Fukuda, H., Aoki, M., Itoyama, Y., Mori, E. and Takeda, A. (2012) 'Severe olfactory dysfunction is a prodromal symptom of dementia associated with Parkinson's disease: a 3 year longitudinal study', *Brain*, 135, pp. 161-169.

Badawy, R.A.B., Tarletti, R., Mula, M., Varrasi, C. and Cantello, R. (2011) 'The routine circular coil is reliable in paired-TMS studies', *Clinical Neurophysiology*, 122(4), pp. 784-8.

Baker, S.C., Rogers, R.D., Owen, A.M., Frith, C.D., Dolan, R.J., Frackowiak, R.S.J. and Robbins, T.W. (1996) 'Neural systems engaged by planning: A PET study of the Tower of London task', *Neuropsychologia*, 34(6), pp. 515-526.

Ballard, C., Jones, E.L., Londos, E., Minthon, L., Francis, P. and Aarsland, D. (2010) 'alpha-Synuclein antibodies recognize a protein present at lower levels in the CSF of patients with dementia with Lewy bodies', *International Psychogeriatrics*, 22(2), pp. 321-7.

Ballard, C., Ziabreva, I., Perry, R., Larsen, J.P., O'Brien, J., McKeith, I., Perry, E. and Aarsland, D. (2006) 'Differences in neuropathologic characteristics across the Lewy body dementia spectrum', *Neurology*, 67(11), pp. 1931-4.

Ballard, C.G., Aarsland, D., McKeith, I., O'Brien, J., Gray, A., Cormack, F., Burn, D., Cassidy, T., Starfeldt, R., Larsen, J.P., Brown, R. and Tovee, M. (2002) 'Fluctuations in attention: PD dementia vs DLB with parkinsonism', *Neurology*, 59(11), pp. 1714-20.

Barone, P., Aarsland, D., Burn, D., Emre, M., Kulisevsky, J. and Weintraub, D. (2011) 'Cognitive impairment in nondemented Parkinson's disease', *Movement Disorders*, 26(14), pp. 2483-2495.

Barone, P., Antonini, A., Colosimo, C., Marconi, R., Morgante, L., Avarello, T.P., Bottacchi, E., Cannas, A., Ceravolo, G., Ceravolo, R., Cicarelli, G., Gaglio, R.M., Giglia, R.M., Iemolo, F., Manfredi, M., Meco, G., Nicoletti, A., Pederzoli, M., Petrone, A., Pisani,

A., Ponfieri, F.E., Quatrale, R., Ramat, S., Scala, R., Volpe, G., Zappulla, S., Bentivoglio, A.R., Stocchi, F., Trianni, G., Del Dotto, P. and Grp, P.S. (2009) 'The Priamo Study: A Multicenter Assessment of Nonmotor Symptoms and Their Impact on Quality of Life in Parkinson's Disease', *Movement Disorders*, 24(11), pp. 1641-1649.

Barone, P., Poewe, W., Albrecht, S., Debieuvre, C., Massey, D., Rascol, O., Tolosa, E. and Weintraub, D. (2010) 'Pramipexole for the treatment of depressive symptoms in patients with Parkinson's disease: a randomised, double-blind, placebo-controlled trial', *Lancet Neurology*, 9(6), pp. 573-580.

Bateman, R.J., Wen, G., Morris, J.C. and Holtzman, D.M. (2007) 'Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker', *Neurology*, 68(9), pp. 666-9.

Beach, T.G., Kuo, Y.M., Spiegel, K., Emmerling, M.R., Sue, L.I., Kokjohn, K. and Roher, A.E. (2000) 'The cholinergic deficit coincides with Abeta deposition at the earliest histopathologic stages of Alzheimer disease', *Journal of Neuropathology & Experimental Neurology*, 59(4), pp. 308-13.

Bedard, M.A., Pillon, B., Dubois, B., Duchesne, N., Masson, H. and Agid, Y. (1999) 'Acute and long-term administration of anticholinergics in Parkinson's disease: specific effects on the subcortico-frontal syndrome', *Brain and cognition*, 40(2), pp. 289-313.

Benton, A.L. (1968) 'Differential behavioural effects of frontal lobe disease', *Neuropsychologia*, 6, pp. 53-60.

Berardelli, A., Wenning, G.K., Antonini, A., Berg, D., Bloem, B.R., Bonifati, V., Brooks, D., Burn, D., Colosimo, C., Fanciulli, J., Ferreira, J., Gasser, T., Grandas, F., Kanovsky, P., Kostic, V., Kulisevsky, J., Oertel, W.H., Poewe, W., Reese, J.P., Relja, M., Ruzicka, E., Schrag, A., Seppi, K., Taba, P. and Vidailhet, M. (2013) 'EFNS/MDS-ES recommendations for the diagnosis of Parkinson's disease', *European Journal of Neurology*, 20, pp. 16-34.

Berg, D., Godau, J. and Walter, U. (2008) 'Transcranial sonography in movement disorders', *Lancet Neurology*, 7(11), pp. 1044-1055.

Beyer, M.K., Herlofson, K., Arsland, D. and Larsen, J.P. (2001) 'Causes of death in a community-based study of Parkinson's disease', *Acta Neurologica Scandinavica*, 103(1), pp. 7-11.

Beyer, M.K., Janvin, C.C., Larsen, J.P. and Aarsland, D. (2007) 'A magnetic resonance imaging study of patients with Parkinson's disease with mild cognitive impairment and dementia using voxel-based morphometry', *Journal of Neurology Neurosurgery and Psychiatry*, 78(3), pp. 254-9.

Bibl, M., Esselmann, H., Lewczuk, P., Trenkwalder, C., Otto, M., Kornhuber, J., Wiltfang, J. and Mollenhauer, B. (2010) 'Combined Analysis of CSF Tau, Abeta42, Abeta1-42% and Abeta1-40% in Alzheimer's Disease, Dementia with Lewy Bodies and Parkinson's Disease Dementia', *International journal of Alzheimer's disease*, 2010.

Bibl, M., Mollenhauer, B., Esselmann, H., Lewczuk, P., Klafki, H.W., Sparbier, K., Smirnov, A., Cepek, L., Trenkwalder, C., Ruther, E., Kornhuber, J., Otto, M. and Wiltfang, J. (2006) 'CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia', *Brain*, 129, pp. 1177-1187.

Bibl, M., Mollenhauer, B., Lewczuk, P., Esselmann, H., Wolf, S., Trenkwalder, C., Otto, M., Stiens, G., Ruther, E., Kornhuber, J. and Wiltfang, J. (2007) 'Validation of amyloid-beta peptides in CSF diagnosis of neurodegenerative dementias', *Molecular psychiatry*, 12(7), pp. 671-80.

Blackwell, A.D., Sahakian, B.J., Vesey, R., Semple, J.M., Robbins, T.W. and Hodges, J.R. (2004) 'Detecting dementia: novel neuropsychological markers of preclinical Alzheimer's disease', *Dementia and Geriatric Cognitive Disorders*, 17(1-2), pp. 42-8.

Blennow, K., Zetterberg, H., Minthon, L., Lannfelt, L., Strid, S., Annas, P., Basun, H. and Andreasen, N. (2007) 'Longitudinal stability of CSF biomarkers in Alzheimer's disease', *Neuroscience Letters*, 419(1), pp. 18-22.

Boddy, F., Rowan, E.N., Lett, D., O'Brien, J.T., McKeith, I.G. and Burn, D.J. (2007) 'Subjectively reported sleep quality and excessive daytime somnolence in Parkinson's

disease with and without dementia, dementia with Lewy Bodies and Alzheimer's disease', *International Journal of Geriatric Psychiatry*, 22(6), pp. 529-535.

Boeve, B.F., Silber, M.H., Ferman, T.J., Kokmen, E., Smith, G.E., Ivnik, R.J., Parisi, J.E., Olson, E.J. and Petersen, R.C. (1998) 'REM sleep behavior disorder and degenerative dementia - An association likely reflecting Lewy body disease', *Neurology*, 51(2), pp. 363-370.

Bohnen, N.I. and Albin, R.L. (2011) 'The cholinergic system and Parkinson disease', *Behavioural brain research*, 221(2), pp. 564-73.

Bohnen, N.I., Albin, R.L., Koeppe, R.A., Wernette, K.A., Kilbourn, M.R., Minoshima, S. and Frey, K.A. (2006a) 'Positron emission tomography of monoaminergic vesicular binding in aging and Parkinson disease', *Journal of Cerebral Blood Flow and Metabolism*, 26(9), pp. 1198-1212.

Bohnen, N.I., Kaufer, D.I., Hendrickson, R., Constantine, G.M., Mathis, C.A. and Moore, R.Y. (2007) 'Cortical cholinergic denervation is associated with depressive symptoms in Parkinson's disease and parkinsonian dementia', *Journal of Neurology Neurosurgery and Psychiatry*, 78(6), pp. 641-3.

Bohnen, N.I., Kaufer, D.I., Hendrickson, R., Ivanco, L.S., Lopresti, B.J., Constantine, G.M., Mathis, C.A., Davis, J.G., Moore, R.Y. and Dekosky, S.T. (2006b) 'Cognitive correlates of cortical cholinergic denervation in Parkinson's disease and parkinsonian dementia', *Journal of Neurology*, 253(2), pp. 242-7.

Bohnen, N.I., Kaufer, D.I., Ivanco, L.S., Lopresti, B., Koeppe, R.A., Davis, J.G., Mathis, C.A., Moore, R.Y. and DeKosky, S.T. (2003) 'Cortical cholinergic function is more severely affected in parkinsonian dementia than in Alzheimer disease: an in vivo positron emission tomographic study', *Archives of Neurology*, 60(12), pp. 1745-8.

Bohnen, N.I., Koeppe, R.A., Minoshima, S., Giordani, B., Albin, R.L., Frey, K.A. and Kuhl, D.E. (2011) 'Cerebral glucose metabolic features of Parkinson disease and incident dementia: longitudinal study', *Journal of Nuclear Medicine*, 52(6), pp. 848-55.

Bohnen, N.I., Muller, M.L.T.M., Koeppe, R.A., Studenski, S.A., Kilbourn, M.A., Frey, K.A. and Albin, R.L. (2009) 'History of falls in Parkinson disease is associated with reduced cholinergic activity', *Neurology*, 73(20), pp. 1670-6.

Bohnen, N.I., Muller, M.L.T.M., Kotagal, V., Koeppe, R.A., Kilbourn, M.A., Albin, R.L. and Frey, K.A. (2010) 'Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease', *Brain*, 133(Pt 6), pp. 1747-54.

Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J.W., Vanacore, N., van Swieten, J.C., Brice, A., Meco, G., van Duijn, C.M., Oostra, B.A. and Heutink, P. (2003) 'Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism', *Science*, 299(5604), pp. 256-9.

Borghi, R., Marchese, R., Negro, A., Marinelli, L., Forloni, G., Zaccheo, D., Abbruzzese, G. and Tabaton, M. (2000) 'Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects', *Neuroscience letters*, 287(1), pp. 65-7.

Braak, H., de Vos, R.A.I., Bohl, J. and Del Tredici, K. (2006) 'Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology', *Neuroscience Letters*, 396(1), pp. 67-72.

Braak, H., Del Tredici, K., Rub, U., de Vos, R.A., Jansen Steur, E.N. and Braak, E. (2003) 'Staging of brain pathology related to sporadic Parkinson's disease', *Neurobiology of Aging*, 24(2), pp. 197-211.

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H. and Del Tredici, K. (2004) 'Stages in the development of Parkinson's disease-related pathology', *Cell and tissue research*, 318(1), pp. 121-34.

Braak, H., Rub, U., Jansen Steur, E.N.H., Del Tredici, K. and de Vos, R.A.I. (2005) 'Cognitive status correlates with neuropathologic stage in Parkinson disease', *Neurology*, 64(8), pp. 1404-10.

Bronnick, K., Alves, G., Aarsland, D., Tysnes, O.B. and Larsen, J.P. (2011) 'Verbal Memory in Drug-Naive, Newly Diagnosed Parkinson's Disease. The Retrieval Deficit Hypothesis Revisited', *Neuropsychology*, 25(1), pp. 114-124.

Bronnick, K., Ehrt, U., Emre, M., De Deyn, P.P., Wesnes, K., Tekin, S. and Aarsland, D. (2006) 'Attentional deficits affect activities of daily living in dementia-associated with Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 77(10), pp. 1136-1142.

Bronnick, K., Emre, M., Lane, R., Tekin, S. and Aarsland, D. (2007) 'Profile of cognitive impairment in dementia associated with Parkinson's disease compared with Alzheimer's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 78(10), pp. 1064-1068.

Bruck, A., Kurki, T., Kaasinen, V., Vahlberg, T. and Rinne, J.O. (2004) 'Hippocampal and prefrontal atrophy in patients with early non-demented Parkinson's disease is related to cognitive impairment', *Journal of Neurology Neurosurgery and Psychiatry*, 75(10), pp. 1467-9.

Buerger, K., Ewers, M., Pirttila, T., Zinkowski, R., Alafuzoff, I., Teipel, S.J., DeBernardis, J., Kerkman, D., McCulloch, C., Soininen, H. and Hampel, H. (2006) 'CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease', *Brain*, 129(Pt 11), pp. 3035-41.

Buongiorno, M., Compta, Y. and Marti, M.J. (2011) 'Amyloid-beta and tau biomarkers in Parkinson's disease-dementia', *Journal of the neurological sciences*, 310(1-2), pp. 25-30.

Burke, R.E., Dauer, W.T. and Vonsattel, J.P.G. (2008) 'A Critical Evaluation of the Braak Staging Scheme for Parkinson's Disease', *Annals of Neurology*, 64(5), pp. 485-491.

Burn, D.J., Rowan, E.N., Allan, L.M., Molloy, S., O'Brien, J.T. and McKeith, I.G. (2006) 'Motor subtype and cognitive decline in Parkinson's disease, Parkinson's disease with

dementia, and dementia with Lewy bodies', *Journal of Neurology Neurosurgery and Psychiatry*, 77(5), pp. 585-9.

Burton, E.J., McKeith, I.G., Burn, D.J., Williams, E.D. and O'Brien, J.T. (2004) 'Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls', *Brain*, 127(Pt 4), pp. 791-800.

Buter, T.C., van den Hout, A., Matthews, F.E., Larsen, J.P., Brayne, C. and Aarsland, D. (2008) 'Dementia and survival in Parkinson disease - A 12-year population study', *Neurology*, 70(13), pp. 1017-1022.

Butterfield, L.C., Cimino, C.R., Oelke, L.E., Hauser, R.A. and Sanchez-Ramos, J. (2010) 'The Independent Influence of Apathy and Depression on Cognitive Functioning in Parkinson's Disease', *Neuropsychology*, 24(6), pp. 721-730.

Calabresi, P., Picconi, B., Parnetti, L. and Di Filippo, M. (2006) 'A convergent model for cognitive dysfunctions in Parkinson's disease: the critical dopamine-acetylcholine synaptic balance', *Lancet Neurology*, 5(11), pp. 974-83.

Cantello, R., Tarletti, R. and Civardi, C. (2002) 'Transcranial magnetic stimulation and Parkinson's disease', *Brain Research Reviews*, 38(3), pp. 309-327.

Caughey, B. and Lansbury, P.T. (2003) 'Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders', *Annual review of neuroscience*, 26, pp. 267-98.

Caviness, J.N., Driver-Dunckley, E., Connor, D.J., Sabbagh, M.N., Hentz, J.G., Noble, B., Evidente, V.G., Shill, H.A. and Adler, C.H. (2007) 'Defining mild cognitive impairment in Parkinson's disease', *Movement Disorders*, 22(9), pp. 1272-7.

Celebi, O., Temucin, C.M., Elibol, B. and Saka, E. (2012) 'Short latency afferent inhibition in Parkinson's disease patients with dementia', *Movement Disorders*, 27(8), pp. 1052-5.

Chaudhuri, K.R., Prieto-Jurcynska, C., Naidu, Y., Mitra, T., Frades-Payo, B., Tluk, S., Ruessmann, A., Odin, P., Macphee, G., Stocchi, F., Ondo, W., Sethi, K., Schapira, A.H.V. and Martinez-Martin, P. (2010) 'The Nondeclaration of Nonmotor Symptoms of Parkinson's Disease to Health Care Professionals: An International Study Using the Nonmotor Symptoms Questionnaire', *Movement Disorders*, 25(6), pp. 704-709.

Chaudhuri, K.R. and Schapira, A.H.V. (2009) 'Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment', *Lancet Neurology*, 8(5), pp. 464-474.

Cheesman, A.L., Barker, R.A., Lewis, S.J.G., Robbins, T.W., Owen, A.M. and Brooks, D.J. (2005) 'Lateralisation of striatal function: evidence from F-18-dopa PET in Parkinsion's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 76(9), pp. 1204-1210.

Chen-Plotkin, A.S., Hu, W.T., Siderowf, A., Weintraub, D., Goldmann Gross, R., Hurtig, H.I., Xie, S.X., Arnold, S.E., Grossman, M., Clark, C.M., Shaw, L.M., McCluskey, L., Elman, L., Van Deerlin, V.M., Lee, V.M., Soares, H. and Trojanowski, J.Q. (2011) 'Plasma epidermal growth factor levels predict cognitive decline in Parkinson disease', *Annals of neurology*, 69(4), pp. 655-63.

Chen, R. (2004) 'Interactions between inhibitory and excitatory circuits in the human motor cortex', *Experimental Brain Research*, 154(1), pp. 1-10.

Chen, R. (2013) 'Biomarker for Mild Cognitive Impairment: Is Short Latency Afferent Inhibition the Answer?', *Movement Disorders*, DOI: 10.1002/mds.25401.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.P., Magistris, M.R., Mills, K., Roesler, K.M., Triggs, W.J., Ugawa, Y. and Ziemann, U. (2008) 'The clinical diagnostic utility of transcranial magnetic stimulation: Report of an IFCN committee', *Clinical Neurophysiology*, 119(3), pp. 504-532.

Chipchase, L., Schabrun, S., Cohen, L., Hodges, P., Ridding, M., Rothwell, J., Taylor, J. and Ziemann, U. (2012) 'A checklist for assessing the methodological quality of studies

using transcranial magnetic stimulation to study the motor system: an international consensus study', *Clinical neurophysiology*, 123(9), pp. 1698-704.

Cirrito, J.R., Disabato, B.M., Restivo, J.L., Verges, D.K., Goebel, W.D., Sathyan, A., Hayreh, D., D'Angelo, G., Benzinger, T., Yoon, H., Kim, J., Morris, J.C., Mintun, M.A. and Sheline, Y.I. (2011) 'Serotonin signaling is associated with lower amyloid-beta levels and plaques in transgenic mice and humans', *Proceedings of the National Academy of Sciences of the United States of America*, 108(36), pp. 14968-14973.

Clark, C.M., Xie, S., Chittams, J., Ewbank, D., Peskind, E., Galasko, D., Morris, J.C., McKeel, D.W., Jr., Farlow, M., Weitlauf, S.L., Quinn, J., Kaye, J., Knopman, D., Arai, H., Doody, R.S., DeCarli, C., Leight, S., Lee, V.M.Y. and Trojanowski, J.Q. (2003) 'Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses?', *Archives of neurology*, 60(12), pp. 1696-702.

Clark, L.N., Kartsaklis, L.A., Gilbert, R.W., Dorado, B., Ross, B.M., Kisselev, S., Verbitsky, M., Mejia-Santana, H., Cote, L.J., Andrews, H., Vonsattel, J.P., Fahn, S., Mayeux, R., Honig, L.S. and Marder, K. (2009) 'Association of Glucocerebrosidase Mutations With Dementia With Lewy Bodies', *Archives of Neurology*, 66(5), pp. 578-583.

Clinton, L.K., Blurton-Jones, M., Myczek, K., Trojanowski, J.Q. and LaFerla, F.M. (2010) 'Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline', *Journal of Neuroscience*, 30(21), pp. 7281-9.

Compta, Y., Ezquerra, M., Munoz, E., Tolosa, E., Valldeoriola, F., Rios, J., Camara, A., Fernandez, M., Buongiorno, M.T. and Marti, M.J. (2011a) 'High cerebrospinal tau levels are associated with the rs242557 tau gene variant and low cerebrospinal beta-amyloid in Parkinson disease', *Neuroscience letters*, 487(2), pp. 169-73.

Compta, Y., Ibarretxe-Bilbao, N., Pereira, J.B., Junque, C., Bargallo, N., Tolosa, E., Valldeoriola, F., Munoz, E., Camara, A., Buongiorno, M. and Marti, M.J. (2012) 'Grey matter volume correlates of cerebrospinal markers of Alzheimer-pathology in Parkinson's disease and related dementia', *Parkinsonism & related disorders*, 18(8), pp. 941-7.

Compta, Y., Marti, M.J., Ibarretxe-Bilbao, N., Junque, C., Valldeoriola, F., Munoz, E., Ezquerra, M., Rios, J. and Tolosa, E. (2009) 'Cerebrospinal tau, phospho-tau, and beta-amyloid and neuropsychological functions in Parkinson's disease', *Movement Disorders*, 24(15), pp. 2203-10.

Compta, Y., Parkkinen, L., O'Sullivan, S.S., Vandrovcova, J., Holton, J.L., Collins, C., Lashley, T., Kallis, C., Williams, D.R., de Silva, R., Lees, A.J. and Revesz, T. (2011b) 'Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important?', *Brain*, 134(Pt 5), pp. 1493-505.

Cooper, J.A., Sagar, H.J., Doherty, S.M., Jordan, N., Tidswell, P. and Sullivan, E.V. (1992) 'Different effects of dopaminergic and anticholinergic therapies on cognitive and motor function in Parkinson's disease. A follow-up study of untreated patients', *Brain : a journal of neurology*, 115 (Pt 6), pp. 1701-25.

Cruise, K.E., Bucks, R.S., Loftus, A.M., Newton, R.U., Pegoraro, R. and Thomas, M.G. (2011) 'Exercise and Parkinson's: benefits for cognition and quality of life', *Acta Neurologica Scandinavica*, 123(1), pp. 13-19.

Crum, R.M., Anthony, J.C., Bassett, S.S. and Folstein, M.F. (1993) 'Population-based norms for the Mini-Mental State Examination by age and educational level', *JAMA*, 269(18), pp. 2386-91.

Cummings, J.L., Vinters, H.V., Cole, G.M. and Khachaturian, Z.S. (1998) 'Alzheimer's disease - Etiologies, pathophysiology, cognitive reserve, and treatment opportunities', *Neurology*, 51(1), pp. S2-S17.

Dalaker, T.O., Zivadinov, R., Larsen, J.P., Beyer, M.K., Cox, J.L., Alves, G., Bronnick, K., Tysnes, O.B., Antulov, R., Dwyer, M.G. and Aarsland, D. (2010) 'Gray matter correlations of cognition in incident Parkinson's disease', *Movement Disorders*, 25(5), pp. 629-33.

Dalrymple-Alford, J.C., Livingston, L., MacAskill, M.R., Graham, C., Melzer, T.R., Porter, R.J., Watts, R. and Anderson, T.J. (2011) 'Characterizing mild cognitive impairment in Parkinson's disease', *Movement Disorders*, 26(4), pp. 629-36.

Dalrymple-Alford, J.C., MacAskill, M.R., Nakas, C.T., Livingston, L., Graham, C., Crucian, G.P., Melzer, T.R., Kirwan, J., Keenan, R., Wells, S., Porter, R.J., Watts, R. and Anderson, T.J. (2010) 'The MoCA: well-suited screen for cognitive impairment in Parkinson disease', *Neurology*, 75(19), pp. 1717-25.

Damier, P., Hirsch, E.C., Agid, Y. and Graybiel, A.M. (1999) 'The substantia nigra of the human brain - II. Patterns of loss of dopamine-containing neurons in Parkinson's disease', *Brain*, 122, pp. 1437-1448.

de Lau, L.M.L., Schipper, C.M.A., Hofman, A., Koudstaal, P.J. and Breteler, M.M.B. (2005) 'Prognosis of Parkinson disease - Risk of dementia and mortality: The Rotterdam Study', *Archives of Neurology*, 62(8), pp. 1265-1269.

de Rijk, M.C., Breteler, M.M., Graveland, G.A., Ott, A., Grobbee, D.E., van der Meche, F.G. and Hofman, A. (1995) 'Prevalence of Parkinson's disease in the elderly: the Rotterdam Study', *Neurology*, 45(12), pp. 2143-6.

Degardin, A., Devos, D., Cassim, F., Bourriez, J.-L., Defebvre, L., Derambure, P. and Devanne, H. (2011) 'Deficit of sensorimotor integration in normal aging', *Neuroscience Letters*, 498(3), pp. 208-12.

Desikan, R.S., McEvoy, L.K., Thompson, W.K., Holland, D., Brewer, J.B., Aisen, P.S., Sperling, R.A., Dale, A.M., Alzheimers Disease Neuroimaging, I., Weiner, M., Aisen, P., Weiner, M., Aisen, P., Petersen, R., Jack, C.R., Jr., Jagust, W., Trojanowki, J.Q., Toga, A.W., Beckett, L., Green, R.C., Saykin, A.J., Morris, J., Liu, E., Green, R.C., Montine, T., Petersen, R., Aisen, P., Gamst, A., Thomas, R.G., Donohue, M., Walter, S., Gessert, D., Sather, T., Beckett, L., Harvey, D., Gamst, A., Donohue, M., Kornak, J., Jack, C.R., Jr., Dale, A., Bernstein, M., Felmlee, J., Fox, N., Thompson, P., Schuff, N., Alexander, G., DeCarli, C., Jagust, W., Bandy, D., Koeppe, R.A., Foster, N., Reiman, E.M., Chen, K., Mathis, C., Morris, J., Cairns, N.J., Taylor-Reinwald, L., Trojanowki, J.Q., Shaw, L., Lee,

V.M.Y., Korecka, M., Toga, A.W., Crawford, K., Neu, S., Saykin, A.J., Foroud, T.M., Potkin, S., Shen, L., Kachaturian, Z., Frank, R., Snyder, P.J., Molchan, S., Kaye, J., Quinn, J., Lind, B., Dolen, S., Schneider, L.S., Pawluczyk, S., Spann, B.M., Brewer, J., Vanderswag, H., Heidebrink, J.L., Lord, J.L., Petersen, R., Johnson, K., Doody, R.S., Villanueva-Meyer, J., Chowdhury, M., Stern, Y., Honig, L.S., Bell, K.L., Morris, J.C., Ances, B., Carroll, M., Leon, S., Mintun, M.A., Schneider, S., Marson, D., Griffith, R., Clark, D., et al. (2012) 'Amyloid-beta--associated clinical decline occurs only in the presence of elevated P-tau', *Archives of neurology*, 69(6), pp. 709-13.

Di Fonzo, A., Dekker, M.C., Montagna, P., Baruzzi, A., Yonova, E.H., Correia Guedes, L., Szczerbinska, A., Zhao, T., Dubbel-Hulsman, L.O., Wouters, C.H., de Graaff, E., Oyen, W.J., Simons, E.J., Breedveld, G.J., Oostra, B.A., Horstink, M.W. and Bonifati, V. (2009) 'FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome', *Neurology*, 72(3), pp. 240-5.

Di Fonzo, A., Rohe, C.F., Ferreira, J., Chien, H.F., Vacca, L., Stocchi, F., Guedes, L., Fabrizio, E., Manfredi, M., Vanacore, N., Goldwurm, S., Breedveld, G., Sampaio, C., Meco, G., Barbosa, E., Oostra, B.A., Bonifati, V. and Italian Parkinson Genetics, N. (2005) 'A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease', *Lancet*, 365(9457), pp. 412-5.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Bentivoglio, A.R. and Tonali, P.A. (2004a) 'Normal or enhanced short-latency afferent inhibition in Parkinson's disease?', *Brain*, 127(Pt 4), p. E8; author reply E9.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C., Daniele, A., Ghirlanda, S., Gainotti, G. and Tonali, P.A. (2004b) 'Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 75(4), pp. 555-9.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C., Ghirlanda, S., Ranieri, F., Gainotti, G. and Tonali, P. (2005a) 'Neurophysiological predictors of long term response to AChE inhibitors in AD patients', *Journal of neurology, neurosurgery, and psychiatry*, 76(8), pp. 1064-9.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M.A., Di Giovanni, S., Zito, G., Tonali, P. and Rothwell, J.C. (2000) 'Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex', *Experimental brain research* 135(4), pp. 455-61.

Di Lazzaro, V., Oliviero, A., Saturno, E., Dileone, M., Pilato, F., Nardone, R., Ranieri, F., Musumeci, G., Fiorilla, T. and Tonali, P. (2005b) 'Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans', *Journal of Physiology*, 564(Pt 2), pp. 661-8.

Di Lazzaro, V., Oliviero, A., Tonali, P.A., Marra, C., Daniele, A., Profice, P., Saturno, E., Pilato, F., Masullo, C. and Rothwell, J.C. (2002) 'Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation', *Neurology*, 59(3), pp. 392-7.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Marra, C., Ranieri, F., Quaranta, D., Gainotti, G. and Tonali, P.A. (2008) 'In vivo functional evaluation of central cholinergic circuits in vascular dementia', *Clinical neurophysiology*, 119(11), pp. 2494-500.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Ranieri, F., Ricci, V., Bria, P., Tonali, P.A. and Ziemann, U. (2007a) 'Segregating two inhibitory circuits in human motor cortex at the level of GABAA receptor subtypes: a TMS study', *Clinical Neurophysiology*, 118(10), pp. 2207-14.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Oliviero, A., Marra, C., Daniele, A., Ranieri, F., Gainotti, G. and Tonali, P.A. (2006) 'In vivo cholinergic circuit evaluation in frontotemporal and Alzheimer dementias', *Neurology*, 66(7), pp. 1111-3.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Profice, P., Marra, C., Daniele, A., Ranieri, F., Quaranta, D., Gainotti, G. and Tonali, P.A. (2007b) 'Functional evaluation of cerebral cortex in dementia with Lewy bodies', *Neuroimage*, 37(2), pp. 422-9.

Di Lazzaro, V., Pilato, F., Dileone, M., Tonali, P.A. and Ziemann, U. (2005c) 'Dissociated effects of diazepam and lorazepam on short-latency afferent inhibition', *The Journal of physiology*, 569(Pt 1), pp. 315-23.

Diez-Ariza, M., Garcia-Alloza, M., Lasheras, B., Del Rio, J. and Ramirez, M.J. (2002) 'GABA(A) receptor antagonists enhance cortical acetylcholine release induced by 5-HT(3) receptor blockade in freely moving rats', *Brain research*, 956(1), pp. 81-5.

Dubois, B., Burn, D., Goetz, C., Aarsland, D., Brown, R.G., Broe, G.A., Dickson, D., Duyckaerts, C., Cummings, J., Gauthier, S., Korczyn, A., Lees, A., Levy, R., Litvan, I., Mizuno, Y., McKeith, I.G., Olanow, C.W., Poewe, W., Sampaio, C., Tolosa, E. and Emre, M. (2007) 'Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force', *Movement disorders*, 22(16), pp. 2314-24.

Dubois, B., Danze, F., Pillon, B., Cusimano, G., Lhermitte, F. and Agid, Y. (1987) 'Cholinergic-Dependent Cognitive Deficits in Parkinsons-Disease', *Annals of neurology*, 22(1), pp. 26-30.

Dubois, B., Feldman, H.H., Jacova, C., Cummings, J.L., Dekosky, S.T., Barberger-Gateau, P., Delacourte, A., Frisoni, G., Fox, N.C., Galasko, D., Gauthier, S., Hampel, H., Jicha, G.A., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Sarazin, M., de Souza, L.C., Stern, Y., Visser, P.J. and Scheltens, P. (2010) 'Revising the definition of Alzheimer's disease: a new lexicon', *Lancet neurology*, 9(11), pp. 1118-27.

Dubois, B., Tolosa, E., Katzenschlager, R., Emre, M., Lees, A.J., Schumann, G., Pourcher, E., Gray, J., Thomas, G., Swartz, J., Hsu, T. and Moline, M.L. (2012) 'Donepezil in Parkinson's disease dementia: A randomized, double-blind efficacy and safety study', *Movement Disorders*, 27(10), pp. 1230-1238.

Dujardin, K., Sockeel, P., Delliaux, M., Destee, A. and Defebvre, L. (2009) 'Apathy May Herald Cognitive Decline and Dementia in Parkinson's Disease', *Movement Disorders*, 24(16), pp. 2391-2397.

Duncan, G.W., Khoo, T.K., Yarnall, A.J., O'Brien, J., Coleman, S.Y., Brooks, D., Barker, R.A. and Burn, D.J. (2013a) 'Health-related quality of life in early Parkinson's disease: the impact of non-motor symptoms', *Movement Disorders*, Submitted.

Duncan, G.W., Khoo, T.K., Yarnall, A.J., O'Brien, J.T., Brayne, C.E., Coleman, S.Y., Brooks, D.J., Barker, R.A. and Burn, D.J. (2013b) 'The Incidence of Parkinson's Disease in the North-East of England', *Age & Ageing*, In Press.

Ehrt, U., Broich, K., Larsen, J.P., Ballard, C. and Aarsland, D. (2010) 'Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study', *Journal of Neurology Neurosurgery and Psychiatry*, 81(2), pp. 160-5.

Ekman, U., Eriksson, J., Forsgren, L., Mo, S.J., Riklund, K. and Nyberg, L. (2012) 'Functional brain activity and presynaptic dopamine uptake in patients with Parkinson's disease and mild cognitive impairment: a cross-sectional study', *Lancet Neurology*, 11(8), pp. 679-687.

El-Agnaf, O.M.A., Walsh, D.M. and Allsop, D. (2003) 'Soluble oligomers for the diagnosis of neurodegenerative diseases', *Lancet neurology*, 2(8), pp. 461-2.

Elgh, E., Domellof, M., Linder, J., Edstrom, M., Stenlund, H. and Forsgren, L. (2009) 'Cognitive function in early Parkinson's disease: a population-based study', *European Journal of Neurology*, 16(12), pp. 1278-84.

Emir, U.E., Tuite, P.J. and Oz, G. (2012) 'Elevated pontine and putamenal GABA levels in mild-moderate Parkinson disease detected by 7 tesla proton MRS', *PloS one*, 7(1), p. e30918.

Emre, M., Aarsland, D., Albanese, A., Byrne, E.J., Deuschl, G., De Deyn, P.P., Durif, F., Kulisevsky, J., van Laar, T., Lees, A., Poewe, W., Robillard, A., Rosa, M.M., Wolters, E., Quarg, P., Tekin, S. and Lane, R. (2004) 'Rivastigmine for dementia associated with Parkinson's disease', *New England Journal of Medicine*, 351(24), pp. 2509-18.

Emre, M., Aarsland, D., Brown, R., Burn, D.J., Duyckaerts, C., Mizuno, Y., Broe, G.A., Cummings, J., Dickson, D.W., Gauthier, S., Goldman, J., Goetz, C., Korczyn, A., Lees, A., Levy, R., Litvan, I., McKeith, I., Olanow, W., Poewe, W., Quinn, N., Sampaio, C., Tolosa, E. and Dubois, B. (2007) 'Clinical diagnostic criteria for dementia associated with Parkinson's disease', *Movement Disorders*, 22(12), pp. 1689-707; quiz 1837.

Emre, M., Poewe, W., De Deyn, P.P., Barone, P., Kulisevsky, J., Pourcher, E., van Laar, T., Callegari, F., Tenenbaum, N. and Graf, A. (2011) 'A 76-week study on the long-term safety of rivastigmine capsules and patch in patients with dementia associated with Parkinson's disease', *Movement Disorders*, 26, pp. S125-S125.

Emre, M., Tsolaki, M., Bonuccelli, U., Destee, A., Tolosa, E., Kutzelnigg, A., Ceballos-Baumann, A., Zdravkovic, S., Bladstrom, A., Jones, R. and Study, I. (2010) 'Memantine for patients with Parkinson's disease dementia or dementia with Lewy bodies: a randomised, double-blind, placebo-controlled trial', *Lancet neurology*, 9(10), pp. 969-77.

Evans, A.H., Lawrence, A.D., Potts, J., MacGregor, L., Katzenschlager, R., Shaw, K., Zijlmans, J. and Lees, A.J. (2006) 'Relationship between impulsive sensation seeking traits, smoking, alcohol and caffeine intake, and Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 77(3), pp. 317-21.

Evatt, M.L. (2010) 'Beyond vitamin status: is there a role for vitamin d in Parkinson disease?', *Archives of neurology*, 67(7), pp. 795-7.

Eyles, D.W., Smith, S., Kinobe, R., Hewison, M. and McGrath, J.J. (2005) 'Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain', *Journal of chemical neuroanatomy*, 29(1), pp. 21-30.

Fagan, A.M., Mintun, M.A., Mach, R.H., Lee, S.Y., Dence, C.S., Shah, A.R., LaRossa, G.N., Spinner, M.L., Klunk, W.E., Mathis, C.A., DeKosky, S.T., Morris, J.C. and Holtzman, D.M. (2006) 'Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid A beta(42) in humans', *Annals of Neurology*, 59(3), pp. 512-519.

Fang, F., Chen, H., Feldman, A.L., Kamel, F., Ye, W. and Wirdefeldt, K. (2012) 'Head injury and Parkinson's disease: A population-based study', *Movement disorders*, 27(13), pp. 1632-5.

Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., PericakVance, M.A., Risch, N. and vanDuijn, C.M. (1997) 'Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease - A meta-analysis', *JAMA*, 278(16), pp. 1349-1356.

Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L., Gwinn-Hardy, K., Petrucelli, L., Hussey, J., Singleton, A., Tanner, C., Hardy, J. and Langston, J.W. (2001) 'Lewy bodies and parkinsonism in families with parkin mutations', *Annals of Neurology*, 50(3), pp. 293-300.

Fearnley, J.M. and Lees, A.J. (1991) 'Aging and Parkinsons-Disease - Substantia-Nigra Regional Selectivity', *Brain*, 114, pp. 2283-2301.

Feise, R.J. (2002) 'Do multiple outcome measures require p-value adjustment?', *BMC medical research methodology*, 2, p. 8.

Fenelon, G. and Alves, G. (2010) 'Epidemiology of psychosis in Parkinson's disease', *Journal of the Neurological Sciences*, 289(1-2), pp. 12-17.

Fenelon, G., Mahieux, F., Huon, R. and Ziegler, M. (2000) 'Hallucinations in Parkinson's disease: prevalence, phenomenology and risk factors', *Brain*, 123 (Pt 4), pp. 733-45.

Firbank, M.J., Colloby, S.J., Burn, D.J., McKeith, I.G. and O'Brien, J.T. (2003) 'Regional cerebral blood flow in Parkinson's disease with and without dementia', *NeuroImage*, 20(2), pp. 1309-19.

Fisher, A., Pittel, Z., Haring, R., Bar-Ner, N., Kliger-Spatz, M., Natan, N., Egozi, I., Sonego, H., Marcovitch, I. and Brandeis, R. (2003) 'M1 muscarinic agonists can modulate some of the hallmarks in Alzheimer's disease: implications in future therapy', *Journal of Molecular Neuroscience*, 20(3), pp. 349-56.

Folstein, M.F., Folstein, S.E. and McHugh, P.R. (1975) '"Mini-mental state". A practical method for grading the cognitive state of patients for the clinician', *Journal of Psychiatric Research*, 12(3), pp. 189-98.

Foltynie, T., Brayne, C. and Barker, R.A. (2002) 'The heterogeneity of idiopathic Parkinson's disease', *Journal of Neurology*, 249(2), pp. 138-145.

Foltynie, T., Brayne, C.E.G., Robbins, T.W. and Barker, R.A. (2004) 'The cognitive ability of an incident cohort of Parkinson's patients in the UK. The CamPaIGN study', *Brain*, 127, pp. 550-560.

Foltynie, T., Lewis, S.G.J., Goldberg, T.E., Blackwell, A.D., Kolachana, B.S., Weinberger, D.R., Robbins, T.W. and Barker, R.A. (2005) 'The BDNF Val(66)Met polymorphism has a gender specific influence on planning ability in Parkinson's disease', *Journal of Neurology*, 252(7), pp. 833-838.

Forno, L.S. (1996) 'Neuropathology of Parkinson's disease', *Journal of Neuropathology and Experimental Neurology*, 55(3), pp. 259-272.

Foster, E.R., Campbell, M.C., Burack, M.A., Hartlein, J., Flores, H.P., Cairns, N.J., Hershey, T. and Perlmutter, J.S. (2010) 'Amyloid Imaging of Lewy Body-Associated Disorders', *Movement Disorders*, 25(15), pp. 2516-2523.

Francis, P.T. (2009) 'Biochemical and pathological correlates of cognitive and behavioural change in DLB/PDD', *Journal of Neurology*, 256 Suppl 3, pp. 280-5.

Freitas, C., Mondragon-Llorca, H. and Pascual-Leone, A. (2011) 'Noninvasive brain stimulation in Alzheimer's disease: Systematic review and perspectives for the future', *Experimental Gerontology*, 46(8), pp. 611-627.

Fuchs, J., Nilsson, C., Kachergus, J., Munz, M., Larsson, E.M., Schule, B., Langston, J.W., Middleton, F.A., Ross, O.A., Hulihan, M., Gasser, T. and Farrer, M.J. (2007) 'Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication', *Neurology*, 68(12), pp. 916-22.

Gaenslen, A., Unmuth, B., Godau, J., Liepelt, I., Di Santo, A., Schweitzer, K.J., Gasser, T., Machulla, H.J., Reimold, M., Marek, K. and Berg, D. (2008) 'The specificity and sensitivity of transcranial ultrasound in the differential diagnosis of Parkinson's disease: a prospective blinded study', *Lancet Neurology*, 7(5), pp. 417-424.

Galvin, J.E., Pollack, J. and Morris, J.C. (2006) 'Clinical phenotype of Parkinson disease dementia', *Neurology*, 67(9), pp. 1605-1611.

Gasser, T., Hardy, J. and Mizuno, Y. (2011) 'Milestones in PD genetics', *Movement Disorders*, 26(6), pp. 1042-8.

Gilks, W.P., Abou-Sleiman, P.M., Gandhi, S., Jain, S., Singleton, A., Lees, A.J., Shaw, K., Bhatia, K.P., Bonifati, V., Quinn, N.P., Lynch, J., Healy, D.G., Holton, J.L., Revesz, T. and Wood, N.W. (2005) 'A common LRRK2 mutation in idiopathic Parkinson's disease', *Lancet*, 365(9457), pp. 415-6.

Giorgetti, M., Bacciottini, L., Giovannini, M.G., Colivicchi, M.A., Goldfarb, J. and Blandina, P. (2000) 'Local GABAergic modulation of acetylcholine release from the cortex of freely moving rats', *The European journal of neuroscience*, 12(6), pp. 1941-8.

Glenner, G.G. and Wong, C.W. (1984) 'Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein', *Biochemical and biophysical research communications*, 120(3), pp. 885-90.

Goetz, C.G., Emre, M. and Dubois, B. (2008a) 'Parkinson's Disease Dementia: Definitions, Guidelines, and Research Perspectives in Diagnosis', *Annals of Neurology*, 64(6), pp. S81-S92.

Goetz, C.G., Tilley, B.C., Shaftman, S.R., Stebbins, G.T., Fahn, S., Martinez-Martin, P., Poewe, W., Sampaio, C., Stern, M.B., Dodel, R., Dubois, B., Holloway, R., Jankovic, J., Kulisevsky, J., Lang, A.E., Lees, A., Leurgans, S., LeWitt, P.A., Nyenhuis, D., Olanow, C.W., Rascol, O., Schrag, A., Teresi, J.A., van Hilten, J.J., LaPelle, N. and Movement Disorder Society, U.R.T.F. (2008b) 'Movement Disorder Society-sponsored revision of the

Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results', *Movement Disorders*, 23(15), pp. 2129-70.

Goldman, J.G., Weis, H., Stebbins, G., Bernard, B. and Goetz, C.G. (2012a) 'Clinical differences among mild cognitive impairment subtypes in Parkinson's disease', *Movement disorders*, 27(9), pp. 1129-36.

Goldman, S.M., Quinlan, P.J., Ross, G.W., Marras, C., Meng, C., Bhudhikanok, G.S., Comyns, K., Korell, M., Chade, A.R., Kasten, M., Priestley, B., Chou, K.L., Fernandez, H.H., Cambi, F., Langston, J.W. and Tanner, C.M. (2012b) 'Solvent exposures and Parkinson disease risk in twins', *Annals of neurology*, 71(6), pp. 776-84.

Gomperts, S.N., Locascio, J.J., Marquie, M., Santarlasci, A.L., Rentz, D.M., Maye, J., Johnson, K.A. and Growdon, J.H. (2012) 'Brain amyloid and cognition in Lewy body diseases', *Movement Disorders*, 27(8), pp. 965-973.

Gomperts, S.N., Locascio, J.J., Rentz, D., Santarlasci, A., Marquie, M., Johnson, K.A. and Growdon, J.H. (2013) 'Amyloid is linked to cognitive decline in patients with Parkinson disease without dementia', *Neurology*, 80(1), pp. 85-91.

Gomperts, S.N., Rentz, D.M., Moran, E., Becker, J.A., Locascio, J.J., Klunk, W.E., Mathis, C.A., Elmaleh, D.R., Shoup, T., Fischman, A.J., Hyman, B.T., Growdon, J.H. and Johnson, K.A. (2008) 'Imaging amyloid deposition in Lewy body diseases', *Neurology*, 71(12), pp. 903-910.

Goodglass, H. (1972) *The assessment of aphasia and related disorders*. Philadelphia: Lea and Febiger.

Goris, A., Williams-Gray, C.H., Clark, G.R., Foltynie, T., Lewis, S.J.G., Brown, J., Ban, M., Spillantini, M.G., Compston, A., Burn, D.J., Chinnery, P.F., Barker, R.A. and Sawcer, S.J. (2007) 'Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease', *Annals of Neurology*, 62(2), pp. 145-153.

Graham, J.M. and Sagar, H.J. (1999) 'A data-driven approach to the study of heterogeneity in idiopathic Parkinson's disease: Identification of three distinct subtypes', *Movement Disorders*, 14(1), pp. 10-20.

Green, J., McDonald, W.M., Vitek, J.L., Evatt, M., Freeman, A., Haber, M., Bakay, R.A.E., Triche, S., Sirockman, B. and DeLong, M.R. (2002) 'Cognitive impairments in advanced PD without dementia', *Neurology*, 59(9), pp. 1320-1324.

Grilli, M., Lagomarsino, F., Zappettini, S., Preda, S., Mura, E., Govoni, S. and Marchi, M. (2010) 'Specific inhibitory effect of amyloid-beta on presynaptic muscarinic receptor subtypes modulating neurotransmitter release in the rat nucleus accumbens', *Neuroscience*, 167(2), pp. 482-9.

Grimmer, T., Riemenschneider, M., Forstl, H., Henriksen, G., Klunk, W.E., Mathis, C.A., Shiga, T., Wester, H.-J., Kurz, A. and Drzezga, A. (2009) 'Beta amyloid in Alzheimer's disease: increased deposition in brain is reflected in reduced concentration in cerebrospinal fluid', *Biological psychiatry*, 65(11), pp. 927-34.

Guehl, D., Bezard, E., Dovero, S., Boraud, T., Bioulac, B. and Gross, C. (1999) 'Trichloroethylene and parkinsonism: a human and experimental observation', *European journal of neurology*, 6(5), pp. 609-11.

Hall, C.B., Derby, C., LeValley, A., Katz, M.J., Verghese, J. and Lipton, R.B. (2007) 'Education delays accelerated decline on a memory test in persons who develop dementia', *Neurology*, 69(17), pp. 1657-64.

Hall, C.B., Lipton, R.B., Sliwinski, M., Katz, M.J., Derby, C.A. and Verghese, J. (2009) 'Cognitive activities delay onset of memory decline in persons who develop dementia', *Neurology*, 73(5), pp. 356-61.

Hall, S., Ohrfelt, A., Constantinescu, R., Andreasson, U., Surova, Y., Bostrom, F., Nilsson, C., Hakan, W., Decraemer, H., Nagga, K., Minthon, L., Londos, E., Vanmechelen, E., Holmberg, B., Zetterberg, H., Blennow, K. and Hansson, O. (2012) 'Accuracy of a panel

of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders', *Archives of neurology*, 69(11), pp. 1445-52.

Halliday, G., Hely, M., Reid, W. and Morris, J. (2008) 'The progression of pathology in longitudinally followed patients with Parkinson's disease', *Acta Neuropathologica*, 115(4), pp. 409-415.

Halliday, G.M. (2013) 'Motor deficits associated with changes in beta-amyloid in Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 84(5), pp. 475-476.

Halliday, G.M., Macdonald, V. and Henderson, J.M. (2005) 'A comparison of degeneration in motor thalamus and cortex between progressive supranuclear palsy and Parkinson's disease', *Brain*, 128(Pt 10), pp. 2272-80.

Halliday, G.M. and McCann, H. (2010) 'The progression of pathology in Parkinson's disease', *Year in Neurology 2*, 1184, pp. 188-195.

Han, L., McCusker, J., Cole, M., Abrahamowicz, M., Primeau, F. and Elie, M. (2001) 'Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients', *Archives of internal medicine*, 161(8), pp. 1099-105.

Hawkes, C.H., Del Tredici, K. and Braak, H. (2007) 'Parkinson's disease: a dual-hit hypothesis', *Neuropathology and Applied Neurobiology*, 33(6), pp. 599-614.

Healy, D.G., Falchi, M., O'Sullivan, S.S., Bonifati, V., Durr, A., Bressman, S., Brice, A., Aasly, J., Zabetian, C.P., Goldwurm, S., Ferreira, J.J., Tolosa, E., Kay, D.M., Klein, C., Williams, D.R., Marras, C., Lang, A.E., Wszolek, Z.K., Berciano, J., Schapira, A.H., Lynch, T., Bhatia, K.P., Gasser, T., Lees, A.J., Wood, N.W. and International, L.C. (2008) 'Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study', *Lancet Neurology*, 7(7), pp. 583-90.

Hellstrom-Lindahl, E., Moore, H. and Nordberg, A. (2000) 'Increased levels of tau protein in SH-SY5Y cells after treatment with cholinesterase inhibitors and nicotinic agonists', *Journal of neurochemistry*, 74(2), pp. 777-84.

Helmich, R.C., Baumer, T., Siebner, H.R., Bloem, B.R. and Munchau, A. (2005) 'Hemispheric asymmetry and somatotopy of afferent inhibition in healthy humans', *Experimental Brain Research*, 167(2), pp. 211-9.

Hely, M.A., Morris, J.G., Traficante, R., Reid, W.G., O'Sullivan, D.J. and Williamson, P.M. (1999) 'The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years', *Journal of Neurology Neurosurgery and Psychiatry*, 67(3), pp. 300-7.

Hely, M.A., Morris, J.G.L., Reid, W.G.J. and Trafficante, R. (2005) 'Sydney multicenter study of Parkinson's disease: non-L-dopa-responsive problems dominate at 15 years', *Movement Disorders*, 20(2), pp. 190-199.

Hely, M.A., Reid, W.G., Adena, M.A., Halliday, G.M. and Morris, J.G. (2008) 'The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years', *Movement Disorders*, 23(6), pp. 837-44.

Henchcliffe, C., Dodel, R. and Beal, M.F. (2011) 'Biomarkers of Parkinson's disease and Dementia with Lewy bodies', *Progress in neurobiology*, 95(4), pp. 601-13.

Henry, J.D. and Crawford, J.R. (2004a) 'A meta-analytic review of verbal fluency performance following focal cortical lesions', *Neuropsychology*, 18(2), pp. 284-295.

Henry, J.D. and Crawford, J.R. (2004b) 'Verbal fluency deficits in Parkinson's disease: A meta-analysis', *Journal of the International Neuropsychological Society*, 10(4), pp. 608-622.

Herlofson, K., Lie, S.A., Arsland, D. and Larsen, J.P. (2004) 'Mortality and Parkinson disease - A community based study', *Neurology*, 62(6), pp. 937-942.

Hernan, M.A., Zhang, S.M., Rueda-deCastro, A.M., Colditz, G.A., Speizer, F.E. and Ascherio, A. (2001) 'Cigarette smoking and the incidence of Parkinson's disease in two prospective studies', *Annals of Neurology*, 50(6), pp. 780-6.

Hesse, C., Rosengren, L., Andreasen, N., Davidsson, P., Vanderstichele, H., Vanmechelen, E. and Blennow, K. (2001) 'Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke', *Neuroscience Letters*, 297(3), pp. 187-190.

Himeno, E., Ohyagi, Y., Ma, L., Nakamura, N., Miyoshi, K., Sakae, N., Motomura, K., Soejima, N., Yamasaki, R., Hashimoto, T., Tabira, T., LaFerla, F.M. and Kira, J. (2011) 'Apomorphine treatment in Alzheimer mice promoting amyloid-beta degradation', *Annals of neurology*, 69(2), pp. 248-56.

Hindle, J.V., Petrelli, A., Clare, L. and Kalbe, E. (2013) 'Nonpharmacological Enhancement of Cognitive Function in Parkinson's Disease: A Systematic Review', *Movement Disorders*, DOI: 10.1002/mds.25377.

Hobson, P. and Meara, J. (1999) 'The detection of dementia and cognitive impairment in a community population of elderly people with Parkinson's disease by use of the CAMCOG neuropsychological test', *Age & Ageing*, 28(1), pp. 39-43.

Hobson, P. and Meara, J. (2004) 'Risk and incidence of dementia in a cohort of older subjects with Parkinson's disease in the United Kingdom', *Movement Disorders*, 19(9), pp. 1043-1049.

Hoehn, M.M. and Yahr, M.D. (1967) 'Parkinsonism: onset, progression and mortality', *Neurology*, 17(5), pp. 427-42.

Holmberg, B., Johnels, B., Blennow, K. and Rosengren, L. (2003) 'Cerebrospinal fluid Abeta42 is reduced in multiple system atrophy but normal in Parkinson's disease and progressive supranuclear palsy', *Movement disorders*, 18(2), pp. 186-90.

Holroyd, S., Currie, L. and Wooten, G.F. (2001) 'Prospective study of hallucinations and delusions in Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 70(6), pp. 734-8.

Hong, Z., Shi, M., Chung, K.A., Quinn, J.F., Peskind, E.R., Galasko, D., Jankovic, J., Zabetian, C.P., Leverenz, J.B., Baird, G., Montine, T.J., Hancock, A.M., Hwang, H., Pan, C., Bradner, J., Kang, U.J., Jensen, P.H. and Zhang, J. (2010) 'DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease', *Brain*, 133(Pt 3), pp. 713-26.

Hoops, S., Nazem, S., Siderowf, A.D., Duda, J.E., Xie, S.X., Stern, M.B. and Weintraub, D. (2009) 'Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson disease', *Neurology*, 73(21), pp. 1738-45.

Hosokai, Y., Nishio, Y., Hirayama, K., Takeda, A., Ishioka, T., Sawada, Y., Suzuki, K., Itoyama, Y., Takahashi, S., Fukuda, H. and Mori, E. (2009) 'Distinct patterns of regional cerebral glucose metabolism in Parkinson's disease with and without mild cognitive impairment', *Movement Disorders*, 24(6), pp. 854-62.

Hu, W.T., Chen-Plotkin, A., Arnold, S.E., Grossman, M., Clark, C.M., Shaw, L.M., McCluskey, L., Elman, L., Karlawish, J., Hurtig, H.I., Siderowf, A., Lee, V.M.Y., Soares, H. and Trojanowski, J.Q. (2010) 'Biomarker discovery for Alzheimer's disease, frontotemporal lobar degeneration, and Parkinson's disease', *Acta neuropathologica*, 120(3), pp. 385-99.

Huang, C., Mattis, P., Perrine, K., Brown, N., Dhawan, V. and Eidelberg, D. (2008) 'Metabolic abnormalities associated with mild cognitive impairment in Parkinson disease', *Neurology*, 70(16 Pt 2), pp. 1470-7.

Huang, C., Mattis, P., Tang, C., Perrine, K., Carbon, M. and Eidelberg, D. (2007) 'Metabolic brain networks associated with cognitive function in Parkinson's disease', *Neuroimage*, 34(2), pp. 714-23.

Hughes, A.J., Daniel, S.E., Ben-Shlomo, Y. and Lees, A.J. (2002) 'The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service', *Brain*, 125, pp. 861-870.

Hughes, A.J., Daniel, S.E., Kilford, L. and Lees, A.J. (1992) 'Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases', *Journal of Neurology Neurosurgery and Psychiatry*, 55(3), pp. 181-4.

Hughes, T.A., Ross, H.F., Musa, S., Bhattacherjee, S., Nathan, R.N., Mindham, R.H.S. and Spokes, E.G.S. (2000) 'A 10-year study of the incidence of and factors predicting dementia in Parkinson's disease', *Neurology*, 54(8), pp. 1596-1602.

Hurtig, H.I., Trojanowski, J.Q., Galvin, J., Ewbank, D., Schmidt, M.L., Lee, V.M., Clark, C.M., Glosser, G., Stern, M.B., Gollomp, S.M. and Arnold, S.E. (2000) 'Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease', *Neurology*, 54(10), pp. 1916-21.

Ikeuchi, T., Kakita, A., Shiga, A., Kasuga, K., Kaneko, H., Tan, C.F., Idezuka, J., Wakabayashi, K., Onodera, O., Iwatsubo, T., Nishizawa, M., Takahashi, H. and Ishikawa, A. (2008) 'Patients homozygous and heterozygous for SNCA duplication in a family with parkinsonism and dementia', *Archives of Neurology*, 65(4), pp. 514-519.

Imperato, A., Dazzi, L., Obinu, M.C., Gessa, G.L. and Biggio, G. (1993) 'Inhibition of Hippocampal Acetylcholine-Release by Benzodiazepines - Antagonism by Flumazenil', *European Journal of Pharmacology*, 238(1), pp. 135-137.

Irvine, G.B., El-Agnaf, O.M., Shankar, G.M. and Walsh, D.M. (2008) 'Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's diseases', *Molecular medicine*, 14(7-8), pp. 451-64.

Jack, C.R., Jr., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C. and Trojanowski, J.Q. (2010) 'Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade', *Lancet neurology*, 9(1), pp. 119-28.

Jankovic, J. (2008) 'Are adenosine antagonists, such as istradefylline, caffeine, and chocolate, useful in the treatment of Parkinson's disease?', *Annals of Neurology*, 63(3), pp. 267-9.

Janvin, C.C., Aarsland, D. and Larsen, J.P. (2005) 'Cognitive predictors of dementia in Parkinson's disease: A community-based, 4-year longitudinal study', *Journal of Geriatric Psychiatry and Neurology*, 18(3), pp. 149-154.

Janvin, C.C., Larsen, J.P., Aarsland, D. and Hugdahl, K. (2006) 'Subtypes of mild cognitive impairment in Parkinson's disease: progression to dementia', *Movement disorders*, 21(9), pp. 1343-9.

Jarrett, J.T., Berger, E.P. and Lansbury, P.T., Jr. (1993) 'The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease', *Biochemistry*, 32(18), pp. 4693-7.

Jean, L., Bergeron, M.E., Thivierge, S. and Simard, M. (2010) 'Cognitive Intervention Programs for Individuals With Mild Cognitive Impairment: Systematic Review of the Literature', *American Journal of Geriatric Psychiatry*, 18(4), pp. 281-296.

Jellinger, K. (1988) 'The pedunculopontine nucleus in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 51(4), pp. 540-3.

Jellinger, K.A. (2006) 'The morphological basis of mental dysfunction in Parkinson's disease', *Journal of the neurological sciences*, 248(1-2), pp. 167-72.

Jellinger, K.A. (2010a) 'The neuropathologic substrate of Parkinson disease dementia', *Acta neuropathologica*, 119(1), pp. 151-3.

Jellinger, K.A. (2010b) 'Neuropathology in Parkinson's disease with mild cognitive impairment', *Acta Neuropathologica*, 120(6), pp. 829-830.

Jellinger, K.A. (2012) 'CSF biomarkers in different phenotypes of Parkinson disease', *Journal of Neural Transmission*, 119(4), pp. 455-456.

Jellinger, K.A. and Attems, J. (2008) 'Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease', *Acta neuropathologica*, 115(4), pp. 427-36.

Jenkinson, N., Nandi, D., Muthusamy, K., Ray, N.J., Gregory, R., Stein, J.F. and Aziz, T.Z. (2009) 'Anatomy, physiology, and pathophysiology of the pedunculopontine nucleus', *Movement disorders*, 24(3), pp. 319-28.

Jokinen, P., Scheinin, N., Aalto, S., Nagren, K., Savisto, N., Parkkola, R., Rokka, J., Haaparanta, M., Roytta, M. and Rinne, J.O. (2010) '[C-11]PIB-, [F-18]FDG-PET and MRI imaging in patients with Parkinson's disease with and without dementia', *Parkinsonism & Related Disorders*, 16(10), pp. 666-670.

Kanemaru, K., Kameda, N. and Yamanouchi, H. (2000) 'Decreased CSF amyloid beta 42 and normal tau levels in dementia with Lewy bodies', *Neurology*, 54(9), pp. 1875-1876.

Kar, S., Slowikowski, S.P.M., Westaway, D. and Mount, H.T.J. (2004) 'Interactions between beta-amyloid and central cholinergic neurons: implications for Alzheimer's disease', *Journal of Psychiatry & Neuroscience*, 29(6), pp. 427-441.

Kehagia, A.A., Barker, R.A. and Robbins, T.W. (2010) 'Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease', *Lancet neurology*, 9(12), pp. 1200-13.

Kempster, P.A., O'Sullivan, S.S., Holton, J.L., Revesz, T. and Lees, A.J. (2010) 'Relationships between age and late progression of Parkinson's disease: a clinico-pathological study', *Brain*, 133(Pt 6), pp. 1755-62.

Khoo, T.K., Yarnall, A.J., Duncan, G.W., Coleman, S., O'Brien, J., Brooks, D., Barker, R.A. and Burn, D.J. (2013) 'The spectrum of nonmotor symptoms in early Parkinson disease', *Neurology*, 80, pp. 1-6.

Kim, J.W., Cheon, S.M., Park, M.J., Kim, S.Y. and Jo, H.Y. (2009) 'Cognitive Impairment in Parkinson's Disease without Dementia: Subtypes and Influences of Age', *Journal of Clinical Neurology*, 5(3), pp. 133-138.

Kish, S.J., Rajput, A., Gilbert, J., Rozdilsky, B., Chang, L.J., Shannak, K. and Hornykiewicz, O. (1986) 'Elevated gamma-aminobutyric acid level in striatal but not extrastriatal brain regions in Parkinson's disease: correlation with striatal dopamine loss', *Annals of neurology*, 20(1), pp. 26-31.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) 'Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism', *Nature*, 392(6676), pp. 605-8.

Klassen, B.T., Hentz, J.G., Shill, H.A., Driver-Dunckley, E., Evidente, V.G.H., Sabbagh, M.N., Adler, C.H. and Caviness, J.N. (2011) 'Quantitative EEG as a predictive biomarker for Parkinson disease dementia', *Neurology*, 77(2), pp. 118-124.

Klein, J.C., Eggers, C., Kalbe, E., Weisenbach, S., Hohmann, C., Vollmar, S., Baudrexel, S., Diederich, N.J., Heiss, W.D. and Hilker, R. (2010) 'Neurotransmitter changes in dementia with Lewy bodies and Parkinson disease dementia in vivo', *Neurology*, 74(11), pp. 885-892.

Klepac, N., Trkulja, V., Relja, M. and Babic, T. (2008) 'Is quality of life in non-demented Parkinson's disease patients related to cognitive performance? A clinic-based cross-sectional study', *European Journal of Neurology*, 15(2), pp. 128-133.

Knekt, P., Kilkkinen, A., Rissanen, H., Marniemi, J., Saaksjarvi, K. and Heliovaara, M. (2010) 'Serum vitamin D and the risk of Parkinson disease', *Archives of neurology*, 67(7), pp. 808-11.

Kordower, J.H., Chu, Y.P., Hauser, R.A., Freeman, T.B. and Olanow, C.W. (2008) 'Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease', *Nature Medicine*, 14(5), pp. 504-506.

Kotagal, V., Albin, R.L., Muller, M.L.T.M., Koeppe, R.A., Chervin, R.D., Frey, K.A. and Bohnen, N.I. (2012a) 'Symptoms of rapid eye movement sleep behavior disorder are associated with cholinergic denervation in Parkinson disease', *Annals of neurology*, 71(4), pp. 560-8.

Kotagal, V., Bohnen, N.I., Muller, M.L.T.M., Koeppe, R.A., Frey, K.A. and Albin, R.L. (2012b) 'Cerebral Amyloid Deposition and Serotoninergic Innervation in Parkinson Disease', *Archives of Neurology*, 69(12), pp. 1628-1631.

Koyama, A., Okereke, O.I., Yang, T., Blacker, D., Selkoe, D.J. and Grodstein, F. (2012) 'Plasma amyloid-beta as a predictor of dementia and cognitive decline: a systematic review and meta-analysis', *Archives of neurology*, 69(7), pp. 824-31.

Kramer, M.L. and Schulz-Schaeffer, W.J. (2007) 'Presynaptic alpha-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies', *The Journal of Neuroscience*, 27(6), pp. 1405-10.

Kruse, N., Schulz-Schaeffer, W.J., Schlossmacher, M.G. and Mollenhauer, B. (2012) 'Development of electrochemiluminescence-based singleplex and multiplex assays for the quantification of alpha-synuclein and other proteins in cerebrospinal fluid', *Methods*, 56(4), pp. 514-518.

Lane, R., He, Y.S., Morris, C., Leverenz, J.B., Emre, M. and Ballard, C. (2009) 'BuChE-K and APOE epsilon 4 Allele Frequencies in Lewy Body Dementias, and Influence of Genotype and Hyperhomocysteinemia on Cognitive Decline', *Movement Disorders*, 24(3), pp. 392-400.

Lange, K.W., Robbins, T.W., Marsden, C.D., James, M., Owen, A.M. and Paul, G.M. (1992) 'L-dopa withdrawal in Parkinson's disease selectively impairs cognitive performance in tests sensitive to frontal lobe dysfunction', *Psychopharmacology*, 107(2-3), pp. 394-404.

Langlais, P.J., Thal, L., Hansen, L., Galasko, D., Alford, M. and Masliah, E. (1993) 'Neurotransmitters in basal ganglia and cortex of Alzheimer's disease with and without Lewy bodies', *Neurology*, 43(10), pp. 1927-34.

Lashley, T., Holton, J.L., Gray, E., Kirkham, K., O'Sullivan, S.S., Hilbig, A., Wood, N.W., Lees, A.J. and Revesz, T. (2008) 'Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients', *Acta Neuropathologica*, 115(4), pp. 417-25.

Lashuel, H.A., Hartley, D.M., Balakhaneh, D., Aggarwal, A., Teichberg, S. and Callaway, D.J. (2002) 'New class of inhibitors of amyloid-beta fibril formation. Implications for the mechanism of pathogenesis in Alzheimer's disease', *Journal of Biological Chemistry*, 277(45), pp. 42881-90.

Lees, A.J., Hardy, J. and Revesz, T. (2009) 'Parkinson's disease', *Lancet*, 373(9680), pp. 2055-2066.

Leroi, I., McDonald, K., Pantula, H. and Harbishettar, V. (2012) 'Cognitive impairment in Parkinson disease: impact on quality of life, disability, and caregiver burden', *Journal of geriatric psychiatry and neurology*, 25(4), pp. 208-14.

Leroi, I., Overshott, R., Byrne, E.J., Daniel, E. and Burns, A. (2009) 'Randomized Controlled Trial of Memantine in Dementia Associated with Parkinson's Disease', *Movement Disorders*, 24(8), pp. 1217-1221.

Leverenz, J.B., Watson, G.S., Shofer, J., Zabetian, C.P., Zhang, J. and Montine, T.J. (2011) 'Cerebrospinal fluid biomarkers and cognitive performance in non-demented patients with Parkinson's disease', *Parkinsonism & Related Disorders*, 17(1), pp. 61-4.

Levy, G., Jacobs, D.M., Tang, M.X., Cote, L.J., Louis, E.D., Alfaro, B., Mejia, H., Stern, Y. and Marder, K. (2002a) 'Memory and executive function impairment predict dementia in Parkinson's disease', *Movement Disorders*, 17(6), pp. 1221-1226.

Levy, G., Schupf, N., Tang, M.X., Cote, L.J., Louis, E.D., Mejia, H., Stern, Y. and Marder, K. (2002b) 'Combined effect of age and severity on the risk of dementia in Parkinson's disease', *Annals of Neurology*, 51(6), pp. 722-729.

Levy, G., Tang, M.X., Louis, E.D., Cote, L.J., Alfaro, B., Mejia, H., Stern, Y. and Marder, K. (2002c) 'The association of incident dementia with mortality in PD', *Neurology*, 59(11), pp. 1708-13.

Lewis, S.J., Cools, R., Robbins, T.W., Dove, A., Barker, R.A. and Owen, A.M. (2003) 'Using executive heterogeneity to explore the nature of working memory deficits in Parkinson's disease', *Neuropsychologia*, 41(6), pp. 645-54.

Lewis, S.J.G., Foltynie, T., Blackwell, A.D., Robbins, T.W., Owen, A.M. and Barker, R.A. (2005) 'Heterogeneity of Parkinson's disease in the early clinical stages using a data driven approach', *Journal of Neurology Neurosurgery and Psychiatry*, 76(3), pp. 343-348.

Li, J.Y., Englund, E., Holton, J.L., Soulet, D., Hagell, P., Lees, A.J., Lashley, T., Quinn, N.P., Rehncrona, S., Bjorklund, A., Widner, H., Revesz, T., Lindvall, O. and Brundin, P. (2008) 'Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation', *Nature Medicine*, 14(5), pp. 501-503.

Lins, H., Wichart, I., Bancher, C., Wallesch, C.W., Jellinger, K.A. and Rosler, N. (2004) 'Immunoreactivities of amyloid beta peptide((1-42)) and total tau protein in lumbar cerebrospinal fluid of patients with normal pressure hydrocephalus', *Journal of Neural Transmission*, 111(3), pp. 273-280.

Lippa, C.F., Duda, J.E., Grossman, M., Hurtig, H.I., Aarsland, D., Boeve, B.F., Brooks, D.J., Dickson, D.W., Dubois, B., Emre, M., Fahn, S., Farmer, J.M., Galasko, D., Galvin, J.E., Goetz, C.G., Growdon, J.H., Gwinn-Hardy, K.A., Hardy, J., Heutink, P., Iwatsubo, T., Kosaka, K., Lee, V.M., Leverenz, J.B., Masliah, E., McKeith, I.G., Nussbaum, R.L., Olanow, C.W., Ravina, B.M., Singleton, A.B., Tanner, C.M., Trojanowski, J.Q., Wszolek, Z.K. and Group, D.P.W. (2007) 'DLB and PDD boundary issues: diagnosis, treatment, molecular pathology, and biomarkers', *Neurology*, 68(11), pp. 812-9.

Liskowsky, W. and Schliebs, R. (2006) 'Muscarinic acetylcholine receptor inhibition in transgenic Alzheimer-like Tg2576 mice by scopolamine favours the amyloidogenic route of processing of amyloid precursor protein', *International Journal of Developmental Neuroscience*, 24(2-3), pp. 149-56.

Litvan, I., Aarsland, D., Adler, C.H., Goldman, J.G., Kulisevsky, J., Mollenhauer, B., Rodriguez-Oroz, M.C., Troster, A.I. and Weintraub, D. (2011) 'MDS Task Force on Mild Cognitive Impairment in Parkinson's Disease: Critical Review of PD-MCI', *Movement Disorders*, 26(10), pp. 1814-1824.

Litvan, I., Bhatia, K.P., Burn, D.J., Goetz, C.G., Lang, A.E., McKeith, I., Quinn, N., Sethi, K.D., Shults, C. and Wenning, G.K. (2003) 'SIC Task Force appraisal of clinical diagnostic criteria for Parkinsonian disorders', *Movement Disorders*, 18(5), pp. 467-486.

Litvan, I., Goldman, J.G., Troster, A.I., Schmand, B.A., Weintraub, D., Petersen, R.C., Mollenhauer, B., Adler, C.H., Marder, K., Williams-Gray, C.H., Aarsland, D., Kulisevsky, J., Rodriguez-Oroz, M.C., Burn, D.J., Barker, R.A. and Emre, M. (2012) 'Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines', *Movement Disorders*, 27(3), pp. 349-56.

Liu, M., Choi, D.-Y., Hunter, R.L., Pandya, J.D., Cass, W.A., Sullivan, P.G., Kim, H.-C., Gash, D.M. and Bing, G. (2010) 'Trichloroethylene induces dopaminergic neurodegeneration in Fisher 344 rats', *Journal of neurochemistry*, 112(3), pp. 773-83.

Maetzler, W., Liepelt, I., Reimold, M., Reischl, G., Solbach, C., Becker, C., Schulte, C., Leyhe, T., Keller, S., Melms, A., Gasser, T. and Berg, D. (2009) 'Cortical PIB binding in Lewy body disease is associated with Alzheimer-like characteristics', *Neurobiology of disease*, 34(1), pp. 107-12.

Mamikonyan, E., Moberg, P.J., Siderowf, A., Duda, J.E., Have, T.T., Hurtig, H.I., Stern, M.B. and Weintraub, D. (2009) 'Mild cognitive impairment is common in Parkinson's disease patients with normal Mini-Mental State Examination (MMSE) scores', *Parkinsonism & Related Disorders*, 15(3), pp. 226-31.

Manganelli, F., Ragno, M., Cacchio, G., Iodice, V., Trojano, L., Silvaggio, F., Scarcella, M., Grazioli, M., Santoro, L. and Perretti, A. (2008) 'Motor cortex cholinergic dysfunction in CADASIL: a transcranial magnetic demonstration', *Clinical Neurophysiology*, 119(2), pp. 351-5.

Manganelli, F., Vitale, C., Santangelo, G., Pisciotta, C., Iodice, R., Cozzolino, A., Dubbioso, R., Picillo, M., Barone, P. and Santoro, L. (2009) 'Functional involvement of central cholinergic circuits and visual hallucinations in Parkinsons disease', *Brain*, 132, pp. 2350-2355.

Marder, K., Tang, M.X., Cote, L., Stern, Y. and Mayeux, R. (1995) 'The Frequency and Associated Risk-Factors for Dementia in Patients with Parkinsons-Disease', *Archives of Neurology*, 52(7), pp. 695-701.

Marra, C., Quaranta, D., Profice, P., Pilato, F., Capone, F., Iodice, F., Di Lazzaro, V. and Gainotti, G. (2011) 'Central cholinergic dysfunction measured "in vivo" correlates with different behavioural disorders in Alzheimer's disease and dementia with Lewy body', *Brain Stimulation*, doi:10.1016/j.brs.2011.08.009.

Marras, C., Armstrong, M.J., Meaney, C.A., Fox, S.H., Rothberg, B., Reginold, W., Tang-Wai, D.F., Gill, D., Eslinger, P.J., Zadikoff, C., Kennedy, N., Marshall, F.J., Mapstone, M., Chou, K.L., Persad, C., Litvan, I., Mast, B.T., Gerstenecker, A.T., Weintraub, S. and Duff-Canning, S. (2013) 'Measuring Mild Cognitive Impairment in Patients With Parkinson's Disease', *Movement Disorders*, DOI: 10.1002/mds.25426.

Marras, C., McDermott, M.P., Rochon, P.A., Tanner, C.M., Naglie, G., Rudolph, A., Lang, A.E. and Grp, P.S. (2005) 'Survival in Parkinson disease - Thirteen-year follow-up of the DATATOP cohort', *Neurology*, 64(1), pp. 87-93.

Martinez-Martin, P., Rodriguez-Blazquez, C., Kurtis, M.M., Chaudhuri, K.R. and Grp, N.V. (2011) 'The Impact of Non-Motor Symptoms on Health-Related Quality of Life of Patients with Parkinson's Disease', *Movement Disorders*, 26(3), pp. 399-406.

Martorana, A., Di Lorenzo, F., Esposito, Z., Lo Giudice, T., Bernardi, G., Caltagirone, C. and Koch, G. (2013) 'Dopamine D-2-agonist Rotigotine effects on cortical excitability and central cholinergic transmission in Alzheimer's disease patients', *Neuropharmacology*, 64, pp. 108-113.

Martorana, A., Esposito, Z., Di Lorenzo, F., Giacobbe, V., Sancesario, G.M., Bucchi, G., Bonni, S., Bernardini, S., Sorge, R., Sancesario, G., Bernardi, G., Caltagirone, C. and Koch, G. (2012) 'Cerebrospinal fluid levels of Abeta42 relationship with cholinergic cortical activity in Alzheimer's disease patients', *Journal of neural transmission*, 119(7), pp. 771-8.

Martorana, A., Mori, F., Esposito, Z., Kusayanagi, H., Monteleone, F., Codeca, C., Sancesario, G., Bernardi, G. and Koch, G. (2009) 'Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients', *Neuropsychopharmacology*, 34(10), pp. 2323-8.

Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M. and Mucke, L. (2001) 'beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease', *Proceedings of the National Academy of Sciences of the United States of America*, 98(21), pp. 12245-50.

Mattila, P.M., Rinne, J.O., Helenius, H., Dickson, D.W. and Roytta, M. (2000) 'Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease', *Acta neuropathologica*, 100(3), pp. 285-90.

Mattsson, N., Zetterberg, H., Hansson, O., Andreasen, N., Parnetti, L., Jonsson, M., Herukka, S.-K., van der Flier, W.M., Blankenstein, M.A., Ewers, M., Rich, K., Kaiser, E., Verbeek, M., Tsolaki, M., Mulugeta, E., Rosen, E., Aarsland, D., Visser, P.J., Schroder, J., Marcusson, J., de Leon, M., Hampel, H., Scheltens, P., Pirttila, T., Wallin, A., Jonhagen, M.E., Minthon, L., Winblad, B. and Blennow, K. (2009) 'CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment', *JAMA : the journal of the American Medical Association*, 302(4), pp. 385-93.

McColgan, P., Evans, J.R., Breen, D.P., Mason, S.L., Barker, R.A. and Williams-Gray, C.H. (2012) 'Addenbrooke's Cognitive Examination-Revised for Mild Cognitive Impairment in Parkinson's Disease', *Movement Disorders*, 27(9), pp. 1172-1176.

McKeith, I.G., Dickson, D.W., Lowe, J., Emre, M., O'Brien, J.T., Feldman, H., Cummings, J., Duda, J.E., Lippa, C., Perry, E.K., Aarsland, D., Arai, H., Ballard, C.G., Boeve, B., Burn, D.J., Costa, D., Del Ser, T., Dubois, B., Galasko, D., Gauthier, S., Goetz, C.G., Gomez-Tortosa, E., Halliday, G., Hansen, L.A., Hardy, J., Iwatsubo, T., Kalaria, R.N., Kaufer, D., Kenny, R.A., Korczyn, A., Kosaka, K., Lee, V.M.Y., Lees, A., Litvan, I., Londos, E., Lopez, O.L., Minoshima, S., Mizuno, Y., Molina, J.A., Mukaetova-Ladinska, E.B., Pasquier, F., Perry, R.H., Schulz, J.B., Trojanowski, J.Q. and Yamada, M. (2005) 'Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium', *Neurology*, 65(12), pp. 1863-72.

Melzer, T.R., Watts, R., MacAskill, M.R., Pitcher, T.L., Livingston, L., Keenan, R.J., Dalrymple-Alford, J.C. and Anderson, T.J. (2012) 'Grey matter atrophy in cognitively impaired Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 83(2), pp. 188-94.

Meng, X.F. and D'Arcy, C. (2012) 'Education and Dementia in the Context of the Cognitive Reserve Hypothesis: A Systematic Review with Meta-Analyses and Qualitative Analyses', *Plos One*, 7(6).

Merims, D., Balas, M., Peretz, C., Shabtai, H. and Giladi, N. (2006) 'Rater-blinded, prospective comparison: Quetiapine versus clozapine for Parkinson's disease psychosis', *Clinical Neuropharmacology*, 29(6), pp. 331-337.

Meyer, P.M., Strecker, K., Kendziorra, K., Becker, G., Hesse, S., Woelpl, D., Hensel, A., Patt, M., Sorger, D., Wegner, F., Lobsien, D., Barthel, H., Brust, P., Gertz, H.J., Sabri, O. and Schwarz, J. (2009) 'Reduced alpha 4 beta 2*-Nicotinic Acetylcholine Receptor Binding and Its Relationship to Mild Cognitive and Depressive Symptoms in Parkinson Disease', *Archives of General Psychiatry*, 66(8), pp. 866-877.

Milber, J.M., Noorigian, J.V., Morley, J.F., Petrovitch, H., White, L., Ross, G.W. and Duda, J.E. (2012) 'Lewy pathology is not the first sign of degeneration in vulnerable neurons in Parkinson disease', *Neurology*, 79(24), pp. 2307-2314.

Miller, E. (1985) 'Possible Frontal Impairments in Parkinsons-Disease - a Test Using a Measure of Verbal Fluency', *British Journal of Clinical Psychology*, 24(Sep), pp. 211-212.

Moghekar, A., Goh, J., Li, M., Albert, M. and O'Brien, R.J. (2012) 'Cerebrospinal fluid Abeta and tau level fluctuation in an older clinical cohort', *Archives of neurology*, 69(2), pp. 246-50.

Molina, J.A., Benito-Leon, J., Jimenez-Jimenez, F.J., Orti-Pareja, M., Berbel, A., Tallon-Barranco, A., de Bustos, F. and Hernanz, A. (1997) 'Tau protein concentrations in cerebrospinal fluid of non-demented Parkinson's disease patients', *Neuroscience Letters*, 238(3), pp. 139-141.

Mollenhauer, B., Bibl, M., Wiltfang, J., Steinacker, P., Ciesielczyk, B., Neubert, K., Trenkwalder, C. and Otto, M. (2006a) 'Total tau protein, phosphorylated tau (181p) protein, beta-amyloid(1-42), and beta-amyloid(1-40) in cerebrospinal fluid of patients with dementia with Lewy bodies', *Clinical Chemistry and Laboratory Medicine*, 44(2), pp. 192-195.

Mollenhauer, B., Cepek, L., Bibl, M., Wiltfang, J., Schulz-Schaeffer, W.J., Ciesielczyk, B., Neumann, M., Steinacker, P., Kretzschmar, H.A., Poser, S., Trenkwalderf, C. and Otto, M. (2005) 'Tau protein, A beta 42 and S-100B protein in cerebrospinal fluid of patients with dementia with Lewy bodies', *Dementia and Geriatric Cognitive Disorders*, 19(2-3), pp. 164-170.

Mollenhauer, B., Cullen, V., Kahn, I., Krastins, B., Outeiro, T.F., Pepivani, I., Ng, J., Schulz-Schaeffer, W., Kretzschmar, H.A., McLean, P.J., Trenkwalder, C., Sarracino, D.A., Vonsattel, J.-P., Locascio, J.J., El-Agnaf, O.M.A. and Schlossmacher, M.G. (2008) 'Direct quantification of CSF alpha-synuclein by ELISA and first cross-sectional study in patients with neurodegeneration', *Experimental neurology*, 213(2), pp. 315-25.

Mollenhauer, B., Esselmann, H., Roeber, S., Schulz-Schaeffer, W.J., Trenkwalder, C., Bibl, M., Steinacker, P., Kretzschmar, H.A., Wiltfang, J. and Otto, M. (2011a) 'Different CSF beta-amyloid processing in Alzheimer's and Creutzfeldt-Jakob disease', *Journal of neural transmission*, 118(5), pp. 691-7.

Mollenhauer, B., Locascio, J.J., Schulz-Schaeffer, W., Sixel-Doring, F., Trenkwalder, C. and Schlossmacher, M.G. (2011b) 'alpha-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study', *Lancet neurology*, 10(3), pp. 230-40.

Mollenhauer, B., Trautmann, E., Taylor, P., Manninger, P., Sixel-Doring, F., Ebentheuer, J., Trenkwalder, C. and Schlossmacher, M.G. (2013) 'Total CSF alpha-synuclein is lower in de novo Parkinson patients than in healthy subjects', *Neuroscience Letters*, 532, pp. 44-48.

Mollenhauer, B. and Trenkwalder, C. (2009) 'Neurochemical Biomarkers in the Differential Diagnosis of Movement Disorders', *Movement Disorders*, 24(10), pp. 1411-1426.

Mollenhauer, B., Trenkwalder, C., von Ahsen, N., Bibl, M., Steinacker, P., Brechlin, P., Schindehuette, J., Poser, S., Wiltfang, J. and Otto, M. (2006b) 'Beta-amlyoid 1-42 and tau-protein in cerebrospinal fluid of patients with Parkinson's disease dementia', *Dementia and Geriatric Cognitive Disorders*, 22(3), pp. 200-208.

Molloy, S., McKeith, I.G., O'Brien, J.T. and Burn, D.J. (2005) 'The role of levodopa in the management of dementia with Lewy bodies', *Journal of Neurology Neurosurgery and Psychiatry*, 76(9), pp. 1200-1203.

Montine, T.J., Shi, M., Quinn, J.F., Peskind, E.R., Craft, S., Ginghina, C., Chung, K.A., Kim, H., Galasko, D.R., Jankovic, J., Zabetian, C.P., Leverenz, J.B. and Zhang, J. (2010) 'CSF Abeta(42) and tau in Parkinson's disease with cognitive impairment', *Movement Disorders*, 25(15), pp. 2682-5.

Morgante, L., Epifanio, A., Spina, E., Zappia, M., Di Rosa, A.E., Marconi, R., Basile, G., Di Raimondo, G., La Spina, P. and Quattrone, A. (2004) 'Quetiapine and clozapine in parkinsonian patients with dopaminergic psychosis', *Clinical Neuropharmacology*, 27(4), pp. 153-156.

Morley, J.F. and Duda, J.E. (2012) 'Head injury and the risk of Parkinson's disease', *Movement disorders*, 27(13), pp. 1592-4.

Morley, J.F., Xie, S.X., Hurtig, H.I., Stern, M.B., Colcher, A., Horn, S., Dahodwala, N., Duda, J.E., Weintraub, D., Chen-Plotkin, A.S., Van Deerlin, V., Falcone, D. and Siderowf, A. (2012) 'Genetic influences on cognitive decline in Parkinson's disease', *Movement disorders : official journal of the Movement Disorder Society*, 27(4), pp. 512-8.

Morris, J.C., Roe, C.M., Xiong, C., Fagan, A.M., Goate, A.M., Holtzman, D.M. and Mintun, M.A. (2010) 'APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging', *Annals of neurology*, 67(1), pp. 122-31.

Mosimann, U.P., Mather, G., Wesnes, K.A., O'Brien, J.T., Burn, D.J. and McKeith, I.G. (2004) 'Visual perception in Parkinson disease dementia and dementia with Lewy bodies', *Neurology*, 63(11), pp. 2091-2096.

Mosimann, U.P., Rowan, E.N., Partington, C.E., Collerton, D., Littlewood, E., O'Brien, J.T., Burn, D.J. and McKeith, I.G. (2006) 'Characteristics of visual hallucinations in Parkinson disease dementia and dementia with Lewy bodies', *American Journal of Geriatric Psychiatry*, 14(2), pp. 153-160.

Muller-Dahlhaus, J.F.M., Liu, Y. and Ziemann, U. (2008) 'Inhibitory circuits and the nature of their interactions in the human motor cortex - a pharmacological TMS study', *Journal of Physiology*, 586(2), pp. 495-514.

Mulsant, B.H., Pollock, B.G., Kirshner, M., Shen, C., Dodge, H. and Ganguli, M. (2003) 'Serum anticholinergic activity in a community-based sample of older adults: relationship with cognitive performance', *Archives of General Psychiatry*, 60(2), pp. 198-203.

Mulugeta, E., Blennow, K., Zetterberg, H., Ballard, C., Minthon, L., Londos, E., Hansson, O. and Aarsland, D. (2011a) 'CSF levels of sAPP-alpha and sApp-beta levels in dementia with Lewy body and Parkinson's disease dementia', *Movement Disorders*, 26, pp. S321-S321.

Mulugeta, E., Londos, E., Ballard, C., Alves, G., Zetterberg, H., Blennow, K., Skogseth, R., Minthon, L. and Aarsland, D. (2011b) 'CSF amyloid beta 38 as a novel diagnostic marker for dementia with Lewy bodies', *Journal of Neurology Neurosurgery and Psychiatry*, 82(2), pp. 160-164.

Mura, E., Lanni, C., Preda, S., Pistoia, F., Sara, M., Racchi, M., Schettini, G., Marchi, M. and Govoni, S. (2010a) 'Beta-amyloid: a disease target or a synaptic regulator affecting age-related neurotransmitter changes?', *Current Pharmaceutical Design*, 16(6), pp. 672-83.

Mura, E., Preda, S., Govoni, S., Lanni, C., Trabace, L., Grilli, M., Lagomarsino, F., Pittaluga, A. and Marchi, M. (2010b) 'Specific neuromodulatory actions of amyloid-beta on dopamine release in rat nucleus accumbens and caudate putamen', *Journal of Alzheimer's Disease*, 19(3), pp. 1041-53.

Mura, E., Zappettini, S., Preda, S., Biundo, F., Lanni, C., Grilli, M., Cavallero, A., Olivero, G., Salamone, A., Govoni, S. and Marchi, M. (2012) 'Dual effect of beta-amyloid on 7 and 42 nicotinic receptors controlling the release of glutamate, aspartate and GABA in rat hippocampus', *PLoS ONE [Electronic Resource]*, 7(1), p. e29661.

Muslimovic, D., Post, B., Speelman, J.D. and Schmand, B. (2005) 'Cognitive profile of patients with newly diagnosed Parkinson disease', *Neurology*, 65(8), pp. 1239-45.

Naismith, S.L., Mowszowski, L., Diamond, K. and Lewis, S.J.G. (2013) 'Memory in Parkinson’s Disease: A Healthy Brain Ageing Cognitive Training Program', *Movement Disorders*, DOI: 10.1002/mds.25457.

Nakano, I. and Hirano, A. (1984) 'Parkinson's disease: neuron loss in the nucleus basalis without concomitant Alzheimer's disease', *Annals of neurology*, 15(5), pp. 415-8.

Nardone, R., Bergmann, J., Brigo, F., Christova, M., Kunz, A., Seidl, M., Tezzon, F., Trinka, E. and Golaszewski, S. (2012a) 'Functional evaluation of central cholinergic circuits in patients with Parkinson's disease and REM sleep behavior disorder: a TMS study', *Journal of Neural Transmission*.

Nardone, R., Bergmann, J., Christova, M., Caleri, F., Tezzon, F., Ladurner, G., Trinka, E. and Golaszewski, S. (2012b) 'Short latency afferent inhibition differs among the subtypes of mild cognitive impairment', *Journal of neural transmission*, 119(4), pp. 463-71.

Nardone, R., Bergmann, J., Kronbichler, M., Kunz, A., Klein, S., Caleri, F., Tezzon, F., Ladurner, G. and Golaszewski, S. (2008a) 'Abnormal short latency afferent inhibition in early Alzheimer's disease: a transcranial magnetic demonstration', *Journal of Neural Transmission*, 115(11), pp. 1557-62.

Nardone, R., Bergmann, J., Kunz, A., Christova, M., Brigo, F., Tezzon, F., Trinka, E. and Golaszewski, S. (2012c) 'Cortical afferent inhibition is reduced in patients with idiopathic REM sleep behavior disorder and cognitive impairment: a TMS study', *Sleep medicine*, 13(7), pp. 919-25.

Nardone, R., Bergmann, J., Tezzon, F., Ladurner, G. and Golaszewski, S. (2008b) 'Cholinergic dysfunction in subcortical ischaemic vascular dementia: a transcranial magnetic stimulation study', *Journal of neural transmission*, 115(5), pp. 737-43.

Nardone, R., Bratti, A. and Tezzon, F. (2006) 'Motor cortex inhibitory circuits in dementia with Lewy bodies and in Alzheimer's disease', *Journal of neural transmission*, 113(11), pp. 1679-84.

Nardone, R., Florio, I., Lochner, P. and Tezzon, F. (2005) 'Cholinergic cortical circuits in Parkinson's disease and in progressive supranuclear palsy: a transcranial magnetic stimulation study', *Experimental brain research*, 163(1), pp. 128-31.

Nasreddine, Z.S., Phillips, N.A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L. and Chertkow, H. (2005) 'The Montreal Cognitive Assessment, MoCA: a

brief screening tool for mild cognitive impairment', *Journal of the American Geriatrics Society*, 53(4), pp. 695-9.

Nazem, S., Siderowf, A.D., Duda, J.E., Have, T.T., Colcher, A., Horn, S.S., Moberg, P.J., Wilkinson, J.R., Hurtig, H.I., Stern, M.B. and Weintraub, D. (2009) 'Montreal cognitive assessment performance in patients with Parkinson's disease with "normal" global cognition according to mini-mental state examination score', *Journal of the American Geriatrics Society*, 57(2), pp. 304-8.

Nebes, R.D., Pollock, B.G., Halligan, E.M., Kirshner, M.A. and Houck, P.R. (2007) 'Serum anticholinergic activity and motor performance in elderly persons', *The journals of gerontology*, 62(1), pp. 83-5.

Nelson, H.E. and O'Connell, A. (1978) 'Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test', *Cortex*, 14(2), pp. 234-44.

Neumann, J., Bras, J., Deas, E., O'Sullivan, S.S., Parkkinen, L., Lachmann, R.H., Li, A., Holton, J., Guerreiro, R., Paudel, R., Segarane, B., Singleton, A., Lees, A., Hardy, J., Houlden, H., Revesz, T. and Wood, N.W. (2009) 'Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease', *Brain*, 132, pp. 1783-1794.

Newmark, H.L. and Newmark, J. (2007) 'Vitamin D and Parkinson's disease--a hypothesis', *Movement disorders*, 22(4), pp. 461-8.

Ni, Z., Muller-Dahlhaus, F., Chen, R. and Ziemann, U. (2011) 'Triple-pulse TMS to study interactions between neural circuits in human cortex', *Brain Stimulation*, 4(4), pp. 281-293.

Nobili, F., Abbruzzese, G., Morbelli, S., Marchese, R., Girtler, N., Dessi, B., Brugnolo, A., Canepa, C., Drosos, G.C., Sambuceti, G. and Rodriguez, G. (2009) 'Amnestic mild cognitive impairment in Parkinson's disease: a brain perfusion SPECT study', *Movement disorders : official journal of the Movement Disorder Society*, 24(3), pp. 414-21.

Noe, E., Marder, K., Bell, K.L., Jacobs, D.M., Manly, J.J. and Stern, Y. (2004) 'Comparison of dementia with Lewy bodies to Alzheimer's disease and Parkinson's disease with dementia', *Movement Disorders*, 19(1), pp. 60-67.

Nussbaum RL, E.C. (2003) 'Alzheimer's disease and Parkinson's disease', *New England Journal of Medicine*, 348, pp. 1356–64.

Ohrfelt, A., Grognet, P., Andreasen, N., Wallin, A., Vanmechelen, E., Blennow, K. and Zetterberg, H. (2009) 'Cerebrospinal fluid alpha-synuclein in neurodegenerative disorders-a marker of synapse loss?', *Neuroscience letters*, 450(3), pp. 332-5.

Olanow, C.W. (2012) 'A colonic biomarker of Parkinson's disease?', *Movement Disorders*, 27(6), pp. 674-676.

Olanow, C.W. and McNaught, K. (2011) 'Parkinson's disease, proteins, and prions: milestones', *Movement Disorders*, 26(6), pp. 1056-71.

Olanow, C.W., Perl, D.P., DeMartino, G.N. and McNaught, K.S. (2004) 'Lewy-body formation is an aggresome-related process: a hypothesis', *Lancet Neurology*, 3(8), pp. 496-503.

Oliviero, A., Profice, P., Tonali, P.A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F. and Di Lazzaro, V. (2006) 'Effects of aging on motor cortex excitability', *Neuroscience research*, 55(1), pp. 74-7.

Otto, M., Wiltfang, J., Cepek, L., Neumann, M., Mollenhauer, B., Steinacker, P., Ciesielczyk, B., Schulz-Schaeffer, W., Kretzschmar, H.A. and Poser, S. (2002) 'Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease', *Neurology*, 58(2), pp. 192-7.

Owen, A.M., Beksinska, M., James, M., Leigh, P.N., Summers, B.A., Marsden, C.D., Quinn, N.P., Sahakian, B.J. and Robbins, T.W. (1993) 'Visuospatial memory deficits at different stages of Parkinson's disease', *Neuropsychologia*, 31(7), pp. 627-44.

Owen, A.M., Sahakian, B.J., Hodges, J.R., Summers, B.A., Polkey, C.E. and Robbins, T.W. (1995a) 'Dopamine-Dependent Frontostriatal Planning Deficits in Early Parkinsons-Disease', *Neuropsychology*, 9(1), pp. 126-140.

Owen, A.M., Sahakian, B.J., Semple, J., Polkey, C.E. and Robbins, T.W. (1995b) 'Visuo-spatial short-term recognition memory and learning after temporal lobe excisions, frontal lobe excisions or amygdalo-hippocampectomy in man', *Neuropsychologia*, 33(1), pp. 1-24.

Pai, M.C. and Chan, S.H. (2001) 'Education and cognitive decline in Parkinson's disease: a study of 102 patients', *Acta Neurologica Scandinavica*, 103(4), pp. 243-247.

Paleologou, K.E., Kragh, C.L., Mann, D.M.A., Salem, S.A., Al-Shami, R., Allsop, D., Hassan, A.H., Jensen, P.H. and El-Agnaf, O.M.A. (2009) 'Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies', *Brain*, 132(Pt 4), pp. 1093-101.

Palomar, F.J., Suarez, A., Franco, E., Carrillo, F., Gil-Neciga, E. and Mir, P. (2013) 'Abnormal sensorimotor plasticity in CADASIL correlates with neuropsychological impairment', *Journal of neurology, neurosurgery, and psychiatry*, 84(3), pp. 329-36.

Pan, S., Shi, M., Jin, J., Albin, R.L., Lieberman, A., Gearing, M., Lin, B., Pan, C., Yan, X., Kashima, D.T. and Zhang, J. (2007) 'Proteomics identification of proteins in human cortex using multidimensional separations and MALDI tandem mass spectrometer', *Molecular & cellular proteomics : MCP*, 6(10), pp. 1818-23.

Pappata, S., Santangelo, G., Aarsland, D., Vicidomini, C., Longo, K., Bronnick, K., Amboni, M., Erro, R., Vitale, C., Caprio, M.G., Pellecchia, M.T., Brunetti, A., De Michele, G., Salvatore, M. and Barone, P. (2011) 'Mild cognitive impairment in drug-naive patients with PD is associated with cerebral hypometabolism', *Neurology*, 77(14), pp. 1357-62.

Paris, A.P., Saleta, H.G., Maraver, M.D.C., Silvestre, E., Freixa, M.G., Torrellas, C.P., Pont, S.A., Nadal, M.F., Garcia, S.A., Bartolome, M.V.P., Fernandez, V.L. and Rusinol, A.B.

(2011) 'Blind Randomized Controlled Study of the Efficacy of Cognitive Training in Parkinson's Disease', *Movement Disorders*, 26(7), pp. 1251-1258.

Parkinson, J. (1817) *Essay on the Shaking Palsy*. London: Sherwood, Neely and Jones.

Parkkinen, L., Pirttila, T. and Alafuzoff, I. (2008) 'Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance', *Acta Neuropathologica*, 115(4), pp. 399-407.

Parnetti, L., Chiasserini, D., Bellomo, G., Giannandrea, D., De Carlo, C., Qureshi, M.M., Ardah, M.T., Varghese, S., Bonanni, L., Borroni, B., Tambasco, N., Eusebi, P., Rossi, A., Onofrj, M., Padovani, A., Calabresi, P. and El-Agnaf, O. (2011) 'Cerebrospinal fluid Tau/alpha-synuclein ratio in Parkinson's disease and degenerative dementias', *Movement disorders*, 26(8), pp. 1428-35.

Parnetti, L., Tiraboschi, P., Lanari, A., Peducci, M., Padiglioni, C., D'Amore, C., Pierguidi, L., Tambasco, N., Rossi, A. and Calabresi, P. (2008) 'Cerebrospinal fluid biomarkers in Parkinson's disease with dementia and dementia with Lewy bodies', *Biological Psychiatry*, 64(10), pp. 850-5.

Paulus, W., Classen, J., Cohen, L.G., Large, C.H., Di Lazzaro, V., Nitsche, M., Pascual-Leone, A., Rosenow, F., Rothwell, J.C. and Ziemann, U. (2008) 'State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation', *Brain Stimulation*, 1(3), pp. 151-163.

Payami, H., Larsen, K., Bernard, S. and Nutt, J. (1994) 'Increased risk of Parkinson's disease in parents and siblings of patients', *Annals of Neurology*, 36(4), pp. 659-61.

Pedersen, K.F., Larsen, J.P., Tysnes, O.B. and Alves, G. (2013) 'Prognosis of Mild Cognitive Impairment in Early Parkinson Disease. The Norwegian ParkWest Study', *JAMA Neurology*, doi:10.1001/jamaneurol.2013.2110.

Pellecchia, M.T., Santangelo, G., Picillo, M., Pivonello, R., Longo, K., Pivonello, C., Vitale, C., Amboni, M., De Rosa, A., Moccia, M., Erro, R., De Michele, G., Santoro, L., Colao, A.

and Barone, P. (2013) 'Serum epidermal growth factor predicts cognitive functions in early, drug-naive Parkinson's disease patients', *Journal of neurology*, 260(2), pp. 438-44.

Perneger, T.V. (1998) 'What's wrong with Bonferroni adjustments', *BMJ (Clinical research ed)*, 316(7139), pp. 1236-8.

Perry, E.K., Kilford, L., Lees, A.J., Burn, D.J. and Perry, R.H. (2003) 'Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs', *Annals of neurology*, 54(2), pp. 235-238.

Petersen, R.C. (2004) 'Mild cognitive impairment as a diagnostic entity', *Journal of Internal Medicine*, 256(3), pp. 183-194.

Petersen, R.C., Roberts, R.O., Knopman, D.S., Boeve, B.F., Geda, Y.E., Ivnik, R.J., Smith, G.E. and Jack, C.R., Jr. (2009) 'Mild cognitive impairment: ten years later', *Archives of Neurology*, 66(12), pp. 1447-55.

Peto, V., Jenkinson, C., Fitzpatrick, R. and Greenhall, R. (1995) 'The development and validation of a short measure of functioning and well being for individuals with Parkinson's disease', *Quality of Life Research*, 4(3), pp. 241-8.

Petrou, M., Bohnen, N.I., Muller, M.L.T.M., Koeppe, R.A., Albin, R.L. and Frey, K.A. (2012) 'A beta-amyloid deposition in patients with Parkinson disease at risk for development of dementia', *Neurology*, 79(11), pp. 1161-1167.

Pickart, C.M. (2001) 'Mechanisms underlying ubiquitination', *Annual Review of Biochemistry*, 70, pp. 503-33.

Pihlajamaki, M., Tanila, H., Hanninen, T., Kononen, M., Laakso, M., Partanen, K., Soininen, H. and Aronen, H.J. (2000) 'Verbal fluency activates the left medial temporal lobe: A functional magnetic resonance imaging study', *Annals of Neurology*, 47(4), pp. 470-476.

Pletnikova, O., West, N., Lee, M.K., Rudow, G.L., Skolasky, R.L., Dawson, T.M., Marsh, L. and Troncoso, J.C. (2005) 'Abeta deposition is associated with enhanced cortical alpha-synuclein lesions in Lewy body diseases', *Neurobiology of Aging*, 26(8), pp. 1183-92.

Poewe, W., Wolters, E., Emre, M., Onofrj, M., Hsu, C.C., Tekin, S., Lane, R. and Investigators, E. (2006) 'Long-term benefits of rivastigmine in dementia associated with Parkinson's disease: An active treatment extension study', *Movement Disorders*, 21(4), pp. 456-461.

Poletti, M., Emre, M. and Bonuccelli, U. (2011) 'Mild cognitive impairment and cognitive reserve in Parkinson's disease', *Parkinsonism & Related Disorders*, 17(8), pp. 579-586.

Poletti, M., Frosini, D., Pagni, C., Baldacci, F., Nicoletti, V., Tognoni, G., Lucetti, C., Del Dotto, P., Ceravolo, R. and Bonuccelli, U. (2012) 'Mild cognitive impairment and cognitive-motor relationships in newly diagnosed drug-naive patients with Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 83(6), pp. 601-6.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I. and Nussbaum, R.L. (1997) 'Mutation in the alpha-synuclein gene identified in families with Parkinson's disease', *Science*, 276(5321), pp. 2045-7.

Post, B., Merkus, M.P., De Haan, R.J. and Speelman, J.D. (2007) 'Prognostic factors for the progression of Parkinson's disease: A systematic review', *Movement Disorders*, 22(13), pp. 1839-1851.

Postuma, R.B., Bertrand, J.A., Montplaisir, J., Desjardins, C., Vendette, M., Romenets, S.R., Panisset, M. and Gagnon, J.F. (2012) 'Rapid eye movement sleep behavior disorder and risk of dementia in Parkinson's disease: A prospective study', *Movement Disorders*, 27(6), pp. 720-726.

Preda, S., Govoni, S., Lanni, C., Racchi, M., Mura, E., Grilli, M. and Marchi, M. (2008) 'Acute beta-amyloid administration disrupts the cholinergic control of dopamine release in the nucleus accumbens', *Neuropsychopharmacology*, 33(5), pp. 1062-70.

Quik, M., Perez, X.A. and Bordia, T. (2012) 'Nicotine as a potential neuroprotective agent for Parkinson's disease', *Movement Disorders*, 27(8), pp. 947-57.

Qureshi, I.A. and Mehler, M.F. (2011) 'Advances in epigenetics and epigenomics for neurodegenerative diseases', *Current Neurology & Neuroscience Reports*, 11(5), pp. 464-73.

Ramirez, A., Heimbach, A., Grundemann, J., Stiller, B., Hampshire, D., Cid, L.P., Goebel, I., Mubaidin, A.F., Wriekat, A.L., Roeper, J., Al-Din, A., Hillmer, A.M., Karsak, M., Liss, B., Woods, C.G., Behrens, M.I. and Kubisch, C. (2006) 'Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase', *Nature Genetics*, 38(10), pp. 1184-91.

Reesink, F.E., Lemstra, A.W., van Dijk, K.D., Berendse, H.W., van de Berg, W.D.J., Klein, M., Blankenstein, M.A., Scheltens, P., Verbeek, M.M. and van der Flier, W.M. (2010) 'CSF alpha-synuclein does not discriminate dementia with Lewy bodies from Alzheimer's disease', *Journal of Alzheimer's disease*, 22(1), pp. 87-95.

Rinne, J.O., Ma, S.Y., Lee, M.S., Collan, Y. and Roytta, M. (2008) 'Loss of cholinergic neurons in the pedunculopontine nucleus in Parkinson's disease is related to disability of the patients', *Parkinsonism & related disorders*, 14(7), pp. 553-7.

Robbins, T.W., James, M., Owen, A.M., Sahakian, B.J., McInnes, L. and Rabbitt, P. (1994) 'Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers', *Dementia (Basel, Switzerland)*, 5(5), pp. 266-81.

Rochester, L., Yarnall, A.J., Baker, M.R., David, R.V., Lord, S., Galna, B. and Burn, D.J. (2012) 'Cholinergic dysfunction contributes to gait disturbance in early Parkinson's disease', *Brain*, 135, pp. 2779-2788.

Rogasch, N.C. and Fitzgerald, P.B. (2013) 'Assessing Cortical Network Properties Using TMS-EEG', *Human Brain Mapping*, DOI: 10.1002/hbm.22016.

Rolinski, M., Fox, C., Maidment, I. and McShane, R. (2012) 'Cholinesterase inhibitors for dementia with Lewy bodies, Parkinson's disease dementia and cognitive impairment in Parkinson's disease', *Cochrane database of systematic reviews (Online)*, 3, p. CD006504.

Rosenthal, E., Brennan, L., Xie, S., Hurtig, H., Milber, J., Weintraub, D., Karlawish, J. and Siderowf, A. (2010) 'Association Between Cognition and Function in Patients With Parkinson Disease With and Without Dementia', *Movement Disorders*, 25(9), pp. 1170-1176.

Ross, G.W., Abbott, R.D., Petrovitch, H., Morens, D.M., Grandinetti, A., Tung, K.H., Tanner, C.M., Masaki, K.H., Blanchette, P.L., Curb, J.D., Popper, J.S. and White, L.R. (2000) 'Association of coffee and caffeine intake with the risk of Parkinson disease', *JAMA*, 283(20), pp. 2674-9.

Rothman, K.J. (1990) 'No adjustments are needed for multiple comparisons', *Epidemiology*, 1(1), pp. 43-6.

Rowan, E., McKeith, I.G., Saxby, B.K., O'Brien, J.T., Burn, D., Mosimann, U., Newby, J., Daniel, S., Sanders, J. and Wesnes, K. (2007) 'Effects of donepezil on central processing speed and attentional measures in Parkinson's disease with dementia and dementia with Lewy bodies', *Dementia and Geriatric Cognitive Disorders*, 23(3), pp. 161-7.

Sabbagh, M.N., Adler, C.H., Lahti, T.J., Connor, D.J., Vedders, L., Peterson, L.K., Caviness, J.N., Shill, H.A., Sue, L.I., Ziabreva, I., Perry, E., Ballard, C.G., Aarsland, D., Walker, D.G. and Beach, T.G. (2009) 'Parkinson disease with dementia: comparing patients with and without Alzheimer pathology', *Alzheimer disease and associated disorders*, 23(3), pp. 295-7.

Sadot, E., Gurwitz, D., Barg, J., Behar, L., Ginzburg, I. and Fisher, A. (1996) 'Activation of m1 muscarinic acetylcholine receptor regulates tau phosphorylation in transfected PC12 cells', *Journal of neurochemistry*, 66(2), pp. 877-80.

Sahakian, B.J., Morris, R.G., Evenden, J.L., Heald, A., Levy, R., Philpot, M. and Robbins, T.W. (1988) 'A comparative study of visuospatial memory and learning in Alzheimer-type dementia and Parkinson's disease', *Brain*, 111 (Pt 3), pp. 695-718.

Sailer, A., Cunic, D.I., Paradiso, G.O., Gunraj, C.A., Wagle-Shukla, A., Moro, E., Lozano, A.M., Lang, A.E. and Chen, R. (2007) 'Subthalamic nucleus stimulation modulates afferent inhibition in Parkinson disease', *Neurology*, 68(5), pp. 356-63.

Sailer, A., Molnar, G.F., Paradiso, G., Gunraj, C.A., Lang, A.E. and Chen, R. (2003) 'Short and long latency afferent inhibition in Parkinson's disease', *Brain : a journal of neurology*, 126(Pt 8), pp. 1883-94.

Sakuma, K., Murakami, T. and Nakashima, K. (2007) 'Short latency afferent inhibition is not impaired in mild cognitive impairment', *Clinical neurophysiology*, 118(7), pp. 1460-3.

Samaranch, L., Lorenzo-Betancor, O., Arbelo, J.M., Ferrer, I., Lorenzo, E., Irigoyen, J., Pastor, M.A., Marrero, C., Isla, C., Herrera-Henriquez, J. and Pastor, P. (2010) 'PINK1-linked parkinsonism is associated with Lewy body pathology', *Brain*, 133, pp. 1128-1142.

Sardi, S.P., Clarke, J., Kinnecom, C., Tamsett, T.J., Li, L.Y., Stanek, L.M., Passini, M.A., Grabowski, G.A., Schlossmacher, M.G., Sidman, R.L., Cheng, S.H. and Shihabuddin, L.S. (2011) 'CNS expression of glucocerebrosidase corrects alpha-synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy', *Proceedings of the National Academy of Sciences of the United States of America*, 108(29), pp. 12101-12106.

Scatton, B., Javoy-Agid, F., Rouquier, L., Dubois, B. and Agid, Y. (1983) 'Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease', *Brain research*, 275(2), pp. 321-8.

Schliebs, R. and Arendt, T. (2006) 'The significance of the cholinergic system in the brain during aging and in Alzheimer's disease', *Journal of Neural Transmission*, 113(11), pp. 1625-44.

Schneider, S.A., Paisan-Ruiz, C., Quinn, N.P., Lees, A.J., Houlden, H., Hardy, J. and Bhatia, K.P. (2010) 'ATP13A2 mutations (PARK9) cause neurodegeneration with brain iron accumulation', *Movement Disorders*, 25(8), pp. 979-84.

Schrag, A., Hovris, A., Morley, D., Quinn, N. and Jahanshahi, M. (2006) 'Caregiver-burden in Parkinson's disease is closely associated with psychiatric symptoms, falls, and disability', *Parkinsonism & Related Disorders*, 12(1), pp. 35-41.

Schrag, A., Jahanshahi, M. and Quinn, N. (2000a) 'How does Parkinson's disease affect quality of life? A comparison with quality of life in the general population', *Movement Disorders*, 15(6), pp. 1112-1118.

Schrag, A., Jahanshahi, M. and Quinn, N. (2000b) 'What contributes to quality of life in patients with Parkinson's disease?', *Journal of Neurology Neurosurgery and Psychiatry*, 69(3), pp. 308-12.

Selikhova, M., Williams, D.R., Kempster, P.A., Holton, J.L., Revesz, T. and Lees, A.J. (2009) 'A clinico-pathological study of subtypes in Parkinson's disease', *Brain*, 132, pp. 2947-2957.

Selkoe, D.J. (1996) 'Amyloid beta-protein and the genetics of Alzheimer's disease', *The Journal of biological chemistry*, 271(31), pp. 18295-8.

Selkoe, D.J. (2003) 'Aging, amyloid, and Alzheimer's disease: a perspective in honor of Carl Cotman', *Neurochemical Research*, 28(11), pp. 1705-13.

Seppi, K., Weintraub, D., Coelho, M., Perez-Lloret, S., Fox, S.H., Katzenschlager, R., Hametner, E.M., Poewe, W., Rascol, O., Goetz, C.G. and Sampaio, C. (2011) 'The Movement Disorder Society Evidence-Based Medicine Review Update: Treatments for

the Non-Motor Symptoms of Parkinson's Disease', *Movement Disorders*, 26, pp. S42-S80.

Seto-Salvia, N., Clarimon, J., Pagonabarraga, J., Pascual-Sedano, B., Campolongo, A., Combarros, O., Mateo, J.I., Regana, D., Martinez-Corral, M., Marquie, M., Alcolea, D., Suarez-Calvet, M., Molina-Porcel, L., Dols, O., Gomez-Isla, T., Blesa, R., Lleo, A. and Kulisevsky, J. (2011) 'Dementia Risk in Parkinson Disease Disentangling the Role of MAPT Haplotypes', *Archives of Neurology*, 68(3), pp. 359-364.

Seto-Salvia, N., Pagonabarraga, J., Houlden, H., Pascual-Sedano, B., Dols-Icardo, O., Tucci, A., Paisan-Ruiz, C., Campolongo, A., Anton-Aguirre, S., Martin, I., Munoz, L., Bufill, E., Vilageliu, L., Grinberg, D., Cozar, M., Blesa, R., Lleo, A., Hardy, J., Kulisevsky, J. and Clarimon, J. (2012) 'Glucocerebrosidase mutations confer a greater risk of dementia during Parkinson's disease course', *Movement Disorders*, 27(3), pp. 393-399.

Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J. and Swindlehurst, C. (1992) 'Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids', *Nature*, 359(6393), pp. 325-7.

Shannon, K.M., Keshavarzian, A., Dodiya, H.B., Jakate, S. and Kordower, J.H. (2012a) 'Is alpha-synuclein in the colon a biomarker for premotor Parkinson's Disease? Evidence from 3 cases', *Movement Disorders*, 27(6), pp. 716-719.

Shannon, K.M., Keshavarzian, A., Mutlu, E., Dodiya, H.B., Daian, D., Jaglin, J.A. and Kordower, J.H. (2012b) 'Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease', *Movement Disorders*, 27(6), pp. 709-715.

Shaw, L.M., Vanderstichele, H., Knapik-Czajka, M., Clark, C.M., Aisen, P.S., Petersen, R.C., Blennow, K., Soares, H., Simon, A., Lewczuk, P., Dean, R., Siemers, E., Potter, W., Lee, V.M.Y., Trojanowski, J.Q. and Alzheimer's Disease Neuroimaging, I. (2009) 'Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects', *Annals of neurology*, 65(4), pp. 403-13.

Sherer, T.B., Richardson, J.R., Testa, C.M., Seo, B.B., Panov, A.V., Yagi, T., Matsuno-Yagi, A., Miller, G.W. and Greenamyre, J.T. (2007) 'Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease', *Journal of neurochemistry*, 100(6), pp. 1469-79.

Shi, M., Bradner, J., Hancock, A.M., Chung, K.A., Quinn, J.F., Peskind, E.R., Galasko, D., Jankovic, J., Zabetian, C.P., Kim, H.M., Leverenz, J.B., Montine, T.J., Ginghina, C., Kang, U.J., Cain, K.C., Wang, Y., Aasly, J., Goldstein, D. and Zhang, J. (2011) 'Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression', *Annals of neurology*, 69(3), pp. 570-80.

Shi, M. and Zhang, J. (2011) 'CSF alpha-synuclein, tau, and amyloid beta in Parkinson's disease', *Lancet Neurology*, 10(8), pp. 681-681.

Shimada, H., Hirano, S., Shinotoh, H., Aotsuka, A., Sato, K., Tanaka, N., Ota, T., Asahina, M., Fukushi, K., Kuwabara, S., Hattori, T., Suhara, T. and Irie, T. (2009) 'Mapping of brain acetylcholinesterase alterations in Lewy body disease by PET', *Neurology*, 73(4), pp. 273-8.

Shoji, M., Golde, T.E., Ghiso, J., Cheung, T.T., Estus, S., Shaffer, L.M., Cai, X.D., McKay, D.M., Tintner, R. and Frangione, B. (1992) 'Production of the Alzheimer amyloid beta protein by normal proteolytic processing', *Science*, 258(5079), pp. 126-9.

Shukla, A.W., Moro, E., Gunraj, C., Lozano, A., Hodaie, M., Lang, A.E. and Chen, R. (2013) 'Long-term subthalamic nucleus stimulation improves sensorimotor integration and proprioception', *Journal of Neurology Neurosurgery and Psychiatry*, doi:10.1136/jnnp-2012-304102.

Siderowf, A., Xie, S.X., Hurtig, H., Weintraub, D., Duda, J., Chen-Plotkin, A., Shaw, L.M., Van Deerlin, V., Trojanowski, J.Q. and Clark, C. (2010) 'CSF amyloid {beta} 1-42 predicts cognitive decline in Parkinson disease', *Neurology*, 75(12), pp. 1055-61.

Sidransky, E., Nalls, M.A., Aasly, J.O., Aharon-Peretz, J., Annesi, G., Barbosa, E.R., Bar-Shira, A., Berg, D., Bras, J., Brice, A., Chen, C.M., Clark, L.N., Condroyer, C., De Marco,

E.V., Durr, A., Eblan, M.J., Fahn, S., Farrer, M.J., Fung, H.C., Gan-Or, Z., Gasser, T., Gershoni-Baruch, R., Giladi, N., Griffith, A., Gurevich, T., Januario, C., Kropp, P., Lang, A.E., Lee-Chen, G.J., Lesage, S., Marder, K., Mata, I.F., Mirelman, A., Mitsui, J., Mizuta, I., Nicoletti, G., Oliveira, C., Ottman, R., Orr-Urtreger, A., Pereira, L.V., Quattrone, A., Rogaeva, E., Rolfs, A., Rosenbaum, H., Rozenberg, R., Samii, A., Samaddar, T., Schulte, C., Sharma, M., Singleton, A., Spitz, M., Tan, E.K., Tayebi, N., Toda, T., Troiano, A.R., Tsuji, S., Wittstock, M., Wolfsberg, T.G., Wu, Y.R., Zabetian, C.P., Zhao, Y. and Ziegler, S.G. (2009) 'Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease', *New England Journal of Medicine*, 361(17), pp. 1651-1661.

Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M.R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J. and Gwinn-Hardy, K. (2003) 'alpha-Synuclein locus triplication causes Parkinson's disease', *Science*, 302(5646), p. 841.

Sjogren, M., Minthon, L., Davidsson, P., Granerus, A.K., Clarberg, A., Vanderstichele, H., Vanmechelen, E., Wallin, A. and Blennow, K. (2000) 'CSF levels of tau, beta-amyloid(1-42) and GAP-43 in frontotemporal dementia, other types of dementia and normal aging', *Journal of Neural Transmission*, 107(5), pp. 563-579.

Slawek, J., Derejko, M. and Lass, P. (2005) 'Factors affecting the quality of life of patients with idiopathic Parkinson's disease - a cross-sectional study in an outpatient clinic attendees', *Parkinsonism & Related Disorders*, 11(7), pp. 465-468.

Sollinger, A.B., Goldstein, F.C., Lah, J.J., Levey, A.I. and Factor, S.A. (2010) 'Mild cognitive impairment in Parkinson's disease: Subtypes and motor characteristics', *Parkinsonism & Related Disorders*, 16(3), pp. 177-180.

Song, S.K., Lee, J.E., Park, H.J., Sohn, Y.H., Lee, J.D. and Lee, P.H. (2011) 'The pattern of cortical atrophy in patients with Parkinson's disease according to cognitive status', *Movement Disorders*, 26(2), pp. 289-96.

Sonnen, J.A., Postupna, N., Larson, E.B., Crane, P.K., Rose, S.E., Montine, K.S., Leverenz, J.B. and Montine, T.J. (2010) 'Pathologic correlates of dementia in individuals with Lewy body disease', *Brain pathology*, 20(3), pp. 654-9.

Spira, P.J., Sharpe, D.M., Halliday, G., Cavanagh, J. and Nicholson, G.A. (2001) 'Clinical and pathological features of a Parkinsonian syndrome in a family with an Ala53Thr alpha-synuclein mutation', *Annals of Neurology*, 49(3), pp. 313-9.

Spitsyna, G., Warren, J.E., Scott, S.K., Turkheimer, F.E. and Wise, R.J.S. (2006) 'Converging language streams in the human temporal lobe', *Journal of Neuroscience*, 26(28), pp. 7328-7336.

Starkstein, S.E., Mayberg, H.S., Leiguarda, R., Preziosi, T.J. and Robinson, R.G. (1992) 'A Prospective Longitudinal-Study of Depression, Cognitive Decline, and Physical Impairments in Patients with Parkinsons-Disease', *Journal of Neurology Neurosurgery and Psychiatry*, 55(5), pp. 377-382.

Stebbins, G.T., Goetz, C.G., Burn, D.J., Jankovic, J., Khoo, T.K. and Tilley, B.C. (2013) 'How to Identify Tremor Dominant and Postural Instability/Gait Difficulty Groups With the Movement Disorder Society Unified Parkinson's Disease Rating Scale: Comparison With the Unified Parkinson's Disease Rating Scale', *Movement Disorders*, DOI: 10.1002/mds.25383.

Stern, M.B. and Siderowf, A. (2010) 'Parkinson's At Risk Syndrome: Can Parkinson's Disease Be Predicted?', *Movement Disorders*, 25(3), pp. S89-S93.

Steur, E.J., Vermes, J. and de Vos, R.A.I. (1998) 'Cerebrospinal-fluid tau protein and aspartate aminotransferase in Parkinson's disease', *Lancet*, 351(9109), pp. 1105-1106.

Strozyk, D., Blennow, K., White, L.R. and Launer, L.J. (2003) 'CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study', *Neurology*, 60(4), pp. 652-6.

Svenningsson, P., Westman, E., Ballard, C. and Aarsland, D. (2012) 'Cognitive impairment in patients with Parkinson's disease: diagnosis, biomarkers, and treatment', *Lancet Neurology*, 11(8), pp. 697-707.

Swainson, R., Hodges, J.R., Galton, C.J., Semple, J., Michael, A., Dunn, B.D., Iddon, J.L., Robbins, T.W. and Sahakian, B.J. (2001) 'Early detection and differential diagnosis of Alzheimer's disease and depression with neuropsychological tasks', *Dementia and geriatric cognitive disorders*, 12(4), pp. 265-80.

Tanaka, K., de Quadros, A.C., Santo, R.F., Stella, F., Gobbi, L.T.B. and Gobbi, S. (2009) 'Benefits of physical exercise on executive functions in older people with Parkinson's disease', *Brain and Cognition*, 69(2), pp. 435-441.

Tanaka, M., Kim, Y.M., Lee, G., Junn, E., Iwatsubo, T. and Mouradian, M.M. (2004) 'Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective', *Journal of Biological Chemistry*, 279(6), pp. 4625-31.

Tapiola, T., Alafuzoff, I., Herukka, S.-K., Parkkinen, L., Hartikainen, P., Soininen, H. and Pirttila, T. (2009) 'Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain', *Archives of neurology*, 66(3), pp. 382-9.

Tapiola, T., Overmyer, M., Lehtovirta, M., Helisalmi, S., Ramberg, J., Alafuzoff, I., Riekkinen, P., Sr. and Soininen, H. (1997) 'The level of cerebrospinal fluid tau correlates with neurofibrillary tangles in Alzheimer's disease', *Neuroreport*, 8(18), pp. 3961-3.

Tateno, F., Sakakibara, R., Kawai, T., Kishi, M. and Murano, T. (2012) 'Alpha-synuclein in the cerebrospinal fluid differentiates synucleinopathies (Parkinson Disease, dementia with Lewy bodies, multiple system atrophy) from Alzheimer disease', *Alzheimer disease and associated disorders*, 26(3), pp. 213-6.

Taylor, J.P., Rowan, E.N., Lett, D., O'Brien, J.T., McKeith, I.G. and Burn, D.J. (2008) 'Poor attentional function predicts cognitive decline in patients with non-demented

Parkinson's disease independent of motor phenotype', *Journal of Neurology Neurosurgery and Psychiatry*, 79(12), pp. 1318-23.

Taylor, K.S., Cook, J.A. and Counsell, C.E. (2007) 'Heterogeneity in male to female risk for Parkinson's disease', *Journal of Neurology Neurosurgery and Psychiatry*, 78(8), pp. 905-6.

Taylor, K.S.M., Counsell, C.E., Harris, C.E., Gordon, J.C. and Smith, W.C.S. (2006) 'Pilot study of the incidence and prognosis of degenerative Parkinsonian disorders in Aberdeen, United Kingdom: methods and preliminary results', *Movement disorders : official journal of the Movement Disorder Society*, 21(7), pp. 976-82.

Teunissen, C.E., Petzold, A., Bennett, J.L., Berven, F.S., Brundin, L., Comabella, M., Franciotta, D., Frederiksen, J.L., Fleming, J.O., Furlan, R., Hintzen, R.Q., Hughes, S.G., Johnson, M.H., Krasulova, E., Kuhle, J., Magnone, M.C., Rajda, C., Rejdak, K., Schmidt, H.K., van Pesch, V., Waubant, E., Wolf, C., Giovannoni, G., Hemmer, B., Tumani, H. and Deisenhammer, F. (2009) 'A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking', *Neurology*, 73(22), pp. 1914-22.

Thathiah, A. and De Strooper, B. (2009) 'G protein-coupled receptors, cholinergic dysfunction, and Abeta toxicity in Alzheimer's disease', *Science signaling*, 2(93), p. re8.

Tiraboschi, P., Hansen, L.A., Alford, M., Sabbagh, M.N., Schoos, B., Masliah, E., Thal, L.J. and Corey-Bloom, J. (2000) 'Cholinergic dysfunction in diseases with Lewy bodies', *Neurology*, 54(2), pp. 407-11.

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., Insola, A., Mazzone, P., Tonali, P. and Rothwell, J.C. (2000) 'Short latency inhibition of human hand motor cortex by somatosensory input from the hand', *The Journal of physiology*, 523 Pt 2, pp. 503-13.

Tokuda, T., Qureshi, M.M., Ardah, M.T., Varghese, S., Shehab, S.A.S., Kasai, T., Ishigami, N., Tamaoka, A., Nakagawa, M. and El-Agnaf, O.M.A. (2010) 'Detection of elevated

levels of alpha-synuclein oligomers in CSF from patients with Parkinson disease', *Neurology*, 75(20), pp. 1766-72.

Tokuda, T., Salem, S.A., Allsop, D., Mizuno, T., Nakagawa, M., Qureshi, M.M., Locascio, J.J., Schlossmacher, M.G. and El-Agnaf, O.M.A. (2006) 'Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease', *Biochemical and biophysical research communications*, 349(1), pp. 162-6.

Tomlinson, C.L., Stowe, R., Patel, S., Rick, C., Gray, R. and Clarke, C.E. (2010) 'Systematic review of levodopa dose equivalency reporting in Parkinson's disease', *Movement Disorders*, 25(15), pp. 2649-53.

Trompetto, C., Assini, A., Buccolieri, A., Marchese, R. and Abbruzzese, G. (1999) 'Intracortical inhibition after paired transcranial magnetic stimulation depends on the current flow direction', *Clinical Neurophysiology*, 110(6), pp. 1106-1110.

Troster, A.I. (2008) 'Neuropsychological characteristics of dementia with Lewy bodies and Parkinson's disease with dementia: differentiation, early detection, and implications for "mild cognitive impairment" and biomarkers', *Neuropsychology review*, 18(1), pp. 103-19.

Tsutsumi, R., Hanajima, R., Hamada, M., Shirota, Y., Matsumoto, H., Terao, Y., Ohminami, S., Yamakawa, Y., Shimada, H., Tsuji, S. and Ugawa, Y. (2012a) 'Reduced interhemispheric inhibition in mild cognitive impairment', *Experimental brain research*, 218(1), pp. 21-6.

Tsutsumi, R., Shirota, Y., Ohminami, S., Terao, Y., Ugawa, Y. and Hanajima, R. (2012b) 'Conditioning intensity-dependent interaction between short-latency interhemispheric inhibition and short-latency afferent inhibition', *Journal of Neurophysiology*, 108(4), pp. 1130-1137.

Tune, L.E. (2001) 'Anticholinergic effects of medication in elderly patients', *The Journal of clinical psychiatry*, 62 Suppl 21, pp. 11-4.

Twelves, D., Perkins, K.S. and Counsell, C. (2003) 'Systematic review of incidence studies of Parkinson's disease', *Movement Disorders*, 18(1), pp. 19-31.

Uc, E.Y., McDermott, M.P., Marder, K.S., Anderson, S.W., Litvan, I., Como, P.G., Auinger, P., Chou, K.L., Growdon, J.C. and Invest, P.S.G.D. (2009) 'Incidence of and risk factors for cognitive impairment in an early Parkinson disease clinical trial cohort', *Neurology*, 73(18), pp. 1469-1477.

Udupa, K., Ni, Z., Gunraj, C. and Chen, R. (2009) 'Interactions between short latency afferent inhibition and long interval intracortical inhibition', *Experimental Brain Research*, 199(2), pp. 177-183.

Urdinguio, R.G., Sanchez-Mut, J.V. and Esteller, M. (2009) 'Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies', *Lancet Neurology*, 8(11), pp. 1056-72.

Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G., Albanese, A., Nussbaum, R., Gonzalez-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W.P., Latchman, D.S., Harvey, R.J., Dallapiccola, B., Auburger, G. and Wood, N.W. (2004a) 'Hereditary early-onset Parkinson's disease caused by mutations in PINK1', *Science*, 304(5674), pp. 1158-60.

Valente, E.M., Bentivoglio, A.R., Dixon, P.H., Ferraris, A., Ialongo, T., Frontali, M., Albanese, A. and Wood, N.W. (2001) 'Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36', *American Journal of Human Genetics*, 68(4), pp. 895-900.

Valente, E.M., Salvi, S., Ialongo, T., Marongiu, R., Elia, A.E., Caputo, V., Romito, L., Albanese, A., Dallapiccola, B. and Bentivoglio, A.R. (2004b) 'PINK1 mutations are associated with sporadic early-onset parkinsonism', *Annals of Neurology*, 56(3), pp. 336-41.

van der Hoorn, A., Burger, H., Leenders, K.L. and de Jong, B.M. (2012) 'Handedness correlates with the dominant parkinson side: A systematic review and meta-analysis', *Movement Disorders*, 27(2), pp. 206-210.

van Dijk, K.D., Teunissen, C.E., Drukarch, B., Jimenez, C.R., Groenewegen, H.J., Berendse, H.W. and van de Berg, W.D.J. (2010) 'Diagnostic cerebrospinal fluid biomarkers for Parkinson's disease: a pathogenetically based approach', *Neurobiology of disease*, 39(3), pp. 229-41.

van Duijn, C.M., Dekker, M.C., Bonifati, V., Galjaard, R.J., Houwing-Duistermaat, J.J., Snijders, P.J., Testers, L., Breedveld, G.J., Horstink, M., Sandkuijl, L.A., van Swieten, J.C., Oostra, B.A. and Heutink, P. (2001) 'Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36', *American Journal of Human Genetics*, 69(3), pp. 629-34.

Vemuri, P., Wiste, H.J., Weigand, S.D., Shaw, L.M., Trojanowski, J.Q., Weiner, M.W., Knopman, D.S., Petersen, R.C., Jack, C.R., Jr. and Alzheimer's Disease Neuroimaging, I. (2009) 'MRI and CSF biomarkers in normal, MCI, and AD subjects: diagnostic discrimination and cognitive correlations', *Neurology*, 73(4), pp. 287-93.

Vendette, M., Gagnon, J.F., Decary, A., Massicotte-Marquez, J., Postuma, R.B., Doyon, J., Panisset, M. and Montplaisir, J. (2007) 'REM sleep behavior disorder predicts cognitive impairment in Parkinson disease without dementia', *Neurology*, 69(19), pp. 1843-1849.

Ventruti, A. and Cuervo, A.M. (2007) 'Autophagy and neurodegeneration', *Current Neurology & Neuroscience Reports*, 7(5), pp. 443-51.

Verbeek, M.M., De Jong, D. and Kremer, H.P.H. (2003) 'Brain-specific proteins in cerebrospinal fluid for the diagnosis of neurodegenerative diseases', *Annals of clinical biochemistry*, 40(Pt 1), pp. 25-40.

Verleden, S., Vingerhoets, G. and Santens, P. (2007) 'Heterogeneity of cognitive dysfunction in Parkinson's disease: A cohort study', *European Neurology*, 58(1), pp. 34-40.

Visser, P.J., Verhey, F., Knol, D.L., Scheltens, P., Wahlund, L.-O., Freund-Levi, Y., Tsolaki, M., Minthon, L., Wallin, A.K., Hampel, H., Burger, K., Pirttila, T., Soininen, H., Rikkert, M.O., Verbeek, M.M., Spiru, L. and Blennow, K. (2009) 'Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study', *Lancet neurology*, 8(7), pp. 619-27.

von Campenhausen, S., Bornschein, B., Wick, R., Botzel, K., Sampaio, C., Poewe, W., Oertel, W., Siebert, U., Berger, K. and Dodel, R. (2005) 'Prevalence and incidence of Parkinson's disease in Europe', *European neuropsychopharmacology*, 15(4), pp. 473-90.

Vossius, C., Larsen, J.P., Janvin, C. and Aarsland, D. (2011) 'The Economic Impact of Cognitive Impairment in Parkinson's Disease', *Movement Disorders*, 26(8), pp. 1541-1544.

Vranova, H.P., Mares, J., Hlustik, P., Nevrly, M., Stejskal, D., Zapletalova, J., Obereigneru, R. and Kanovsky, P. (2012) 'Tau protein and beta-amyloid(1-42) CSF levels in different phenotypes of Parkinson's disease', *Journal of Neural Transmission*, 119(3), pp. 353-362.

Vranova, H.P., Mares, J., Nevrly, M., Stejskal, D., Zapletalova, J., Hlustik, P. and Kanovsky, P. (2010) 'CSF markers of neurodegeneration in Parkinson's disease', *Journal of Neural Transmission*, 117(10), pp. 1177-1181.

Weintraub, D., Dietz, N., Duda, J.E., Wolk, D.A., Doshi, J., Xie, S.X., Davatzikos, C., Clark, C.M. and Siderowf, A. (2012) 'Alzheimer's disease pattern of brain atrophy predicts cognitive decline in Parkinson's disease', *Brain*, 135(Pt 1), pp. 170-80.

Weintraub, D., Doshi, J., Koka, D., Davatzikos, C., Siderowf, A.D., Duda, J.E., Wolk, D.A., Moberg, P.J., Xie, S.X. and Clark, C.M. (2011) 'Neurodegeneration across stages of cognitive decline in Parkinson disease', *Archives of Neurology*, 68(12), pp. 1562-8.

Weintraub, D., Mavandadi, S., Mamikonyan, E., Siderowf, A.D., Duda, J.E., Hurtig, H.I., Colcher, A., Horn, S.S., Nazem, S., Ten Have, T.R. and Stern, M.B. (2010) 'Atomoxetine for depression and other neuropsychiatric symptoms in Parkinson disease', *Neurology*, 75(5), pp. 448-455.

Weintraub, D., Moberg, P.J., Culbertson, W.C., Duda, J.E. and Stern, M.B. (2004a) 'Evidence for impaired encoding and retrieval memory profiles in Parkinson disease', *Cognitive & Behavioral Neurology*, 17(4), pp. 195-200.

Weintraub, D., Moberg, P.J., Duda, J.E., Katz, I.R. and Stern, M.B. (2004b) 'Effect of psychiatric and other nonmotor symptoms on disability in Parkinson's disease', *Journal of the American Geriatrics Society*, 52(5), pp. 784-788.

Wesnes, K.A., McKeith, I., Edgar, C., Emre, M. and Lane, R. (2005) 'Benefits of rivastigmine on attention in dementia associated with Parkinson disease', *Neurology*, 65(10), pp. 1654-6.

Wesnes, K.A., McKeith, I.G., Ferrara, R., Emre, M., Del Ser, T., Spano, P.F., Cicin-Sain, A., Anand, R. and Spiegel, R. (2002) 'Effects of rivastigmine on cognitive function in dementia with lewy bodies: a randomised placebo-controlled international study using the cognitive drug research computerised assessment system', *Dementia and geriatric cognitive disorders*, 13(3), pp. 183-92.

Whittington, C.J., Podd, J. and Stewart-Williams, S. (2006) 'Memory deficits in Parkinson's disease', *Journal of Clinical & Experimental Neuropsychology*, 28(5), pp. 738-54.

Williams-Gray, C.H., Evans, J.R., Goris, A., Foltynie, T., Ban, M., Robbins, T.W., Brayne, C., Kolachana, B.S., Weinberger, D.R., Sawcer, S.J. and Barker, R.A. (2009a) 'The distinct

cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort', *Brain*, 132(Pt 11), pp. 2958-69.

Williams-Gray, C.H., Foltynie, T., Brayne, C.E.G., Robbins, T.W. and Barker, R.A. (2007a) 'Evolution of cognitive dysfunction in an incident Parkinson's disease cohort', *Brain*, 130, pp. 1787-1798.

Williams-Gray, C.H., Goris, A., Saiki, M., Foltynie, T., Compston, D.A.S., Sawcer, S.J. and Barker, R.A. (2009b) 'Apolipoprotein E genotype as a risk factor for susceptibility to and dementia in Parkinson's Disease', *Journal of Neurology*, 256(3), pp. 493-498.

Williams-Gray, C.H., Hampshire, A., Robbins, T.W., Owen, A.M. and Barker, R.A. (2007b) 'Catechol O-methyltransferase val(158)met genotype influences frontoparietal activity during planning in patients with Parkinson's disease', *Journal of Neuroscience*, 27(18), pp. 4832-4838.

Williams, D.R. and Lees, A.J. (2005) 'Visual hallucinations in the diagnosis of idiopathic Parkinson's disease: a retrospective autopsy study', *Lancet neurology*, 4(10), pp. 605-10.

Winder-Rhodes, S.E., Evans, J.R., Ban, M., Mason, S.L., Williams-Gray, C.H., Foltynie, T., Duran, R., Mencacci, N.E., Sawcer, S.J. and Barker, R.A. (2013) 'Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort', *Brain* 136, pp. 392-399.

Winter, Y., Bezdolnyy, Y., Katunina, E., Avakjan, G., Reese, J.P., Klotsche, J., Oertel, W.H., Dodel, R. and Gusev, E. (2010) 'Incidence of Parkinson's disease and atypical parkinsonism: Russian population-based study', *Movement disorders*, 25(3), pp. 349-56.

Woods, S.P. and Troster, A.I. (2003) 'Prodromal frontal/executive dysfunction predicts incident dementia in Parkinson's disease', *Journal of the International Neuropsychological Society*, 9(1), pp. 17-24.

Yarnall, A.J., Rochester, L., Baker, M.R., David, R., Khoo, T.K., Duncan, G.W., Galna, B. and Burn, D.J. (2013) 'Short Latency Afferent Inhibition: A Biomarker for Mild Cognitive Impairment in Parkinson's Disease?', *Movement Disorders*, DOI: 10.1002/mds.25360.

Yarnall, A.J., Rochester, L. and Burn, D.J. (2011) 'The interplay of cholinergic function, attention, and falls in Parkinson's disease', *Movement disorders*, 26(14), pp. 2496-503.

Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M. and Leirer, V.O. (1983) 'Development and Validation of a Geriatric Depression Screening Scale - a Preliminary-Report', *Journal of Psychiatric Research*, 17(1), pp. 37-49.

Young-Bernier, M., Davidson, P.S.R. and Tremblay, F. (2012a) 'Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age', *Neurobiology of aging*, 33(4), pp. 835.e1-11.

Young-Bernier, M., Kamil, Y., Tremblay, F. and Davidson, P.S.R. (2012b) 'Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and older adults', *Behavioral and brain functions*, 8, p. 17.

Yu, R.L., Wu, R.M., Tai, C.H., Lin, C.H., Cheng, T.W. and Hua, M.S. (2012) 'Neuropsychological profile in patients with early stage of Parkinson's disease in Taiwan', *Parkinsonism & Related Disorders*, 18(10), pp. 1067-1072.

Zaccai, J., Brayne, C., McKeith, I., Matthews, F., Ince, P.G. and Mrc Cognitive Function, A.N.S. (2008) 'Patterns and stages of alpha-synucleinopathy: Relevance in a population-based cohort', *Neurology*, 70(13), pp. 1042-8.

Zadikoff, C., Fox, S.H., Tang-Wai, D.F., Thomsen, T., de Bie, R.M.A., Wadia, P., Miyasaki, J., Duff-Canning, S., Lang, A.E. and Marras, C. (2008) 'A comparison of the mini mental state exam to the Montreal cognitive assessment in identifying cognitive deficits in Parkinson's disease', *Movement disorders*, 23(2), pp. 297-9.

Zesiewicz, T.A., Sullivan, K.L., Arnulf, I., Chaudhuri, K.R., Morgan, J.C., Gronseth, G.S., Miyasaki, J., Iverson, D.J., Weiner, W.J. and Neurology, A.A. (2010) 'Practice Parameter:

Treatment of nonmotor symptoms of Parkinson disease Report of the Quality Standards Subcommittee of the American Academy of Neurology', *Neurology*, 74(11), pp. 924-931.

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R.J., Calne, D.B., Stoessl, A.J., Pfeiffer, R.F., Patenge, N., Carbajal, I.C., Vieregge, P., Asmus, F., Muller-Myhsok, B., Dickson, D.W., Meitinger, T., Strom, T.M., Wszolek, Z.K. and Gasser, T. (2004) 'Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology', *Neuron*, 44(4), pp. 601-7.

Ziropadja, L., Stefanova, E., Petrovic, M., Stojkovic, T. and Kostic, V.S. (2012) 'Apathy and depression in Parkinson's disease: The Belgrade PD study report', *Parkinsonism & Related Disorders*, 18(4), pp. 339-342.

Zoghi, M., Pearce, S.L. and Nordstrom, M.A. (2003) 'Differential modulation of intracortical inhibition in human motor cortex during selective activation of an intrinsic hand muscle', *The Journal of physiology*, 550(Pt 3), pp. 933-46.

Zweig, R.M., Cardillo, J.E., Cohen, M., Giere, S. and Hedreen, J.C. (1993) 'The locus ceruleus and dementia in Parkinson's disease', *Neurology*, 43(5), pp. 986-91.

Zweig, R.M., Jankel, W.R., Hedreen, J.C., Mayeux, R. and Price, D.L. (1989) 'The pedunculopontine nucleus in Parkinson's disease', *Annals of neurology*, 26(1), pp. 41-6.