

Targeting Cognitive Impairment in Parkinson's Disease: Novel Pharmacological and Non-
Pharmacological Approaches

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Table of Contents

TABLE OF CONTENTS	III
ABSTRACT	VIII
DECLARATION	IX
ACKNOWLEDGEMENTS	X
CONTRIBUTIONS MADE BY CO-AUTHORS	XII
PRESENTATIONS ARISING FROM THIS THESIS	XIII
THESIS EXPLANATION	XIV
ABBREVIATIONS	XV
LIST OF FIGURES	XVIII
LIST OF TABLES	XX
STYLE CONVENTIONS	XXI
FINANCIAL SUPPORT	XXII
CHAPTER 1 GENERAL INTRODUCTION	1
1.0 INTRODUCTION	2
1.1 COGNITIVE IMPAIRMENT IN PARKINSON’S DISEASE	3
1.1.1 EVOLUTION AND PRESENTATION OF COGNITIVE IMPAIRMENT IN PD.....	3
1.2 PATHOPHYSIOLOGY OF COGNITIVE IMPAIRMENT IN PD	7
1.2.1 PATHOLOGICAL PROTEIN AGGREGATION	7
1.2.2 DOPAMINE AND THE DOPAMINERGIC PATHWAYS	10
1.2.2.1 <i>Dopamine Pathways and the Basal Ganglia</i>	10
1.2.2.2 <i>Striatal Loops of the Basal Ganglia</i>	15
1.3 NEUROTRANSMITTER DYSFUNCTION AND COGNITIVE IMPAIRMENT IN PD	17
1.3.1 DOPAMINE	17
1.3.2 NORADRENALINE.....	18
1.3.3 SEROTONIN	20
1.3.4 ACETYLCHOLINE	21
1.4 TARGETING COGNITIVE IMPAIRMENT IN PARKINSON’S DISEASE	24
1.4.1 PHARMACOLOGICAL APPROACHES.....	24
1.4.1.1 <i>Dopamine Replacement Therapy</i>	24
1.4.1.2 <i>Cholinesterase Therapy</i>	26
1.4.1.3 <i>Noradrenaline Therapy</i>	28
1.4.1.4 <i>Serotonergic Therapy</i>	30
1.4.1.5 <i>Glutamatergic Therapy</i>	31
1.4.1.6 <i>Limitations of Pharmacological Approaches</i>	32
1.4.2 NON-PHARMACOLOGICAL APPROACHES	33
1.4.2.1 <i>Diet</i>	33
1.4.2.2 <i>Aerobic exercise and physical activity</i>	37
1.4.2.3 <i>tDCS/rTMS</i>	39
1.4.2.4 <i>Cognitive training</i>	40
1.5 CONCLUSION	43

CHAPTER 2 STUDY SYNOPSIS, AIMS AND HYPOTHESES.....	44
2.0 SYNOPSIS, HYPOTHESES AND AIMS.....	45
CHAPTER 3 FYN KINASE ACTIVITY AND ITS ROLE IN NEURODEGENERATIVE DISEASE PATHOLOGY: A POTENTIAL UNIVERSAL TARGET?	48
3.0 ABSTRACT.....	49
3.1 INTRODUCTION	50
3.2 STRUCTURE OF FYN.....	51
3.3 MULTIPLE SCLEROSIS.....	54
3.3.1 ROLE OF FYN IN T-CELL REGULATION IN MS.....	59
3.4 ALZHEIMER'S DISEASE.....	61
3.4.1 ROLE OF FYN IN REGULATING AMYLOID-BETA AND HYPERPHOSPHORYLATED TAU PRODUCTION IN AD	62
3.4.2 ROLE OF FYN KINASE IN NMDA RECEPTOR DYSFUNCTION AND PrPc INTERACTIONS IN AD.....	64
3.5 PARKINSON'S DISEASE.....	68
3.5.1 ROLE OF FYN KINASE IN INDUCING NEUROINFLAMMATION IN PD.....	68
3.6 FYN: A COMMON THERAPEUTIC TARGET ACROSS MULTIPLE NEURODEGENERATIVE DISORDERS	74
3.7 CONCLUSIONS AND FUTURE INVESTIGATIONS	77
CHAPTER 4 ANATOMICAL DISTRIBUTION OF FYN KINASE IN THE PARKINSON'S DISEASE BRAIN.....	80
4.0 ABSTRACT.....	81
4.1 INTRODUCTION	82
4.2 METHODS.....	84
4.2.1 POST-MORTEM SAMPLES.....	84
4.2.2 FIXED TISSUE COLLECTION AND PROCESSING	85
4.2.3 IMMUNOHISTOCHEMISTRY.....	86
4.2.4 WESTERN BLOT ANALYSIS.....	87
4.2.5 ELISA	88
4.2.6 STATISTICAL ANALYSIS.....	88
4.3 RESULTS	89
4.3.1 NO DIFFERENCE IN TOTAL FYN	89
4.3.2 SIGNIFICANT INCREASE IN FYN PHOSPHORYLATION IN THE STRIATUM AND SN.....	90
4.3.3 NO INCREASED ASTROCYTIC REACTIVITY IN PD COMPARED TO CONTROLS	91
4.3.4 NO INCREASE IN MICROGLIAL POPULATION OR ACTIVATION STATE IN PD COMPARED TO CONTROLS	93
4.3.5 NO DIFFERENCES OBSERVED IN RELATIVE EXPRESSION OF NeuN BETWEEN PD AND CONTROLS..	95
4.4 DISCUSSION.....	96
4.5 CONCLUSION	100
CHAPTER 5 FYN KINASE INHIBITION USING AZD0530 IMPROVES RECOGNITION MEMORY AND REDUCES DEPRESSIVE-LIKE BEHAVIOUR IN AN EXPERIMENTAL MODEL OF EARLY-STAGE PARKINSON'S DISEASE.....	102
5.0 ABSTRACT.....	103

5.1	INTRODUCTION	104
5.2	MATERIALS AND METHODS	107
5.2.1	ANIMALS	107
5.2.2	STEREOTACTIC SURGERY	108
5.2.3	EXPERIMENTAL DESIGN AND DRUG ADMINISTRATION	109
5.2.4	FUNCTIONAL TESTING	109
5.2.4.1	<i>Rotarod</i>	110
5.2.4.2	<i>Adjusting Step Test</i>	110
5.2.4.3	<i>Open Field Test</i>	110
5.2.4.4	<i>Tremulous Jaw Movement</i>	111
5.2.4.5	<i>Elevated Plus Maze</i>	111
5.2.4.6	<i>Novel Object Recognition</i>	111
5.2.4.7	<i>Barnes Maze</i>	112
5.2.4.8	<i>Forced Swim Test</i>	113
5.2.5	TISSUE COLLECTION AND PROCESSING.....	113
5.2.6	IMMUNOHISTOCHEMICAL ANALYSIS	114
5.2.7	WESTERN BLOT ANALYSIS.....	115
5.2.8	STATISTICAL ANALYSIS.....	116
5.3	RESULTS	117
5.3.1	IMPAIRMENTS IN VOLITIONAL LOCOMOTION AND AST WERE PROGRESSIVE IN NATURE, BUT NO IMPROVEMENTS IN MOTOR FUNCTION WERE OBSERVED WITH AZD0530 TREATMENT.....	117
5.3.2	INTRASTRIATAL INJECTION OF 6-OHDA LED TO DEPRESSIVE-LIKE BEHAVIOUR, WHICH WAS REDUCED BY AZD0530 TREATMENT.....	120
5.3.3	AZD0530 ADMINISTRATION IMPROVED RECOGNITION MEMORY AS MEASURED BY PREFERENCE FOR THE NOVEL OBJECT.	121
5.3.4	AZD0530 DID NOT RESCUE DOPAMINERGIC INTEGRITY IN THE STRIATUM	123
5.3.5	NO ALTERATIONS TO MEASURES OF INFLAMMATION WERE OBSERVED WITH AZD0530 ADMINISTRATION.....	124
5.4	DISCUSSION.....	127
5.6	CONCLUSION	133
CHAPTER 6 OPTIMISING COGNITIVE TRAINING FOR THE TREATMENT OF COGNITIVE DYSFUNCTION IN PARKINSON'S DISEASE: CURRENT LIMITATIONS AND FUTURE DIRECTIONS		
135		
6.0	ABSTRACT.....	136
6.1	INTRODUCTION	137
6.2	PD AND COGNITIVE DYSFUNCTION.....	138
6.2.1	PHARMACOLOGICAL TREATMENT OF COGNITIVE IMPAIRMENT IN PD: LIMITATIONS	139
6.3	COGNITIVE TRAINING IN PD.....	141
6.4	CONSIDERATION 1: METHOD OF DELIVERY	159
6.4.1	PEN AND PAPER-BASED CT.....	159
6.4.2	COMPUTER-BASED CT.....	161
6.4.3	COMBINATION OF PEN AND PAPER AND COMPUTER-BASED CT	164
6.5	CONSIDERATION 2: STANDARD VS TAILORED DELIVERY	166
6.6	CONSIDERATION 3: GROUP-BASED VS HOME-BASED ADMINISTRATION.....	169
6.7	CONSIDERATION 4: STANDARDISATION OF ASSESSMENT BATTERIES.....	171
6.8	SPECIFIC RECOMMENDATIONS FOR THE DELIVERY OF CT IN THE PD POPULATION.....	173
6.9	THE FUTURE OF CT FOR PD.....	174

6.9.1	MULTIMODAL DELIVERY: CT + TRANSCRANIAL DIRECT-CURRENT STIMULATION (tDCS).....	174
6.9.2	MULTIMODAL DELIVERY: CT + EXERCISE	176
6.10	THE USE OF ADAPTIVE AND ASSISTIVE TECHNOLOGY TO DELIVER CT IN PD..	178
6.11	CONCLUSIONS.....	183
CHAPTER 7 DEVELOPMENT AND CO-DESIGN OF ORBIT: A NOVEL ‘SERIOUS GAMING’ SYSTEM TARGETING COGNITIVE IMPAIRMENT IN PARKINSON’S DISEASE		184
7.0	ABSTRACT.....	185
7.1	INTRODUCTION	186
7.2	PARTICIPANTS AND METHODS	190
7.2.1	PARTICIPANTS	190
7.2.2	THE ORBIT SYSTEM	191
7.2.3	CT GAMES CATALOGUE.....	192
7.2.3.1	<i>A Bridge Too Far</i>	193
7.2.3.2	<i>Farm Quest</i>	194
7.2.3.3	<i>Snake</i>	195
7.2.3.4	<i>Squirrel</i>	195
7.2.3.5	<i>Sunday Driver</i>	196
7.2.3.6	<i>Marine Life</i>	197
7.2.3.7	<i>Driving Maniac</i>	198
7.2.3.8	<i>Swimma</i>	199
7.2.3.9	<i>Whack-A-Mole</i>	199
7.2.3.10	<i>Munchkinis</i>	200
7.2.3.11	<i>Who Stole the Coin/Who’s the Boss?</i>	201
7.2.3.12	<i>Chow Time</i>	202
7.2.4	PROCEDURE	203
7.2.5	OUTCOME MEASURES.....	203
7.2.6	OUTCOME MEASURE INTERPRETATION AND STATISTICS	204
7.3	RESULTS	205
7.3.1	QUALITY OF LIFE AND DEPRESSION	206
7.3.2	GAME EXPERIENCE.....	207
7.3.3	SYSTEM FEEDBACK	207
7.3.3.1	<i>Session 1 - Initial feedback</i>	207
7.3.4	INDIVIDUAL GAME FEEDBACK	208
7.3.5	OVERALL USABILITY.....	210
7.3.6	FOLLOW-UP.....	211
7.4	DISCUSSION.....	212
7.4.1	SAMPLE POPULATION.....	212
7.4.2	INITIAL FEEDBACK ON THE ORBIT SYSTEM	213
7.4.3	FEEDBACK ON THE ORBIT SYSTEM FOLLOWING ONE WEEK OF EXPOSURE	215
7.4.3.1	<i>The Orby Controller</i>	215
7.4.3.2	<i>Gaming Suite Software</i>	217
7.4.4	IMPLEMENTATION OF PARTICIPANT FEEDBACK TO ALTER THE ORBIT SYSTEM	220
7.4.4.1	<i>Ergonomics of the controller setup</i>	221
7.4.4.2	<i>Modulation of task difficulty</i>	222
7.4.4.3	<i>Improved clarity of instructions</i>	223
7.4.4.4	<i>Change to game objectives</i>	225
7.4.4.5	<i>Improved controller sensitivity/responsiveness</i>	227
 CHAPTER 8 GENERAL DISCUSSION		230
8.0	INTRODUCTION	231
8.1	PHARMACOLOGICAL INTERVENTION	232

8.1.1	FYN IN PARKINSON’S DISEASE: POTENTIAL IMPLICATIONS FOR COGNITIVE IMPAIRMENT	234
8.1.2	AZD0530 (SARACATINIB) AS A NOVEL THERAPEUTIC STRATEGY FOR PD	238
8.2	NON-PHARMACOLOGICAL INTERVENTION	242
8.2.1	COGNITIVE TRAINING IN PD	243
8.2.2	ORBIT DEVELOPMENT	248
8.3	CONCLUSION	252
CHAPTER 9 REFERENCE LIST		256
CHAPTER 10 APPENDICES		378

Abstract

Whilst Parkinson's disease (PD) is primarily characterised as a motor disorder, non-motor symptoms represent a significant burden to the PD population. These symptoms include anxiety, depression, fatigue and cognitive impairment (CI), with CI representing the single biggest predictor of quality of life, mortality and caregiver burden for PD patients. Impairments range from mild-severe and dementia and involve dysfunction in domains such as learning, memory, response inhibition, visuospatial function and executive function. Unfortunately, current treatments for CI in PD provide variable symptomatic relief but do nothing to alter disease progression. To this end, the aim of this thesis is to explore novel pharmacological and non-pharmacological treatment strategies for cognitive dysfunction in PD. The first part of the thesis will investigate the role of a potential novel pathological target, Fyn kinase, in the pathophysiology of neurological disease, examine its distribution in key anatomical regions of the PD brain, and evaluate the potential of inhibition of this target for the treatment of cognitive impairment in PD. The second part of this thesis will instead turn to a non-pharmacological strategy, exploring the utility of cognitive training (CT) in PD and engaging in a co-design process for a "Serious Games" based strategy to deliver CT in this population.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Contributions made by co-authors

The following people have contributed to authorship of the manuscripts enclosed in this thesis (in alphabetical order): Lyndsey Collins-Praino, Laura Carr, Frances Corrigan, Lisa Drew, Simon Drum, Ben Ellul, David Hobbs, Sanam Mustafa, Kavi Sivasankar, Emma Thornton and Bradley Wesson. The individual contributions of each author can be summarised as:

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Thesis explanation

All chapters are prepared in publication format. Chapter 1 is a general introduction to cognitive impairment in Parkinson's and a review of current treatment strategies, Chapter 2 states the doctoral aims and hypotheses. Chapter 3 is a review of Fyn kinase and its role in the pathophysiology of neurodegenerative disease. Chapters 4 and 5 are experimental chapters investigating the anatomical distribution of Fyn kinase in the human PD brain (Chapter 4) and the potential therapeutic efficacy of Fyn kinase inhibition in a pre-clinical model of PD (Chapter 5). Chapter 6 is a critical review of the literature on the use of CT for the treatment of CT in PD, concluding with specific recommendations for use of CT in this population. Chapter 7 is an experimental chapter describing the reiterative co-design process for the development of a novel CT serious gaming program, OrbIT. Finally, Chapter 8 presents a discussion of the work presented in this thesis as a whole. References and appendices follow.

Chapters that have been prepared in publication format are prefaced by a signed statement of authorship.

Abbreviations

5-HT	5-hydroxytryptamine
6-OHDA	6 hydroxy-dopamine
A β	amyloid-beta
α -syn	alpha-synuclein
AD	Alzheimer's disease
ADL	activities of daily living
ALS	amyotrophic lateral sclerosis
APP	amyloid precursor protein
APT-II	Attention Process Training II
AST	adjusting step test
BDNF	brain-derived neurotrophic factor
BG	basal ganglia
CI	cognitive impairment
CNS	central nervous system
COMT	catechol-o-methyl transferase
CoRe	Cognitive Rehabilitation System
CT	cognitive training
DA	dopamine
DALY	disability adjusted life years
DAMP	danger associated molecular patterns
DAT	dopamine transporter
DCC	deleted in colorectal cancer
DLPFC	dorsolateral prefrontal cortex
DMN	default mode network
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EPM	elevated plus maze
Erk	extracellular signal-regulated kinases
fMRI	functional magnetic resonance imaging
GTPase	guanosine triphosphatase
Hcy	homocysteine

IFN γ	interferon gamma
IL	interleukin
iNOS	inducible nitric oxide synthase
KD	ketogenic diet
LC	locus coeruleus
L-dopa	Levodopa
LID	Levodopa-induced dyskinesia
LTP	long-term potentiation
LDP	long-term depression
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
MBP	myelin basic protein
MCI	mild cognitive impairment
MD	Mediterranean diet
MoCA	Montreal Cognitive Assessment
MMSE	Mini-Mental State Exam
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
MS	multiple sclerosis
MTL	medial temporal lobe
NHS	normal horse serum
NLRP3	Nod-Like Receptor Protein 3
NMDA	N-Methyl-D-Aspartic
NFT's	neurofibrillary tangles
OPC	oligodendrocyte precursor cells
ORT	object recognition test
PBS	phosphate buffered solution
PD	Parkinson's disease
PD-D	Parkinson's disease dementia
PD-MCI	Parkinson's disease mild cognitive impairment
PDGF	platelet derived growth factor
PKC δ	protein kinase C delta
PrPc	prion protein

QoL	quality of life
QKI	quaking protein
RPM	revolutions per minute
rTMS	repetitive transcranial magnetic stimulation
SFK	SRC family kinase
SH	SRC homology
SOPT	speed of processing training
SN	substantia nigra
SNpc	substantia nigra <i>pars compacta</i>
SP	synaptic plasticity
SSRI	serotonin re-uptake inhibitor
STEP	striatal-enriched tyrosine phosphatase
SVZ	subventricular zone
TBI	traumatic brain injury
Th17	t-helper 17
TNF α	tumour necrosis factor alpha
tDCS	transcranial Direct-Current Stimulation
UPDRS	Unified Parkinson's Disease Rating Scale
VR	virtual reality
VTA	ventral tegmental area

List of Figures

Figure 1.1 Overview of the dopaminergic and functional striatal pathways.....	11
Figure 1.2 Overview of the direct and indirect pathways of the basal ganglia.	13
Figure 3.1 Schematic representation of Fyn.....	52
Figure 3.2 Overview of the role of Fyn in physiological processes associated with myelination and T-cell mediated inflammation.	55
Figure 3.3 Overview of the role of Fyn in physiological and pathological processes in Alzheimer's Disease.....	62
Figure 3.4. Overview of the role of Fyn in microglia under inflammatory conditions.....	70
Figure 3.5. Overview of Fyn dysfunction in neurodegenerative diseases and potential commonalities.....	76
Figure 4.1. Fyn levels as measured by Fyn Western Blot in the (A) Prefrontal (n=4/7) (B) Hippocampus (n=5/8) (C) Striatum (n=4/8) and (D) Substantia Nigra (n=5/8).....	89
Figure 4.2. Phosphorylated to Pan Fyn Ratio as measured by ELISA in the (A) Prefrontal (n=4/7) (B) Hippocampus (n=5/8) (C) Striatum (n=5/8) and (D) Substantia Nigra (n=4/8).	90
Figure 4.3. Graphs and representative images of glial fibrillary acidic protein (GFAP) staining in the (A) prefrontal (n=6/10) (B) hippocampus (n=7/10) (C) striatum (n=7/9) and (D) substantia nigra (n=7/9).....	92
Figure 4.5. NeuN expression as examined via Western Blot in the (A) prefrontal (n=5/7) (B) hippocampus (n=5/8) (C) striatum (n=5/7) and (D) substantia nigra (n=5/8).....	95
Figure 5.1. Overview of Experimental Design Timeline	109
Figure 5.2.....	118
Figure 5.3. Neuropsychiatric outcomes.....	121
Figure 5.4. Cognitive outcomes.....	122
Figure 5.5. Representative images of TH staining within the striatum (A-D) of Sham (A), Vehicle (B), 6mg/kg (C) and 12mg/kg (D), as well as respective graph (E).....	124
Figure 5.6. Representative images of IBA1 staining within the striatum (A-D) of Sham (A), Vehicle (B), 6mg/kg (C) and 12mg/kg (D), as well as respective graph (E).	125
Figure 5.7. Representative images of morphological appearance of IBA1 staining of (A) resting, (B) activated and (C) amoeboid.....	126
Figure 5.8. Astrocytic protein levels assessed by GFAP reactivity.....	126
Figure 7.1. Orby controller and set up.....	192
Figure 7.2. A Bridge Too Far	194
Figure 7.3 Farm Quest.....	195
Figure 7.4. Snake.....	195

Figure 7.5. Squirrel.....	196
Figure 7.6. Sunday Driver.	197
Figure 7.7. Marine Life.	198
Figure 7.8. Driving Maniac.	198
Figure 7.9. Swimma.	199
Figure 7.10. Whack-A-Mole.	200
Figure 7.11 Munchkinis.....	201
Figure 7.12 Who Stole the Coin?/Who’s the Boss?.....	202
Figure 7.13 Chow Time.....	202
Figure 7.14 Images depicting variations in controller grip (A) one handed, (B) upper holder, (C) lower hold, (D) fingertip and (E) bear grip.	216
Figure 7.15 Orby controller set-up - Featuring addition of a grip mat and straps.....	221
Figure 7.16 A Bridge Too Far	223
Figure 7.17 Sunday Driver.	224
Implementation of instructions triggered by activity during gameplay in Sunday Driver.....	224
Figure 7.18 Farm Quest.....	225
Implementation of a voluntary ‘hint’ feature in Farm Quest.....	225

List of Tables

Table 1.1 Neuropsychological deficits in Parkinson’s Disease Cognitive Impairment and Parkinson’s Disease Dementia	3
Table 4.1. Clinicopathological Data for controls and PD cases	85
Table 4.2. HALO Microglial Detection Parameter Summary.....	87
Table 6.1: Study methodology details of Cognitive training in Parkinson’s disease.....	143
Table 6.2 Summary of Cognitive training in Parkinson’s disease study outcomes	157
Table 7.1 Overview of primary and supplementary cognitive domains trained in gaming suite...	193
Table 7.2 Demographic and patient data ($n=13$). $Mean \pm SD$	205
Table 7.3 WHO-QOL-BREF, GDS and PDQ-39 ($n=12$). $Mean \pm SD$	206
Table 7.4. Session 1 – Initial feedback. $\pm = SD$	208
Table 7.5 Individual Game Feedback.....	209
Table 7.6. Results of the System Usability Scale Questionnaire. Scores are based on a scale of 1-5 (1=Strongly Disagree, 5 = Strongly Agree). $\pm = SD$	210
Table 7.7. Results of a Follow-Up Survey.	211

Style Conventions

The abbreviations, punctuations and reference style used in this thesis conform with the *Oxford style manual* and *Harvard referencing style*. The spelling is British English and conforms with the *Oxford English Dictionary*.

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01

General Introduction

1.0 INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD) and the fastest growing neurological disorder, affecting 1 in every 350 Australians and more than 6.1 million people globally in 2016 (Parkinson's, 2011, Feigin et al., 2019). From 1990 to 2016, prevalence, disability adjusted life years (DALYs) and death rates of the disease all increased, with a global burden which has more than doubled (Dorsey et al., 2018). Age is the biggest risk factor for PD (Reeve et al., 2014) and, in part due to the ageing population, prevalence is further projected to more than double by the year 2030 (Dorsey et al., 2007).

Clinically, the cardinal motor symptoms include muscle rigidity, akinesia, resting tremor, bradykinesia and postural/gait changes (Barnum and Tansey, 2012). In addition, PD patients also experience non-motor symptoms, such as anxiety, depression, fatigue and cognitive impairments (Barnum and Tansey, 2012). Cognitive impairment is a disabling comorbidity in PD, representing the single biggest predictor of quality of life (QoL), mortality and caregiver burden (Duncan et al., 2014). Despite the prevalence and debilitating nature of these impairments, treatment options are limited, and efficacy is significantly variable within the population (Goldman and Weintraub, 2015). Currently, the most commonly used strategy targeting cognitive impairment in PD are the cholinesterase inhibitors used for AD, which may paradoxically worsen motor deficits (Collins-Praino et al., 2011). Accordingly, new therapeutic strategies to address this gap are imperative. Attempts to develop such treatments have, however, been limited due to an incomplete understanding of the mechanisms underpinning cognitive impairment in PD. This review will outline the nature of these impairments, including structural and functional changes observed, and discuss the current literature regarding both pharmacological and non-pharmacological interventions and their efficacy in addressing cognitive impairment in PD.

1.1 COGNITIVE IMPAIRMENT IN PARKINSON'S DISEASE

1.1.1 Evolution and Presentation of Cognitive Impairment in PD

Cognitive impairment in PD can range from mild cognitive impairment (PD-MCI) to Parkinson's disease dementia (PD-D). Impairments manifest in multiple domains, including executive function, attention, learning, memory, speed of processing, response inhibition and visuospatial functioning, with not all domains equally affected, particularly early on in the disease course (see Table 1.1 for summary) (Williams-Gray et al., 2009, Kehagia et al., 2010, O'Callaghan and Lewis, 2017).

Table 1.1 Neuropsychological deficits in Parkinson's Disease Cognitive Impairment and Parkinson's Disease Dementia

Neuropsychological Deficits	PD-CI/MCI	PD Dementia
Planning	+	+++
Attention	+	+++
Working memory	+	+++
Set-shifting	+	+++
Response inhibition	+	++
Reinforcement learning	+	++
Visuospatial	-	+++
Memory	-	++
Semantic fluency	-	++

Extent of deficits; + mild, ++ moderate, +++ severe, - absent. *Table adapted from O'Callaghan 2017*

Early in the pathogenesis of PD, cognitive changes occur in many individuals, although they are usually subtle and detectable only with formal neuropsychological testing (Levin and Katzen, 2005). Newly diagnosed, untreated PD patients demonstrate impairments on tests of immediate verbal recall, language production/semantic fluency, set formation, cognitive

sequencing, working memory and visuomotor construction compared to healthy, non-demented controls (Cooper et al., 1991). These cognitive deficits are estimated to occur in 20-40% of individuals early in PD, although they are often overshadowed by motor features (Benito-Leon et al., 2011, Elgh et al., 2009, Foltynie et al., 2004, Muslimovic et al., 2005, Williams-Gray et al., 2009). In a population-based case-control study of individuals with early PD (<5 years duration), subjective memory complaints were present in 59% of PD patients, compared to 37% of controls (Benito-Leon et al., 2011). Similarly, in a longitudinal population-based study, 30% of patients were impaired in one or more cognitive domains (episodic memory, executive function and verbal function) (Elgh et al., 2009), indicating cognitive dysfunction may already be a source of considerable concern for PD patients at time of diagnosis.

Many individuals are also at increased risk of progression to PD-MCI. In a meta-analysis involving over 1,346 PD patients, the prevalence of MCI was 26% (Aarsland et al., 2010). The Movement Disorder Society criteria for diagnosis of MCI in PD (Litvan et al., 2012) includes (1) a diagnosis of PD as based on United Kingdom Brain Bank criteria; (2) gradual decline in cognitive ability in the context of established PD, as reported by the individual/informant or by the clinician; (3) cognitive deficits on formal neuropsychological testing or a scale of global cognitive abilities; and (4) cognitive deficits that do not significantly interfere with functional independence, although subtle difficulties on complex functional tasks may be present (Litvan et al., 2012). These deficits must occur independently of a diagnosis of PD-D, another explanation for the cognitive impairment (e.g., stroke or delirium), or another PD-associated co-morbid condition that could influence cognitive performance (e.g., depression, anxiety or excessive daytime sleepiness)(Litvan et al., 2012). At time of PD diagnosis, 15-40% of patients already meet criteria for diagnosis of PD-MCI (Aarsland, 2016, Monastero et al., 2018). By 3-5 years post-diagnosis, 20%-

57% of individuals qualify for a diagnosis (Caviness et al., 2007, Williams-Gray et al., 2007).

The clinical presentation of PD-MCI can be heterogeneous, leading to the proposal of multiple sub-types of MCI (Janvin et al., 2006). Kalbe and colleagues examined 269 patients with PD-MCI and concluded that 39.4% suffered from non-amnesic single domain, 30.5% from amnesic multiple domains, 23.4% from non-amnesic multiple domain and 6.7% amnesic single domain (Kalbe et al., 2016). Despite this heterogeneity, of all cognitive functions, deficits in executive function (e.g., impairment in the ability to plan and to inhibit behaviours or deficits in attention and working memory) are most commonly seen in PD-MCI (Kalbe et al., 2016) and can severely impact an individual's ability to carry out activities of daily living (ADLs) (Pagonabarraga and Kulisevsky, 2012). These cognitive changes resemble those seen in patients with frontal lobe lesions, and may be concomitant with dopaminergic fronto-striatal function (Owen et al., 1992). Interestingly, MCI can also be a fluctuating state, with a reported 9-28% of PD-MCI patients reverting to a state of normal cognitive function in a 5-year study (Pedersen et al., 2017), however; a recent meta-analysis found, of those with PD-MCI, 20% converted to PD-D with-in 3 years. This increased to 34% with follow-up >3 years (Saredakis et al., 2019). In a prospective population-based study of 224 PD patients, Aarsland and colleagues (2003) found that 78% of individuals with PD developed dementia after 8 years of follow-up, a prevalence that was nearly three times that of the non-PD group (Aarsland et al., 2003). Furthermore, within 20 years of diagnosis, over 80% of individuals with PD are reported to progress to PD-D (Hely et al., 2008).

According to Movement Disorder Society criteria, PD-D consists of: (1) a diagnosis of PD according to Queen Square Brain Bank criteria and (2) a diagnosed dementia syndrome, developing within the context of established PD, that leads to impairment in more

than one cognitive domain, represents a decline from premorbid level and causes deficits in ADL (e.g. social, occupational or personal care) (Emre et al., 2007). PD-D includes both cognitive features (e.g., impairments in attention, executive function, visuospatial function and memory), and behavioural features (e.g., apathy, changes in personality and mood, hallucinations, delusions and excessive daytime sleepiness) (Emre et al., 2007). A number of risk factors are known to be associated with the development of PD-D, including higher age, motor symptom severity and the presence of mild cognitive impairment at baseline (Aarsland and Kurz, 2010).

Individuals with PD carry six times the risk of dementia compared to the general population (Aarsland et al., 2001). Patients suffering from specific subtypes of PD are also more at risk for dementia than others. While patients with tremor-dominant PD are relatively spared from dementia development, patients of the akinetic/rigid subtype with akinetic-rigid PD have been shown to be at increased risk of cognitive and neuropsychiatric impairments, including working memory, executive function and depression (Aarsland et al., 2003, Alves et al., 2006, Burn et al., 2006, Karunanayaka et al., 2016, Williams-Gray et al., 2007, Weintraub et al., 2010a, Kelly et al., 2015). A recent study comparing cognitive decline in the 2 subtypes reported worsening in working memory, verbal fluency, attention, and visuospatial abilities with akinetic-rigid PD compared to tremor-dominant PD (Wojtala et al., 2019). Furthermore, akinetic-rigid PD is also associated with more rapid cognitive decline and development of dementia (Alves et al., 2006). This has led to key questions about the pathophysiological changes that may drive the emergence and progression of cognitive impairment in PD.

1.2 PATHOPHYSIOLOGY OF COGNITIVE IMPAIRMENT IN PD

Pathologically, PD is characterised by the presence of Lewy Bodies (LB), intracellular aggregates of alpha-synuclein (α -syn) and ubiquitin, and the loss of dopaminergic (DA) neurons in the substantia nigra (SN) *pars compacta* (pc) (Pollanen et al., 1993). Although risk factors such as age, family history and pesticide exposure have been identified, it is unclear exactly what leads to the development of PD (Noyce et al., 2012). The following will explore the contribution of these pathologies to the presentation of cognitive impairment in PD.

1.2.1 *Pathological Protein Aggregation*

Under normal physiological conditions, α -syn is an intracellular protein predominantly localised within the nucleus and presynaptic terminals (Maroteaux et al., 1988, Bendor et al., 2013). Mechanistically, α -syn is necessary for compartmentalisation, storage and recycling of neurotransmitters (Allen Reish and Standaert, 2015). In PD, for reasons yet unknown, disordered α -syn monomers begin to misfold and form oligomers, which in turn aggregate, forming LBs in the soma of affected neurons resulting in synaptic dysfunction and cell death (Meade et al., 2019). Some neuronal populations are more vulnerable than others to LB pathology, with DA neurons in the midbrain particularly susceptible (Halliday et al., 2005, Mori et al., 2002).

In 2003, Heiko Braak and colleagues developed a highly influential theory proposing that the progression of α -syn/LB spread may actually begin in the medulla, causing autonomic and olfactory disturbances (stage I-II). As LBs spread up the brainstem, affecting midbrain nuclei such as the SN, this coincides with the appearance of sleep and motor disturbances (stage III). LB pathology then spreads to the thalamus and hippocampus (stage

VI), followed by widespread deposition in high order cortical areas (stage V-VI) (Braak et al., 2003, Braak et al., 2004). Moreover, increasing evidence suggests α -syn in the brain may be derived from the periphery, with α -syn pathogenic species shown to present in the enteric nervous system (ENS) (Fitzgerald et al., 2019). Given the bi-directional communication between the ENS and CNS, the gastrointestinal system is suggested to serve as a conduit for transmission of pathological proteins such as α -syn from the gut to the brain (Chalazonitis and Rao, 2018). Given the heterogeneity observed in PD patients, however, there appears to be considerable variation with regards to conformity of pathological progression (Zaccai et al., 2008, Kalaitzakis et al., 2008), suggesting both propagation and aggregation of α -syn in the CNS is pivotal to disease genesis and symptomology (Xu and Pu, 2016).

Given the prevalence of cognitive impairment in PD, a growing number of studies have looked at the relationship between α -syn pathology and cognition. Recent evidence suggests that the presence of α -syn in the plasma of PD patients may serve as a predictor of cognitive decline, with a 2017 study demonstrating an increase in α -syn plasma levels in patients with PD-D compared to those with normal cognition (Lin et al., 2017). Interestingly, α -syn levels correlated with poorer cognitive function (as assessed by the Mini-Mental State Exam (MMSE)), but not degree of motor impairment. This finding was, however, not observed in the PD-MCI cohort (Lin et al., 2017). This suggested the magnitude of α -syn may be indicative of the extent of cognitive deficit. In support of these findings, increased levels of α -syn have recently been shown to be a significant predictor of cognitive impairment, as measured using the MMSE and the Clinical Dementia Rating scale, in PD, further enabling the distinction between participants with normal cognition and PD patients with cognitive impairment (Chen et al., 2020).

While the exact mechanisms that link α -syn to cognitive impairment are still unknown, it was initially postulated that the aforementioned progression of LB pathology into areas of the brain critical for cognitive function may be responsible (Kim et al., 2014, Dickson et al., 2009). Braak and colleagues (2005) reported that one-third of patients with sporadic PD at stage III of the Braak neuropathological staging already showed significant cognitive decline, even though α -syn aggregations are not yet present in the cortex during this early pathological stage. This number rose to two-thirds of the cohort at stage IV, was greater than 90% at stage V and reached 100% at stage VI, suggesting that the spread of LB into areas of the cortex critical for cognition may be driving the emergence of impairment (Braak et al., 2005). Interestingly, however, the combined results of two studies investigating the relationship between LB stage and cognitive impairment revealed only 3 of 16 PD-MCI participants demonstrated stage IV neocortical spread (Adler et al., 2010, Jellinger, 2010). Interpretation of this is limited, however, by the small number of PD-MCI cases investigated to date. Evidence is more promising in PD-D, where LB related pathology appears to be an important factor in the development and presentation of dementia in PD, with studies demonstrating the presence and severity of cortical LB pathology to be the most correlated with cognitive impairment in PD-D (Braak et al., 1996, Dickson et al., 2009). Furthermore, neuroanatomical location of LB pathology was also important, with a study demonstrating increased odds of dementia and more rapid decline was associated with neocortical distribution of LB, whereas distribution in limbic structures was associated with dysfunction in visuospatial skills (Schneider et al., 2012). Despite these findings, however, the link between LB pathology and risk of cognitive impairment, particularly mild CI, appears to be highly variable (Adler et al., 2010, Jellinger, 2010, Goldman et al., 2014). This may reflect the inherent pathophysiological heterogeneity of PD and is likely to reflect a relationship between CI and other neuropathologies observed in the disease.

Other pathologically aggregated proteins, such as amyloid beta ($A\beta$) and tau, have consequently been shown to play a role in CI in PD. In a study of individuals with PD, PD-D and PD with cognitive impairment, not dementia, 1/3 of individuals with PD with cognitive impairment and 50% of individuals with PD-D had the same biomarker signature of CSF levels of amyloid beta 42 ($A\beta_{42}$), total tau and phospho-tau 181 as observed in individuals with Alzheimer's disease (Montine et al., 2010). Similarly, pathological inversion of the microtubule associated protein tau (*MAPT*) gene has been shown to influence the development of cognitive impairment and dementia in PD (Goris et al., 2007). Interestingly, however, not all literature is consistent. Several previous positron emission tomography (PET) imaging studies have failed to find evidence for significant amyloid or tau pathology in individuals with PD-MCI compared to cognitively healthy individuals with PD (Gomperts et al., 2016, Winer et al., 2018, Lee et al., 2018). This is consistent with the results of a recent study that reported no relationship between serum levels of either $A\beta_{42}$ or tau and performance on a series of neuropsychological tests (Chojdak-Lukasiewicz et al., 2020). Thus, factors beyond pathological protein aggregation alone may be responsible for the emergence of cognitive impairment in PD. One such factor may be dysfunction in dopaminergic signalling pathways.

1.2.2 Dopamine and the Dopaminergic Pathways

1.2.2.1 Dopamine Pathways and the Basal Ganglia

DA is a modulatory neurotransmitter best known for its facilitation in the selection of movement. DA exerts its effect by binding to high affinity dopamine receptors, with 5 dopamine receptors identified in the CNS, termed D1 to D5 respectively (Thal et al., 2018). Upon receptor binding, in addition to motor function, DA is also involved in cognitive processes, such as long-term potentiation (LTP) and synaptic plasticity (SP) (i.e. the

strengthening of synapses over time in response to increases in activity), processes which underpin learning and memory (Centonze et al., 2001). Other cognitive functions, such as attention and decision making (Calabresi et al., 2013), are also regulated by DA signalling. In order to understand the implications of DA loss for cognitive function in PD, it is first necessary to understand the role of DA in normal brain physiology.

The SN and the ventral tegmental area (VTA) are midbrain structures which house the largest group of DA-producing neurons in the brain (Lanciego et al., 2012). Functionally, DA projections from these regions include two main pathways; the nigrostriatal and mesocorticolimbic (further subdivided into the mesolimbic and mesocortical) (Figure 1.1).

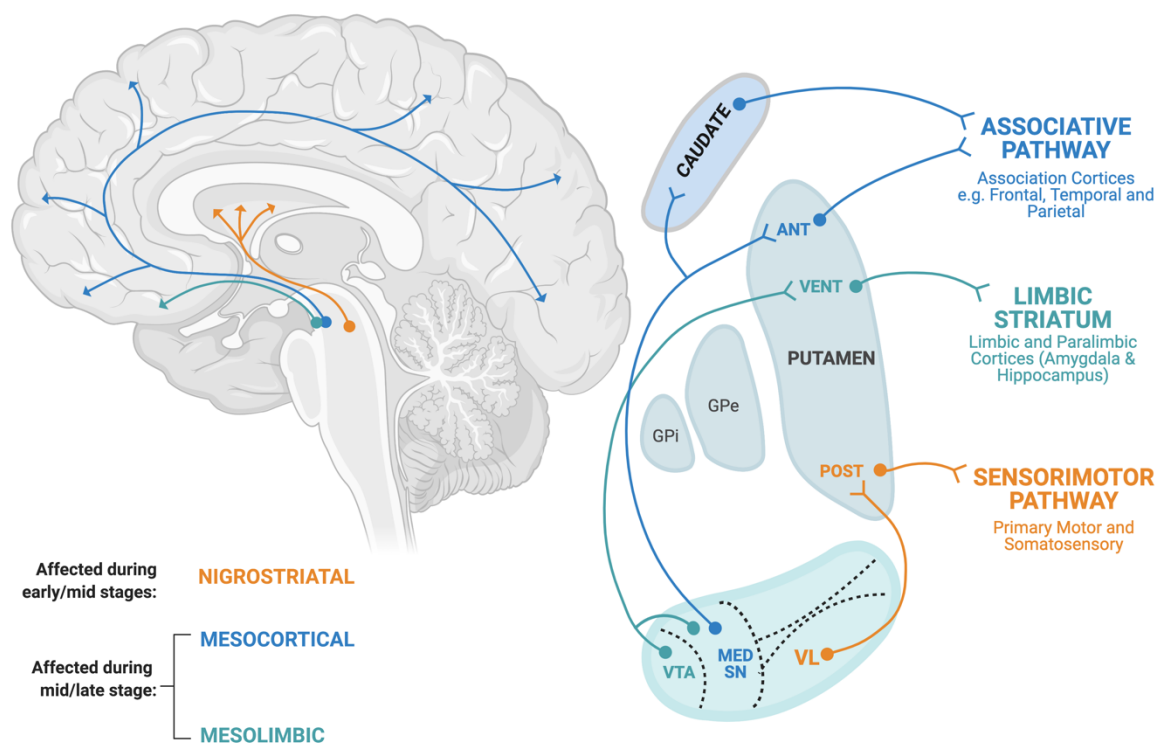


Figure 1.1 Overview of the dopaminergic and functional striatal pathways. ANT – anterior putamen, GPi – globus pallidus interna, GPe – globus pallidus externa, MED SN – medial substantia nigra, POST – posterior putamen, VENT – ventral putamen, VL – venterolateral SN, VTA – ventral tegmental area

The nigrostriatal pathway projects from the SN pars compacta (SNpc) to the dorsal striatum (caudate and putamen) and is primarily involved in motor function (Dickson et al., 2009). In terms of the mesocorticolimbic pathways, the mesolimbic pathway projects from the medial SN and VTA to limbic structures, such as the nucleus accumbens and hippocampus, and is associated with emotional regulation, behavioural selection, impulsivity and reward behaviours (Wise, 2004, Fields et al., 2007, Wise, 2000). DA neurons of the VTA primarily project to the cortex, particularly the prefrontal cortex, to form the mesocortical pathway which is primarily associated with cognitive functions such as motivation and executive function (Lapish et al., 2007, Morales and Margolis, 2017, Lammel et al., 2014). In PD, the nigrostriatal pathway appears to be particularly vulnerable, whereas DA neurons of the VTA, which ultimately innervate the mesocorticolimbic pathways, exhibit reduced vulnerability (Koltun et al., 2018). Despite this, however, a recent review concluded unequivocal degeneration of the DA neurons in the VTA in PD (Alberico et al., 2015). Although a direct relationship between VTA degeneration and cognitive decline in PD remains unclear, observations of changes to the mesocorticolimbic pathway have been strongly implicated in cognitive impairment in PD, likely associated with the extensive connections to frontal brain regions (Kish et al., 1988, Rosvold, 1972, Swanson et al., 2000, Cools, 2001).

The DA pathways projecting from the SN and the VTA to the striatum and cortical regions together form a series of pathways termed the cortico-basal ganglia-thalamo-cortical loop (Haber, 2014). The Basal Ganglia (BG) part of this loop involves a series of intricate connections between specific nuclei (Hornykiewicz, 2002). These nuclei comprise of the putamen, caudate (neo-striatum) globus pallidus (interna and externa) and subthalamic nucleus, which subsequently innervate 2 main functional pathways: the direct and indirect.

The direct pathway *promotes* the execution of planned motor action by exciting cortical neurons. Conversely, the indirect pathway *inhibits* unwanted motor action by inhibiting cortical neurons (Gerfen and Surmeier, 2011) (pathways summarised in Figure 1.2). In PD, the loss of DA neurons results in reduced binding and activation of D1 and D2 receptors, intrinsically involved in the regulation of the direct (D1) and indirect (D2) pathways. DA binds with a higher affinity to D2 receptors, which predominantly regulate activity of the indirect pathway. Consequently, loss of DA preferentially impairs the direct pathway due to lower affinity of DA to D1, leading to a favouring of the indirect (inhibitory) pathway (Gerfen et al., 1990, Albin et al., 1995) (Helmich et al., 2012).

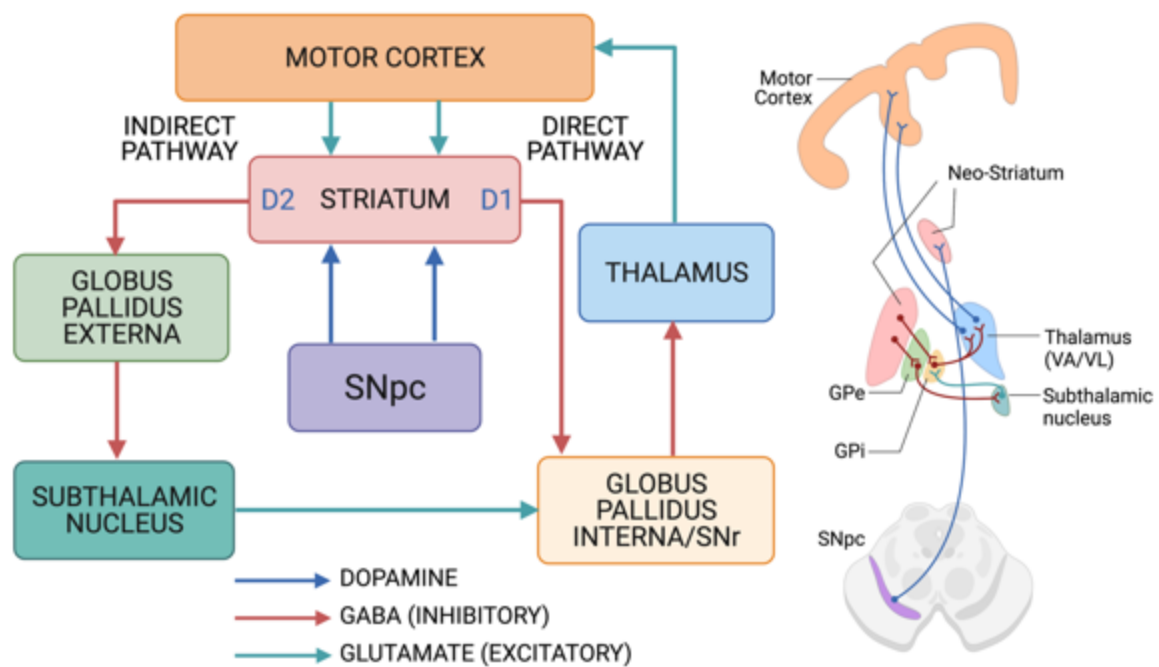


Figure 1.2 Overview of the direct and indirect pathways of the basal ganglia. D1 receptors in the direct pathway are excitatory and, in conjunction with excitatory input from the cortex, release the thalamus from inhibition, allowing excitation of the motor cortex and the subsequent facilitation of motor plan selection (Helmich et al., 2012). On the other hand, D2 receptors are inhibitory, working to counteract the excitation from the cortex. Consequently, the activation of D2 receptors works to refine movement by preventing the execution of motor plans that are irrelevant for the current task (Helmich et al., 2012). In a healthy individual, these pathways are balanced for smooth execution of movement. In PD, a loss of the DA neurons in the SNpc results in a loss of binding to D1 and D2

receptors, leading to an imbalance of the pathways in favour of the inhibitory indirect pathway. GPi – internal globus pallidus, GPe – external globus pallidus, SNpc– substantia nigra pars compacta, SNr– substantia nigra reticulata, VA – ventral anterior nucleus, VL – venterolateral nucleus,

Degeneration of DA neurons in the SNpc ultimately compromises the integrity of the nigrostriatal/sensorimotor pathways, with significant functional consequences for individuals affected (Cheng et al., 2010). Whilst primarily associated with sensory and motor deficits, this has implications for cognitive function, particularly relating to the role of the BG in executive functions (Robbins and Arnsten, 2009, Robbins and Cools, 2014). For example, the shift of balance towards the indirect pathway is linked to cognitive dysfunction in reinforcement learning, with PD patients impaired in cognitive tasks which require learning from positive feedback and overly proficient at tasks which require learning from negative feedback (Knowlton et al., 1996, Shohamy et al., 2004, Ashby et al., 1998). Mechanistically, during positive reinforcement learning, phasic bursts of DA increase SP to stimulate the learning of rewarding behaviours via the direct pathway; conversely, dips in phasic DA bursts in the indirect pathway facilitate learning from negatively feedback (Frank et al., 2004). Thus, in PD, reductions in DA impairs positive feedback learning in the direct pathway, while enhancing the ability to learn from negative consequences mediated by the indirect pathway. As positive motivation is an important feature of reinforcement learning, motivational deficit and shift towards negative reinforcement in PD patients increases the likelihood of depression, apathy, and reduced likelihood for novelty seeking behaviour (Foerde et al., 2015, Niv et al., 2006). Although PD is characterised by the loss of DA neurons in the SNpc, recent evidence suggests the primary site of neurodegeneration may in fact be the striatum, with studies in early PD identifying more severe degeneration of DA striatal axon terminals than SNpc cell bodies (Kordower et al., 2013, Fazio et al., 2018).

1.2.2.2 Striatal Loops of the Basal Ganglia

The striatum is the largest component and primary afferent structure of the BG. Anatomically, the striatum is linked to several cortical regions crucial in cognition via the BG neurocircuitry (Macpherson and Hikida, 2019). Functionally, the striatum can be subdivided into three circuits the associative, sensorimotor and limbic striatum (Figure 1.2). The **associative striatum** includes projections from the medial SN to the caudate and anterior putamen, which are connected to various association areas in the frontal, temporal and parietal lobes. The **sensorimotor striatum** involves projections from the venterolateral SN to the posterior putamen, which projects to the primary motor and somatosensory cortices. Finally, the **limbic striatum** connects the medial SN and VTA to the ventral striatum, which then projects to limbic and paralimbic structures, the amygdala and the hippocampus (Chung et al., 2018). A 2018 study found striatal DAT availability was closely linked to cognitive deficits in patients with early-stage PD, with DAT availability in the caudate and anterior putamen (associative striatum) and ventral striatum (limbic striatum) positively associated with cognitive functions in multiple domains, including attention/working memory, frontal/executive function, visuospatial function, memory and language (Chung et al., 2018). Accordingly, a decrease in DAT availability in the associative and limbic striatum was more prominent in PD-MCI than PD patients with normal cognition (Chung et al., 2018).

Changes in fronto-striatal circuitry are amongst the most prolific in PD and are directly linked to executive dysfunctions, including impairments in planning, working memory and attentional set-shifting (Robbins and Cools, 2014). Specifically, goal-directed behaviour involves connections between the ventral striatum and medial orbitofrontal cortex, attentional set-shift involves connections between the ventral and dorsal striatum and the dorsolateral prefrontal cortex (DLPFC), and working memory between the dorsal

striatum and DLPFC (O'Callaghan et al., 2014, Morris et al., 2016, Leh et al., 2010). Studies have observed reductions in the associative fronto-striatal loop when performing attentional set-shift tasks in PD patients with CI, but not those without (Nagano-Saito et al., 2014).

Deficits in encoding and retrieval in PD have also been related to dysfunction in frontal circuitry (Drag et al., 2009). In terms of their relationship to disease progression, despite the dominance of nigrostriatal motor deficits, executive function and working memory impairments associated with the dorsolateral pre-frontal cortex (DLPFC) are also known to be affected early in the disease course (Miah et al., 2012). Deficits in memory such as learning and delayed recall also known to be impaired in early disease stages, however, recognition memory is believed to be affected in later stages (Segura et al., 2013).

Cortical regions implicated in the pathogenesis of PD have been reinforced with investigations using *in vivo* neuroimaging (magnetic resonance imaging (MRI) and PET) (Niethammer et al., 2012). Structural neuroimaging studies have revealed changes in volume and cortical thickness in various brain regions, with changes to striatal volume observed specifically in patients with PD-MCI (Apostolova et al., 2010, Hanganu et al., 2014, Melzer et al., 2012). Furthermore, grey matter atrophy in PD does not appear to be impacted in cognitively intact PD patients compared to controls despite a marked reduction in volume was observed in PD-MCI in the temporal, parietal and frontal cortex and the bilateral caudal hippocampus, amygdala and right putamen (Melzer et al., 2012). Grey matter loss was even more significant in the PD-D population, in addition to deterioration in the intracalcarine and lingual gyri, posterior cingulate gyrus, frontal regions and bilateral caudate, with a correlation between grey matter loss and global cognitive scores (Melzer et al., 2012). Functional imaging (fMRI) studies have revealed cognitive domains directly influenced by PD, with under-recruitment of the dorsal and prefrontal regions (Segura et al., 2013) during

tasks of working memory (Ekman et al., 2012, Lewis et al., 2003) and planning observed (Dagher et al., 2001), Karunanayaka and colleagues (2016) further demonstrated that patients of the akinetic/rigid subtype had decreased activity in both the left inferior parietal cortex and the left posterior cingulate cortex, two nodes of the default mode network (DMN), a key resting state network in cognitive processing (Raichle et al., 2001), compared to either healthy controls or tremor-dominant PD patients (Karunanayaka et al., 2016). Compared to tremor-dominant patients, reduced DA projections from the ventrolateral SNpc to the dorsal striatum have been observed in akinetic/rigid patients, a pathway associated with inhibitory control and impulsivity (Eggers et al., 2011). More recently, a long-term study over 4 years identified worse symptoms in akinetic/rigid PD compared to tremor-dominant patients were associated with more significant gray matter loss in the superiorparietal lobule via structural imaging, and altered connectivity in areas such as the DLPFC observed via resting state fMRI, which authors linked to the strong relationship between akinetic-rigid symptoms and impairments in executive function in PD (Kann et al., 2020).

Cumulative evidence suggests structural and functional deficits between cortical regions are central to the genesis of PD. Such deficits, however, are indicative of underlying abnormalities in key neurotransmitter systems which have significant repercussions for cognitive impairment.

1.3 NEUROTRANSMITTER DYSFUNCTION AND COGNITIVE IMPAIRMENT IN PD

1.3.1 Dopamine

Due to the prolific cortical connections throughout the brain outlined above, loss of striatal DA has been identified as a strong causative candidate for cognitive impairment in PD. A 2012 study established a reduced striatal DA uptake in those with PD-MCI than those without (Ekman et al., 2012). Furthermore, a greater degeneration of DA neurons in the medial SN and loss of presynaptic striatal (associative and limbic) DA has been observed in PD-D (Paulus and Jellinger, 1991, Rinne et al., 1989).

Loss of DA within the striatum has also been significantly linked to dysfunction of these fronto-striatal circuits due to the abundant feedforward projections such as those described above. In support of this, reduced DA uptake in the frontal cortex has been related to impairments in verbal fluency, working memory and attention (Rinne et al., 2000). More specifically, DA loss has been linked to dysfunction of the orbitofrontal and prefrontal cortex, which are involved in emotional regulation planning, reasoning, working memory, problem solving and impulse control (Kehagia et al., 2013). Furthermore, loss of striatal DA tone has been implicated in the hippocampal dysfunction observed in PD due to its role in LTP, a process which underlies cellular mechanisms of memory (Calabresi et al., 2013). Taken together, these results support a role for DA dysfunction in cognitive impairment in PD. However, given its heterogenous nature, it is not surprising that cognitive impairment in PD has also been linked to changes in multiple other neurotransmitter circuits, such as the adrenergic, cholinergic and serotonergic systems, with evidence these occur both dependently and independently of DA dysfunction (Lee et al., 2015, Aosaki et al., 2010).

1.3.2 Noradrenaline

Changes to adrenergic activity in the brain have been associated with DA dysfunction, with degeneration of noradrenaline neurons in the locus coeruleus (LC) correlating with DA density in the SN in non-PD individuals with parkinsonian features (Paredes-Rodriguez et

al., 2020). Adrenergic LC degeneration also appears to occur independent of DA dysfunction, with LB pathology and neuronal loss noted to precede degeneration of the SNpc by years (Braak et al., 2003, van Dijk et al., 2012).

The LC is the main noradrenergic nucleus of the brain, providing innervation to widespread regions such as the VTA, SN, amygdala, hippocampus, thalamus and basal forebrain and the neocortex (Bari et al., 2020, Moore and Bloom, 1979). Accordingly, LC noradrenergic excitatory input is believed to be involved in cognitive functions, such as attention, learning, memory encoding and modulation of SP, in addition to sleep and mood disturbances (Chamberlain and Robbins, 2013, Remy et al., 2005, Ishihara-Paul et al., 2008, Alonso et al., 2009, Chaudhuri and Odin, 2010). The LC supplies a substantial proportion of noradrenergic innervation to most of the thalamic nuclei (Lindvall et al., 1974). Whilst a reduction of noradrenaline in motor-associated thalamic nuclei is to be expected, a marked reduction has also been observed in thalamic nuclei (anteroventral and mediodorsal) associated with learning, memory, emotion and motivation in PD cases compared to controls (Piffl et al., 2012). This reduction appears to be in contrast with early PD, where an upregulation of noradrenaline LC transmission is observed in an effort to compensate for striatal DA loss (Isaias et al., 2011).

The impact of a reduction in LC noradrenergic input has not been directly assessed in PD-MCI or PD-D to date; however, degeneration of LC neurons has been found to be more marked in AD patients with MCI than without, a finding which has been correlated with worsening global cognitive function, as well as performance on tests of memory, executive function and visuospatial ability (Grudzien et al., 2007, Kelly et al., 2017). Animal models (transgenic A53T and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)) of PD have also observed a reduction in NA associated with α -syn pathology and cognitive dysfunction in domains of learning, memory, attention and executive function which precede

DA loss and motor deficits (Sotiriou et al., 2010, Xu et al., 2012, Decamp and Schneider, 2004). Finally, MRI imaging in PD patients with and without MCI has found a negative correlation with performance on tests of cognitive flexibility and LC signalling (Li et al., 2019). Further evidence for the link between NA loss and cognitive dysfunction in PD is therapeutic use of selective noradrenaline reuptake inhibitors, which have been found to improve performance in tasks related to response inhibition, attention and problem solving in PD patients (Kehagia et al., 2014); however, efficacy appears to be dependent on disease severity (Kehagia et al., 2014, Ye et al., 2015). Interestingly, LC integrity was also recently shown to be predictive of response of cognitive impairment to the noradrenaline reuptake inhibitor atomoxetine in individuals with PD, with lower LC integrity associated with improvements in response inhibition (O'Callaghan et al., 2021).

1.3.3 Serotonin

Alterations to serotonin (5-Hydroxytryptamine – 5-HT) signalling have been strongly tied to DA changes, with increased levels of striatal serotonin transporter linked to a reduction in striatal DAT in early-PD (Strecker et al., 2011), indicative of a potential compensatory mechanism. However, the death of DA has also been linked to a reduction of serotonin binding in an animal model of PD and disease progression has been associated with degeneration of serotonergic signalling (Lee et al., 2015, Kerényi et al., 2003). Cortical availability of serotonin is also reduced in PD (Scatton 1983). In particular, 5-HT neurons in the median raphe nucleus, which project to the cortex and hippocampus, have been shown to degenerate by late-stage PD (Halliday et al., 1990). Additionally, reductions of 5-HT in the caudate nucleus, which receives projections from the dorsal raphe nucleus, have also been observed (Kish et al., 2007, Kerényi et al., 2003).

To date, the implications of alterations in serotonin signalling in PD for cognitive function are not well understood; however, a reduction in serotonergic signalling has been correlated with severity of depression in an animal model of PD (Lee et al., 2015) and markers of reduced serotonin innervation have been observed in PD-D (Huot et al., 2010, Perry et al., 1993). A recent imaging study demonstrated extensive reduction in serotonin uptake in areas of the striatum (caudate and putamen), as well as extra-striatal regions (amygdala and pallidum), with pallidum uptake associated with increased depression. However, these were not associated with differences in cognitive assessment (Montreal Cognitive Assessment - MoCA) (Nicastro et al., 2020).

Although evident, the specific effects of 5-HT disruption on alterations to cognitive function, however, appear to be variable and may be dependent on specific receptors. For example, 5-HT_{1A} receptors are localised on glutamatergic pyramidal cells of the HPC, regulating their activity (Azmitia et al., 1996). 5-HT_{1A} agonists have been shown to suppress induction of LTP (Pollandt et al., 2003), whilst antagonists have reported enhancement of LTP. It is postulated a reduction in 5-HT_{1A} results in a concomitant increase in DA and NA levels in the medial prefrontal cortex and hippocampus, facilitating LTP and working memory. A 2019 study in a 6-hydroxy-dopamine (6-OHDA) model of PD revealed DA loss was associated with a decrease in 5-HT_{1A} density in the ventral hippocampus and impaired working memory (Liu et al., 2019). Although this is an emerging area of research, due to the extensive crosstalk between 5-HT, DA and NA, it is difficult to understand the role disruptions in serotonergic signalling specifically have for cognitive impairments in PD; however, preliminary studies warrant future research.

1.3.4 Acetylcholine

The cholinergic system has been well established in mediating memory, attention and neuroplasticity (Hilker et al., 2005, Zmarowski et al., 2005). This system consists of two

neuronal aggregates which project to extensive regions throughout the brain. Extending from the basal forebrain, cholinergic neurons of the nucleus basalis of Meynert (nbM) and medial septal nucleus project to regions of the neocortex, limbic cortex and amygdala. Cholinergic neurons of the pedunculopontine nucleus (PPN) project to the basal forebrain, brain stem, BG and thalamus (Woolf, 2006). In terms of contribution to cognitive function, acetylcholine (ACh) is considered a neuromodulator and is involved in encoding memory via enhancement of feedforward afferent inputs to the cortex to initiate response to sensory stimuli, whilst decreasing excitatory feedback to mediate retrieval (Hasselmo, 2006). Disruption of these two basal forebrain acetylcholine pathways have been implicated in PD (Müller and Bohnen, 2013). Positron Emission Tomography (PET) imaging studies have reported a severe loss of cortical cholinergic activity in PD-D compared to non-demented PD, a response attributed to the loss of ascending projections from the nbM to the cerebral cortex (Hilker et al., 2005). In support of this, a more severe loss of nbM acetylcholine has been observed in PD-D than in late-stage PD without cognitive impairment (Gaspar and Gray, 1984, Tiraboschi et al., 2000, Mattila et al., 2001).

In terms of pathway connectivity, the PPN cholinergic projections have a significant effect on DA systems due to innervations to the SNpc and VTA, where they are believed to be involved in sustaining the phasic bursts and tonic discharge of DA in relation to sensory stimuli (French and Muthusamy, 2018). This alters DA released from the SNpc and VTA to the striatum, modifying BG activity. Accordingly, the PPN is considered crucial for the phasic DA bursts necessary for learning (Scarnati et al., 1986, Futami et al., 1995), with PPN lesions leading to impaired attention and memory learning (Inglis et al., 1994, Inglis et al., 2000, Inglis et al., 2001). Studies have reported degeneration of approximately 50% of cholinergic neurons of the PPN in PD (Jellinger, 1988, Gai et al., 1991, Hirsch et al., 1987, Zweig et al., 1989). This is also supported by the Braak staging model, which suggests LB

and neuronal loss in the SN occurs in conjunction with α -syn deposits in cholinergic neurons of the basal forebrain (Braak et al., 2003). This is believed to influence DA signalling, disrupting the equilibrium and further promoting the shift in balance towards the indirect ‘inhibitory’ pathway (French and Muthusamy, 2018).

Accordingly, this loss of cholinergic tone has significant knock-on effects for cognitive function in PD, with cognitive impairment in PD associated with loss of basal forebrain acetylcholine (Ruberg et al., 1986). In support of this, a reduction in neocortical acetylcholinesterase has been related to lower cognitive performance in cognitively normal PD patients (Bohnen et al., 2006). More specifically, post-mortem data suggests a reduction in acetylcholine in the frontal cortices is almost doubled in PD-D patients relative to non-demented PD patients (Ruberg et al., 1986). In fact, of all neurotransmitters altered in PD-D, cholinergic changes are the most striking, with cholinergic function more severely affected in PD-D than in AD (Bohnen and Albin, 2011, Bohnen et al., 2006, Hilker et al., 2005, Ruberg et al., 1986). Anatomically, studies have revealed a reduction in cortical levels of choline acetyltransferase in the hippocampus, prefrontal cortex and temporal cortex is correlated to extent of cognitive impairment (Gaspar and Gray, 1984, Mattila et al., 2001, Hall et al., 2014). Interestingly, the ‘dual syndrome hypothesis’ suggests that deficits in executive function are related to DA dysfunction, whereas visuospatial deficits may be linked to a depletion of acetylcholine in the posterior cortical loop (Kehagia et al., 2013). This is supported clinically, with cholinergic dysfunction correlating with deficits in memory and visuospatial function (Pillon et al., 1993).

Given the prolific contributions to cognitive impairment in PD-D, many pharmacological therapies have targeted neurotransmitter dysfunctions. Accordingly, the current goal of these therapies has primarily been to restore the balance in neurotransmitter levels in PD. Efficacy

of some of these approaches targeting DA, NA, Ach and 5-HT dysfunction, including their mechanisms of action and limitations, will be discussed below.

1.4 TARGETING COGNITIVE IMPAIRMENT IN PARKINSON'S DISEASE

1.4.1 Pharmacological Approaches

1.4.1.1 Dopamine Replacement Therapy

In terms of current treatment options for PD, the primary approaches aim to increase levels of striatal DA. Levodopa (L-dopa) is a DA precursor developed over 50 years ago and is considered the 'gold-standard' treatment for PD (Hornykiewicz, 2015). Another DA centred approach is the use of dopamine receptor agonists such as bromocriptine, pergolide, ropinirole and pramipexole. These treatments predominantly work as agonists of D2-like receptors, mimicking DA (Brooks, 2000). Finally, MAO and COMT inhibitors, such as Selegiline and Tolcapone (respectively), work by inhibiting enzymes which would otherwise metabolise or degrade DA, slowing the breakdown in the brain (DeMaagd and Philip, 2015). The most common course of treatment in PD involves the use of L-Dopa in conjunction with DA receptor agonists and/or inhibitors of DA breakdown (Brooks, 2000).

Whilst these are considered successful in reducing the motor impairments associated with the disease, results for pharmacotherapies targeting DA dysfunction for cognitive impairment are mixed. Multiple studies have reported that use of DA-targeting medications in PD patients have been associated with improvements in specific cognitive functions, such as set-shifting (Cools, 2001), planning (Owen et al., 1995), working memory (Fournet et al., 2000, Lewis et al., 2005) and spatial working memory strategy (Miah et al., 2012). Accordingly, improvements observed with DA medication have been linked to the reliance of these domains on fronto-striatal activity, with increased DA restoring balance along the

nigrostriatal and mesocortical pathways, more specifically, the dorsal striatal-dorsolateral prefrontal cortex circuit (Cools et al., 2003). In contrast, a MPTP macaque model of PD associated with cognitive deficits revealed no improvement or even worsening of attention/working memory deficits with L-Dopa administration (Schneider et al., 2013). Furthermore, whilst a study assessing blood flow during planning and spatial working memory tasks revealed a significant reduction in blood flow in the DLPFC with L-dopa administration, an effect attributed to increased efficiency of the prefrontal cortex, these changes did not appear to translate to improved cognitive outcomes with patients ON L-dopa compared with performance OFF L-dopa, likely related to a lack of impairment in the PD population compared with controls (Cools et al., 2002). This is consistent with a study by Poston and colleagues (2016), which found that DA replacement resulted in slower cognitive reaction time during working memory tasks, with individual differences correlated with loss of compensatory hyperactivation in the putamen in PD (Poston et al., 2016).

Even more concerning, several studies have also supported worsening of specific cognitive functions with DA therapy in areas such as decision-making (Torta et al., 2009), impulsivity (Cools et al., 2003), reversal/procedural learning (Cools, 2001, Frank et al., 2004) and visual memory (Miah et al., 2012). This appears to be dose-dependent, with increased dosage correlating with worsened cognitive function in these domains (Torta et al., 2009). This is particularly concerning, as dosage generally increases as disease progresses (Brooks, 2008). Rather than dorsal fronto-striatal activity, worsening of these specific cognitive functions appears to be related to areas dependant on ventral fronto-striatal circuitry (Sohn et al., 2000). This may result in the constant stream of DA from DA replacement medication ‘flooding’ the ventral striatum and mesolimbic streams connecting to the hippocampus, amygdala and prefrontal cortex (Gotham et al., 1998). This ‘flooding’ reduces the peaks and troughs of phasic DA bursts that are required for mechanisms

underlying learning, such as SP (Frank et al., 2004). Subsequently, whilst increased DA in the dorsal stream compensates for the loss of DA, increased impulsivity and impaired learning may be due to an ‘overdose’ of the ventral streams (Gotham et al., 1998, Fern-Pollak et al., 2004, Cools et al., 2007, Cools et al., 2001). Thus, caution must be used when considering the use of dopamine-targeting medications for the treatment of cognitive dysfunction in individuals with PD, particularly early on in disease course.

With regards to the efficacy of dopamine-targeting medications for treating cognitive dysfunction in patients diagnosed with PD-MCI or PD-D, the literature to date is limited. Studies have mainly focused on function in specific cognitive domains, with mixed effects noted (Cools, 2001, Cools, 2006, Rowe et al., 2008, Aarts et al., 2014, Hughes et al., 2013, Hughes et al., 2010, MacDonald et al., 2011, Nombela et al., 2014, Obeso et al., 2011, Maril et al., 2013, Palminteri et al., 2009). For example, the use of a selective MAO inhibitor (rasagiline) in patients with PD-MCI found no improvement in cognition (Weintraub et al., 2016, Frakey and Friedman, 2017). Conversely, treatment with a new MAO inhibitor (Safinamide) has demonstrated improvements in attention and memory and QoL with 6-months of treatment; however, participants were not considered cognitively impaired (MMSE >26) (Santos García et al., 2021). Well-controlled, longitudinal clinical trials are necessary in order to more fully assess the potential benefits of dopamine-targeting therapies for the treatment of PD-MCI and PD-D, but are currently lacking. Accordingly, the pharmacological treatment of cognitive impairment in PD has predominantly focused on two main areas, cholinesterase inhibitors and noradrenaline reuptake inhibitors.

1.4.1.2 Cholinesterase Therapy

The use of cholinesterase inhibitors are currently the preferred, and only, pharmacological treatment strategy for cognitive impairment in PD (Akbar and Friedman, 2015), with

approval of these drugs initially based on the efficacy of these treatments in AD. The most common medications are donepezil, rivastigmine and galantamine, with only rivastigmine currently approved for the treatment of mild-severe dementia in PD (for review see (Pagano et al., 2015)).

Rivastigmine is a cholinesterase inhibitor that increases acetylcholine availability at the synapse by inhibiting its breakdown (Enz et al., 1991, Reingold et al., 2007). As discussed, deficits in cholinergic activity are more pronounced in PD-D than non-demented PD patients and acetylcholine inhibition has proven detrimental for cognitive function, indicating a role for cholinergic dysfunction in cognitive impairment in PD (Hilker et al., 2005). Several studies have supported the use of rivastigmine in PD-D (McKeith et al., 2000, Reading et al., 2001, Giladi et al., 2003). For example, a study in participants with PD-D demonstrated moderate improvements compared with a placebo in measures of cognition (including orientation, memory, language and visuospatial function), executive function and attention, ADLs and neuropsychiatric symptoms (Emre et al., 2004). A subsequent study with donepezil, another cholinesterase inhibitor, confirmed similar dose-dependent results in cognitive outcomes; however, no significant improvements were observed in either ADLs or behaviour, and adverse events were more common (Dubois et al., 2012). With regards to use in PD-MCI, potential improvements appear more subtle, with a randomised double-blind, placebo-controlled study noting an improvement in cognitive function and a trend for improvement in global cognition and anxiety; however, no treatment effect for measures of depression, apathy or ADL (Mamikonyan et al., 2015). This is in keeping with a Cochrane review, which determined cholinesterase inhibitor treatment was not supported for patients with cognitive impairment that does not reach threshold for dementia (Rolinski et al., 2012). More recent drugs, such as apomorphine, which acts as a DA agonist and cholinergic antagonist, have shown more promising results, with a recent 2019 study demonstrating

improvements in measures of mood/cognition and attention/memory on the Nonmotor Symptom Scale with treatment; however, participants baseline cognitive status was not considered and it is therefore difficult to draw parallels for the treatment of either PD-MCI or PD-D (Dafsari et al., 2019).

Whilst the use of cholinesterase inhibitors appears promising, at least for PD-D, efficacy is variable and treatment is associated with significant side effects (Emre et al., 2014). Side effects include gastrointestinal symptoms (Aarsland, 2016), nausea (29%), vomiting (16.6%) and even tremor (10.2%) (Emre et al., 2014), leading to concerns that long-term use of cholinesterase inhibitors could worsen motor symptoms in some PD patients. In support of this, both rivastigmine and donepezil can induce tremor in response to their administration in a subset of individuals (Gurevich et al., 2006, McCain et al., 2007, Song et al., 2008), and administration of the anticholinesterase galantamine can potentially induce tremor (Collins et al., 2011).

1.4.1.3 Noradrenaline Therapy

More recently, the use of pharmacotherapies targeting noradrenaline have been trialled for the treatment of cognitive impairment in PD (Borchert et al., 2016, Ghosh et al., 2020, Kehagia et al., 2014, Marsh et al., 2009, Ye et al., 2015, Ye et al., 2016). This predominantly involves atomoxetine, a selective reuptake inhibitor of noradrenaline (Bymaster et al., 2002, Hinson et al., 2017). Due to the role of noradrenaline in the inhibition of neural systems and its subsequent involvement in executive function, most studies have focused on outcomes relating to executive dysfunction in PD. A 2009 study in patients with PD and executive dysfunction revealed improvement on measures of executive function (Marsh et al., 2009). Additionally, a study in PD without cognitive impairment demonstrated a reduction in impulsivity and risk-taking behaviour and improvements in problem solving and attention

(Kehagia et al., 2014). However, a study in PD-MCI patients observed improvements on subjective measures of attention and impulsivity with treatment, but not on objective measures of attention, working memory, processing speed or set-shifting (Hinson et al., 2017), indicating potentially mixed benefits for executive function later on in disease course. Improvements in response inhibition with atomoxetine have been linked to increased fronto-striatal connectivity (Ye et al., 2015); however, it is worth noting that this response may be dependent on dose and disease stage, with replacement therapy for those with significant noradrenergic loss likely to improve with treatment, while those with mild changes potentially experiencing an ‘overdose’ effect associated with impaired response inhibition (Ye et al., 2015).

With regards to other cognitive domains, Weintraub and colleagues (2010) investigated the efficacy of atomoxetine for global cognition in addition to neuropsychiatric symptoms (Weintraub et al., 2010b). Whilst improvement was noted for global cognition, with a trend for anxiolytic effect, there were no significant improvements noted in measures of depression, apathy or motor function (Weintraub et al., 2010b). Overall, a recent systematic review concluded there may be benefits of atomoxetine for the treatment of executive dysfunction in PD; however, it is difficult to determine the full efficacy given patients in the majority of studies were still receiving standard DA therapy (Warner et al., 2018). With regards to efficacy in cognitively impaired patients, a 2020 meta-analysis of the use of atomoxetine in cognitively impaired PD patients determined that use in PD-MCI participants did not result in improvements in attention, executive function, perceptual-motor function, language or learning and (Ghosh et al., 2020, Hinson et al., 2017, Marsh et al., 2009). Furthermore, significant adverse events have been noted, with results across studies noting patients experiencing reduced sleep (50%), constipation (42%), confusion

(25%), slowed movement (25%), jitteriness (20%) and even atrial fibrillation (7%) and chest pain (7%) (Hinson et al., 2017, Marsh et al., 2009).

1.4.1.4 Serotonergic Therapy

Given the role of serotonergic dysfunction in PD, the use of anti-depressants such as serotonin re-uptake inhibitors (SSRIs) and tricyclics, have also been proposed for use in PD (Barone et al., 2006, Hauser and Zesiewicz, 1997, Liu et al., 2013, Menza et al., 2009, Leentjens et al., 2003b). These most notably include sertraline and citalopram. In particular, efficacy has been proposed for neuropsychiatric symptoms, such as depression, with tricyclics demonstrating superiority to SSRIs; however, results appear variable (Rocha et al., 2013). A 2013 systematic review and meta-analysis indicated no significant superiority of antidepressants over placebo for the treatment of depression in PD (Rocha et al., 2013). With regards to targeting serotonergic transmission for the treatment of cognitive impairment, this has primarily focused on targeting serotonin receptors, such as 5-HT₆ and 5-HT_{2A}, involved in cognition, particularly learning and memory (Upton et al., 2008). Intepirdine is a selective 5-HT₆ receptor antagonist and a recent study investigated the efficacy in patients with PD; however, results indicated a lack of efficacy on all outcomes, including cognition (Taylor et al., 2020). Furthermore, SYN120, a 5-HT₆/5-HT_{2A} antagonist, also indicated no improvements in those with PD-D, as well as a worsening in motor symptoms (Fernandez, 2019). The investigation of the role of serotonin receptors in cognition in PD is an emerging field and, whilst other 5-HT receptor antagonists are currently being trialled in animal models, the majority have focused on motor impairments and have not been trialled in humans (Kwan et al., 2020, Hamadjida et al., 2018, Liu et al., 2019). Accordingly, preliminary evidence does not, at this stage, indicate serotonergic therapy as an efficacious avenue for treatment of cognitive impairment in PD.

1.4.1.5 Glutamatergic Therapy

The most commonly trialled pharmacotherapy targeting glutamatergic dysfunction in PD are N-Methyl-D-Aspartic (NMDA) antagonists (Hallett and Standaert, 2004, Greenamyre and O'Brien, 1991, Vanle et al., 2018). NMDA is a glutamate receptor channel strongly involved in processes underpinning learning and memory. Accordingly, overactivation of NMDA receptors has been observed in PD and dysfunction of these receptors has been associated with cognitive impairment in the disease (Aarsland et al., 2009, Wesnes et al., 2015, Bandini et al., 2002, Vanle et al., 2018). In an animal model of PD, blockade of NMDA receptor phosphorylation was able to rescue cognitive deficits (Ferreira et al., 2017b); however, evidence of efficacy in human populations is still ongoing. Two NMDA antagonists currently under investigation are memantine and amantadine. The use of memantine in PD-D has noted slight, yet significant, improvements in cognition, but not in neuropsychiatric measures (Aarsland et al., 2009). This has been supported in subsequent studies, which have noted improvements in attention and recognition memory (Wesnes et al., 2015), decreased impulsivity and improved cognition (Litvinenko et al., 2010). However, a 2010 trial of memantine in patients with PD-D revealed no significant improvement in CogState performance across domains assessing attention, executive function, set-shifting, language and memory (Emre et al., 2010). Although literature appears mixed, a recent meta-analysis concluded memantine treatment results in significant improvements in attention processing speed and executive function (Meng et al., 2019). Most excitingly, due to the proposed role of NMDA dysfunction in the pathological progression of PD, it has been theorised that NMDA antagonists may represent a disease-modifying therapeutic target based on results in animal models (Ferro et al., 2007). However, follow-up studies suggest memantine discontinuation in PD-D may be associated with increased deterioration in global function compared to placebo (Leroi et al., 2009), in addition to increased anxiety and depression

(Johansson et al., 2011). Accordingly, preliminary results recommend continuation of treatment is required and do not support a disease-modifying effect of NMDA antagonists. This is likely due to the disease-stage, with PD-D patients likely to have already undergone considerable neurodegeneration at time of diagnosis.

1.4.1.6 Limitations of Pharmacological Approaches

Several strategies discussed have indicated potential therapeutic benefits of pharmacotherapies for the treatment of cognitive impairment in PD, but this literature is quite mixed. Furthermore, pharmacological treatments have proven largely ineffective to date for their ability to lead to long-term improvement in cognitive function (Seppi et al., 2011). Even DA therapies, considered the ‘gold-standard’ approach for the treatment of motor symptoms in PD, are not without their significant limitations. For example, DA therapies become less effective overtime, with dementia in PD correlated with a loss of response to DA therapy and, in particular, a loss of D3 receptors in the nucleus accumbens, caudal striatum and globus pallidus (Joyce et al., 2002). In support of this, although dopamine replacement with levodopa and pergolide was capable of leading to significant, but incomplete, initial improvements on measures of learning, long-term memory, visuospatial and executive function, these benefits were not sustained at a two-year follow-up testing point (Kulisevsky et al., 2000). This suggests that dopamine replacement alone is not able to target all cognitive domains affected in PD and may not be able to compensate as the disease progresses and additional neurotransmitter systems are further impacted. Levodopa-induced dyskinesia (LID) is also a complication of long-term levodopa use, with impairment in attention and executive function both predictive of LID development (Luca et al., 2021). Although several other potential therapeutic targets have been discussed, the only treatment currently approved for the treatment of cognitive impairment in PD are

cholinesterase inhibitors, which have been shown to worsen motor impairments (Collins et al., 2011), and memantine, which may have limitations for long-term use (Leroi et al., 2009). Accordingly, a range of non-pharmacological therapies have been investigated with the hope of either identifying synergistic therapies that may improve the efficacy of existing pharmacological approaches or indeed even prevent the emergence of cognitive impairment in PD.

1.4.2 Non-Pharmacological Approaches

Due in large part to the heterogeneity of cognitive decline, associations have been made with the rate and severity of decline and individual factors, such as healthy lifestyles, weight, nutrition, metabolic syndromes and cardiovascular risk factors (Martín-Fernández et al., 2015). These are considered modifiable risk factors, which may be amenable to intervention with non-pharmacological therapies, such as diet and exercise. Cognitive stimulation approaches, including transcranial direct current stimulation (tDCS) and cognitive training (CT), are also commonly investigated potential approaches (Lawrence et al., 2018).

1.4.2.1 Diet

The role of diet has gained significant attention recently in understanding the pathogenesis of PD (Seidl et al., 2014, Agim and Cannon, 2015), with PD associated with a range of vitamin deficiencies, including vitamin B and vitamin D (Sato et al., 1997, Iacopetta et al., 2020). A correlation has been found between serum Vitamin D levels and cognitive function, with higher vitamin D concentrations associated with higher scores on cognitive tests of verbal memory and verbal fluency amongst PD patients (Peterson et al., 2013). This finding may have a genetic basis, with polymorphisms in the vitamin D Receptor (VDR) gene linked to PD (Li et al., 2015). Specifically, additional copies of the FokIA allele, a functional VDR

polymorphism, was associated with a decrease in cognitive function in a long-term study of PD patients (Gatto et al., 2016). Mechanistically, it is believed vitamin D may provide a neuroprotective function, due to its involvement in the regulation of neurotrophic factors, antioxidative properties, immunoregulation and modulation of neuronal excitability (Khairy and Attia, 2019, Garcion et al., 2002). Promisingly, vitamin D supplementation was shown to prevent disease deterioration compared to placebo (Suzuki et al., 2013); however, a recent meta-analysis concluded vitamin D supplementation did not reduce the motor symptoms of PD (Zhou et al., 2019). Nevertheless, given the link between cognitive decline and vitamin D levels, vitamin D supplementation for the treatment or prevention of cognitive impairment may represent an area of future research.

In addition to vitamin D, vitamins B-12 and folate may also represent targets for dietary intervention in PD. Homocysteine (Hcy) is a metabolite involved in methylation processes which impact cellular growth and homeostasis, with high levels of Hcy indicating deficiency in vitamins B-12 and folate (Gilfix, 2005), Levodopa therapy has been shown to increase production of Hcy and subsequent methylation linked to increased deoxyribonucleic acid (DNA) defects and oxidative damage (Martín-Fernández et al., 2015). Studies have demonstrated an association between cognitive impairment and elevated levels of Hcy (Smith, 2008, Harris et al., 2012), and this has also been linked specifically to CI in PD patients (Harris et al., 2012), suggesting Hcy may facilitate the progression of disease and cognitive impairment. In fact, a study administering vitamin B-12 and folate supplementation noted improvements in cognitive performance on an episodic memory task, which were associated with a decrease in Hcy levels and increase in B-12 and folate in an older population (Harris et al., 2012). Accordingly, a reduction of Hcy levels via vitamin B and folate supplementation has been proposed, however, larger, more well-controlled and

more long-term studies are necessary in order to determine if supplementation may improve outcomes or reduce the risk of cognitive impairment in PD (Martín-Fernández et al., 2015).

Neurodegeneration in PD has also been strongly associated with mitochondrial dysfunction and oxidative stress (Hwang, 2013). Ketones are water-soluble molecules with a ketone group produced by the liver from fatty acids when glucose is in short supply and may be able to restore these processes in PD. In support of this, ketones have been linked to enhancement of neuronal energy metabolism through stimulation of mitochondrial biogenesis, in addition to antioxidant processes (Bough et al., 2006). Additionally, high-carbohydrate diets and associated glucose hypometabolism/insulin resistance have been linked to acceleration of PD progression, MCI and dementia (Krikorian et al., 2019, Ou et al., 2021, Chohan et al., 2021). Accordingly, the low-carb, high-fat ketogenic diet (KD) has been proposed as a potential therapeutic intervention for PD, particularly linked to neuroprotection of the hippocampus (Ziegler et al., 2003). Studies have demonstrated neuroprotective effects of a KD in animal models of PD; however, cognitive outcomes were not assessed (Cheng et al., 2009a, Yang and Cheng, 2010). More recently, implementation of a KD for 8 weeks in PD patients revealed a significant decrease in non-motor symptoms, including cognitive impairment as assessed by the MoCA, compared to a low-fat diet, high-carbohydrate diet (Phillips et al., 2018). Additionally, a study comparing a high-carb diet to a KD regime in patients with PD-MCI observed enhanced cognitive performance in measures of language and memory (Krikorian et al., 2019). This continues to be an emerging area of research; however, due to difficulties associated with compliance in implementing strict dietary changes, future investigations appear to focus on maintaining a therapeutic level of ketosis via pharmacological intervention through the use of ketone ester supplementation (Thickbroom, 2021).

Finally, adherence to the Mediterranean diet (MD) has been strongly associated with reduced risk and later onset of neurodegenerative diseases, including PD (Maraki et al., 2019, Alcalay et al., 2012). The positive effects of the MD are attributed to its increased proportion of foods containing anti-inflammatories and antioxidants, such as vitamin C, vitamin E and carotenoid (Visioli and Galli, 2001), in addition to its reduced consumption of foods considered risk factors in PD, such as dairy, meat and saturated fatty acids (Godos et al., 2020, Alcalay et al., 2012). Regarding its potential therapeutic efficacy in targeting cognitive impairment, multiple studies have linked the MD to improved cognitive health, with a systematic review concluding improved cognitive function and dementia in AD (Pettersson and Philippou, 2016). Accordingly, a promising 2020 study probed this relationship in PD, demonstrating more significant improvements in executive function, language, attention, concentration and active memory in those adhering to a MD than a control diet (Paknahad et al., 2020). Future investigations are needed to establish the efficacy and feasibility of the MD for the treatment of already established PD-MCI and PD-D specifically.

Overall, diet manipulation may represent a patient-motivated approach to treatment targeting cognitive impairment in PD; however, there is a significant lack of studies investigating their efficacy in PD-MCI and PD-D. In addition, implementation and compliance with strict diets may represent a significant barrier to the feasibility of the approach, however this may be able to be overcome with development of diet plans or services which tailor diets specifically for PD patients.

1.4.2.2 Aerobic exercise and physical activity

Although diet is one modifiable lifestyle factor, physical activity may also represent a promising patient-driven approach to targeting CI in PD. Physical activity has been linked to a decreased risk of PD (Ascherio and Schwarzschild, 2016). Aerobic training is believed to promote neural rearrangement (El-Sayes et al., 2019) and, while the cellular mechanisms via which these neuroplastic effects occur are still unclear, they may involve enhanced neurogenesis/synaptogenesis (Valkanova et al., 2014) or increases in myelination (Song et al., 2005). Physical exercise leads to increased levels of neural growth factors, including BDNF, which is essential for facilitating neurogenesis, cell survival and SP (Gomez-Pinilla et al., 2008). Thus, physical exercise may promote neurogenesis/synaptogenesis, improving the survival of cells and synapses (Nuechterlein et al., 2016). Furthermore, exercise has also been shown to protect against oxidative stress and mitochondrial dysfunction (Svensson et al., 2015).

Whilst the efficacy of exercise therapy on motor function is well-established (Perrey, 2013), less is known about the cognitive benefits. With regards to studies demonstrating improvement in cognitive function in PD, a single case study demonstrated aerobic exercise produced improvement in executive function, working memory and language (Nocera et al., 2010). Other studies have noted improvements specifically in executive function, which were linked to improved cerebral blood flow (Ridgel et al., 2011, Tanaka et al., 2009). Specifically, a 2011 study noted improvements in frontal lobe dependent tasks, such as spatial working memory and verbal fluency, compared to fronto-temporal/temporal tasks, such as spatial and pattern recognition memory (Cruise et al., 2011). A long-term study also demonstrated sustained improvement in cognitive function at 12- and 24-months post-intervention with two different exercise programs, suggesting a neuroprotective effect in a

disease that would otherwise be expected to likely to demonstrate decline (David et al., 2015). With regards to PD-MCI and PD-D specifically, a study combining aerobic exercise in a virtual environment noted improvements in executive functions and attention in a cohort of both PD and PD-MCI patients (Mirelman et al., 2016). However, a systematic review of the use of exercise in the treatment of Lewy Body Dementias, encompassing both PD-D and dementia with Lewy bodies, pointed out that, to date, there has been a significant lack of studies looking at benefits of exercise in these populations, as individuals with established dementia are often excluded from study designs (Inskip et al., 2016). This suggests an important area for future research, particularly given that benefits of exercise have been demonstrated in both pre-clinical models of Alzheimer's (Vasconcelos-Filho et al., 2021, Yu et al., 2021) and individuals with dementia (Liu-Ambrose et al., 2021). The use of home-based multimodal exercise programs may be of particular use for the investigation of cognitive benefits in PD-D, based on similar proposals put forward for those with Alzheimer's disease (Cezar et al., 2021).

While exercise does appear to have beneficial effects for cognitive function in PD, questions remain about what type of exercise is likely to be most efficacious. A recent meta-analysis of RCTs evaluating cognitive outcomes in PD patients engaging in different modalities of exercise, including dance, treadmill, cycling and multimodal approaches, showed that high-intensity treadmill training demonstrated the largest effect size, although improvements were seen with multiple types of exercise (Silva et al., 2019). Despite the promising results overall, however, there are important gaps in the literature that still remain. Specifically, as outlined above, further studies are needed in individuals with PD with established cognitive dysfunction (that is, PD-MCI and PD-D). Additionally, there are methodological limitations in existing studies of the effects of exercise on cognition function in PD more broadly, including small sample sizes, limited durations of the trial and lack of

appropriate control groups. Finally, it is critical that future studies investigate changes in biomarkers related to disease progression, including neuroinflammation, levels of growth factors and alterations in pathological proteins, in order to more fully elucidate the potential mechanisms underlying beneficial effects. This is a significant limitation of the field more broadly, with many studies on the effects of physical activity on dementia progression in individuals with Alzheimer's disease, MCI and other dementias lacking effective biomarker assessment (Moniruzzaman et al., 2020).

1.4.2.3 tDCS/rTMS

Non-invasive brain stimulation via transcranial Direct-Current Stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS) may prove to be effective non-pharmacological approaches to the treatment of cognitive dysfunction in PD, due to their potential to facilitate neuronal plasticity, amongst several other proposed mechanisms (Mohammadi, 2016). Location of the stimulation depends on the outcomes targeted and assessed; however, studies evaluating cognitive impairment primarily involve stimulation of the DLPFC (Manenti et al., 2016, Randver, 2018, Jiang et al., 2020). Initial studies have reported improved cognition following tDCS of the DLPFC in non-demented individuals with PD, including improvements in working memory (Boggio et al., 2006) and executive function (Pereira et al., 2013). In a comparable study by Doruk and colleagues (2014), results also appear to be maintained up to at least 1 month following intervention (Doruk et al., 2014). It is theorised that tDCS of the prefrontal cortex may enhance declarative and long-term memory consolidation (Javadi et al., 2014). rTMS of the DLPFC and brainstem, another type of non-invasive brain stimulation, has also been shown to improve executive dysfunction at 1-month in PD patients >65 compared to baseline (Mally et al., 2017, Khedr et al., 2003, Khedr et al., 2006). A long-term follow-up study combining rTMS (DLPFC and

brainstem) and tDCS (cerebellum) demonstrated improvements in executive function dependent on age. For patients >65 years, executive dysfunction improved gradually over 3.5 years; however, those <65 years showed a slower deterioration of function, likely attributed to the lack of initial impairments in those <65 at time of baseline (Málly et al., 2018).

These previous studies were in cohorts of relatively cognitively healthy PD patients; however, a study combining tDCS (DLPFC) with physical rehabilitation (PR) in PD-MCI reported reduction of depressive symptoms, as well as improvements in motor ability (PR +/- tDCS), cognition and verbal fluency (PR + tDCS only), which were all stable at 3-month follow-up (Manenti et al., 2016). In contrast, a 2017 study in PD-D patients involving a single session of tDCS of the DLPFC found no post-stimulation attentional improvements compared to placebo, with the authors concluding multiple sessions or higher-density tDCS may be required to elicit cognitive benefits in those with established dementia (Elder et al., 2017). Taken together, these results suggest a promising outlook for tDCS for improving cognitive outcomes in both cognitively healthy PD and PD-MCI patients; however, efficacy in PD-D is yet to be determined.

1.4.2.4 Cognitive training

Cognitive training (CT) is defined as training programs that provide structured practice on specific cognitive tasks, designed to improve performance in one or more cognitive domains, such as memory, attention or executive function (Clare and Woods, 2004). Although studies on the neural basis of CT in PD are sparse, and have included only a handful of subjects, studies in healthy aging may also provide insight. Systematic review of various CT and magnetic resonance imaging (MRI) modalities suggest structural changes in both grey and white matter occur following CT, particularly in the hippocampus (Belleville and Bherer, 2012, Valkanova et al., 2014). There is also evidence to suggest benefits may be due to

neuroplasticity, with an increase in cerebral blood flow and neural connectivity in the default mode network and central executive network following CT (Chapman et al., 2015).

Many studies have investigated the benefits of CT for cognitive function in PD, with a comprehensive review of the literature to follow in **Chapter 6**. As a brief overview, the use of CT programs to target cognitive impairment in PD is generally well established, with improvements noted in global cognition (Bernini et al., 2019, Folkerts et al., 2018, Petrelli et al., 2015, Pompeu et al., 2012), general executive function (Bernini et al., 2019, Mohlman et al., 2011, Petrelli et al., 2014, Petrelli et al., 2015, Sammer et al., 2006, Sinforiani et al., 2004, Vlagsma et al., 2020), attention (Bernini et al., 2019, Mohlman et al., 2011, Nombela et al., 2011, París et al., 2011, Zimmermann et al., 2014), processing speed (París et al., 2011, Pena et al., 2014), learning (Naismith et al., 2013), memory (Naismith et al., 2013), verbal fluency (Sinforiani et al., 2004), verbal memory (Bernini et al., 2019, Díez-Cirarda et al., 2018, Pena et al., 2014, Sinforiani et al., 2004), visuospatial memory/ability (Díez-Cirarda et al., 2018, París et al., 2011, Pena et al., 2014, Zimmermann et al., 2014) and theory of mind (Pena et al., 2014). Importantly, several of these improvements also translated to improvements in ADLs (Díez-Cirarda et al., 2018, Pena et al., 2014) and improvements in certain domains were maintained at various follow-up periods (ranging from 6 weeks to 18 months) (Bernini et al., 2019, Díez-Cirarda et al., 2018, Petrelli et al., 2015, Pompeu et al., 2012, Sinforiani et al., 2004). Collectively, these results are very promising for the utility of CT in the PD population; however, several of these studies either did not differentiate between PD and PD-MCI, or specifically excluded patients with PD-MCI or PD-D. This is a significant limitation of current studies and, given the benefits of cognitive training reported in other populations with cognitive impairments such as AD (Kallio et al., 2017), it is necessary to include these populations in order to fully understand the benefits.

With regards to CT in patients with MCI, there are several studies which have observed improved outcomes. These include improvements in global cognition (Reuter et al., 2012, Alloni et al., 2018, Van De Weijer et al., 2020, Maggio et al., 2018), executive function (Reuter et al., 2012, Costa et al., 2014, Alloni et al., 2018, Lawrence et al., 2018, Maggio et al., 2018, Cerasa et al., 2014), attention (Alloni et al., 2018, Cerasa et al., 2014, Lawrence et al., 2018, Maggio et al., 2018), processing speed (Reuter et al., 2012), memory (Costa et al., 2014, Maggio et al., 2018), verbal fluency (Costa et al., 2014) (Maggio et al., 2018), verbal memory (Alloni et al., 2018, Lawrence et al., 2018), visuospatial memory/ability (Alloni et al., 2018) and language (Lawrence et al., 2018, Maggio et al., 2018). Only 2 of these studies assessed patients after a follow-up period, with only Reuter and colleagues (2012) observing maintained improvements at 6-months follow-up (Reuter et al., 2012). Although these results seem promising, a recent Cochrane review evaluating the effectiveness of CT for PD-MCI and PD-D identified 7 studies fitting their criteria, culminating in a total of 225 participants with variable intervention lengths (Orgeta et al., 2020). The review found ‘no difference between CT intervention and control groups in global cognition and no convincing evidence of benefit in specific cognitive skills or measures of either ADL or quality of life (QoL)’ (Orgeta et al., 2020). This review, however, only included randomized control trials and specifically excluded multimodal studies. One of the benefits of CT as a therapeutic intervention is the ability to pair its implementation with other interventions, both pharmacological and non-pharmacological, such as exercise and tDCS. For example, Lawrence and colleagues integrated tDCS and a tailored CT program, observing improvements in executive function and language beyond those observed with just tDCS, with follow-up noting maintained improvements in executive function, attention, verbal memory and language (Lawrence et al., 2018). Additionally, studies have found combining aerobic exercise and CT may have an additive effect (Shatil,

2013). CT techniques are also evolving, with technological advances able to personally tailor and optimise implementation of CT programs specifically for the needs of the PD population and, thus, represent a promising non-pharmacological approach to addressing cognitive impairment in PD.

1.5 CONCLUSION

Whilst there are many approaches for the treatment of cognitive impairment in PD currently under investigation, to date, the only pharmacological interventions known to target cognitive symptoms are cholinesterase inhibitors, which may be exacerbating the motor symptoms of the disease, or memantine, which may have limitations associated with long-term use. With regards to non-pharmacological therapies, interventions are promising, and negative side-effects are minimal, however, due to variability in study design and populations, it is difficult to ascertain the ideal treatment regimens and implementation can be challenging.

02

Study Synopsis, Aims and Hypotheses

2.0 SYNOPSIS, HYPOTHESES AND AIMS

Currently, there are no interventions known to slow or halt cognitive decline in PD, with current treatments restricted to symptomatic relief of primarily the motor symptoms. This is due, in large part, to the unknown pathogenesis of PD, limiting the development of efficacious therapeutic strategies. To this end, the aim of this thesis is to explore novel treatment strategies for cognitive dysfunction in PD in hopes of addressing a significant unmet clinical need.

The first part of the thesis will focus on pharmacological targets of cognitive impairment in PD by exploring the role of a kinase implicated in the pathological processes of neurodegenerative diseases, Fyn (Chapter 3). This will be further explored by evaluating the distribution of Fyn kinase in key anatomical regions of the PD brain (Chapter 4) and evaluating the potential of Fyn inhibition for the treatment of cognitive impairment in PD (Chapter 5). The second part of this thesis will instead turn to a non-pharmacological strategy, exploring the utility of CT in PD (Chapter 6) and engaging in a co-design process for a ‘serious games’-based strategy to deliver CT in the PD population (Chapter 7). Based on reviews conducted, the specific aims and respective hypotheses were as follows:

AIM 1. Assess and investigate levels of Fyn kinase expression and markers of neuroinflammation in the brains of PD patients compared with controls

Rationale

Fyn kinase has been implicated in pathological processes observed in neurodegenerative diseases such as PD, however, its role in the disease is not clear. This aim sought to investigate if human PD patients exhibit greater levels of Fyn kinase activity than neurologically healthy aged-matched controls and if this was associated with increased markers of neuroinflammation in brain areas associated with cognitive dysfunction in PD.

Hypothesis

Fyn kinase activity will be upregulated in the post-mortem brains of PD patients compared with controls and this upregulation will be associated with increased markers of neuroinflammation

AIM 2. Determine if Fyn kinase inhibition is capable of preventing or reducing motor, cognitive and neuropsychiatric impairment in an early experimental model of PD

Rationale

Current research has established the efficacy of Fyn kinase inhibition in an animal model of Alzheimer's Disease (AD), observing improvements in memory impairments. Much like AD, PD is associated with similar pathological processes, in addition to a novel role of Fyn in neuroinflammation in PD; however, efficacy of Fyn inhibition has not been assessed in PD. Therefore, the second aim of this thesis was to assess if Fyn kinase inhibition translates to an improvement in motor, cognitive and neuropsychiatric outcomes in an early-stage 6-OHDA animal model of PD.

Hypothesis

Fyn kinase inhibition via daily administration of 6mg/kg or 12 mg/kg will reduce impairments in motor, cognitive and neuropsychiatric outcomes.

AIM 3. Establish if Fyn kinase inhibition is capable of reducing dopaminergic loss and neuroinflammation in an early experimental model of PD

Rationale

Dopaminergic loss is a key pathological hallmark of PD and it has been established DA neurons are particularly vulnerable to the stressors brought about by Fyn mediated microglial activation. Consequently, by reducing microglial activation Fyn inhibition Accordingly, the

relationship between degree of inhibition, DA integrity and neuroinflammation were analysed to gain a full understanding of the effects.

Hypothesis

Fyn kinase inhibition will be associated with reduced loss of DA, an increase in markers of neuroinflammation in a dose-dependent manner.

AIM 4. Develop and assess the feasibility of a novel serious gaming system (OrbIT) for cognitive training (CT) for the treatment of CI in PD in a co-design pilot study

Rationale

Several studies have demonstrated CT may improve cognitive outcomes in PD patients. Unfortunately, there are limitations with regard to current techniques used to administer and assess CT in the PD population, in part due to the severe motor impairments associated, making it difficult to use standard CT equipment. The development of the OrbIT system integrated a number of features addressing hardware barriers and integrating features of gameplay, which may improve outcomes for PD patients. By delivering a serious gaming suite designed to target specific domains affect in PD and the use of a novel controller designed specifically for people with motor impairments, the OrbIT gaming system may improve the delivery of CT to prevent and/or treat cognitive impairments in those with PD. In order to confirm feasibility of the OrbIT platform, a co-design pilot study was conducted, and PD participants were involved in the developmental process.

Hypothesis

The OrbIT platform will demonstrate strong feasibility for use in the PD population based on positive feedback with regards to enjoyment and usability of the system.

03

Fyn Kinase Activity and its Role in Neurodegenerative Disease Pathology: A Potential Universal Target?

Chapter prepared in publication format by **GUGLIETTI, B.**, SIVASANKAR, S.,
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3.0 ABSTRACT

Fyn is a non-receptor tyrosine kinase belonging to the Src family kinases (SFKs) which has been implicated in several integral functions throughout the central nervous system (CNS), including myelination and synaptic transmission. More recently, Fyn dysfunction has been associated with pathological processes observed in neurodegenerative diseases, such as multiple sclerosis (MS), Alzheimer's Disease (AD) and Parkinson's Disease (PD). Neurodegenerative diseases are amongst the leading cause of death and disability worldwide and, due to the ageing population, prevalence is predicated to rise in the coming years. Symptoms across neurodegenerative diseases are both debilitating and degenerative in nature and, concerningly, there are currently no disease modifying therapies to prevent their progression. As such, it is important to identify potential new therapeutic targets. This review will outline the role of Fyn in normal physiological CNS processes associated with neurodegenerative diseases and explore the role Fyn dysfunction may play in the pathological processes observed, such as demyelination, pathological protein aggregation, neuroinflammation and cognitive dysfunction.

3.1 INTRODUCTION

Neurodegenerative diseases are amongst the leading cause of death and disability worldwide (Feigin et al., 2019). They encompass a range of diseases associated with progressive loss of neurons, the most common of which include multiple sclerosis (MS), Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD) and Parkinson's disease (PD). Involving a range of motor and cognitive impairments, neurodegenerative diseases are both progressive and incapacitating in nature. Due to increased life expectancy and population growth worldwide, prevalence of these age-associated diseases is expected to continue to rise (Erkkinen et al., 2018). Accordingly, these represent a considerable burden on worldwide health-care systems and the individuals and carers who experience them. For example, for PD alone, counts of prevalence, mortality and disability-adjusted life years (DALY's) more than doubled from 1990 to 2016, with projected total US economic burden surpassing \$79 billion by 2037 (Dorsey et al., 2018, Yang et al., 2020b).

Perhaps the greatest concern regarding the increasing prevalence of neurodegenerative diseases are the limitations of current therapeutic interventions. At present, there are no available disease-modifying agents for these conditions, with currently available treatments largely restricted to symptomatic relief (Cummings, 2017). These do nothing to halt, delay or slow the inevitable degenerative progression of pathologies observed, highlighting the growing need to identify novel therapeutic targets. A potential approach may be to target common cellular mechanisms underlying several of the major neurodegenerative diseases. Due to its diverse role in the human central nervous system (CNS), one such promising target may be the tyrosine kinase Fyn.

Fyn is one of eleven members of the Src family of tyrosine kinases (SFKs) (Nygaard et al., 2014). Widely expressed in many tissues, in recent years, Fyn has garnered significant attention in cancer research due to its role in the signalling pathways that control cell

proliferation, migration, invasion and apoptosis (Resh, 1998). Excitingly, *in vivo* work demonstrated Src family inhibitors, including Fyn, were able to inhibit solid tumour growth (Green et al., 2009). Unfortunately, the progression to phase II human trials in cancers, such as melanoma, breast cancer and pancreatic cancer, found limited benefit due to the high threshold required for kinase inhibition in order to modify tumour progression (>98%) (Nygaard et al., 2014). Whilst this was a significant setback for Fyn as a target in oncological applications, as research in the area of Src kinases developed, so too did a greater understanding of the diverse role of Fyn in neurological function. A recent review by Matrone and colleagues summarised several of the key functions of Fyn in neurological processes, cementing its pivotal and versatile role in the brain (Matrone et al., 2020). Indeed, Fyn plays a significant role in myelination, neurodevelopment and SP, as well as modulating the neuroinflammatory response, key processes that are disrupted in neurodegenerative disease (Knox and Jiang, 2015, Kramer-Albers and White, 2011, Panicker et al., 2015). This review will critically consider how alterations in Fyn kinase may lead to the common pathological processes that underlie the development of neurodegenerative disease, including MS, ALS, AD and PD, and will explore the potential of Fyn as a potentially universal therapeutic target to prevent, reduce or treat disease progression in these degenerative conditions.

3.2 STRUCTURE OF FYN

Fyn is 59-kDa in size and is encoded by the Fyn gene, situated on chromosome 6q2(Resh, 1998) (Sherratt et al., 1997). Fyn is known to exist in three active isoforms: Fyn-B, Fyn-T and Fyn-D7(Cooke and Perlmutter, 1989) (Goldsmith et al., 2002). Fyn-B is broad in its expression in the body, but it is found in particularly high levels in the brain (Cooke and

Perlmutter, 1989). Meanwhile, Fyn-T and Fyn-D7 are generally found in cells derived from haematopoietic stem cells (astrocytes, microglia, oligodendrocytes, and neurons) and peripheral blood mononuclear cells, respectively (Cooke and Perlmutter, 1989) (Goldsmith et al., 2002). As it is a member of a large SFK family, Fyn also shares similar structural properties with the other SFK members (Src, Lyn, Yes, and Lck) (Boggon and Eck, 2004).

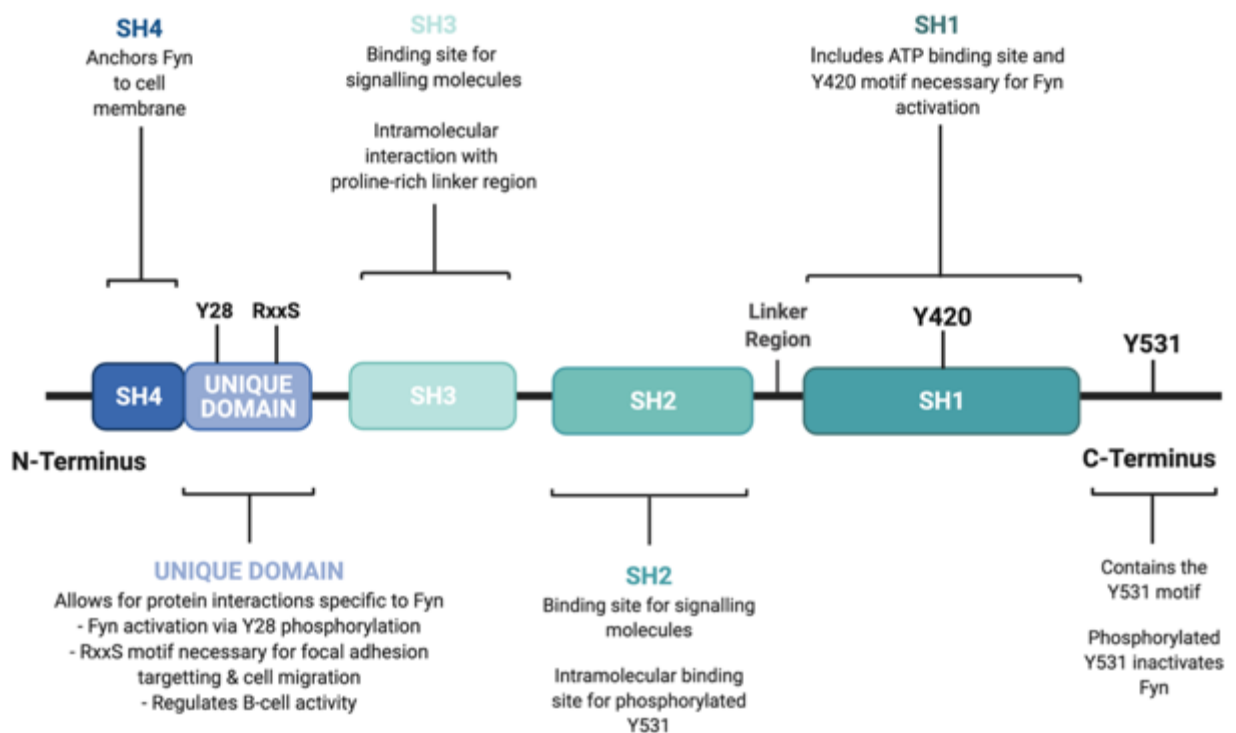


Figure 3.1 Schematic representation of Fyn. The multidomain Fyn structure extends from an N-terminus to a carboxyl (C)-terminus, with multiple domains in between the terminuses, each with its own specific functionality. The Src homology (SH) 4 (SH4) domain at the N-terminus anchors Fyn to the cell membrane (Resh, 1993). The subsequent domain, referred to as the unique region, is poorly conserved amongst SFK members and thus allows for Fyn-specific functions (Hansen et al., 1997, Xu et al., 1997). Both the SH3 domain and its neighbouring SH2 domain are capable of binding to signalling molecules within the cell. However, the SH2 domain also serves as the binding site for a phosphorylated Y531 motif when Fyn is in its inactive state. Connecting the SH2 and SH1 domains is the proline-rich linker region that can interact with the SH3 domain to form an inactive conformation during Fyn's inactive state. The linker region is followed by the catalytic SH1 domain that is well conserved amongst all SFK members. This particular domain includes the Y420 motif; the phosphorylation of which is essential for Fyn activation. Equally important for the regulation of

Fyn activation is the Y531 motif, located on the C-terminus (Knox and Jiang, 2015, Krämer-albers and White, 2011, Resh, 1998, Xu et al., 1997). Figure created in BioRender.com (2021).

Fyn consists of four Src homology (SH) domains (SH1- SH4), which are well conserved between SFK members, in addition to a Fyn-specific unique domain (Figure 1). At the N-terminus end is the very short SH4 domain, capable of undergoing fat modification, which allows Fyn to anchor itself at the hydrophobic cell membrane (Resh, 1993). The SH4 domain is followed by a unique domain, consisting of the Y28 motif, which is phosphorylated by platelet-derived growth factor (PDGF) receptor, leading to activation of Fyn. Interactions within this domain are integral for focal adhesion targeting and cell migration (Yeo et al., 2011), as well as for regulating B-cell activity in the adaptive immune system (Barua et al., 2012, Pleiman et al., 1994). Beyond this lies the catalytic region of Fyn, consisting of the SH1, SH2 and SH3 domains, as well as the regulatory C-terminus end.

The regulation of Fyn activity is facilitated by the dynamic phosphorylation status of both the Y531 motif on the C-terminus and the Y420 motif on the SH1 domain's activation loop (Cheng et al., 1991, Ponniah et al., 1999, Yang et al., 2011). In Fyn's basal state, the Y531 residue on the C-terminus is phosphorylated and forms an intramolecular interaction with the SH2 domain. Similarly, the SH3 domain interacts with the linker region, located between the SH1 and SH2 domains. This results in a folded conformation that restricts both the SH2 and SH3 domains from binding to their substrates, thus preventing any external protein interactions (Knox and Jiang, 2015, Krämer-albers and White, 2011, Resh, 1998, Xu et al., 1997). This inactive Fyn conformation is also aided by the dephosphorylation of Y420 in the SH1 domain (Knox and Jiang, 2015) (Nguyen et al., 2002).

In contrast, upon dephosphorylation of the Y531 motif, the Y531-SH2 intramolecular complex is disrupted, generating an open conformation that allows for Fyn activation (Bhandari et al., 1998). This is further enhanced by the autophosphorylation of

the Y420 motif in the SH1 domain (Xu et al., 1997) (Knox and Jiang, 2015, Nguyen et al., 2002). The open conformation makes the SH2 and SH3 domains accessible to a broad range of substrates for interaction, allowing Fyn to function as a major upstream signalling mediator in various intracellular processes (Panicker et al., 2015, Suzuki and Okumura-Noji, 1995, Wu et al., 2007). Recent investigations have allowed for a deeper understanding of Fyn within the nervous system. As such, it has also unveiled Fyn's potential role in neuronal signalling pathways linked to multiple neurodegenerative conditions.

3.3 MULTIPLE SCLEROSIS

Multiple Sclerosis is a chronic demyelinating disease characterized by progressive loss of the myelin sheath surrounding neurons of the CNS (Maghzi et al., 2011). Although the specific cause remains unclear, studies strongly point to the aberrant activation of both the innate and adaptive immune system in the pathophysiology of the disease (Hemmer et al., 2015). This results in upregulation of the inflammatory response and subsequent autoimmune initiated attack of the myelin sheath, specifically by targeting myelin-forming oligodendrocytes, inducing apoptosis and necrosis (Cudrici et al., 2006). Fyn is known to be expressed by oligodendrocytes (Sperber and McMorris, 2001) and has been implicated in the differentiation and maturation of oligodendrocytes and their subsequent myelinating functions, as well as in modulation of the immune response, suggesting that dysfunction of Fyn may play a role in the pathophysiology of MS (Lau et al., 2016, Palacios and Weiss, 2004, Umemori et al., 1994). In support of this, genome-wide association studies have implicated single-nucleotide polymorphisms of the Fyn gene in susceptibility factors associated with MS (Baranzini et al., 2009). Here, we outline evidence for Fyn kinase modulation as a potential novel therapeutic target for MS.

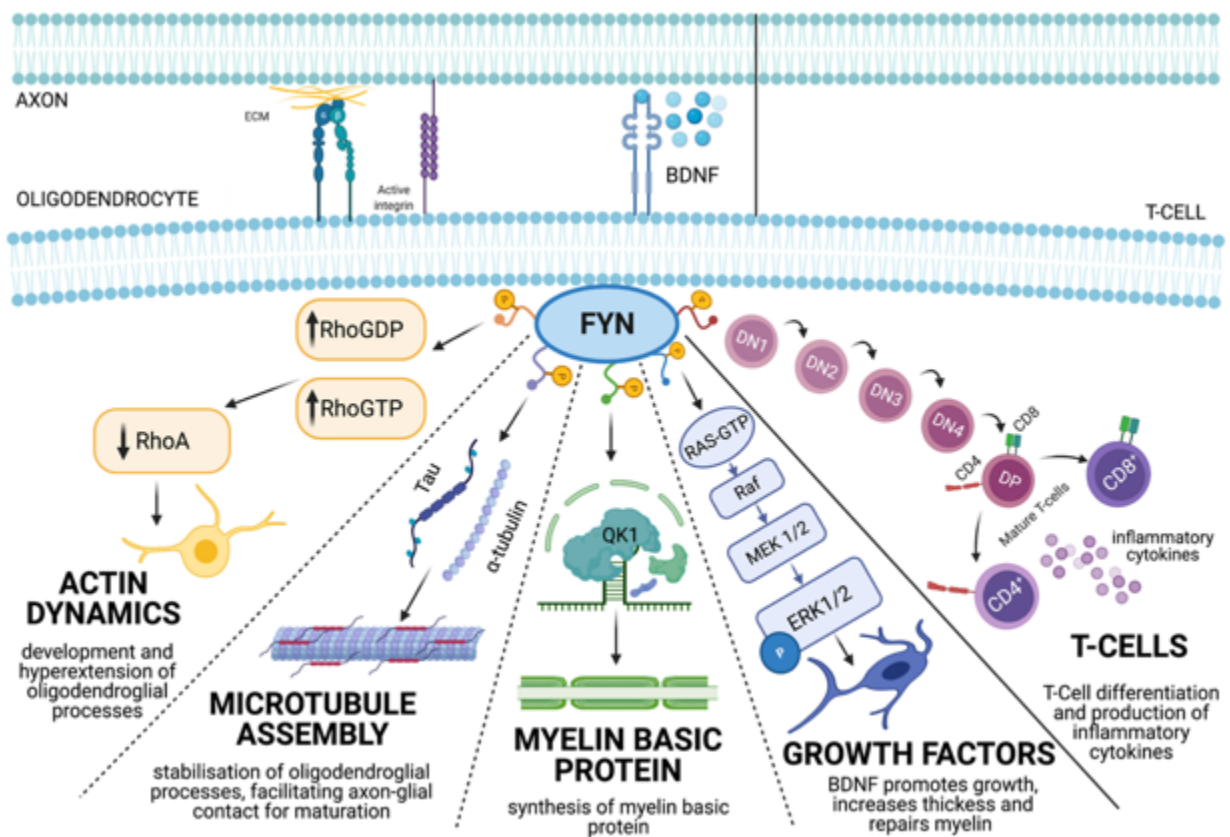


Figure 3.2 Overview of the role of Fyn in physiological processes associated with myelination and T-cell mediated inflammation. Actin Dynamics - Fyn phosphorylation increases RhoGTPases activity, downregulating RhoA and altering actin dynamics to allow hyperextension of oligodendroglial processes, enabling differentiation, maturation and axonal contact. Microtubule Assembly – Fyn tyrosines bind to tau and α -tubulin, stabilising oligodendroglial processes, orchestrating axon-glia contact and facilitating transport of myelin towards the axon. Myelin Basic Protein – contactin/integrin complex activates Fyn in lipid rafts, phosphorylating the MBP mRNA QK1 protein, leading to granular dissociation and subsequent synthesis of MBP. Growth Factors – BDNF phosphorylates Fyn, activating the MAPK signalling pathway and promoting myelin growth. Within T-cells – Fyn is required for T-cell development and subsequent differentiation of CD4+ T-cells, which infiltrate the CNS and differentiate depending on APC to produce inflammatory cytokines. Figure created in BioRender.com (2021).

During normal development, oligodendrocytes proliferate from oligodendrocyte precursor cells (OPCs), which then undergo terminal differentiation and initiate process formation in order to become functional myelinating cells (Osterhout et al., 1999). With demyelination a

key pathological hallmark of MS, remyelination is a vital natural repair mechanism to mitigate extensive loss (Stangel et al., 2011). Initial processes require OPCs to migrate from the subventricular zone (SVZ) to the damaged area and differentiate into mature cells, which are disrupted in MS (Gruchot et al., 2019), and we propose that this may be at least partially driven by alterations in Fyn activity.

Seminal work by Bare and colleagues (1993) identified that the peak of Fyn activity in the CNS corresponds with the peak of myelination in the developing brain (Bare et al., 1993). Subsequent work by Umemori and colleagues revealed Fyn- deficient mice demonstrated reduced myelination, further solidifying a place for Fyn in myelinating mechanisms (Umemori et al., 1994). Fyn activation is one of the earliest differentiation triggers, with Fyn activity 10-30 times higher in mature oligodendrocytes (Osterhout et al., 1999, Scarlato et al., 2000).

Fyn assists in the migration of OPCs, mediating the PDGF activation of Cyclin Dependent kinase 5 (CDK5) and generating the rearrangement of the actin cytoskeleton that facilitates migration (Miyamoto et al., 2008). Indeed, OPC differentiation requires stable axonal contact in order to successfully achieve maturation, which is also largely coordinated by cytoskeleton interactions, such as actin filaments and microtubules (White and Krämer-Albers, 2014). During differentiation, extracellular matrix (ECM) integrins binding on OPCs interact with the cytoplasmic SH2 Fyn domain, leading to Fyn activation (Figure 1) (Wolf et al., 2001). In turn, Fyn activation phosphorylates Rho family guanosine triphosphatase (GTPase) proteins (p190RhoGAP and p250RhoGAP) (Wolf et al., 2001). Rho family GTPases control cellular morphology via ‘molecular switches’, with binding of GDP representing an ‘off’ state and GTP representing an ‘on’ state. Fyn activation promotes increased expression of RhoGDP (or the ‘off’ state’), inactivating RhoA, allowing hyperextension of oligodendroglial processes and thereby enabling oligodendrocyte

differentiation and subsequent maturation (Liang et al., 2004). Netrin- 1 has also been implicated in this process, with the binding of Netrin-1 to its receptor (Deleted in Colorectal Cancer (DCC)) resulting in the recruitment and activation of Fyn and a subsequent decrease in RhoA activity, further facilitating increased process extension and elaboration (Rajasekharan et al., 2010).

Interestingly, in addition to promotion of GDP, Fyn activation also upregulates GTP (or the 'on' state). When an axon is initially linked to an oligodendrocyte for myelination, the axonal ECM protein laminin-2 binds to the extracellular domain of the oligodendroglial integrin. Fyn associates intracellularly with the integrin, resulting in its phosphorylation and the subsequent activation of Rho family GTPases. This cascade facilitates actin dynamics, promoting membrane ruffling and the development of lamellipodia, allowing the oligodendrocytes to make contact with axons (Liang et al., 2004, Ridley et al., 1992). In support of this, inhibition of Fyn activity has been shown to block morphological differentiation of OPCs into mature oligodendrocytes, a process which has been linked to its role in the integrin-Fyn-GTPase transduction pathway (Liang et al., 2004, Osterhout et al., 1999).

In addition to actin dynamics, Fyn activity may also promote oligodendrocyte maturation via its association with tau at the SH3 domain, helping to regulate the assembly of microtubules and consequently contributing to the formation of the cytoskeleton of oligodendrocytes (Gorath et al., 2001, Richter-Landsberg, 2008, White and Krämer-Albers, 2014). During development, the F3 adhesion molecule on oligodendrocytes forms a complex with Fyn within lipid rafts, inducing the phosphorylation of Fyn tyrosines (Tyr-531 and Tyr-420) (Kramer et al., 1999). Fyn tyrosines bind both to tau and another cytoskeleton protein, α -tubulin, which subsequently recruit microtubulin to the contact area, facilitating arborisation and stabilisation of oligodendroglial processes and orchestrating the axon-glia

contact vital for their maturation (Figure 3.2) (Klein et al., 2002, Kramer et al., 1999, Martín-Cófreces et al., 2006). In line with this, overexpression of tau deletion protein in cultured oligodendrocytes has been shown to reduce both the length and number of processes, likely due to disruption of Fyn-tau binding (Klein et al., 2002).

Fyn-Tau interactions mediating microtubule assembly also facilitate movement of cargo, such as myelin, towards the axon and the production of myelin itself (Laursen et al., 2009, White et al., 2008). Specifically, Fyn is prominent in the production of Myelin Basic Protein (MBP), the major protein in the myelin sheath, and is critical for its formation and maintenance (Umemori et al., 1994). Previous work has demonstrated a ~ 50% reduction of MBP in Fyn *-/-* mice compared to wild-type mice (Umemori et al., 1994). Mechanistically, production of MBP is controlled by a contactin/integrin complex within oligodendrocytes, which facilitates the activation of Fyn in lipid rafts (Laursen et al., 2009). Activated Fyn then phosphorylates the MBP mRNA binding quaking (QKI) protein, leading to granular dissociation and subsequent synthesis of MBP (Figure 1) (Zhang et al., 2003). In support of this, suppression of Fyn activity blocked the translation of MBP that would normally be induced by neuronal stimulation (Wake et al., 2011).

Finally, Fyn promotes oligodendrocyte myelination via interaction with growth factors, including BDNF (Peckham et al., 2016). Peckham and colleagues recently demonstrated *in vitro* that, in oligodendrocytes, BDNF stimulates the autophosphorylation of Fyn during myelination, consequently activating extracellular signal-regulated kinases (Erk) 1/2, part of the mitogen-activated protein kinase (MAPK) signalling pathway (Peckham et al., 2016). Erk 1/2 activation promotes rapid myelin growth to increase myelin thickness (Figure 3.2). Whilst the underlying mechanisms are still unclear, this could also link back to Fyn's role in the phosphorylation of the Rho family GTPase protein p190RhoGAP, which also regulates proteins known to influence the Erk1/2 pathway

(Pullikuth and Catling, 2010). Evidence suggests BDNF is critical to repair the structural integrity of damaged myelin observed in MS (Lee et al., 2012).

Given that failure of remyelination in MS is strongly linked to failure of OPC differentiation and subsequent ability to myelinate axons, modulation of Fyn activity may thus represent a novel therapeutic target, capable of facilitating the differentiation and maturation of OPCs via phosphorylation of GDP and GTP protein and decreasing RhoA expression. Indeed, RhoA expression has been spatially associated with MS lesions, with inhibition posed as a potential therapeutic target (Zhang et al., 2008). Fyn may also prevent accumulation of myelin debris, which is linked to disease severity. This is driven by protein-misfolding, including via increased deamination of MBP, where arginine amino acids are converted into citrulline. Increased deamination of MBP is seen in Fyn-deficient mice (Seiwa et al., 2000, Yang et al., 2016), and thus may be prevented with increased Fyn activity.

3.3.1 Role of Fyn in T-Cell regulation in MS

Upregulation of the autoimmune response has been implicated in the attack of the myelin sheath observed in MS (Hemmer et al., 2015). In particular, the adaptive immune response, where T-cells recognise and attack myelin protein, has been strongly implicated (Hemmer et al., 2015). In addition to its role in myelination outlined above, Fyn is perhaps best known for its role in immune function, with extensive evidence establishing Fyn kinase as an upstream regulator of the adaptive immune response of both T-cells and B-cells; however, this review will focus on T-cells specifically, given their pivotal role in MS pathogenesis (Hemmer et al., 2015).

T-cells differentiate from thymocytes, with Fyn and its interactions with another SFK, Lck, involved in nearly all stages of this development (Palacios and Weiss, 2004). In particular, Fyn and Lck are critical in pre-T-Cell Receptor Signalling, allowing the

progression of naïve thymocytes through the developmental stages of T-Cells (Palacios and Weiss, 2004). This includes progression from DN1 through to DN4 and then the eventual proliferation of CD4⁺ or CD8⁺ naïve T-cells, which migrate to scan for foreign peptide ligands, allowing them to become activated during infection (Figure 3.2) (Palacios and Weiss, 2004). Seminal research demonstrated Lck and Fyn-deficient mice exhibited an absolute block in transition between the DN3 and DN4 stages of T-cell development, preventing proliferation of T-cells (Groves et al., 1996, van Oers et al., 1996).

Once matured, CD4⁺ T-cells are able to infiltrate into the CNS, where they can differentiate further, depending on the cytokines produced by Antigen-Presenting Cells (Reboldi et al., 2009). In normal regulatory processes, CD4⁺ -cells are associated with the production of anti-inflammatory cytokines, which help to maintain homeostasis. However, in MS, it is postulated that MBP, as well as other myelin proteins, act as antigens, leading to the priming and activation of autoreactive myelin-specific CD4⁺ T-cells and the release of pro-inflammatory cytokines, such as IL-23 and IL-17 (Murphy et al., 2010). These promote a shift to the T-Helper 17 (Th17) phenotype and an associated upregulation of the inflammatory response exacerbating tissue damage (González and Pacheco, 2014). Fyn has been implicated in the promotion and regulation of Th17 cells, with Fyn ^{-/-} mice exhibiting decreased levels of IL-17 (Ueda et al., 2012). This alone would suggest inhibition of Fyn may assist in down-regulation of the inflammatory response in MS and beneficial effects for the disease; however, as discussed, Fyn is crucial for CD4⁺ differentiation to produce beneficial anti-inflammatory cytokines and complete inhibition may therefore negatively affect this response, suggesting that a more nuanced approach is necessary.

Decades of studies have provided evidence of the relationship between Fyn and healthy neuronal/oligodendroglial connections. At present, the specific role of Fyn in MS

has not been thoroughly characterised; however, parallels may be drawn based on current understanding of its role in myelination and T-cell regulation more broadly. In MS, the best approach to targeting Fyn activity is likely to be highly dependent on the mechanism you aim to modify. For example, attempts to remyelinate via increased OPC migration, maturation and myelination, or to reduce the inhibitory effects of pathological MBP aggregation, suggest upregulation of Fyn may be beneficial. In contrast, to mitigate the inflammatory-induced neurodegeneration observed in MS, Fyn inhibition may be required. Thus, it is necessary to better understand the pathogenesis of MS and the specific role Fyn plays in the time course of the disease, in order to develop an optimal therapeutic approach.

3.4 ALZHEIMER'S DISEASE

Pathologically, Alzheimer's Disease (AD) is characterised by the abnormal misfolding and aggregation of two key proteins, A β and hyper-phosphorylated tau (Association, 2020). Pathological accumulation of these proteins promotes excitotoxicity in AD, leading to the subsequent neurodegeneration observed in the disease (Cheng et al., 2009b, Selkoe, 2008). Shirazi and colleagues first evaluated the role of Fyn in the context of human AD and demonstrated elevated levels of Fyn kinase in the hippocampus of AD patients (Shirazi and Wood, 1993). More recently, increased levels of FynT have been observed in the neocortex of AD patients, a finding which was associated with neurodegenerative pathology and cognitive impairment (Lee et al., 2016, Low et al., 2021). Since, subsequent studies have demonstrated a key role of Fyn kinase in the regulation of A β , tau and NMDA signalling, leading to the identification of Fyn as a potential novel therapeutic target for the disease (Figure 3.3) (Yang et al., 2011).

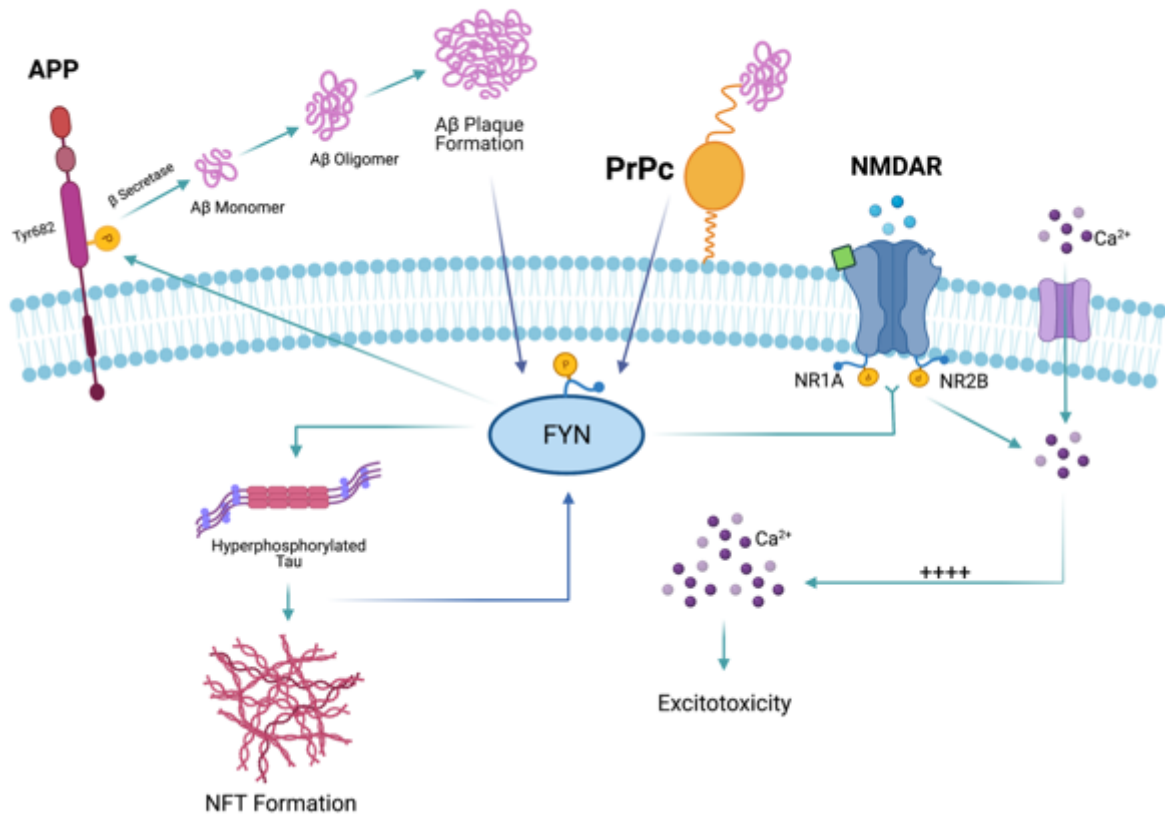


Figure 3.3 Overview of the role of Fyn in physiological and pathological processes in Alzheimer's Disease. Fyn phosphorylates the Tyr682 subunit of APP, promoting amyloidogenic signaling and the aggregation of A β . A β aggregates in turn phosphorylate Fyn, where, in the AD brain, Fyn phosphorylation promotes hyperphosphorylation of Tau and the formation of NFTs (neurofibrillary tangles). This process can result in further upregulation of Fyn. A β aggregates also bind to PrPc (cellular prion receptor protein), which phosphorylate Fyn. Collectively, in AD, increased Fyn activity results in hyperphosphorylation of the NMDAR2B subunit, leading to increased calcium influx and subsequent excitotoxicity. Figure created in BioRender.com (2021).

3.4.1 Role of Fyn in regulating amyloid-beta and hyperphosphorylated tau production in AD

In AD, cleavage of the APP via the amyloidogenic pathway through the sequential actions of β - and γ -secretion leads to the formation of A β (O'Brien and Wong, 2011). Aggregation of these A β monomers into toxic oligomers in AD results in a series of neurodegenerative alterations throughout the brain that culminate in impaired LTP and subsequent neuronal cell death (Li et al., 2011). Fyn is thought to promote amyloidogenic processing of APP, via increasing phosphorylation of APP at Tyr682 (Iannuzzi et al., 2020). The Tyr682 residue in APP is crucial for APP trafficking in neurons, controlling APP endocytosis and distribution (Poulsen et al., 2017). Increased phosphorylation at Tyr682, such as that induced by Fyn, impairs APP endocytosis, forcing the accumulation of APP into acidic neuronal compartments, like the late endosome and lysosomes, where the β -secretase, BACE-1 is optimally active, promoting the generation of A β peptides. Indeed, overexpression of Fyn promotes amyloidogenic processing of APP (Poulsen et al., 2017) and, recently, an increase in Tyr phosphorylation of APP was found to be correlated with increased Fyn activity in AD patients (Iannuzzi et al., 2020). Importantly, Fyn inhibition was able to prevent increased Tyr phosphorylation of APP, with a concomitant decrease in A β secretion *in vitro* (Poulsen et al., 2017).

The amyloid cascade hypothesis suggests excessive extracellular A β leads to hyperphosphorylation of the tau protein (Gotz et al., 2004). As mentioned earlier, tau is involved in the stabilisation of cytoskeletal microtubules in the healthy brain (Hervy and Bicout, 2019). In AD, tau hyperphosphorylation and aggregation leads to the formation of neurotoxic intraneuronal inclusions called neurofibrillary tangles (NFTs) in the somatodendritic compartment, which destabilise neurons and impair axonal transport (Ittner and Gotz, 2011). Evidence posits that A β -induced tau phosphorylation may be mediated through Fyn kinase interactions (Folch et al., 2016, Li and Götz, 2017). Mechanistically, the SH3 domain of Fyn binds to tau, which contains a Fyn specific phosphorylation site at

TyrY18 (Larson et al., 2012, Liu et al., 2016). The presence of A β dimers sharply increases Fyn phosphorylation and the subsequent Y18 phosphorylation of tau, which abnormally accumulates in dendritic spines, causing NFT formation and eventually leading to neuronal dysfunction and death (Larson et al., 2012) (Hoover et al., 2010). This may occur through the activation of the ERK/S6 pathway by Fyn, with both pharmacological inhibition and genetic deletion of Fyn suppressing ERK/S6 activation and preventing A β -induced hyperphosphorylation of tau (Li and Götz, 2017).

Recently, LM11A-31, a small molecule modulator of the p75 neurotrophin receptor, was found to inhibit tau hyperphosphorylation by reducing excess activation of Fyn kinase both *in vitro* in neurons exposed to oligomeric A β , as well as *in vivo* in the APP mouse model of AD (Yang et al., 2020a). Similarly, the Fyn kinase inhibitor Saracatinib has been shown to inhibit okadaic acid-induced tau hyperphosphorylation in both mouse neuroblastoma and differentiated rat primary cortical neuron cultures (Yadikar et al., 2020), as well as to prevent deficits in spatial memory and passive avoidance learning, with concomitant decreases in hippocampal level of hyperphosphorylated tau, in transgenic P301S tau mice (Tang et al., 2020). These effects may be due, at least in part, to the interaction between the SH3 domain of Fyn and PxxP motifs in the proline rich domain of tau. In support of this, a peptide inhibitor of Tau-SH3 interactions was able to ameliorate A β oligomer-induced toxicity in rat primary hippocampal neurons (Rush et al., 2020).

3.4.2 Role of Fyn kinase in NMDA receptor dysfunction and PrPc interactions in AD

Accumulation of abnormal aggregates of tau and A β is thought to drive synaptic dysfunction via alterations in LTP and long-term depression (LTD), leading to the cognitive dysfunction observed in AD (Luscher and Malenka, 2012). LTP/LTD is largely mediated by NMDA receptors (NMDAR), which are tightly regulated by Fyn (Trepanier et al., 2012) At rest, the

calcium channel of NMDAR is blocked by Mg^{2+} . In LTP, the strong and prolonged release of glutamate activates AMPA receptors, and the subsequent depolarisation removes the magnesium blockage, allowing influx of calcium and activation of calmodulin dependent protein kinase II mediated signalling cascade that enhances synaptic strength. Conversely, a modest activation of NMDARs allows less calcium to enter, triggering long-term depression and weakening synapses (Luscher and Malenka, 2012). Fyn kinase phosphorylates both the NR1A and NR2B subunits of the NMDA receptor (Nakazawa et al., 2001, Suzuki and Okumura-Noji, 1995). There are 7 tyrosine residues on the NR2B C-terminus, with Y1472 the major Fyn kinase site. Phosphorylation at Y1472 stabilises the synaptic localisation of the NMDAR, preventing the interaction with clathrin adaptor protein and consequent internalisation (Trepanier et al., 2012). Increased membrane localisation of NMDAR increases calcium influx and initiates LTP. However, in pathological conditions, such as AD, this can also mediate excitotoxicity, promoting excessive calcium influx in response to unregulated glutamate release (Esposito et al., 2013). $A\beta$ oligomers have been shown to induce increased phosphorylation of NMDAR via Fyn, which is believed to contribute to cell death via excitotoxicity (Um et al., 2012). Similarly, tau is also capable of inducing NMDA receptor-dependent calcium influx and subsequent excitotoxicity, a process which is dependent on Y18 phosphorylation of Tau by Fyn (Miyamoto et al., 2017). Evidence suggests that these processes may be mediated via cellular prion protein (PrPc) interactions (Um and Strittmatter, 2013).

Whilst the role of PrPc under normal physiological conditions is not well characterised, it is suggested to be a response mediator in neurite outgrowth and cell adhesion (Pantera et al., 2009). Like Fyn, PrPc is localised to lipid rafts and clustering of PrPc has been shown to activate Fyn kinase (Pantera et al., 2009, Stuermer et al., 2004). PrPc signalling is dependent on this Fyn activation, leading to phosphorylation of signalling

cascades which activate Erks, as well as focal adhesion kinases, such as caveolin-1, ultimately promoting neurite outgrowth and cell survival (Pantera et al., 2009).

In AD, PrPc has been linked to the binding of A β oligomers to neurons (Poulsen et al., 2017, Schwarze-Eicker et al., 2005). A β oligomers can bind with high affinity to PrPc at the dendritic spines of neurons, leading to Fyn's colocalisation and subsequent phosphorylation via as-yet-unidentified mechanisms (Larson et al., 2012, Laurén et al., 2009, Um et al., 2012). This, in turn, leads to an increase in the Fyn-specific phosphorylation of the glutamatergic NMDA NR2B subunit (Renner et al., 2010). As such, overexpression of PrPc and/or A β results in excessive stimulation of glutamate and subsequent increased calcium influx via Fyn-dependent mechanisms, promoting excitotoxicity and eventually leading to apoptosis (Um et al., 2012). In support of this, Lacor and colleagues identified that Fyn activation by A β -PrPc induces excitotoxicity and destabilises dendritic spines (Lacor et al., 2007). Whilst Fyn has been demonstrated to be overexpressed in AD (Shirazi and Wood, 1993), lending merit to this theory, it is not currently clear if overexpression of Fyn is driving increased PrPc-A β oligomer binding, or if it is rather a consequence of the abnormal clustering of PrPc observed in AD. This represents an area for future investigations; however, given the role of Fyn in the initial cleavage of A β peptides, which predate A β oligomer formation, it may be the former.

Much like amyloid, tau is also known to interact with PrPc in AD (Corbett et al., 2020). In support of this, a recent 2020 study established direct binding of tau to PrPc in a range of *in vivo* experiments (Corbett et al., 2020). Further to this, tau-PrPc binding was also required for disruption of LTP and neurotoxicity. This is in line with previous evidence from a tau deficient mouse model, where the detrimental effects of A β toxicity could be blocked by uncoupling Fyn from NMDA receptors and reducing subsequent neurotoxicity (Ittner et

al., 2010). This may potentially occur by disrupting its co-localisation with tau-PrPc, although this has not yet been investigated.

Given the findings discussed above, it is possible that overactivity of Fyn kinase may play a key role in the pathophysiology of AD via several different mechanisms. Indeed, the literature suggests Fyn gain of function enhances Alzheimer's disease-related phenotypes, whereas Fyn loss of function ameliorates Alzheimer's disease-related phenotypes (Chin et al., 2005, Um et al., 2012). In line with this, Pena and colleagues (2010) found a complete protection of hippocampal neurons from A β -induced toxicity was observed in Fyn $-/-$ mice (Pena et al., 2010). Similarly, over-expression of Fyn has been found to accelerate synapse loss and induce the onset of cognitive impairment in a transgenic murine AD model (Chin et al., 2004, Chin et al., 2005). Consequently, targeting Fyn overexpression may represent a therapeutic window for intervention, with inhibition of Fyn potentially altering early pathological amyloid signalling in AD. There are currently several studies which have investigated the efficacy of Fyn inhibition in AD. Animal studies have identified that the non-selective inhibition of Fyn kinase via Imatinib derivatives increases A β clearance, attenuating spatial learning and memory deficits (Lonskaya et al., 2013). Similarly, Saracatinib, a selective Fyn kinase inhibitor has also demonstrated success in a transgenic mouse model of AD, where it was able to restore spatial memory deficits and synaptic depletion (Kaufman 2015). Excitingly, these beneficial effects were found to persist even after drug washout (Smith et al., 2018). More recently, similarly beneficial effects of Saracatinib for cognitive function were reported in transgenic P301S tau mice (Tang et al., 2020). Saracatinib progressed to a Phase 1b trial in AD, which found the drug was safe and well tolerated across doses (Nygaard et al., 2015). Disappointingly, however, a subsequent phase 2a clinical trial revealed no improvements in cerebral metabolic decline, cognitive

impairments or other biomarkers in patients with mild Alzheimer dementia, although a potential trend for a slowing of hippocampal volume and entorhinal thickness atrophy was observed (Van Dyck 2019). Currently, Mastinib, a less selective Fyn kinase inhibitor, is undergoing Phase 2b/3 clinical trials in AD and preliminary results announced improvements in measures of cognitive and ADL with 4.5mg/kg in mild-moderate AD; however, 13% of participants experienced adverse events (Clinical Trial Identifier: NCT01872598). These preliminary results indicate a promising potential for the utility of targeting Fyn kinase to reduce several pathological pathways observed in AD, proactively improving cognitive dysfunction by potentially stopping or slowing disease progression.

3.5 PARKINSON'S DISEASE

Parkinson's Disease (PD) is a neurodegenerative disease characterized by a loss of dopaminergic (DA) neurons in the substantia nigra (SN) (Pollanen et al., 1993). This is associated with the presentation of motor impairments, such as resting tremor and akinesia, and a range of non-motor impairments, such as executive dysfunction and depression (Barnum and Tansey, 2012). Whilst the aetiology of PD is unknown, mounting evidence has identified long-term chronic neuroinflammation as detrimental long-term, contributing to the neurodegeneration observed in PD (Pajares et al., 2020). Recently, Fyn dysfunction has been associated with this process (Panicker et al., 2015, Panicker et al., 2019).

3.5.1 Role of Fyn kinase in inducing neuroinflammation in PD

Primarily, the neuroinflammatory response in the CNS is mediated by resident immune cells microglia and astrocytes, and, to a lesser extent, T-cells, with Fyn activity established in each of these cellular processes (Ellwardt et al., 2016, Panicker et al., 2015, Ko et al., 2018).

Most influential, the first line of immune defence in the CNS is mediated by microglia (Yang et al., 2021). In a healthy brain, the microglial response is protective and may be down-regulated once damage has been attended to; however, under pathological conditions, microglia become activated, releasing reactive oxygen species (ROS), nitric oxide (NO) and pro-inflammatory cytokines, such as Tumour Necrosis Factor- α (TNF α), IL (Interleukin)-1 β (IL-1 β) and interferon gamma (IFN γ) (Yan et al., 2014). These products further accelerate microglial activation by binding to their microglial-cell surface receptors, sustaining chronic inflammation (Kim and de Vellis, 2005).

The presence of activated microglia in the brains of post-mortem PD patients has been well established (McGeer et al., 1988, Tansey and Goldberg, 2010) and DA neurons have been identified as particularly susceptible to neurodegeneration via protein kinase C delta (PKC δ) upregulation by microglia (Gordon et al., 2016). In support of the role of Fyn in this pathological process in PD, Panicker and colleagues were first to link the two, finding a greater attenuation of the neuroinflammatory response in Fyn and PKC δ $-/-$ mice, indicating a crucial role of Fyn kinase as a major upstream regulator of the inflammatory response in PD and highlighting the potential of Fyn kinase as a target to mediate this response.

Fyn kinase has been demonstrated to be involved in this process through its role in microglial activation via the Fyn-PKC δ signalling axis and Nod-Like Receptor Protein 3 (NLRP3) inflammasome (Figure 3.4).

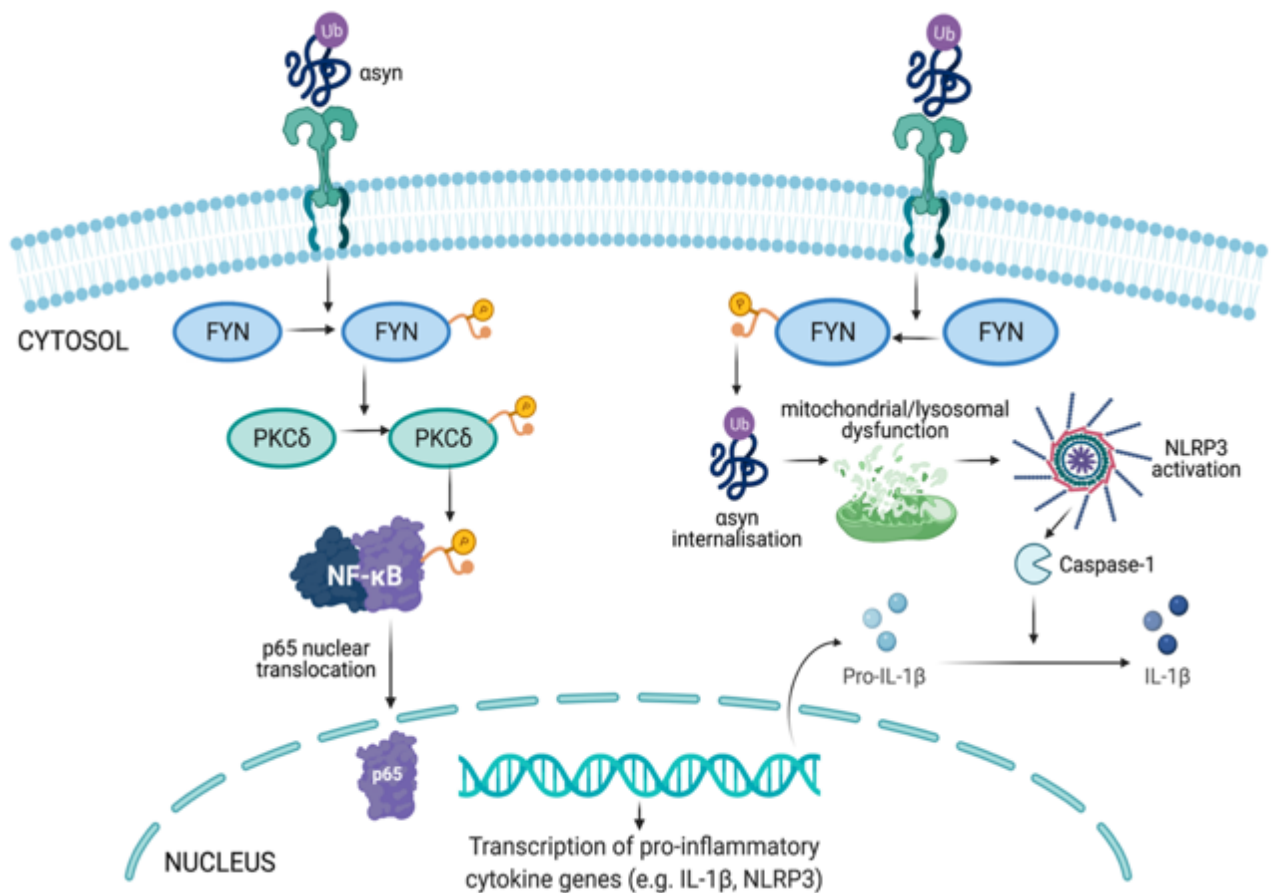


Figure 3.4. Overview of the role of Fyn in microglia under inflammatory conditions. Initiating event (e.g., α -syn presence/pathogen) binds to a membrane receptor, leading to activation of Fyn. Fyn upregulates PKC δ activity, leading to the activation of NF- κ B and causing the translocation of the p65 component into the nucleus, leading to the transcription of pro-inflammatory cytokine genes such as IL-1 β (left). Simultaneously, Fyn activation also leads to the internalisation of α -syn aggregates, priming the NLRP3 inflammasome directly and indirectly (via mitochondrial and lysosomal dysfunction), leading to its activation and conversion of pro-IL-1 β to IL-1 β (Figure adapted from Panicker 2019). Figure created in BioRender.com (2021).

Seminal work by Panicker and colleagues (2015) characterised the preferential expression of Fyn kinase by microglia. When activated, Fyn is localised from the cytoplasm to the microglial plasma membrane (Panicker et al., 2015). Here, an initiating event, such as a pathogen or pro-inflammatory cytokine, binds to a membrane receptor, such as Toll-Like Receptor-4 (TLR4), leading to the activation of Fyn. Fyn associates with PKC δ , upregulating kinase activity and ultimately enhancing NF- κ B activation. When NF- κ B is phosphorylated,

components, such as p65, translocate into the nucleus. p65 translocation and subsequent binding of its subunits to the promoter of genes initiates the transcription of pro-inflammatory cytokines, such as IL-6 and IL-12, and inducible nitric oxide synthase (iNOS) (Gordon et al., 2016). Under pathological conditions, such as those seen in neurodegenerative diseases, these pro-inflammatory cytokines bind to their receptors on the microglial cell surface, leading to further propagation of aberrant microglial activation (Yang et al., 2021). Studies in cell culture and transgenic Fyn ^{-/-} mice have consistently demonstrated Fyn is required for cytokine release and activation of iNOS (Ko et al., 2018, Panicker et al., 2015, Sharma et al., 2018). More recently, Fyn was also shown to be critical for upregulation and post-translational modification of Kv1.3, a voltage-gated calcium channel, in microglia (Sarkar et al., 2020). Kv1.3 may play a key role in sustaining the chronic neuroinflammatory response seen in PD.

In addition to microglia, Fyn is also expressed to a lesser extent in astrocytes, with a key role in astrocytic migration in response to neuronal signals (Dey et al., 2008, Panicker et al., 2015). Astrocytes are neuroglia also involved in the regulation of the CNS immune response and, similar to microglia, play both beneficial and detrimental roles in the brain's response to insult or injury (Giovannoni and Quintana, 2020). Reactive astrocytes have been linked to pathologies in the striatum of animal PD models (6-OHDA and MPTP) and the human PD brain (Charron et al., 2014); however, the role of Fyn in the upregulation of this response in PD has not been directly investigated. This is likely due to the privileged role of microglia in this process where, in addition to increased expression, activated microglia also appear to be required for the initiation of pro-inflammatory astrocyte activity (Liddel et al., 2017).

Fyn kinase, particularly the isoform FynT, plays a role in the astrocytic-mediated production of pro-inflammatory cytokines (IL-1 β and IL-6) via the PKC δ signalling axis,

with Fyn kinase inhibition attenuating the response (Lee et al., 2017). This is associated specifically with chronic exposure to inflammogens, suggesting involvement of astrocytes in a more chronic insult. Fyn also appears to play a regulatory role in astrocytic expression of iNOS following inflammatory stimulation, with increased iNOS expression in Fyn-deficient astrocytes (Ko et al., 2018). Interestingly, this seems to be in contrast to Fyn-deficient microglia, where iNOS expression is decreased (Panicker et al., 2015). These results suggest Fyn kinase is involved in the upregulation of microglial mediated release of iNOS, but may be involved in downregulating the response in astrocytes. Although yet unclear, this discrepancy between the role of Fyn in iNOS production in microglia and astrocytes may be due to their differential expression of Fyn and PKC δ , with microglia expressing significantly more than astrocytes (Panicker et al., 2015). Alternatively, this may be exposure dependent, conditional on the timing of the inflammatory response, or possibly related to differences in Fyn isoform splicing (Lee et al., 2017); however, this remains to be elucidated.

As of yet, a clear pathogenesis of PD has not been elucidated and therefore the initiation of the initial inflammatory response is not clear; however, current evidence posits a potential relationship between decreased clearance and increased spread of α -syn throughout the brain and microglial activation (Lee et al., 2008, Park et al., 2009). In PD, microglia have been recognised as efficient scavengers of misfolded α -syn and recent evidence suggests the uptake of pathological proteins may be facilitated by Fyn via priming and activation of the NLRP3 inflammasome (Figure 3.4). As recently investigated in an elegant series of *in vitro* and *in vivo* experiments by Panicker and colleagues (2019), the NLRP3 acts as an intracellular sensor, detecting danger associated molecular patterns (DAMPs), such as aggregated α -syn, which bind to microglia, leading to Fyn activation, transcription of pro-IL-1 β and subsequent microglial activation (Panicker et al., 2019). This

facilitates the initial uptake of aggregated α -syn by microglia for phagocytic removal, resulting in the release of mitochondrial reactive oxygen species (ROS), lysosomal dysfunction and priming of the NLRP3 inflammasome. Concurrently, the inflammasome is activated, leading to the activation and conversion of pro-IL-1 β to IL-1 β (Panicker et al., 2019). Although this process is intended to assist in the uptake and degradation of aggregated α -syn, ultimately, microglial activation has been shown to hasten α -synuclein dopaminergic neurotoxicity, contributing to disease progression (Zhang et al., 2005). Furthermore, evidence in α -syn cultures suggests the ability of activated microglia to degrade proteins is decreased under inflammatory conditions, resulting in the accumulation of internalised aggregates and perpetuating the response (Lee et al., 2008). As evidence of this, Fyn $-/-$ microglia display significantly reduced uptake of human α -syn compared to WT microglia (Panicker et al., 2019). Accordingly, under normal physiological conditions, Fyn appears to facilitate microglial clearance of aggregated proteins and, as such, Fyn dysfunction may be implicated in the compromised microglial uptake and degradation observed under pathological conditions, contributing to PD disease progression.

Taken together with the Fyn/PKC δ pathway of microglial activation, evidence firmly indicates a key role of Fyn in microglial-mediated neurotoxicity. Thus, Fyn inhibition may represent a potential therapeutic strategy to reduce microglial activation via a reduction of pro-inflammatory cytokine genes and maintain efficiency of microglial-mediated clearance of α -syn. This may also work in tandem to reduce astrocyte-mediated neurotoxicity, cumulatively reducing chronic neuroinflammation and resultant neurodegeneration. To this effect, a crossover study of the phase 2a clinical trial of Fyn inhibition via Saracatinib is currently underway in a PD population (van Dyck et al., 2019). To date, to the best of our knowledge, no other study has investigated Fyn kinase inhibition as a therapeutic strategy for PD.

3.6 FYN: A COMMON THERAPEUTIC TARGET ACROSS MULTIPLE NEURODEGENERATIVE DISORDERS

The literature highlights a specific role of Fyn in pathological processes associated with MS, AD and PD, respectively; however, it is important to note that there are several commonalities between these pathophysiological mechanisms across different neurodegenerative disease (Figure 3.4). For example, although the T-cell immune response is prominent in MS pathology, T cell activity is altered in both AD and PD. Specifically, increased CD4⁺ T cells have been observed in AD (Lombardi et al., 1999), and α -syn pathology in PD has been linked to CD4⁺ T cell infiltration into the brain parenchyma (Olesen et al., 2018). The role of Fyn in T-cell dysfunction in AD and PD is yet to be investigated, however, it is possible that Fyn kinase inhibition may also serve to restore the T-cell response in these diseases.

Similarly, much like AD, PD is also associated with pathological protein aggregation (Pollanen et al., 1993). In PD, α -synuclein monomers misfold, aggregate and form oligomers and fibrils, accumulating intracellularly to form inclusions called Lewy Bodies (LB) which cause progressive dysfunction and neuronal death (Ambrosi et al., 2014, Ferreira et al., 2017b, Pollanen et al., 1993). Interestingly, α -syn is structurally and functionally similar to A β (Resenberger et al., 2011). Consequently, in addition to A β , α -syn oligomers have also been shown to interact with PrPc at the post-synaptic density, forming a complex which phosphorylates Fyn, leading to the activation of the NR2B subunit in the hippocampus and subsequent excitotoxicity (Brás et al., 2018, Ferreira et al., 2017b). Importantly, inactivation of PrPc prevented the toxic effects of α -syn on synaptic function, a response authors attributed to the prevention of Fyn/NR2B signalling and the reestablishment of Ca²⁺ homeostasis (Ferreira et al., 2017b). This suggests that Fyn kinase inhibition in PD, in

addition to reducing neuroinflammation, could also play a key role in preventing pathological PrPc interactions.

Chronic neuroinflammation has also been implicated in pathology across neurodegenerative diseases, including AD and MS (Chen et al., 2016). Although the role of Fyn has not been specifically investigated in neuroinflammatory processes in these diseases, it is likely that, similar to what is observed in PD, the Fyn- PKC δ signalling pathways are also implicated. For example, fibrillar A β has been implicated in the activation of Fyn and the subsequent promotion of microglial activation and macrophage migration (Moore et al., 2002, Stuart et al., 2007). Furthermore, the NLRP3 inflammasome has also been linked to pathology in MS (Barclay and Shinohara, 2017) and AD (Heneka et al., 2013). Thus, Fyn inhibition may also serve to reduce abnormal neuroinflammation observed in these diseases.

Finally, another emerging area of research linking mechanisms common across neurological processes is the role of Fyn in striatal-enriched tyrosine phosphatase (STEP), an enzyme believed to oppose SP by providing a tonic break for synaptic transmission (Pelkey et al., 2002). STEP is highly expressed in the striatum, cortex, hippocampus and amygdala and regulates LTP and LTD through the internalisation of NMDARs and AMPARs (Braithwaite et al., 2006). STEP counteracts Fyn activation, with increased STEP activity leading to the inactivation of Fyn and internalisation of the NR2B subunit, impairing memory consolidation (Nguyen et al., 2002, Zhang and Chergui, 2015). With regards to AD, increased STEP activity in the dentate gyrus of the hippocampus was associated with reduced levels of active Fyn in an animal model of AD (Chin et al., 2005). This is potentially linked to A β , where soluble A β signalling has been shown to dephosphorylate STEP, upregulating its activity (Venkitaramani et al., 2007). Recent studies have linked increased STEP activity to cognitive decline in AD (Chin et al., 2005), with cognitive function in AD mice significantly improved with a decrease in STEP activity (Xu et al., 2014). Similarly,

upregulated STEP activity and subsequent Fyn dysfunction has also been linked to the overexpression of risk genes of PD such as PARK2, with STEP upregulated in the striatum of the human PD brain (Kurup et al., 2015). Given the similarities in pathological protein accumulation in AD and PD, α -syn may interact in a similar fashion to $A\beta$, upregulating STEP, decreasing Fyn kinase activity and increasing NR2B endocytosis. In support of this, overexpression of α -syn in transgenic mice has recently been shown to promote calcineurin activity, one of the proposed mechanisms in AD for $A\beta$ -induced STEP activation (Shi et al., 2018). Despite these initial findings, however, it is still difficult to understand the exact role that STEP-Fyn interactions may play in the pathophysiology of these neurodegenerative disorders. Furthermore, it is difficult to reconcile the cognitive deficits seen due to down-regulation of Fyn in response to increased STEP activity with the results of studies suggesting beneficial effects of Fyn inhibition in these diseases. In light of this, significant research remains to be done to answer these key questions.

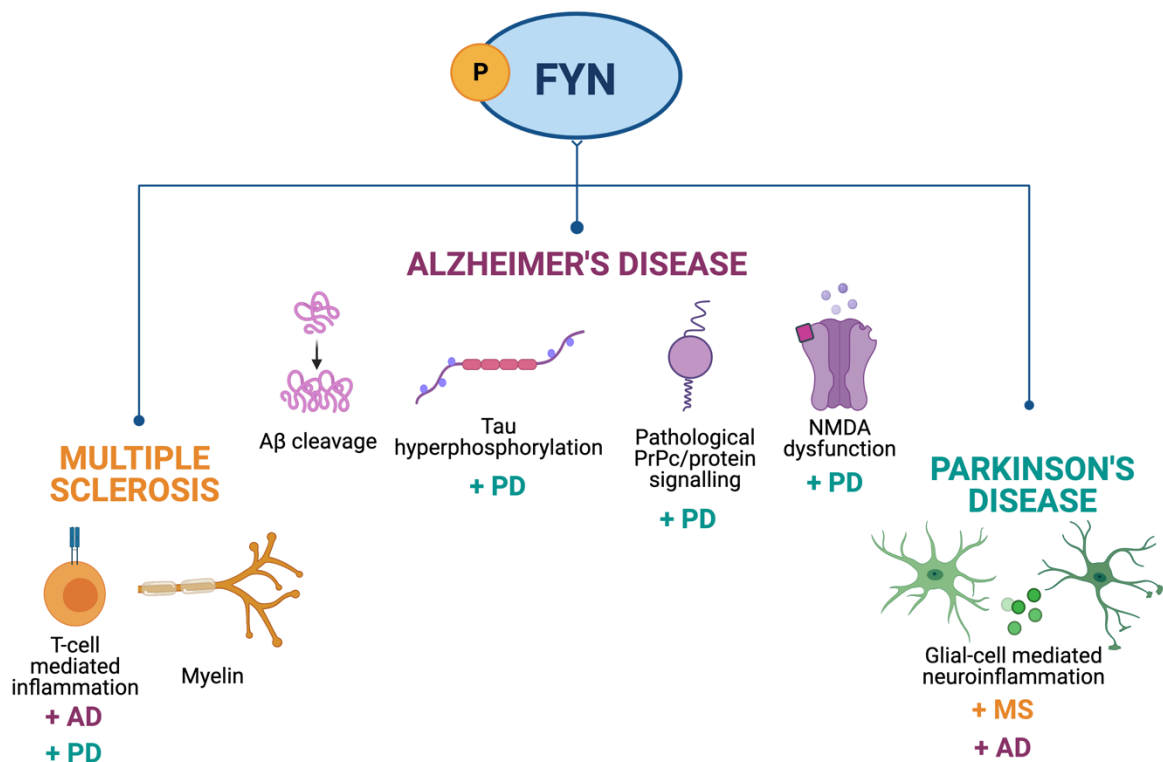


Figure 3.5. Overview of Fyn dysfunction in neurodegenerative diseases and potential commonalities. Multiple Sclerosis - Increased Fyn is implicated in the production of pro-

*inflammatory cytokines via T-Cell mediated inflammation. It's role in the promotion of myelination is well established, however, alterations to Fyn activity in MS are currently unknown. **Alzheimer's disease** – Increased Fyn phosphorylation is associated with pathological A β cleavage which hyperphosphorylate tau, leading to the formations of NFTs. A β aggregates binds to PrPc, phosphorylating Fyn. Collectively, increased Fyn phosphorylation drives NMDA dysfunction and excitotoxicity. **Parkinson's disease** – Increased Fyn phosphorylation is required for the inflammatory response in PD mediated by glial cells (astrocytes and microglia). + = indicates potential common pathological processes observed across diseases in which Fyn may play a role in mediating. Figure created in BioRender.com (2021).*

3.7 CONCLUSIONS AND FUTURE INVESTIGATIONS

Converging lines of evidence suggest Fyn dysfunction may be involved in neurodegenerative disease pathology and, as such, targeting Fyn activity may be a promising strategy for intervention (Figure 3.5).

With regards to MS, pharmacological interventions aimed at remyelination appear to take on two approaches, the neutralisation of differentiation-inhibitors and strategies to promote the stimulation of oligodendrogenesis (Gruchot et al., 2019, Stangel et al., 2011). For the former, pharmaceutical upregulation of Fyn activity may neutralise the inhibitory effects of myelin debris on remyelinating processes. Furthermore, with the latter, upregulation may also be beneficial in several crucial aspects of oligodendrogenesis promotion, with increased Fyn activity required for the migration of OPCs to damaged sites (Rajasekharan et al., 2010, Wolf et al., 2001), the facilitation of actin dynamics permitting oligodendrocyte maturation and contact with axons (Klein et al., 2002, Kramer et al., 1999, Liang et al., 2004), and the subsequent production of myelin proteins (Peckham et al., 2016, Umemori et al., 1994, Zhang et al., 2003). Furthermore, Fyn is also involved in T-cell differentiation, specifically pro-inflammatory cytokine release via Th17 (Ueda et al., 2012).

Taken together, logical therapeutic intervention would aim to increase Fyn activity to promote remyelination in early disease processes to improve functional recovery, particularly in relapsing/remitting MS where it may prevent or shorten the periods of relapse. This needs to be approached with caution, however, given the purported role of Fyn in neuroinflammation, where increased Fyn levels have been associated with increased microglial activation in the PD brain (Panicker et al., 2019). Accordingly, with neuroinflammation prevalent in MS, it is important to characterise the role of Fyn in the time course of MS pathologies specifically, or risk potential exacerbation of disease pathology.

Conversely, in both AD and PD, the current body of evidence promotes the inhibition of Fyn as a therapeutic target for pathological protein-induced toxicity and neuroinflammation. Namely, inhibition of Fyn may interrupt Fyn-PrPc- α -syn/A β signalling, perturbing protein aggregation and oligomeric binding, which would otherwise result in impaired neuronal communication and cell death. Current studies using Fyn kinase inhibitors discussed above have followed this approach, and whilst efficacy has been established in animal models of AD, merits of this approach have not yet been investigated in PD. Furthermore, given the key role that Fyn may play in the upregulation of the microglial and astrocytic activation, Fyn kinase may represent a universal target, not just in AD and PD, but also across multiple conditions known to be associated with a chronic neuroinflammatory response, including ALS (Liu and Wang, 2017), stroke (Wang et al., 2021) and traumatic brain injury (Lozano et al., 2017).

Given the entirety of the literature in the current space, when discussing Fyn as a potential target, it is important to consider and refine dosage due to potential adverse events stemming from its diverse role in normal neurological functions. For example, whilst Fyn inhibition may be an ideal route to target disease progression driven by pathological protein internalisation/aggregation and neuroinflammation, it may not be ideal for other crucial

processes, such as CNS myelination or SP. Furthermore, Fyn's role in SP may mean cognitive function in Fyn inhibition studies needs to be monitored closely, due to the unique balance of NMDA activity required for LTP and LTD and the potential of glutamate-driven excitotoxicity. Fortunately, studies utilising Fyn kinase inhibitors, such as Mastinib and Saracatinib, have demonstrated a much lower level of kinase inhibition is required to modify the inflammatory pathway (Nygaard et al., 2014, Nygaard et al., 2015).

In summary, there are currently no disease modifying treatments for neurodegenerative diseases and symptomatic relief for impairments across the diseases is limited. Given the significant and increasing burden to both the affected individuals and the wider community, it is vital that potential targets are identified for intervention. Evidence highlights Fyn kinase as performing critical roles in the CNS and suggests Fyn dysfunction is involved in pathological signalling cascades underlying several neurodegenerative diseases, including MS, AD and PD. Here, we have highlighted the potential utility of Fyn as a treatment for neurodegenerative diseases by outlining its involvement in pathways associated with pathological disease progression in MS, AD and PD. Subsequently, targeting Fyn kinase activity may represent a promising target for therapeutic intervention. Whilst Fyn's involvement in many neurological signalling cascades has been well defined, future work is needed to characterise Fyn expression and alterations across different stages of pathological progression in neurodegenerative diseases, in order to better understand the ideal therapeutic window and approach

04

Anatomical Distribution of Fyn Kinase in the Parkinson's Disease Brain

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4.0 ABSTRACT

Fyn kinase is an Src family kinase (SFK) widely expressed in many tissues, including the CNS. Recently, alterations to Fyn kinase signalling have been associated with pathological mechanisms underlying neurodegenerative diseases and, as such, the role of Fyn dysfunction is under investigation. In particular, Fyn has been implicated as a major upstream regulator of the neuroinflammatory response in Parkinson's Disease (PD). Chronic neuroinflammation has been observed not just in the substantia nigra (SN), but also in several key regions of the brain, with disruption associated with cognitive dysfunction in PD. This study aimed to characterise the anatomical distribution of Fyn in key brain regions affected in PD, namely the prefrontal cortex, hippocampus, striatum and SN. Fresh and fixed post-mortem PD brain samples ($n=10$) were collected and compared with neurologically healthy age-matched controls ($n=7$) assessing markers of Fyn activity, neuroinflammation and neurodegeneration. Increased Fyn phosphorylation was observed in the SN and striatum of post-mortem PD patients compared with controls. No such increase was observed in the prefrontal cortex or hippocampus. In contrast with previous findings, no increase in microglial or astrocyte reactivity was observed in PD brains across regions and accordingly, increased Fyn phosphorylation was not associated with increased markers of neuroinflammation. Taken together, these results indicate Fyn dysfunction may be involved in the pathological processes observed in PD, however, this appears to be independent of inflammatory mechanisms. Further investigations are required to elucidate if increased Fyn activity is a potential cause or consequence of pathological processing in PD.

4.1 INTRODUCTION

Parkinson's Disease is the second most common neurodegenerative disease after Alzheimer's Disease (AD) and the fastest growing neurological disorder worldwide (Feigin et al., 2019). Clinically, PD is characterised by its cardinal motor symptoms, including resting tremor, muscle rigidity, akinesia, bradykinesia and postural/gait changes. There are also a range of non-motor symptoms associated with the disease, such as depression and cognitive impairment (Barnum and Tansey, 2012). Pathologically, PD is characterised by the presence of Lewy Bodies, intracellular aggregates of alpha-synuclein (α -syn) and ubiquitin, and the loss of dopaminergic (DA) neurons in the substantia nigra *pars compacta* (SNpc) (Pollanen et al., 1993).

Neuroinflammation is thought to be a key driver of this dopaminergic neuronal death, leading to a vicious circle whereby neuronal death drives further microglial activation with the release of inflammatory mediators, exacerbating further cell death (Tansey and Goldberg, 2010, Salvi et al., 2017, Lee et al., 2002) Chronic neuroinflammation is not just localised to the SN in PD, however, with a number of other regions having been identified as affected by microglial-mediated neurodegeneration, including the prefrontal cortex, hippocampus and striatum (caudate+putamen) (Imamura et al., 2003, Doorn et al., 2014a). Just as neuroinflammation and resultant DA cell death in the SN and striatum have been strongly linked to degeneration of the nigrostriatal pathway and the motor impairments of PD (Hunter et al., 2007, Chen and Saez-Atienzar, 2018), neuroinflammation within the prefrontal cortex and hippocampus may contribute to both cognitive dysfunction and depression in PD. In support of this, dysfunction of the mesocorticolimbic pathway, which has extensive projections to areas such as the prefrontal cortex and hippocampus, has been associated with cognitive dysfunction in domains such as executive functioning and memory in PD (Carlesimo et al., 2012, Parker et al., 2013). Similarly, mesocorticolimbic dopamine

loss within the cingulate cortex, as measured by ¹²³I-FP-CIT SPECT imaging, has been shown to be associated with depression in individuals with PD (Frosini et al., 2015). This has been related to abnormal connectivity between the ventral tegmental area and the anterior cingulate cortex (Wei et al., 2018).

Fyn kinase is an Src family kinase (SFK) which is widely expressed in many tissues, including the CNS. Fyn kinase activity has been shown to modulate a number of important neurological processes including axon guidance, oligodendrocyte maturation, myelination and SP (for review, see (Matrone et al., 2020)). More recently, alterations to Fyn kinase signalling have been associated with pathological mechanisms underlying neurodegenerative diseases (**see Chapter 3**) and as such, the role of Fyn dysfunction in these conditions is under investigation. In particular, Fyn has been implicated in signalling cascades associated with Parkinson's Disease. Specifically, a seminal study by Panicker and colleagues outlined the critical role of Fyn in the neuroinflammatory response in PD (Panicker et al., 2015).

Fyn kinase has been shown to be critical in facilitating the microglial-mediated inflammatory response in PD through the nuclear translocation of the p65 component of NFκβ into the nucleus of microglia, and the subsequent up-regulated production of pro-inflammatory cytokines, such as IL-1β and IL-6, promoting chronic inflammation (Panicker et al., 2015). In addition, Fyn is also activated by pathological α-syn oligomers, binding to the cellular prion protein receptor (PrPc), promoting upregulation of NMDA receptors and excitotoxic cell death (Ferreira et al., 2017b). Accordingly, it is hypothesised Fyn dysfunction may be involved in the pathophysiology of PD, perhaps via several molecular mechanisms. In support of this, a GWAS study identified the FYN gene as a novel PD risk locus (Nalls et al., 2019); however, it is unclear if Fyn dysfunction plays a role in the pathogenesis of the disease or is rather a consequence of other pathological alterations

involved. In order to better understand Fyn's role in PD, it is important to characterise the anatomical distribution of Fyn in key brain areas relevant to the pathophysiology of PD to gain a better understanding of the role Fyn may play in pathologies of PD. Specifically, pathological signalling in areas such as the prefrontal cortex, hippocampus, striatum and SN are strongly associated with the often overlooked non-motor symptoms in PD which, to-date, have limited therapeutic interventions for treatment (Carlesimo et al., 2012, Parker et al., 2013). Understanding Fyn activity in these regions may shed light on mechanisms underlying cognitive impairment in PD. Accordingly, this study aims to determine if Fyn kinase is upregulated in the brains of post-mortem PD patients and its relationship to markers of neuroinflammation.

4.2 METHODS

4.2.1 Post-Mortem Samples

Neuropathological screening was carried out and PD diagnosis confirmed by a registered pathologist. Clinical case summary reports were screened for cause of death, medical history and abnormal brain pathology in order to select uncomplicated PD cases or neurologically healthy controls. There were 17 cases in total included for analysis (PD n = 10, controls n = 7 (Table 4.1)). There was no significant difference in age between control (73.43 ± 3.66) and PD (78.20 ± 1.63) cases ($t(15) = 1.32, p = 0.21$). The male to female ratio was 3:4 (M = 3, F = 4) in the control group and 1:1 (M = 5, F = 5) in the PD group and there was no significant difference in post-mortem interval (control = 22.43 ± 6.20 , PD = 14.61 ± 2.61) ($t(14) = 1.27, p = 0.23$).

Table 4.1. Clinicopathological Data for controls and PD cases

<i>Clinicopathological Data</i>	Controls (n=7)	PD (n=10)
Age (Years), Mean (SD)	73.43 (9.69)	78.2 (5.16)
Sex	3 M	5 M
	4 F	5 F
Post-Mortem Interval (Hours)	22.43 (16.39)	14.61 (7.82)

4.2.2 Fixed tissue collection and processing

Tissue collection and subsequent analyses were carried out in accordance with the National Statement on Ethical Conduct in Human Research (2018) and approved by the University of Adelaide Human Research Ethics Committee (H-2016-174). The post-mortem human brain tissue was prepared and provided by the South Australian Brain Bank. Brains were bisected at autopsy, with one half immersion fixed in buffered formalin and the other half coronally sectioned, frozen on a bed of dry ice and stored at -70°C. Fixed tissues were processed and embedded in paraffin wax. For the current study, 5µm coronal sections of the prefrontal cortex, hippocampus, striatum and substantia nigra were prepared from embedded tissue (n = 17), mounted on silane coated slides and allowed to dry at 37°C overnight.

Frozen samples (n = 15, 2 control cases did not have fresh tissue harvested) of the prefrontal cortex, hippocampus, striatum and substantia nigra were placed on dry ice, where ~75mg samples were dissected. Tissue was suspended in buffer solution (20 mM Tris-HCl pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 140 mM 2-mercaptoethanol) with protease and phosphatase inhibitors (Sigma). Samples were then homogenised and sonicated in 3 X 10 s bursts using a sonicator probe and then centrifuged for 15 minutes at 14,000 rpm at 4°C, and supernatant collected. Protein concentrations were estimated with Thermo Pierce BCA

Protein Assay Kit (ThermoScientific) at 562 nm absorbance, in accordance with manufacturer recommendations.

4.2.3 Immunohistochemistry

Immunohistochemical (IHC) analysis was performed according to standard protocols. Briefly, following deparaffinisation in xylene, tissue mounted slides were washed in ethanol and endogenous peroxidases were blocked using methanol with 0.5% hydrogen peroxide. Slides were then washed twice with phosphate buffered saline (PBS). Antigen retrieval was performed (citrate), following which sections were blocked in 3% normal horse serum (NHS) for 30min before overnight incubation at room temperature with IBA-1 (Wako, 019-19741, 1:800) or GFAP (DAKO Z0334, 1:40,000). On the following day, slides were again washed twice in PBS and incubated with secondary antibody (Vector Goat Anti-Rabbit IgG, 1:250 NHS) for 30 minutes at room temperature, followed by two more washes with PBS and incubation with streptavidin peroxidase conjugate (Vector SPC, 1:1000 NHS) for 1 hour. Following a final wash with PBS, the chromogen 3,3-Diaminobenzidine tetrahydrochloride (Vector DAB; 1:50) was applied for 7 minutes. Slides were counterstained with haematoxylin, dehydrated in ethanol and cleared with xylene, prior to being cover slipped. The experimenter was blinded to the group in all following analyses.

IBA1 slides were scanned using a Nanozoomer slide scanner (Hamamatsu, Shizouka, Japan) and analysed using the HALO image analysis platform (Indica Labs, Albuquerque, New Mexico, USA). Analysis settings were based on the microglial activation module (v1.2) for automated counting. Total microglia population and activation state were determined based on morphological parameters of IBA1+ cells (see Table 4.2). Microglial count was divided by total area to determine cells per mm². Activated microglia (per mm²) were divided by total population (per mm²) to determine percent activated.

Table 4.2. HALO Microglial Detection Parameter Summary

Min Microglia Cell Body Diameter	3.4 μm
Microglia Contrast Threshold	0.3 (pixel threshold 0-1)
Minimum Microglia Process OD	0.25 (pixel threshold 0-1)
Max Microglia Process Radius	12 μm
Microglia Max Fragmentation Length	2.5 μm
Activation Process Thickness	2.12 μm

GFAP slides were scanned using a Nanozoomer slide scanner (Hamamatsu, Shizouka, Japan) and the associated software (NDPview) was used to view images. Cells were counted by extracting images using QuPath software across the entire sample, excluding incomplete edges. These were then processed in ImageJ via colour deconvolution, converted to binary and a threshold was applied to demarcate GFAP+ cells. Cell morphology parameters (size and circularity) were used to differentiate cells and an automatic cell counter was used to determine the number of immunopositive cells per mm^2 .

4.2.4 Western Blot Analysis

Western blot analysis was performed using the standard milk method. In brief, 4X Bolt LDS sample buffer and 10X Bolt reducing agent and dH_2O were added to supernatant (30 μg protein), heated at 70° for 10 minutes and then vortexed. Gel electrophoresis was performed using Bolt 4-12% Bis-Tris gels (Life Technologies) with 40 μl of sample loaded per well. Gels were run at 120V for 120 minutes. Following this, gels were transferred to the PVDF membrane using the iBlot 2 Dry Blotting System (Life Technologies). Membranes were washed in 1 \times tris-buffered saline (3 \times 5min). Membrane was blocked in milk (5% w/v dried skim milk in TBS-T) for 2 hours before overnight incubation at 4° in milk (2% w/v dried

skim milk in TBS-T) and primary antibody. Primary antibodies were used at individually optimised concentrations: Fyn (1:500, 4023S, CST), NeuN (1:750, ab177487, Abcam) and housekeeper anti-GAPDH (1:4000, ab83956, Abcam). Following overnight incubation, membranes were washed in TBS-T (3X5min) and incubated in 2% milk and secondary antibodies, according to the species the primary was raised in (donkey anti-rabbit, donkey anti-mouse and donkey anti-chicken, IRDye 800CW; LI-COR, Inc., 1:10000) and incubated for 2 hours at room temperature. Following incubation, each membrane was washed in TBS-T (3×5min) and visualised using an Odyssey Infrared Imaging System (model 9120; software version 3.0.21) (LI-COR, Inc.) at a resolution of 169 μm . Semi-quantitative analysis of band signals was performed using ImageJ version 1.49 and Image Studio Lite version 5.2.

4.2.5 ELISA

Levels of total and phosphorylated Fyn were determined using the Human Phospho-Fyn (Y530) and Total Fyn semi-quantitative ELISA kit from RayBiotech (PEL-FYN-Y530-T-5). ELISA assays were performed in accordance with manufacturers protocol, with all samples run in duplicate. Optical density values were measured at 450nm using a microplate reader to determine a final ratio of Total Fyn:Phosphorylated Fyn.

4.2.6 Statistical Analysis

Data was analysed using Prism software (GraphPad v.9). Statistical outliers were removed based on an interquartile range of 3. In order to compare differences between control and PD samples, data were analysed using an unpaired t-test to compare means. All values are displayed as mean \pm SEM, with significance level set at $p < 0.05$.

4.3 RESULTS

4.3.1 No difference in total Fyn

There was no significant increase in Fyn kinase levels between control and PD cases in the prefrontal cortex (1.1 ± 0.39 vs 2.6 ± 1.1), $t(9) = 0.992$, $p = 0.35$ (Fig 4.1A), hippocampus (1.2 ± 0.27 vs 1.4 ± 0.31), $t(11) = 0.611$, $p = 0.55$ (Fig 4.1B), striatum (0.52 ± 0.086 vs 1 ± 0.19), $t(10) = 1.735$, $p = 0.11$ (Fig 4.1C) or the substantia nigra (2.9 ± 0.91 vs 2.7 ± 0.52), $t(11) = 0.175$, $p = 0.86$ (Fig 4.1D)

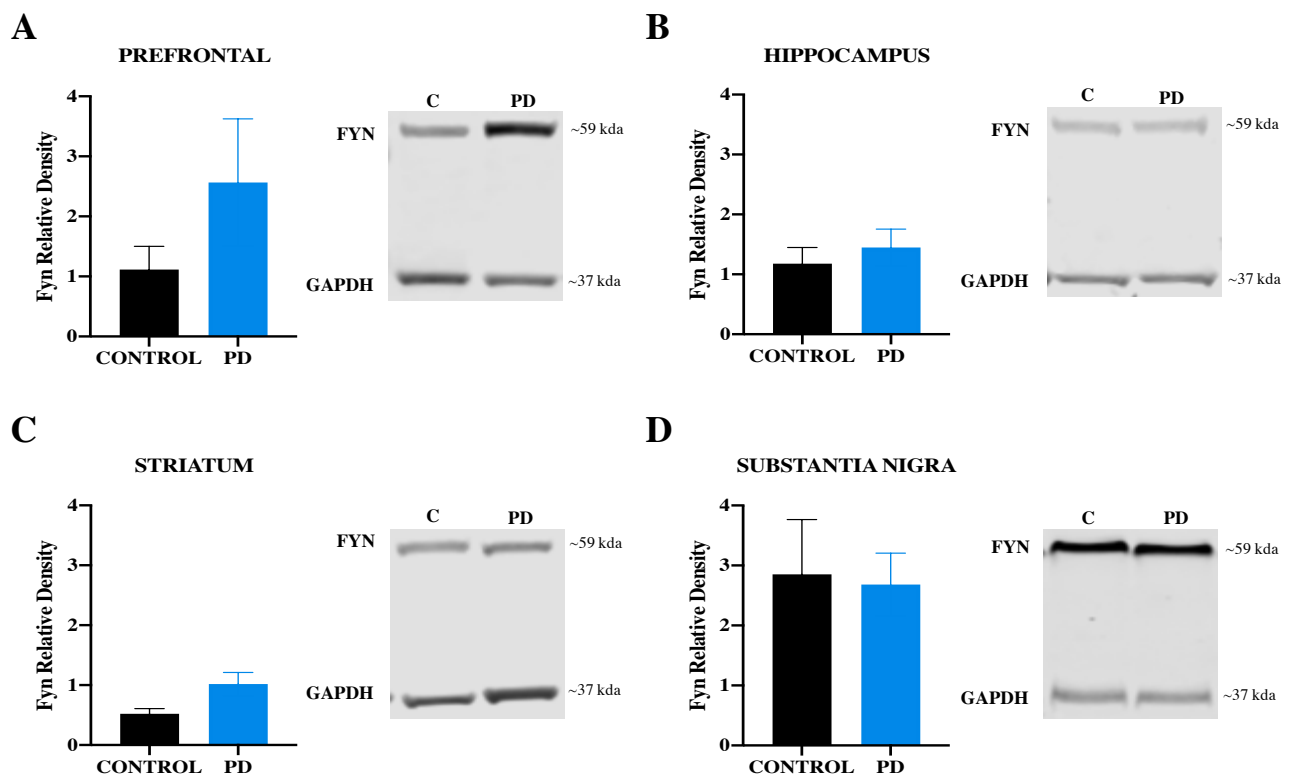


Figure 4.1. Fyn levels as measured by Fyn Western Blot in the (A) Prefrontal ($n=4/7$) (B) Hippocampus ($n=5/8$) (C) Striatum ($n=4/8$) and (D) Substantia Nigra ($n=5/8$). There was no difference in total Fyn level in any of the regions assessed. Graphs represent Mean \pm SEM

4.3.2 Significant increase in Fyn phosphorylation in the striatum and SN

No significant differences were observed between controls and PD in the ratio of Phosphorylated Fyn to Total (Pan) Fyn in either the prefrontal cortex (2.67 ± 0.076 vs 2.77 ± 0.017), $t(9) = 1.756$, $p = 0.11$ (Fig 4.2A) or hippocampus (4.10 ± 0.031 vs 4.11 ± 0.05), $t(11) = 0.1391$, $p=0.89$ (Fig 4.2B). A statistically significant increase in Fyn phosphorylation was however observed in both the striatum (1.397 ± 0.002 vs 1.405 ± 0.002), $t(11) = 2.599$, $p = 0.02$) (Fig 4.2C) and the substantia nigra (1.027 ± 0.0036 vs 1.080 ± 0.0119), $t(10) = 3.056$, $p = 0.01$ (Fig 4.2D) in PD compared to control.

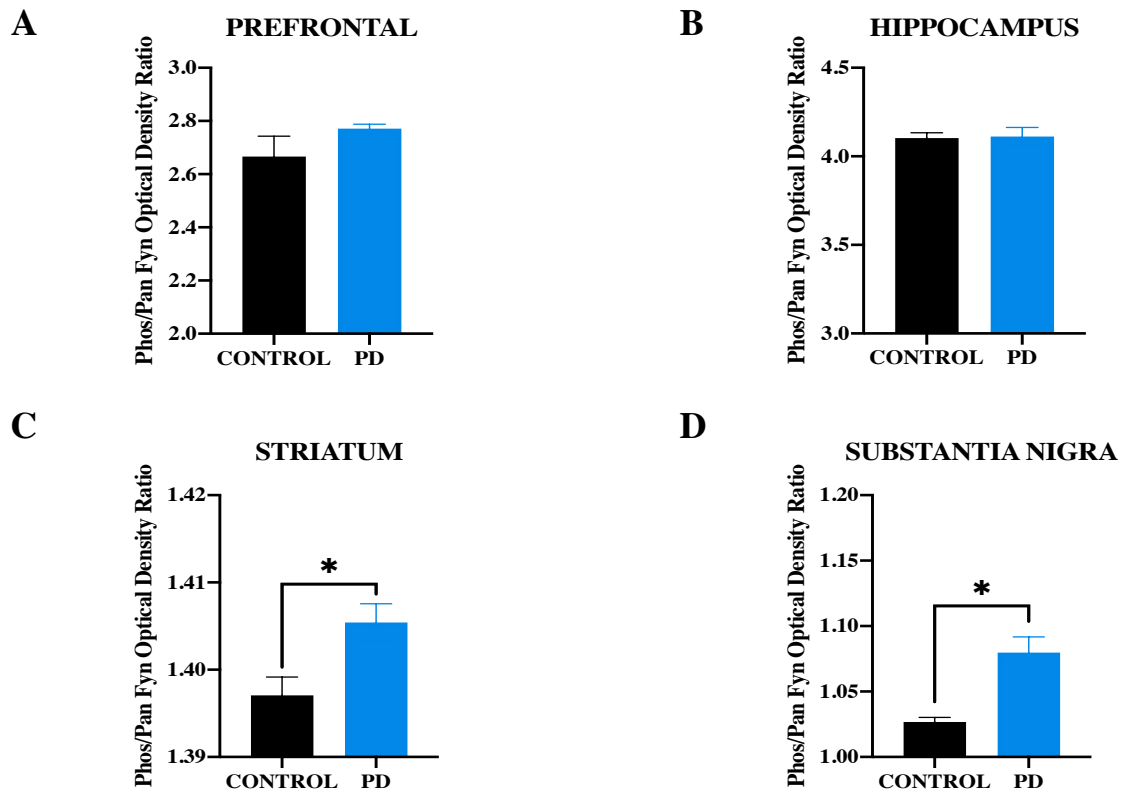


Figure 4.2. Phosphorylated to Pan Fyn Ratio as measured by ELISA in the (A) Prefrontal ($n=4/7$) (B) Hippocampus ($n=5/8$) (C) Striatum ($n=5/8$) and (D) Substantia Nigra ($n=4/8$). There were significant elevations in Fyn phosphorylation in both the striatum and SN, but not in the hippocampus or PFC. Graphs represent Mean \pm SEM. *= $p<0.05$

4.3.3 No increased astrocytic reactivity in PD compared to controls

There was no significant difference in astrocytic number between control and PD cases in the prefrontal cortex (8.96 ± 2.1 vs 14.41 ± 3.43), $t(14) = 1.145$, $p = 0.27$ (Fig 4.3A), hippocampus (12.70 ± 2.74 vs 11.62 ± 3.08), $t(15) = 0.249$, $p = 0.81$ (Fig 4.3B), striatum (18.84 ± 7.08 vs 27.66 ± 8.78), $t(14) = 0.748$, $p = 0.47$ (Fig 4.3C) or substantia nigra (16.02 ± 4.58 vs 18.66 ± 3.6), $t(14) = 0.460$, $p = 0.65$) (Fig 4.3D).

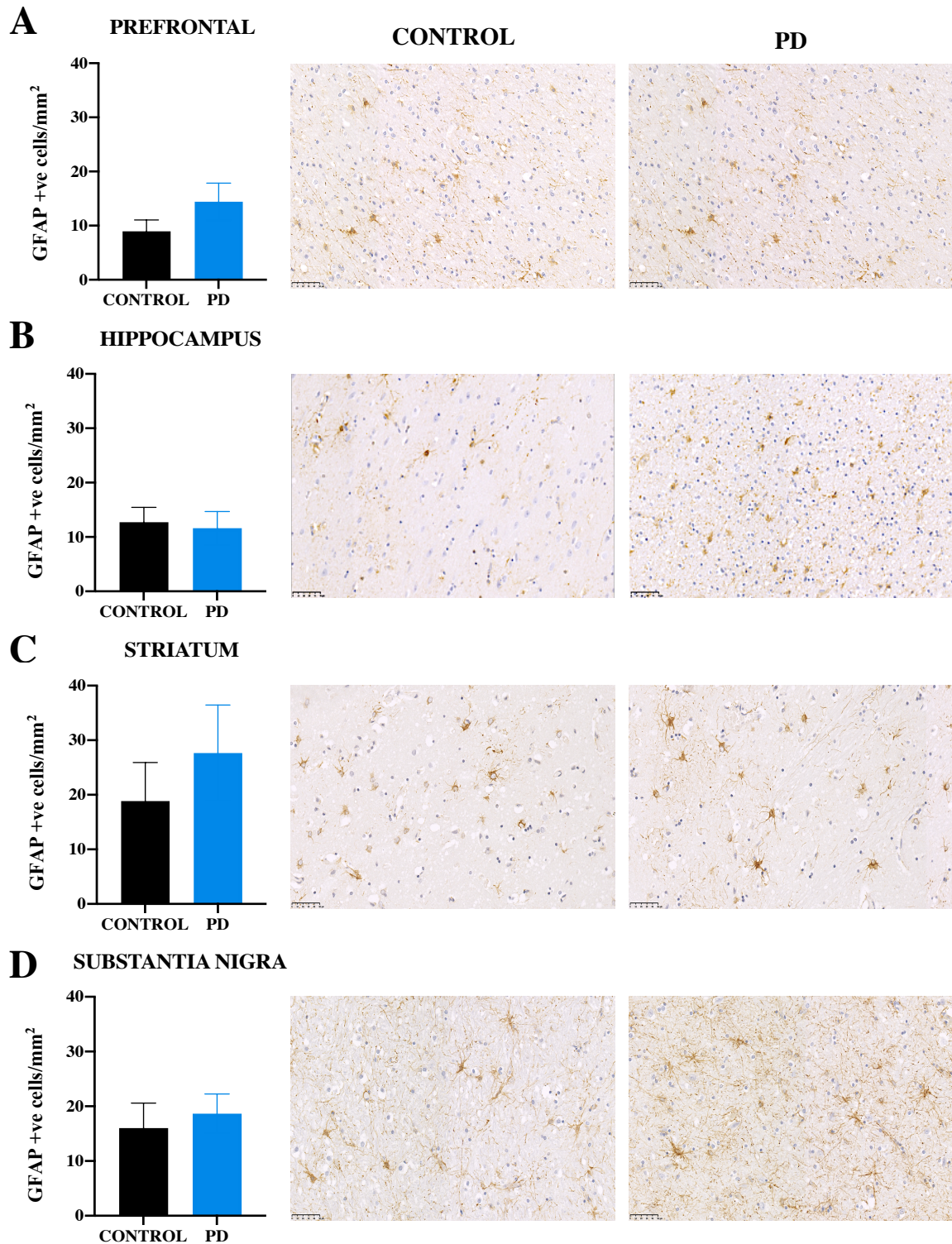


Figure 4.3. Graphs and representative images of glial fibrillary acidic protein (GFAP) staining in the (A) prefrontal ($n=6/10$) (B) hippocampus ($n=7/10$) (C) striatum ($n=7/9$) and (D) substantia nigra ($n=7/9$). There was no difference between PD and controls in any of the regions assessed. Scale bar = 0-50 μ m. Graphs represent Mean \pm SEM

4.3.4 No increase in microglial population or activation state in PD compared to controls

There was no significant difference in total microglial population between control and PD cases in the prefrontal cortex (66.39 ± 17.59 vs 60.13 ± 12.27), $t(14) = 0.30$, $p = 0.768$ (Fig 4.4A), hippocampus (37.11 ± 8.508 vs 49.20 ± 13.38), $t(15) = 0.687$, $p = 0.50$ (Fig 4.4B), striatum (41.20 ± 10.67 vs 38.14 ± 8.137), $t(14) = 0.229$, $p = 0.82$ (Fig 4.4C) or the substantia nigra (88.06 ± 12.71 vs 109.3 ± 20.63), $t(12) = 0.806$, $p = 0.44$ (Fig 4.4D).

When considering percent of the total microglial population that were activated, there was no significant difference between control and PD cases in the prefrontal cortex (42.66 ± 4.59 vs 39.27 ± 2.32), $t(14) = 0.705$, $p = 0.49$, hippocampus (29.03 ± 2.91 vs 32.63 ± 3.0), $t(15) = 0.83$, $p = 0.42$, striatum (21.41 ± 3.61 vs 23.21 ± 1.72), $t(14) = 0.508$, $p = 0.62$, or substantia nigra (24.78 ± 3.79 vs 22.36 ± 4.46), $t(11) = 0.405$, $p = 0.69$.

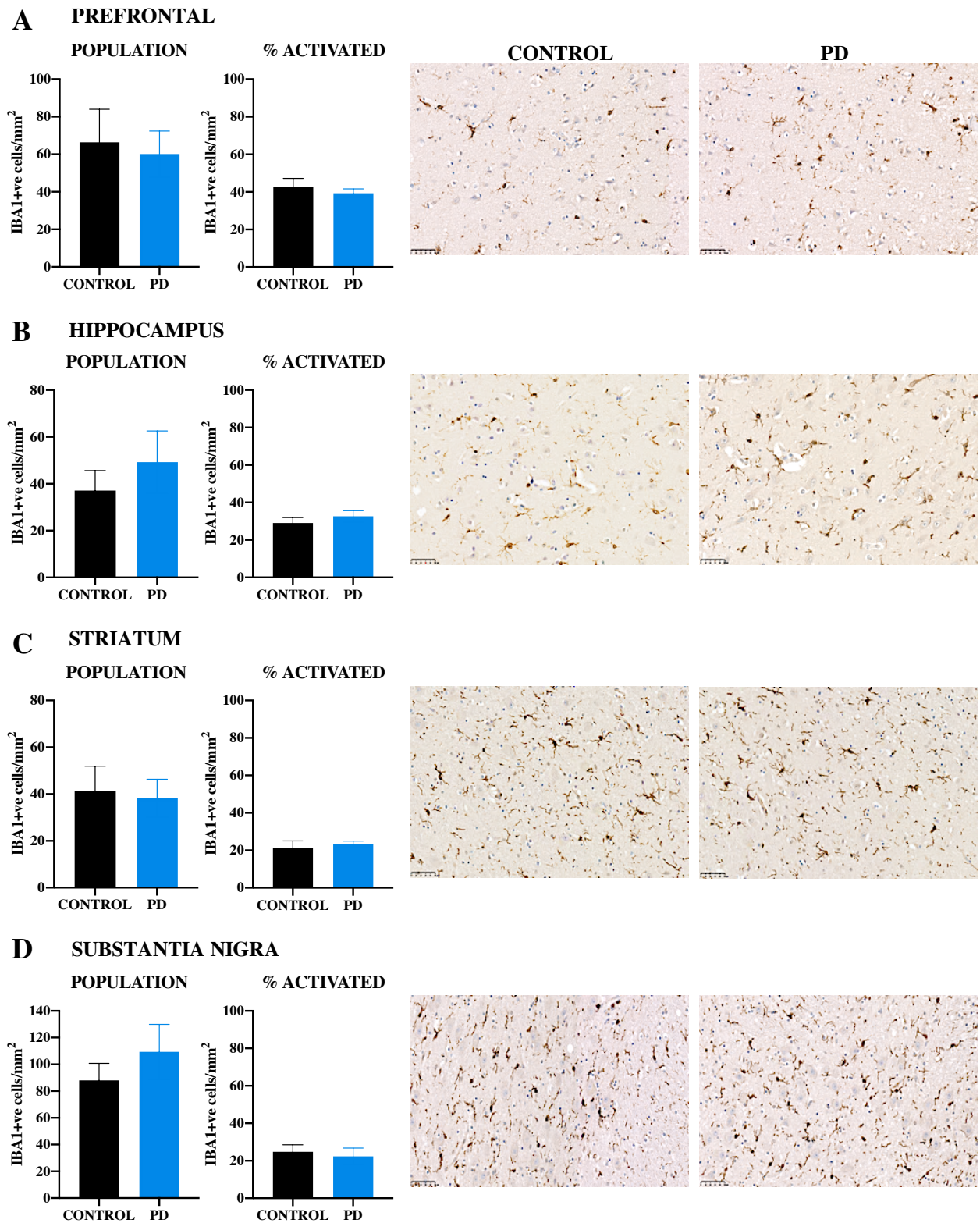


Figure 4.4 Total microglial population and % activated, as measured by IBA1, in the (A) prefrontal (n=7/9) (B)hippocampus (n=7/10) (C) striatum (n=7/10) and (D) substantia nigra (n=6/8). There was no difference in total number of microglia or in % activated microglia in any of the regions assessed. Scale bar = 0-50 μ m. Graphs represent Mean \pm SEM

4.3.5 No differences observed in relative expression of NeuN between PD and controls

There was no significant difference in relative expression of NeuN, suggesting similar neuronal numbers between control and PD cases in the prefrontal cortex (0.85 ± 0.27 vs 0.90 ± 0.18), $t(10) = 0.186$, $p = 0.86$ (Fig 4.5A), hippocampus (1.9 ± 0.86 vs 2.1 ± 0.46), $t(11) = 0.264$, $p = 0.80$ (Fig 4.5B), striatum (1.1 ± 0.61 vs 0.57 ± 0.29), $t(10) = 0.84$, $p = 0.421$ (Fig 4.5C) and substantia nigra (0.29 ± 0.11 vs 0.47 ± 0.22), $t(11) = 0.599$, $p = 0.56$ (Fig 4.5D).

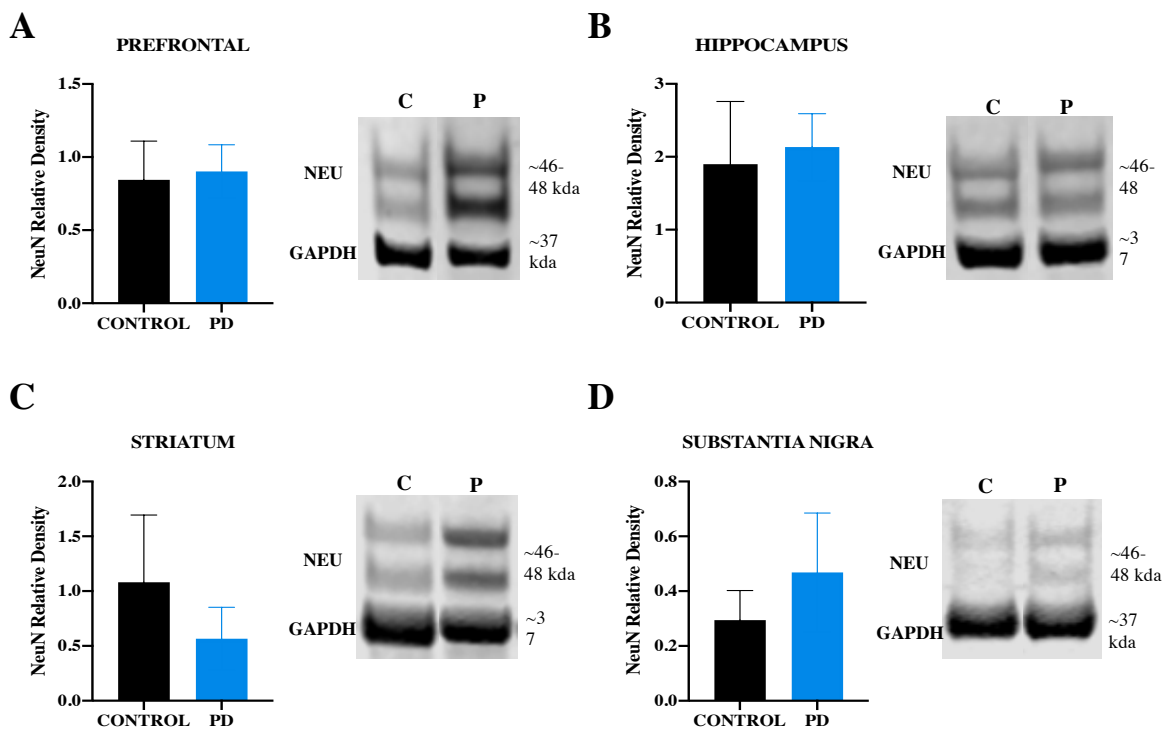


Figure 4.5. NeuN expression as examined via Western Blot in the (A) prefrontal ($n=5/7$) (B) hippocampus ($n=5/8$) (C) striatum ($n=5/7$) and (D) substantia nigra ($n=5/8$). There was no difference in relative NeuN expression in any of the regions assessed. Graphs represent Mean \pm SEM.

4.4 DISCUSSION

This study aimed to investigate Fyn activity in several neuroanatomical regions key to the pathophysiology of PD, as well as its relationship to markers of neuroinflammation. To the best of our knowledge, this is the first study to investigate Fyn phosphorylation in relation to total Fyn levels, as well as Fyn activity in key brain regions affected in PD. There was a significant upregulation of phosphorylated Fyn compared to total Fyn in the striatum and SN of PD patients. This is consistent with the findings of Panicker and colleagues (2019), who observed upregulation of phosphorylated Fyn in the SN in PD compared to controls (Panicker et al., 2019).

Interestingly, increased Fyn phosphorylation in relation to overall Fyn levels was not observed in either the prefrontal cortex or the hippocampus in PD brains compared to controls. This is most likely due to differences in the progression of pathology in these regions in PD. As discussed, PD is primarily associated with loss of DA neurons in the SNpc, which has extensive efferent projections to the striatum, forming the nigrostriatal pathway (Freund et al., 1984, Pollanen et al., 1993). This takes place early in disease progression, with symptomatic presentation of the disease already associated with a 60-80% loss of DA SNpc neurons (Dauer and Przedborski, 2003). Our findings support the up regulation of Fyn phosphorylation within these regions, although how Fyn distribution relates to dopaminergic denervation remains to be investigated. Conversely, efferent projections to the prefrontal cortex and hippocampus, via the ventral striatum, generally originate from the medial SN and/or the VTA, forming the mesocorticolimbic pathway. The medial SN and the VTA are considered to be more preserved in PD (Lammel et al., 2008, Nair-Roberts et al., 2008). Accordingly, these areas may be relatively unaffected by pathological changes induced by the upregulation of Fyn activity. In line with this, it is not until the later Braak stages (V-VI) that Lewy bodies spread to the hippocampus and PFC (Braak et al. 2003). It may be that the

cases included in the current study were too early in disease progression at time of death in order to see involvement of these regions; however, this is difficult to determine given the limited information on disease status of the cases. Future investigations may also want to consider specification of subregions within nuclei, such as distinction between the dorsal and ventral striatum, in order to better understand the nature of Fyn's involvement in PD and its association with disease progression and symptomatic presentation.

The mechanism by which Fyn kinase phosphorylation is up regulated in PD is not yet known. Recently, however, α -syn oligomers have been shown to interact with PrPc at the post-synaptic density, forming a complex which phosphorylates Fyn (Brás et al., 2018, Ferreira et al., 2017b). Accordingly, upregulation of phosphorylated Fyn observed in the striatum and SN in the current study may be related to the typical pathological spread of oligomeric α -syn in the PD brain hypothesised by Braak, with the α -syn/PrPc complex initiating Fyn phosphorylation. As discussed above, this may also explain the anatomical differences observed, with α -syn not reaching the prefrontal and hippocampus until stages V-VI (Braak et al., 2004).

Contrary to our initial hypothesis, upregulation of Fyn phosphorylation was not associated with increases in the neuroinflammatory response as measured by either microglial activation and astrocytic reactivity. The lack of elevation in either total number or percentage of activated microglia observed in our study at first appears contrary to the upregulation of the neuroinflammatory response in PD that has been well-established in the literature (Doorn et al., 2014b). In line with this, previous studies in human post-mortem tissue from PD brains have reported elevations in both total number of microglia (Lastres-Becker et al., 2012, Imamura et al., 2003) and markers of microglial activation (Vroon et al., 2007, Miklossy et al., 2006, Imamura et al., 2003). However, there is recent support for our findings. A 2020 study by Kouli and colleagues utilizing comparable techniques to those

used in our study did not observe increased microglial activation in post-mortem tissue collected from PD patients either with dementia (PDD) or without (PDND), compared to controls, in the SN, hippocampus, or prefrontal cortex (Kouli et al., 2020). These contrasting findings within the literature may be due to inherent limitations in the analysis of post-mortem tissue, including region sampled, post-mortem interval and other confounding factors. It is also worth noting that the current study utilized digital quantification of microglial activation through automated analysis, as opposed to semi-quantitative identification based on manual visual differentiation, which reduced observer bias and allowed for a more objective analysis of activation state. Alternatively, it may be that alternative markers of activation, beyond morphology alone, are more sensitive to detect disease-related alterations in neuroinflammation in post-mortem tissue in PD. For example, levels of IL-1 β , a pro-inflammatory cytokine produced by activated microglia, have consistently been reported to be elevated in the post-mortem PD brain compared to controls, across multiple studies (Mogi et al., 1994, Hunot et al., 1999, Kouli et al., 2020). Recent evidence implicating Fyn in the NLRP3 inflammasome and subsequent conversion of pro-IL-1 β to IL-1 β lends further support for investigation into this relationship in the human PD brain (Panicker et al., 2019).

Similar to our findings for microglia, we also failed to determine a significant increase in astrogliosis, as measured by GFAP, in any of the brain regions analysed. To date, the literature on reactive astrocytes in PD has been quite mixed, with some studies reporting increases in astrogliosis in post-mortem tissue (Lastres-Becker et al., 2012, Mythri et al., 2011) and others not finding such an increase (Tong et al., 2015, Mirza et al., 2000). Most recently, Kouli and colleagues reported increased astrogliosis in the SN of PD cases without dementia compared to controls; however, this was not observed in PD with dementia or across any of the other brain regions investigated, raising questions about whether this

finding might be incidental or due to differences in the region of the SN sampled (Kouli et al., 2020). Furthermore, although astrocytes are implicated in the neuroinflammatory processes in PD, their role may depend on their activation state, with A2 astrocytes considered neuroprotective, whereas A1 astrocytes are associated with neurotoxic activity in neurological diseases (Giovannoni and Quintana, 2020). Whilst overall astrocytic reactivity was not observed, there may have rather been differences in their activation state that was not assessed.

The lack of differences between PD and controls in neuroinflammatory markers observed may also be related to the characteristics of both the PD samples and/or control samples. Although no clinicopathological differences were noted with regards to age or gender between the groups, PD itself has proven heterogenous in nature (Greenland et al., 2019). This heterogeneity appears to be reflected in neuropathology, with neuroimaging studies suggesting, for example, that microglial activation may be more prominent in cases of PD with dementia compared to those without (Edison et al., 2013). Unfortunately, no information on disease duration or severity of motor/cognitive impairments were available for the cases in the current study, and, as such, differences in these may have influenced our pathological findings. Furthermore, in endeavouring to select age-matched controls, samples selected may have been neurologically compromised. For example, neuromelanin deposits were observed in equal numbers across both PD and age-matched controls (results not shown). Neuromelanin can be neuroprotective (Zecca et al., 2008a); however, as it begins to accumulate and occupy the cytoplasm in ageing, this initiates a neuroinflammatory/neurodegenerative cascade that can be observed both in healthy ageing (Fedorow et al., 2006) and in PD (Wilms et al., 2003, Zecca et al., 2008b). Indeed, neuromelanin deposits in older subjects have also been positively correlated with activated microglia and, as such, controls may have exhibited higher than normal neuroinflammatory

markers due to such factors (Beach et al., 2007). Further investigation of this was beyond the scope of this study; however, this exemplifies the fickle nature of obtaining ‘healthy’ age-matched controls for post-mortem analysis. Whilst this may be an initial limitation of this study, the use of an age-matched population is also a strength, as it allows for control for the effects of normal ageing.

While our findings did not support an upregulation of neuroinflammation in areas of increased Fyn phosphorylation in the post-mortem PD brain, this up-regulation may be associated with other pathological changes not assessed in this study. For example, Fyn activity has been associated with N-methyl-d-aspartate (NMDA) dysfunction. Fyn phosphorylates the NR2B subunit of the NMDA glutamate receptor, a function crucial to facilitating SP (Trepanier et al., 2012). Given that increased levels of NMDA receptor NR2B phosphorylation have been observed in the striatum of preclinical models of PD (Menegoz et al., 1995, Dunah et al., 2000, Oh et al., 1998), Fyn may contribute to the glutamate excitotoxicity and subsequent neurodegeneration seen in the disease (Ambrosi et al., 2014, Iovino et al., 2020). Indeed, blockade of Fyn-induced phosphorylation of NMDARs has been shown to rescue α -syn mediated synaptic and cognitive deficits in an α -syn overexpression mouse model of PD (Ferreira et al., 2017b). Future work should investigate whether areas of increased Fyn activity overlap with up-regulation of NMDAR phosphorylation.

4.5 CONCLUSION

In summary, the results of this study have established the presence of increased Fyn phosphorylation in the striatum and SN of PD patients compared with controls, suggesting a potential role in the pathophysiology of the disease. It remains to be seen if Fyn phosphorylation is a driver or a consequence of pathology in PD. Neuroinflammatory

markers assessed in this study did not provide a mechanistic explanation for this upregulation; however, given the diverse role of Fyn in the brain, including its association with other pathological processes observed in PD, further investigations are warranted to elucidate the potential of Fyn as a pathological target for intervention.

05

Fyn Kinase Inhibition Using AZD0530 Improves Recognition Memory and Reduces Depressive-Like Behaviour in an Experimental Model of Early-Stage Parkinson's Disease

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5.0 ABSTRACT

Whilst the complex aetiology of Parkinson's Disease (PD) is not fully understood, a growing body of literature highlights the neuroinflammatory hypothesis, which suggests activation of microglia leads to the release of pro-inflammatory cytokines and subsequent death of neurons in key brain regions. Fyn kinase has recently been established as a major upstream regulator of neuroinflammation in PD due to its role in microglial activation. This study aimed to determine if inhibition of Fyn kinase could lead to reduced neuroinflammation and subsequent improvements in motor and non-motor impairments in an early-stage preclinical model of PD. An experimental model of PD was produced using intra-striatal injection ($4\mu\text{l}$) of the neurotoxin 6-OHDA ($5\mu\text{g}/\mu\text{l}$). Sprague Dawley rats ($n=42$) were given either vehicle, $6\text{mg}/\text{kg}$ or $12\text{mg}/\text{kg}$ of Fyn kinase inhibitor (AZD0530) daily for 32 days via oral gavage and tested on a battery of tasks assessing motor, cognitive and neuropsychiatric outcomes. In terms of motor outcomes, an improvement in volitional locomotion was observed with $6\text{mg}/\text{kg}$ compared with vehicle controls. Animals showed significant improvement in recognition memory and reduction in depressive-like behaviour between $6\text{mg}/\text{kg}$ and $12\text{mg}/\text{kg}$ groups and controls. Pathologically, an inflammatory response was observed; however, there were no significant differences in markers of neuroinflammation between treatment groups. Taken together, results indicate a potential therapeutic benefit for the use of Fyn kinase inhibition to treat non-motor symptoms of PD, although the mechanisms of this remain to be elucidated.

5.1 INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's Disease (AD) and the fastest growing neurological disorder (Dorsey et al., 2018, Feigin et al., 2019). Pathologically, PD is characterised by the presence of Lewy Bodies, intracellular aggregates of alpha-synuclein (α -syn) and ubiquitin, and the loss of dopaminergic (DA) neurons in the substantia nigra *pars compacta* (SNpc) (Pollanen et al., 1993). Clinically, the cardinal motor symptoms include muscle rigidity, akinesia, resting tremor, bradykinesia and postural/gait changes (Barnum and Tansey, 2012). PD patients also experience a variety of non-motor symptoms, such as anxiety, depression and cognitive impairment (Barnum and Tansey, 2012). These neuropsychiatric and cognitive impairments significantly affect quality of life and, unfortunately, current treatment strategies are not effective in addressing these symptoms, which are considered amongst the most debilitating (Duncan et al., 2014). Additionally, the most commonly used strategy targeting cognitive impairment, the cholinesterase inhibitors used for AD, may paradoxically worsen motor deficits (Collins-Praino et al., 2011). Accordingly, new therapeutic strategies to address this gap are necessary; however, to date, attempts to develop such treatments have been limited due to an incomplete understanding of the pathogenesis and the molecular mechanisms underpinning cognitive impairment and mood dysfunction in PD.

Neuroinflammation has been proposed as a key factor driving neurodegeneration in PD (Tansey and Goldberg, 2010). McGeer and colleagues first observed the presence of activated microglia in the SN of post-mortem PD patients (McGeer et al. (1988), and, since this observation, many cell culture, animal and post-mortem studies have confirmed the role of microglial-mediated inflammation in PD (McGeer and McGeer, 2004, Tansey and Goldberg, 2010, Lee et al., 2009). Microglia are the first line of CNS immune defence, acting

as molecular sensors and surveying the environment where they become 'activated' in response to insult or injury, undertaking phagocytosis to maintain homeostasis (Yang et al., 2021). During the early stages of PD, microglia become activated by various signalling pathways in response to stimuli such as protein aggregation and oxidative stress (McGeer and McGeer, 2004). Upon activation, they release ROS, chemokines and pro-inflammatory cytokines, such as TNF α , IL-1 β and IFN γ (Yan et al., 2014). Increases in these detrimental cytokines have consistently been reported in the serum and cerebrospinal fluid of PD patients (Mogi et al., 2000). Under physiological conditions, this response is associated with scavenging and healing and terminates; however, if the stimulus persists, chemokines may initiate an adaptive immune response, recruiting more macrophages, leading to an accumulative increase in pro-inflammatory cytokine release (Salvi et al., 2017). Synergistically, pro-inflammatory cytokines bind to their respective receptors on the microglial surface, further accelerating activation and sustaining a chronic inflammatory state (Lee et al., 2002). This chronic inflammatory state is upregulated in PD, with increased microglial density observed in the SN of PD patients (Beach et al., 2007). Overall, this chronic inflammation is detrimental long-term, initiating cell damage and death and exacerbating disease progression in PD (Tansey and Goldberg, 2010). Although the exact causes of initial microglial stimulation and subsequent upregulation in PD are unknown, an upstream element that may be key to understanding these mechanisms is Fyn kinase.

Fyn is an SRC-family kinase (SFK) involved in several key central nervous system (CNS) functions, including myelination, astrocyte migration and SP (Matrone et al., 2020). Fyn also plays a critical role in both peripheral and central inflammatory processes (Nygaard et al., 2014). This has led to speculation Fyn dysregulation may be implicated in the pathophysiology of multiple neurodegenerative diseases, including PD (Moore et al., 2002,

Stuart et al., 2007, Panicker et al., 2015). In support of this, a seminal study by Panicker and colleagues in both cell culture and animal models of PD, described the critical role of Fyn in regulating the microglial-mediated inflammatory response through nuclear translocation of the p65 component of NF κ B into the nucleus and subsequent up-regulation of pro-inflammatory cytokine production (Panicker et al., 2015). Fyn has also been implicated in the signalling pathway linking α syn oligomers to neurodegenerative disease processes in PD via its binding to the cellular prion protein receptor (PrPc), leading to Fyn activation and subsequent upregulation of NMDA induced excitotoxicity (Ferreira et al., 2017b). Recently, a GWAS study identified the FYN gene as a novel PD risk locus (Nalls et al., 2019), with the Fyn/PKC δ signalling pathway known to contribute to oxidative-stress induced death of dopaminergic neurons (Kaul et al., 2005, Saminathan, 2011). Indeed, in multiple mouse models of PD (LPS, MPTP and 6-OHDA), a greater attenuation of the neuroinflammatory response was seen in Fyn knockout mice compared to wild-type (Panicker et al., 2015), further supporting the role of Fyn as a major upstream regulator of the neuroinflammatory processes in PD. Inhibition of Fyn may therefore represent a novel target for therapeutic intervention. To date, no studies have investigated the benefits of Fyn kinase inhibition in a preclinical model of PD.

AZD0530 (Saracatinib) is an experimental drug which acts as an inhibitor of Src Family Kinases (SFKs), with high specificity for Fyn (Hennequin et al., 2006). Initially developed for the treatment of solid tumours, the drug demonstrated success in animal models; however, was unable to translate to humans, potentially due to the high threshold required for kinase inhibition to modify tumour progression (>98%) (Nygaard et al., 2014). AZD0530 has since been repurposed for its potential therapeutic use in neurodegenerative diseases, which require a much lower level of kinase inhibition to modify the inflammatory pathway

(Nygaard et al., 2014). Recently, AZD0530 was found to be efficacious in a transgenic mouse model of AD, fully rescuing spatial memory deficits and synaptic depletion and dampening microglial activation (Kaufman et al., 2015). The safety, tolerability and availability of AZD0530 has also been assessed in humans with AD and was found to be safe and well tolerated across doses (Nygaard et al., 2015). The drug has not yet been assessed in PD; however, given its utility in other neurodegenerative diseases, AZD0530 may represent a novel candidate for therapeutic use in the disease. Here, we investigate whether AZD0530 can be repurposed for use in PD by evaluating its effect both on parkinsonian motor impairment, as well as non-motor symptoms of the disease, including cognitive and neuropsychiatric dysfunction, using the striatal 6-OHDA model of PD.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Adult male Sprague-Dawley rats weighing between 280-360g ($n=42$) were used in this study. All experimental procedures were approved by the of the University of Adelaide Animal Ethics Committee (M-2015-241a) and conducted according to the Australian National Health and Medical Research Council code of practice for the care and use of animals for scientific purposes (8th edition, 2013). Animals were housed under conventional laboratory conditions with a 12-hour light/dark cycle and access to standard rodent chow and water *ad libitum*. All experiments were conducted in the AM during the light phase of the light/dark cycle.

To represent PD, a unilateral striatal 6-hydroxydopamine (6-OHDA) model was adopted (Schwartz and Huston, 1996). 6-OHDA has a high affinity for dopamine transporters (DAT), which transport the toxin into the cell, causing mitochondrial

dysfunction and oxidative stress and leading to cell death (Ungerstedt et al., 1974). The intrastriatal approach causes 30-40% loss of striatal DA content, representing early-stage PD (Yuan et al., 2005) and is associated with significant increases in neuroinflammatory markers (Ramsey and Tansey, 2014) .

5.2.2 Stereotactic Surgery

Animals were anaesthetised via inhalation of 5% isoflurane under normoxic conditions (0.6 L/min O₂, 1.5 L/min N₂) and mounted securely on a stereotactic frame in a flat skull position with the incisor bar 3.0 mm below horizontal zero. Anaesthesia was maintained with 2.5% inhalational isoflurane via nose cone. Lignocaine (0.25 ml) was administered prior to a midline incision and soft tissue subsequently retracted to expose bregma. Animals were randomly assigned to receive either left or right striatal lesions. Based on methods previously described (Lee et al., 1996, Thornton and Vink, 2012), a 1mm burr hole was made at two separate locations over the striatum using the following standard stereotactic coordinates: (1) AP: 0.5 mm, ML: +/- 2.5 mm, DV: -5.0 mm and (2) AP: -0.5 mm, ML: +/- 4.2, DV: -5.0 mm. Two µl of 6-OHDA (Sigma) (5 µg/µl) was injected at a rate of 1 µl/min into each coordinate (4 µl in total) using a 25 µL Hamilton syringe, which was left in place for 2 minutes to allow for diffusion. Sham animals received equivalent injection of 0.9% sodium chloride (saline). Following injections, anaesthesia was ceased, the incision was closed using 9 mm surgical autoclips and betadine was applied to wound site. Animals were placed on a heatpad to thermostatically maintain body temperature both during and post-surgery and monitored for normal recovery.

5.2.3 Experimental Design and Drug Administration

Animals were randomly assigned into 4 experimental groups ($n=10-11$) and treated with vehicle control (sham + veh; 6-OHDA + veh), 6 mg/kg/day (6-OHDA + 6 mg/kg) or 12 mg/kg/day (6-OHDA + 12 mg/kg) of AZD0530 daily for 33 days via oral gavage. All treatments were administered in a blinded fashion, with allocation of the doses prepared daily by a blinded independent researcher. The vehicle was 0.5% wt/vol hydroxypropylmethylcellulose (HPMC)/0.1% wt/vol polysorbate 80. All animals were dosed in the AM and, on days of functional testing, were treated 1 hour prior to commencement. Treatment doses were chosen based upon efficacy in pilot work and previous studies in preclinical models of AD (Kaufman et al., 2015).

5.2.4 Functional Testing

Animals were tested in the AM for all functional assessments and recorded using the ANY-Maze video tracking system (Stoelting Co. V.4.99m) under the following regime (Figure 5.1). The experimenter was blinded to experimental groups throughout the study duration.

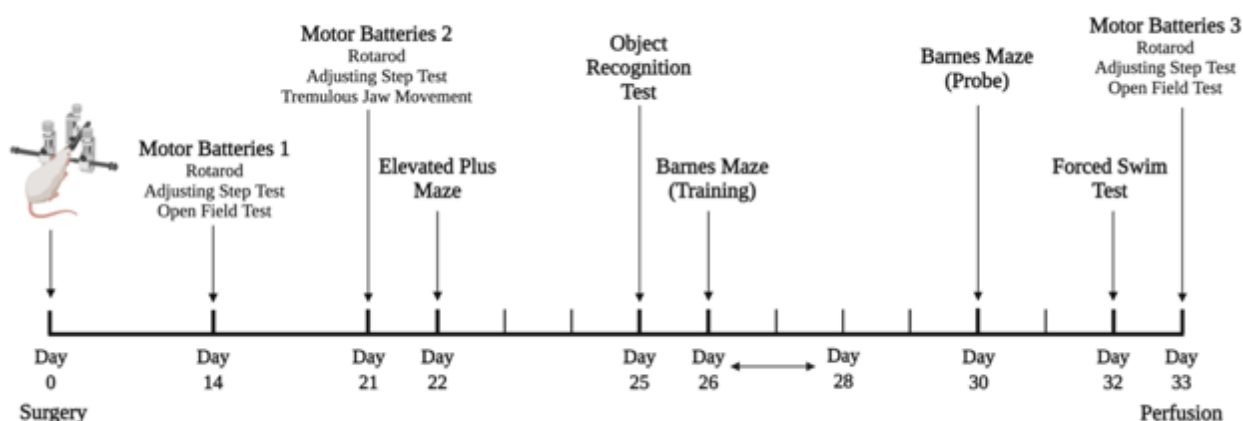


Figure 5.1. Overview of Experimental Design Timeline Figure created in BioRender.com (2021).

5.2.4.1 Rotarod

Rotarod is a common test assessing co-ordination, balance and general locomotion (Deacon, 2013). The device comprises an elevated circular set of metal horizontal bars which rotate on a longitudinal axis between 0-30 revolutions per minute (RPM). Animals were placed on the stationary rotarod for 10 seconds before speed was increased by 3 RPM every 10 seconds until a maximum of 30 RPM. Animals maintained the speed for a further 20 seconds before the speed was decelerated and the animal removed from the test. The test concluded either when the animal fell, gripped without walking for 2 complete revolutions or reached the full 120 seconds and latency was recorded.

5.2.4.2 Adjusting Step Test

The adjusting step test (AST) is a test assessing forelimb akinesia and gait impairments (Olsson et al., 1995). Animals were held so their hind limbs and the ipsilateral forepaw were unable to touch the testing surface. With only the contralateral forepaw touching, animals were moved laterally across a distance of 45 cm. The number of adjusting steps were recorded for contralateral forehand and backhand by slowly moving the animal across the testing surface 3 times in either direction. The average number of adjusting steps taken across the trials was calculated.

5.2.4.3 Open Field Test

The Open Field Test (OFT) incorporates assessment of both motor and anxiety-like behaviour (Leite-Almeida et al., 2009). Animals were placed in the centre of a large square box (95 x 95 cm) with walls at a height of 44.5 cm for 5 minutes. The total distance travelled whilst in this space was measured to assess locomotion and time spent in inner zone measured to assess anxiety.

5.2.4.4 Tremulous Jaw Movement

Tremulous jaw movements (TJM) in rodents occur in the same local frequency as parkinsonian tremor (3-7Hz) (Collins-Praino et al., 2011). The TJM test is thus a validated method of assessing parkinsonian tremor in the 6-OHDA rodent model (Jicha and Salamone, 1991). To assess this, animals were placed in a clear ventilated container (29cm X 20cm X 16cm), allowing continuous visualisation of the lower jaw from all angles. An initial habituation period of 5 minutes was undertaken, followed by two testing phases of 5 minutes. During testing, animals were continuously observed, and any non-directed movement of the lower jaw was recorded via a mechanical hand counter by a trained and blinded observer. An average across both testing phases was taken to indicate tremor severity.

5.2.4.5 Elevated Plus Maze

The elevated plus maze (EPM) is a widely used tool to assess anxiety-like behaviour in rodents (Pego et al., 2008). Animals were placed in the junction of a cross-shaped elevated maze (50cm in height) with two open (50 cm length) and two closed arms (40cm high × 50 cm length) for 5 minutes. Time spent in the closed arms versus open arms was recorded, with increased time spent in the closed arms representing anxiety-like behaviour.

5.2.4.6 Novel Object Recognition

The novel object recognition test (ORT) is a common method to investigate recognition memory in rodents (Ennaceur and Delacour, 1988). For this study, an abridged version of the standard ORT test was used. Here, animals were placed in an empty arena (100cm × 45cm) for 5 minutes to habituate. Thirty minutes later, they were placed back in for 5 minutes with 2 objects. Time spent inspecting each object was measured. Inspection was operationally defined as approaching, looking at and sniffing the object from a distance of

less than or equal to 2cm. In order to assess long-term reference memory, 24 hours later, animals were placed back in the arena with 1 old object and one ‘novel’ object. Time spent inspecting both objects was measured. To determine novel object preference, time spent inspecting the novel object compared to the old object was converted to a preference by dividing time spent with novel object by overall time inspecting both objects and converted to a percentage ($[\text{time}_{\text{novel}} / (\text{time}_{\text{familiar}} + \text{time}_{\text{novel}})] * 100$). A score of greater than 50% indicates a preference for the novel object.

5.2.4.7 Barnes Maze

Barnes Maze is a well-known measure of spatial learning and memory, as well as cognitive flexibility (Sunyer et al., 2007). The apparatus consists of a circular table (1.2 m in diameter) with 18 circular holes equally spaced around the perimeter edge. One of the holes was pre-designated as an escape hole, with a black escape box placed immediately below the hole. The Barnes maze task was performed over the course of five days: three days of acquisition trials (Days 1-3); a rest day, during which there was no interaction with the animals (Day 4) and a probe day, in which the previous location of the escape box was changed to a new location (Day 5). During acquisition days, animals underwent two trials (spaced 15 minutes apart). On all trials, animals were placed in the centre of the Barnes maze with a bright light shone overhead as an aversive stimulus, motivating animals to find and enter the escape box. The latency for animals to find and enter the escape box was recorded. On the probe day, the location of the escape box was changed to a new hole. Two trials were then conducted, one hour apart. In the first trial, the time taken to reach the old location of the escape box was recorded as a measure of long-term spatial reference memory. On both probe day trials, the latency to find and enter the new location of the escape box was recorded as a measure of cognitive flexibility.

5.2.4.8 Forced Swim Test

The forced swim test (FST) is a method for assessing depressive-like behaviour in rats (Slattery and Cryan, 2012). A clear cylindrical vase was half filled with 25 °C water, adjusted for the animal's length so that the hind legs did not touch the bottom of the cylinder. Rats were placed in the tank for 5 minutes and time spent swimming and immobile were measured. Increased time spent immobile indicates behavioural despair, a measure of depressive-like behaviour.

5.2.5 Tissue collection and Processing

At day 33, rats were anaesthetised with 5% isoflurane before being randomly assigned for either immunohistochemical (n=21) or molecular analysis (n=21).

Animals that were to be used for immunohistochemical analysis were transcardially perfused with 0.2mL heparin + 10% formalin. Brains were removed and post-fixed in 10% formalin for one week, then blocked into 2 mm coronal sections and embedded in paraffin-wax. Five µm coronal sections of the striatum beginning +0.5mm from Bregma were prepared from paraffin embedded tissue. Tissue was mounted on silane coated slides and allowed to dry at 37 °C overnight.

Animals that were to be used for molecular analysis were transcardially perfused with 0.9% saline and the brain extracted whole (n=4-6 per group). Brains were snap-frozen in liquid nitrogen, then stored at -80° C. Prior to analysis, brains were placed on dry ice and tissue samples from the striatum were dissected. Tissue was suspended in buffer solution (20 mM Tris-HCl pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 140 mM 2-mercaptoethanol) with protease inhibitor cocktail (Sigma), 10 µg/mL aprotinin, leupeptin, pepstatin A and 10 mM PMSF. Samples were then homogenised and sonicated in 3 × 10 s bursts using a sonicator probe. Following this, homogenised samples were centrifuged for 15 minutes at

14,000 rpm at 4° C, before supernatant was collected. Protein concentrations were estimated with Thermo Pierce BCA Protein Assay Kit (ThermoScientific) at 540 nm absorbance.

5.2.6 Immunohistochemical Analysis

Immunohistochemical analysis was performed using standard protocols. Briefly, following deparaffinization in xylene, tissue mounted slides were rehydrated in ethanol and endogenous peroxidases blocked using methanol with 0.5% hydrogen peroxide. Slides were then washed twice with phosphate buffered saline (PBS). Antigen retrieval was performed (citrate), following which sections were blocked in 3% normal horse serum (NHS) for 30min before overnight incubation with Tyrosine hydroxylase (TH) (Abcam, ab112, 1:750) or IBA-1 (Wako, 019-19741, 1:800). On the following day, slides were again washed twice in PBS and incubated with secondary antibody (Vector Goat Anti-Rabbit IgG, 1:250 NHS) for 30 minutes, followed by two more washes with PBS and incubation with streptavidin peroxidase conjugate (Vector SPC,1:1000 NHS) for 1 hour. Following a final wash with PBS, the chromogen 3,3-Diaminobenzidine tetrahydrochloride (Vector DAB; 1:50) was applied for 7 minutes. Slides were counterstained with haematoxylin, dehydrated in ethanol and cleared with xylene, prior to being coverslipped.

Following staining, slides were scanned using a Nanozoomer slide scanner (Hamamatsu, Shizouka, Japan) and the associated software (NDPview, version 2) was used to view images. TH in the striatum was assessed in ImageJ using colour deconvolution to remove haematoxylin and converted to binary. The striatum was isolated and the % positive area was then calculated for each hemisphere by 2 assessors blinded to experimental conditions. The % difference between ipsilateral and contralateral hemispheres was then calculated for

2 slides per sample to reveal the average % loss (as below). An average of the 2 assessors average % loss was calculated for final analysis.

Average % loss

$$= \frac{\text{Image 1 \% loss (Contra \% - Ipsi \%)} + \text{Image 2 \% loss (Contra \% - Ipsi \%)}}{2}$$

IBA-1 was assessed by counting the reactive and immunopositive cells per mm² in the striatum. Cells were counted by extracting images from the ipsilateral striatum (4 × boxes at 1.04mm² each). These were processed via colour deconvolution, converted to binary and a threshold was applied to demarcate IBA-1+ cells. An automatic cell counter was used and this count was averaged over 2 sections and divided by the total area to generate the number of cells per mm². This was then repeated and compared to the contralateral hemisphere and the difference between the two was calculated. Microglial activation was assessed manually by randomly selecting 6 boxes (3 × 2), across the striatum, each with an area of 0.208mm². Morphological characteristics were assessed, where activated microglia were characterised based on dark nuclear staining, round cell body and short/absent processes. The experimenter was blinded to the experimental group during microglial assessment.

5.2.7 Western Blot Analysis

Western blot analysis was performed using a standard milk method. In brief, 4 × Bolt LDS sample buffer and 10× Bolt reducing agent and dH₂O were added to supernatant (30 µg protein) as per the manufacturer's instructions, heated at 70° C for 10 minutes and then vortexed. Gel electrophoresis was performed using Bolt 4-12% Bis-Tris gels (Life Technologies) with 40µl of sample loaded per well. Gels were run at 120 V for 120 minutes. Following this, gels were transferred to the PVDF membrane using the iBlot 2 Dry Blotting System (Life Technologies). Membranes were washed in 1 × tris-buffered saline (3 washes × 5 min), stained with Ponceau S red solution (Fluka Analytical) for 5 minutes to allow for

protein visualisation (in order to ensure equal protein loading between lanes), and then washed with dH₂O until removal of Ponceau had been achieved.

Membranes were blocked for 2 hours (5% w/v dried skim milk in TBS-T) before overnight incubation at 4° C (2% w/v dried skim milk in TBS-T and primary antibody). Primary antibodies were used at individually optimised concentrations: anti-GFAP (1:40,000, ab7260, Abcam) and the housekeeper chicken anti-GAPDH (1:4000, ab83956, Abcam). Following overnight incubation, membranes were washed in TBS-T (3 × 5 min) and incubated in 2% milk and secondary antibodies, according to the species the primary was raised in (donkey anti-rabbit and donkey anti-chicken, IRDye 800CW; LI-COR, Inc., 1:10000) and incubated for 2 hours at room temperature. Following incubation, each membrane was washed in TBS-T (3 × 5 min) and visualised using an Odyssey Infrared Imaging System (model 9120; software version 3.0.21) (LI-COR, Inc.) at a resolution of 169 μm. Semi-quantitative analysis of band signals was performed using ImageJ version 1.49 and Image Studio Lite version 5.2. Normalisation of blots were performed using a control sample (sham) across the same protein of interest. Relative density of each sample was calculated based on the adjusted density for each blot as described:

$$\text{Adjusted density} = \text{signal of sample/housekeeper} / \text{signal of control protein / housekeeper}$$

$$\text{Relative density} = \text{adjusted density of protein} / \text{adjusted density of housekeeper}$$

5.2.8 Statistical Analysis

Data was analysed using Prism software (GraphPad v.7). For some behavioural tests, animals were excluded if they failed to perform the task (n=3 Rotarod, n=2 TJM, n=1 EPM, n=2 ORT). To compare differences between time and treatment groups on rotarod, AST, OFT and Barnes Maze, a repeated measures two-way Analysis of Variance (ANOVA) was

used with time as a within subjects factor and group as a between subjects factor. Post hoc testing was conducted according to Bonferroni's method. Where a significant main effect of time was noted, a one-way ANOVA was run at each timepoint to further probe effects. For all other measures, in order to compare differences between treatment groups, data were analysed using one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison post-hoc test. All values are displayed as Mean \pm SEM, with significance level set at $p < 0.05$.

5.3 RESULTS

5.3.1 Impairments in volitional locomotion and AST were progressive in nature, but no improvements in motor function were observed with AZD0530 treatment.

General locomotor activity was measured as time on the rotarod at days 14, 21 and 33 post-surgery (Figure 5.2A). A significant main effect of time was observed ($F_{2,68}=28.32$, $p < 0.0001$), with all groups improving from day 14 to day 33. No main effect of treatment was found ($F_{3,34}=0.917$, $p=0.443$), nor was there a significant interaction between testing day and treatment group ($F_{6,68}=1.038$, $p=0.409$).

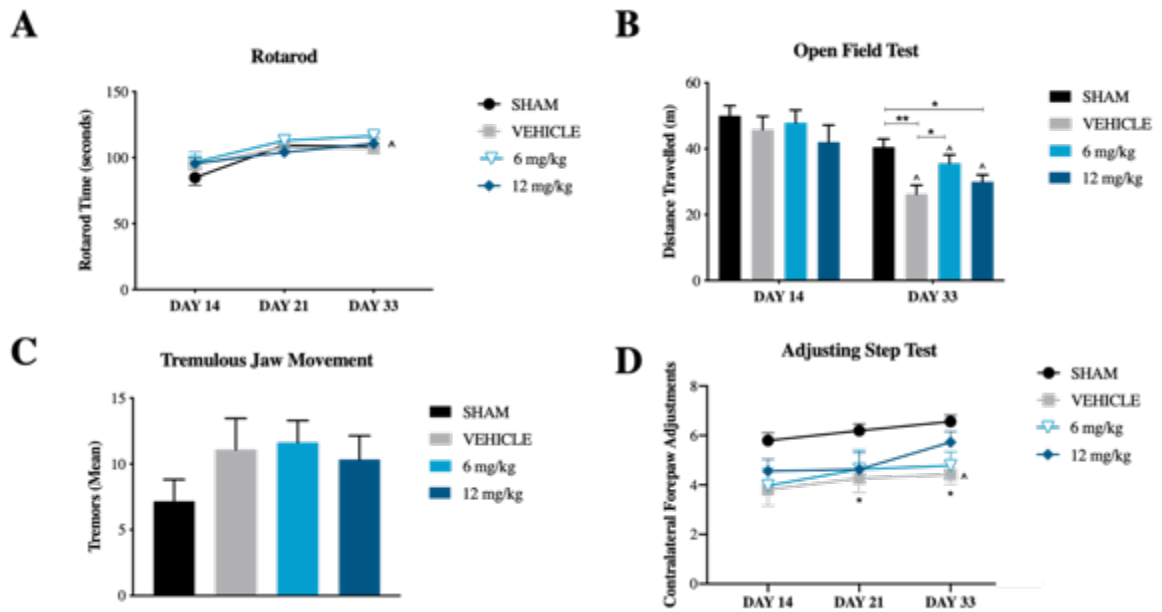


Figure 5.2. Motor outcomes (A) Motor function as measured by time on Rotarod (s) at days 14, 21 and 33 post-surgery. (B) Locomotion as measured by distance travelled (m) on Open Field Test at day 14 and 32. (C) Motor impairments as measured by mean number of tremors in Tremulous Jaw Movement. (D) Motor impairments as measured by Contralateral Forepaw adjustments on Adjusting Step Test at days 14, 21 and 32. Graphs represent the Mean \pm SEM ($n=9-11$ per group). * $p<0.05$, ** $p<0.01$ compared to sham (unless indicated otherwise), ^ denotes significance over time.

Volitional locomotion was also assessed as total distance travelled on day 14 and 33 in the OFT (Figure 5.2B). There was a significant main effect of time ($F_{1, 38}= 40.77$, $p<0.0001$) with vehicle- ($p<0.0001$), 6 mg/kg- ($p<0.05$) and 12 mg/kg- ($p<0.05$) treated animals, but not sham animals, travelling significantly less over time. A main effect of treatment was also observed ($F_{3, 38}= 2.93$, $p<0.05$); however, no significant interaction between time and treatment group was seen ($F_{3, 38}=1.104$, $p=0.360$). Post hoc analysis of treatment effect in the overall model revealed no significant differences between groups ($p > 0.05$), so, to probe further, a one-way ANOVA was conducted for each individual timepoint. At day 14, no significant differences between shams (50.01 ± 3.07), vehicle-treated (45.82 ± 4.0), 6 mg/kg

(47.92±3.79) or 12 mg/kg (42.01±5.18) treated animals were observed ($F_{3,38}=0.68$, $p=0.57$). At day 33, however, there was a significant difference between sham (40.55±2.43) animals and both vehicle- (26.22±2.72) and 12 mg/kg- treated animals (29.96±2.09) ($p<0.001$, $p<0.05$ respectively), indicating an injury effect that was not improved with 12 mg/kg of AZD0530. There was also a significant difference between vehicle and 6 mg/kg (35.57±2.60) treated animals ($p<0.05$); however, 6 mg/kg treated animals actually travelled significantly more, indicating a potential improvement in locomotion ($F_{3,38}=6.34$, $p<0.001$).

Tremor was assessed as the mean number of tremors between 2 trials in the Tremulous Jaw Movement (TJM) test on day 21 (Figure 5.2C). There were no significant differences observed between sham, 6-OHDA vehicle or treatment groups ($F_{3,38}=1.173$, $p=0.368$).

Akinesia was assessed via the AST (Figure 5.2D). Mean contralateral forepaw adjustments at day 14, 21 and 33 were analysed. There was a significant main effect of time ($F_{2,76}=7.8$, $p<0.001$), with post-hoc analysis revealing that, of the 6-OHDA lesioned animals, only 12 mg/kg treated animals demonstrated significantly reduced akinesia over time, with an increased frequency of forepaw adjustments from day 14 (4.56±1.52) to day 33 (5.73±1.377) ($p<0.01$). A main effect of treatment was also observed ($F_{3,38}=3.26$, $p<0.05$), with vehicle-treated animals demonstrating akinesia compared to shams at both day 21 ($p<0.05$) and day 33 ($p<0.05$), but not day 14 ($p>0.05$), indicating a delayed injury effect; however, there was no significant difference between vehicle and any treatment groups ($p>0.05$). No significant interaction between time and treatment group was observed ($F_{6,76}=0.58$, $p=0.75$).

5.3.2 Intrastratial injection of 6-OHDA led to depressive-like behaviour, which was reduced by AZD0530 treatment.

Anxiety-like behaviour was assessed using both the EPM and OFT. There was no significant difference in anxiety-like behaviour on the EPM (Figure 5.3A) between any of the groups on day 22 ($F_{3,37}= 1.14$, $p=0.346$), indicating no injury or treatment effect. Anxiety-like behaviour, as measured by time in centre of the OFT (Figure 5.3B) on days 14 (shams 14.56 ± 8.62 , vehicle-treated 15.54 ± 8.35 , 6 mg/kg 19.72 ± 19.70 or 12 mg/kg 17.15 ± 9.33) and 33 (shams (15.6 ± 8.75), vehicle-treated (8.24 ± 8.87), 6 mg/kg (13.75 ± 10.04) or 12 mg/kg (8.86 ± 4.80)), revealed a significant main effect of time ($F_{1,38}=6.486$, $p<0.05$); however, there were no significant differences observed upon post-hoc analysis. There was no significant main effect of treatment ($F_{3,38}=0.70$, $p=0.558$), nor was there a significant interaction between time point and treatment group ($F_{3,38}=1.06$, $p=0.379$).

Depressive-like behaviour was assessed based on immobility time in the FST (Figure 3C). 6-OHDA vehicle-treated animals spent significantly more time immobile compared to shams (96.41 ± 8.60 vs 65.09 ± 5.81 , $F_{3,38}=6.570$, $p<0.01$). Vehicles also spent significantly more time immobile than either 6mg/kg (65.81 ± 5.58 , $p<0.01$) or 12 mg/kg (60.02 ± 5.309 , $p<0.01$) treated animals. There was no significant difference between shams and either 6 mg/kg or 12 mg/kg-treated animals ($p> 0.05$). Together, results indicate a potential reduction in depressive-like behaviour back to sham equivalent following treatment.

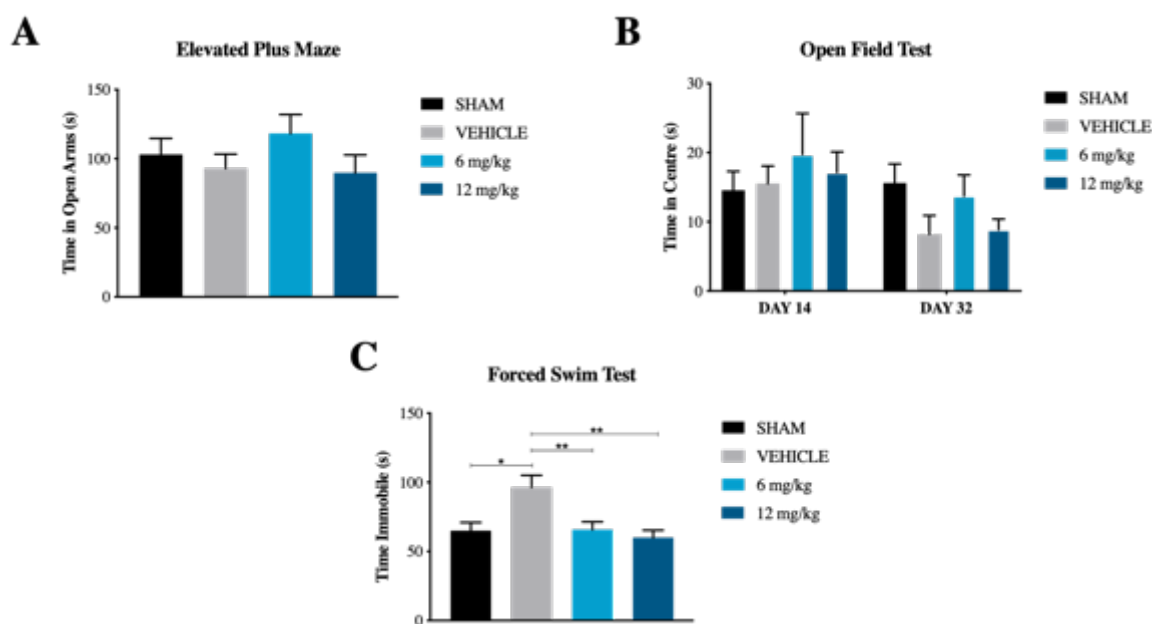


Figure 5.3. Neuropsychiatric outcomes. (A) Anxiety-like behavior as measured by time spent in open arms of Elevated Plus Maze. (B) Anxiety-like behavior as measured by time in centre of Open Field Test at days 14 and 32. (C) Depressive-like behavior as measured by Forced Swim Test. Graphs represent the Mean±SEM (n=10-11 per group). *p<0.05, **p<0.01 compared between injury groups.

5.3.3 AZD0530 administration improved recognition memory as measured by preference for the novel object.

Recognition memory was assessed via novel object preference in the ORT (Figure 5.4A). A significant main effect of time was observed ($F_{1,36}=32.39$, $p<0.0001$), with sham ($p<0.05$), 6 mg/kg ($p<0.05$) and 12 mg/kg ($p<0.001$) treated animals showing an increased preference for the novel object in trial 2, an effect not seen in 6-OHDA vehicle animals. There was also a significant main effect of treatment ($F_{3,36}=3.133$, $p<0.05$). No significant differences were seen between any of the treatment groups during trial 1 (sham: 47.69 ± 2.81 , vehicle: 47.48 ± 3.64 , 6 mg/kg: 50.19 ± 5.54 and 12 mg/kg: 48.23 ± 4.916). In trial 2, however, shams had a higher preference index (69.60 ± 5.82) compared to vehicle-treated 6-OHDA animals (52.70 ± 4.633 , $p<0.05$), indicating an injury effect. A treatment effect was also observed,

with both 6 mg/kg-treated (70.90 ± 3.21 , $p < 0.05$) and 12 mg/kg-treated (75.16 ± 3.90 , $p < 0.01$) animals exhibiting increased preference for the novel object compared to vehicle-treated animals.

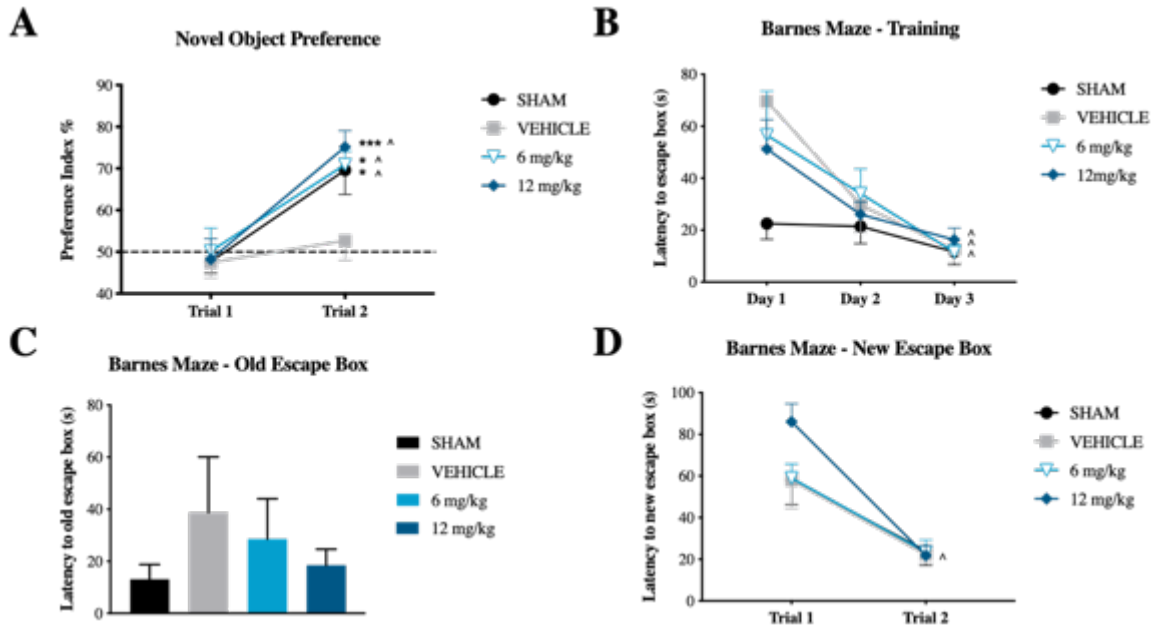


Figure 5.4. Cognitive outcomes (A) Novel object preference as assessed by preference index (%) for object B on the Object Recognition Test (B) Visuo-spatial learning as assessed by latency to escape box on training days 1, 2 and 3 on Barnes Maze. (C) Spatial reference memory as assessed by latency to old escape box. (D) Cognitive flexibility measured by latency to new escape box on probe day. Graphs represent Mean \pm SEM ($n=10-11$ per group). * $p < 0.05$, *** $p < 0.001$ compared to vehicle, ^ denotes significance over time.

The BM was used to assess various elements of visuo-spatial learning and memory. Learning acquisition, as assessed during the BM training phase (Figure 5.4B), demonstrated a significant main effect of time ($F_{2,76}=18.68$, $p < 0.0001$), with vehicle- ($p < 0.0001$), 6 mg/kg- ($p < 0.001$) and 12 mg/kg-treated animals ($p < 0.05$) all improving from day 1 to day 3, demonstrating learning of the task. Shams did not demonstrate significant improvement from day 1 to 3 ($p=0.659$), likely due to their maximal learning of the task on day 1. There was

no significant main effect of treatment ($F_{3,38}=1.398$, $p=0.258$) and no significant interaction between day and treatment group ($F_{6,76}=1.518$, $p=0.184$). Similarly, spatial reference memory, as observed in the latency to old escape box on trial 1 of probe day, revealed no significant differences ($F_{3,38}=0.626$, $p<0.603$) between groups (Figure 5.4C).

Cognitive flexibility was examined via the ability to learn a new location for the escape hole over two trials on probe day (Figure 5.4D). There was a significant main effect of time ($F_{1,38}=52.10$, $p<0.0001$), with all groups demonstrating improved latency to the escape hole from trial 1 to trial 2 ($p<0.05$). There was no significant main effect of treatment ($F_{3,38}=1.180$, $p=0.33$) nor interaction between trial and treatment group ($F_{3,38}=1.50$, $p=0.23$).

5.3.4 AZD0530 did not rescue dopaminergic integrity in the striatum

Dopaminergic integrity was assessed via % area of TH +ve staining in the ipsilateral relative to contralateral striatum following 6-OHDA injection (Figure 5.5). Dopaminergic loss in sham animals was unremarkable. A significant loss of dopaminergic integrity was observed ($F_{3,17}=11.51$, $p<0.001$) in vehicle- ($21.1\pm 2.24\%$), 6 mg/kg- ($34.94\pm 6.71\%$) and 12 mg/kg- treated ($33.46\pm 5.65\%$) animals compared to sham ($-0.92\pm 0.95\%$). There were no significant differences between vehicle and either 6 mg/kg or 12 mg/kg treated animals ($p>0.05$), indicating no prevention of dopaminergic loss with treatment.

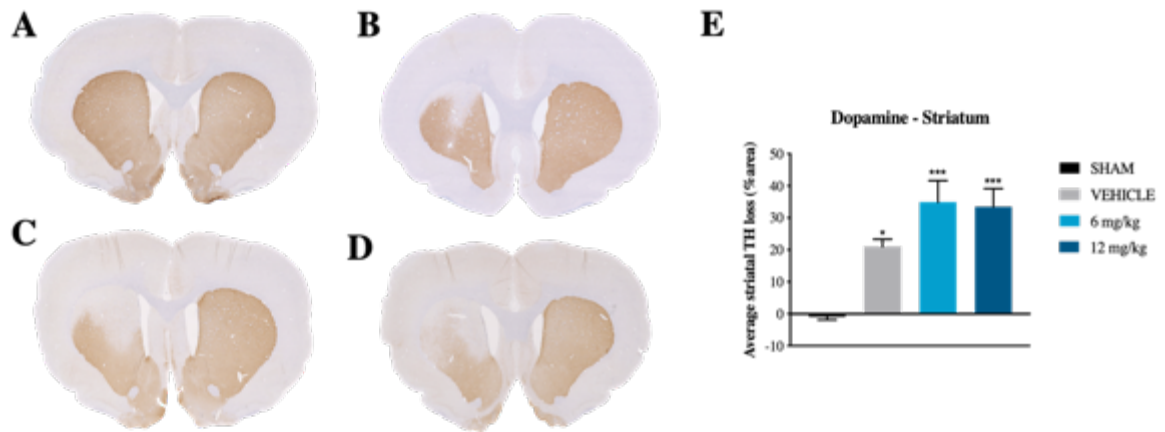


Figure 5.5. Representative images of TH staining within the striatum (A-D) of Sham (A), Vehicle (B), 6mg/kg (C) and 12mg/kg (D), as well as respective graph (E). Graphs represent Mean±SEM (n=5-6 per group). *p<0.05, ***p<0.001 compared to Sham.

5.3.5 No alterations to measures of inflammation were observed with AZD0530 administration

The neuroinflammatory response was assessed both by average number of IBA1 +ve cells (Figure 5.6) and by activation state in the striatum (Figure 5.7). There was a significant main effect of group on total microglial population in the striatum ($F_{3,17}=6.187$, $p=0.01$, with vehicle- (97.14 ± 6.70 , $p<0.05$), 6 mg/kg- (106.9 ± 8.02 , $p<0.01$) and 12 mg/kg-treated animals (108.1 ± 16.16 , $p<0.01$) all displaying a higher average number of microglia compared to shams (49.78 ± 11.47) (Figure 5.6E). There was no significant difference in microglial numbers between vehicle and either 6 mg/kg- or 12 mg/kg-treated animals ($p>0.05$), indicating that AZD0530 treatment did not reduce total microglial population.

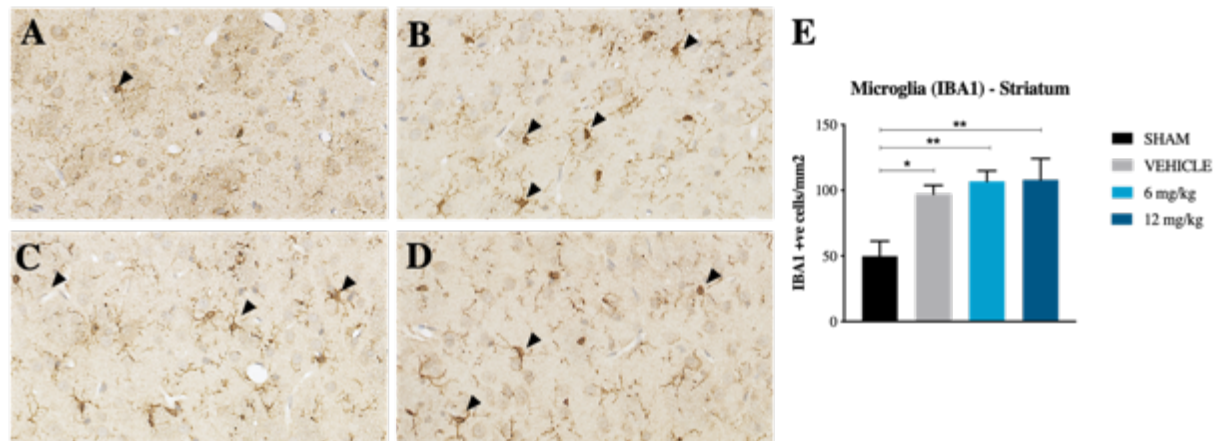


Figure 5.6. Representative images of IBA1 staining within the striatum (A-D) of Sham (A), Vehicle (B), 6mg/kg (C) and 12mg/kg (D), as well as respective graph (E). Graphs represent Mean ± SEM (n=5-6 per group). *p < 0.05, **p < 0.01 compared to Sham.

Activation state was characterised by analysing the cellular morphology of IBA1 +ve cells (Figure 5.7D). There was a significant main effect of group on activation status of the microglia ($F_{3,16}=3.638$, $p=0.04$). Specifically, there was a significant increase in the number of activated microglia observed in vehicle-treated (51.41 ± 6.51 , $p < 0.05$) animals when compared to shams (20.13 ± 4.00). Interestingly, microglial activation was not increased in either the 6 mg/kg- (46.07 ± 5.93 , or 12 mg/kg- (39.10 ± 8.66) treated animals compared to shams ($p > 0.05$). However, the difference in microglial activation between vehicle and either 6 mg/kg- or 12 mg/kg-treated animals was not significant ($p > 0.05$).

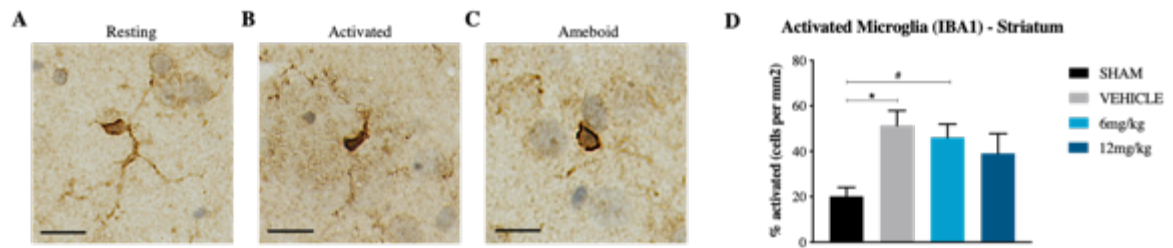


Figure 5.7. Representative images of morphological appearance of IBA1 staining of (A) resting, (B) activated and (C) amoeboid. Scale bar represents 25µm. Respective graph represents activated microglia (B + C relative to total). Graphs represent Mean±SEM (n=5-6 per group). *p=<0.05, #p=<0.01 compared to Sham.

Astrocytic response was assessed via GFAP levels in western blot (Figure 5.8). No significant main effect of group was found ($F_{3,15}=0.865$, $p=0.481$).

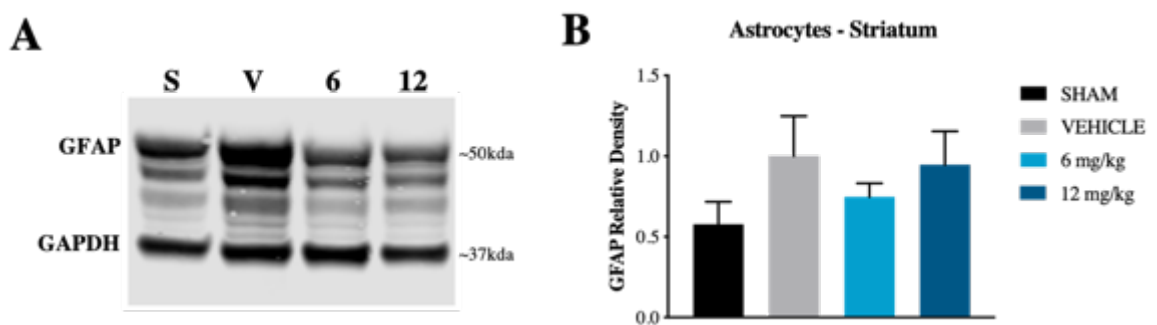


Figure 5.8. Astrocytic protein levels assessed by GFAP reactivity. (A) Western blot images of GFAP and GAPDH (housekeeper protein) markers. (B) Graph illustrates relative density of GFAP. Graph represents Mean±SEM (n=5-6 per group).

5.4 DISCUSSION

This was the first study to assess Fyn kinase inhibition as a therapeutic target for the treatment of PD. In order to trial this novel concept, the study aimed to investigate the efficacy of AZD0530 on a range of motor and non-motor outcomes and to assess the degree of neuroinflammation and dopaminergic integrity in an early stage model of PD. Results indicate animals receiving AZD0530 exhibited no significant changes in neuromuscular coordination, tremor or akinesia; however, they demonstrated improved volitional locomotion with 6 mg/kg and improved recognition memory and reduced depressive-like behaviour with both 6mg/kg and 12 mg/kg AZD0530 treatment. AZD0530 administration did not alter either striatal DA levels or inflammatory markers compared to vehicle-treatment, indicating alternate mechanisms or regionally specific changes may be responsible for improvements seen in behavioural outcomes.

In terms of motor outcomes, no impairments were observed in neuromuscular coordination or tremor in this early-stage model of PD, which corresponded to an average loss of only ~30% of TH staining within the striatum. This is consistent with clinical findings in individuals with PD, where motor deficits manifest only when 70-80% of the DA terminals are destroyed and progressive DA neuronal loss has already occurred (Poston et al., 2016). Conversely, progressive motor impairments in both akinesia and volitional locomotion were observed with this model, appearing after 21 days, with improvements in volitional locomotion observed with AZD0530 treatment at day 33. These disparities in motor impairments and treatment outcomes may be attributed to subtle anatomical differences in striatal dopamine content affected in this model. Striatal dopamine represents the main source of input for the basal ganglia and is therefore crucial for motor behaviour (Haber, 2014). The striatum itself contains 6 anatomical subdivisions, ventro/dorso-lateral,

ventro/dorso-medial and ventro/dorso-central, each with connections to different cortical areas and, subsequently, these subdivisions are often associated with dominance in different motor functionalities (Kirik et al., 1998). For example, tremor is largely dependent on involvement of the ventro-lateral striatum (Collins-Praino et al., 2011), which predominantly remained intact in this early-stage model. In line with this, increases in tremor, as measured by TJMs, were not observed in this model. Similarly, neuromuscular coordination and sensorimotor learning, as measured by rotarod performance, have also been associated with ventro-lateral striatal lesions and also remained unaffected by intrastriatal 6-OHDA administration (Kirik et al., 1998). Conversely, the AST, a measure of akinesia, is dependent on more widespread DA loss in the dorso-lateral, ventro-central and ventro-lateral striatum (Chang et al., 1999) and volitional locomotion in the OFT on dorso-lateral and dorso-medial striatal integrity (Barneoud et al., 2000). An injury effect in the AST was seen at day 21 and in both the OFT and AST at day 33, suggesting that the dorsal DA loss seen in our model may be driving these disparate motor outcomes. Future work should investigate the ability of AZD0530 to treat motor impairments in a preclinical model involving DA loss to the ventro-lateral striatum, in order to more fully assess its benefits for treating motor symptoms of PD. Additionally, a longer time-point may be needed, given that previous literature utilising the intra-striatal 6-OHDA model has reported no changes in locomotion at 3 or 6 weeks post-lesion, but significantly less activity at 9 weeks (Branchi et al., 2008).

Given the significant impact of cognitive and neuropsychiatric impairments on quality of life for individuals with PD and their caregivers, we also assessed the ability of AZD0530 to target these non-motor symptoms. In this early-stage model, however, no significant impairments in learning, cognitive flexibility or visuo-spatial memory were observed on the Barnes Maze. This is in line with previous work in a comparable model, which also reported

sparing of learning and visuo-spatial memory (Branchi et al., 2008, Branchi et al., 2010). Accordingly, the ability of AZD0530 to target such impairments could not be adequately assessed.

There were, however, significant improvements in recognition memory, as observed on the ORT. Compared to spatial or working memory, recognition memory is a relatively simplistic process (Broadbent et al., 2004); hence, it may be more sensitive to the subtle changes in DA signalling seen in this early-stage model. Recognition memory has also been shown to be significantly impaired in PD (Whittington et al., 2000) and connectivity studies have revealed an association with the progressive loss of normal network activity patterns in fronto-parietal connectivity (Segura et al., 2013). Whilst not well understood in the early 6-OHDA model, evidence suggests PD patients may exhibit impairments specifically in familiarity, rather than recollection (Davidson, 2006). Familiarity appears to be dependent on the medial temporal lobe (MTL) and extra-hippocampal structures (parahippocampal/perirhinal cortex) (Gabrieli et al., 1997). Additionally, a case study of a patient with a basal ganglia lesion demonstrated impairments in familiarity, but not recollection, implicating caudate damage may also underlie these impairments (Hay et al., 2002). Therefore, it may be beneficial for future studies to assess markers of inflammation and structural integrity in these areas.

This study also observed a significant decrease in depressive-like behaviour with both 6 mg/kg and 12 mg/kg treatment compared to vehicle. The early 6-OHDA model is known to recapitulate depressive-like behaviour, as was affirmed in our findings (Branchi et al., 2010). This is also in line with clinical data indicating depression may be a prominent non-motor symptom of prodromal PD, even preceding the motor symptoms of the disease (Leentjens

et al., 2003a, Jacob et al., 2010). In fact, depression is experienced by approximately 40% of PD patients early in disease progression (Tolosa et al., 2007). Anatomically, depression in early PD has been linked to dopamine-modulated fronto-striatal network dysfunction (Biundo et al., 2016). This network involves projections from the striatum to the orbitofrontal cortex, which is linked to regulation of emotion; consequently, loss of striatal DA may alter signalling throughout this network (Drevets, 2007). The reduction in depressive behaviour observed with AZD0530 may potentially represent conservation of DA function across this network or in other frontal regions.

Consistent with previous literature in this model (Thornton and Vink, 2012), increases in both microglial number and activation state were observed in all 6-OHDA-lesioned groups compared to shams in this study. Interestingly, however, there was no effect of AZD0530 treatment on either total number of microglia or microglial activation state. This is inconsistent with a previous study by Panicker and colleagues (2015), who established a decrease in microglial response in Fyn^{-/-} 6-OHDA lesioned mice, with associated nigral DA survival (Panicker et al., 2015). This may be dose-related, with a more substantial inhibition of Fyn kinase than that achieved with the dose regime in the current study potentially required to elicit a response. Similarly, a longer time course of treatment may be necessary in order to see improvements. In line with this, a previous study investigating AZD0530 treatment in the APP/PS1 mouse model of Alzheimer's disease reported significant reductions in cortical microglial activation following 7 weeks of daily dosing at 5mg/kg (Kaufman et al. 2015). Alternatively, behavioural results observed in this study may be related to alternate mechanisms involving Fyn signalling. This would be consistent with previous reports from preclinical studies in Alzheimer's disease, which suggest that

improvements seen with AZD0530 treatment are due to the rescue of loss of synaptic density (Smith et al., 2018, Toyonaga et al., 2019, Kaufman et al., 2015).

It is important to note that the role of Fyn in the brain is diverse and widespread (Matrone et al., 2020). Therefore, other molecular mechanisms, particularly effects on specific neurotransmitter systems, may be involved in the beneficial effects of AZD0530 treatment on recognition memory and depressive-like behaviour in our experimental model of early-stage PD. For example, Fyn kinase plays a well-established role in the phosphorylation of N-methyl-d-aspartate (NMDA) glutamate receptors, in particular, the R2A and R2B subunits, and is subsequently implicated in upregulated NMDA function (Trepanier et al., 2012). Although crucial for many normal functions, in PD, hyperphosphorylation and the resulting overactivation of NMDA receptors is a known driver of glutamate excitotoxicity (Iovino et al., 2020, Truong et al., 2009). Additionally, increased levels of NR2B subunit phosphorylation have been observed not only in the striatum (Dunah et al., 2000, Oh et al., 1998), but also in the hippocampus of 6-OHDA lesioned rats (Rostas et al., 1996), which may help to account for the impairments in recognition memory seen in our 6-OHDA model. Whilst Fyn-mediated phosphorylation is vital for normal hippocampal LTP, there appears to be an optimal activity range and excessive activation or inhibition may impact performance (Parsons et al., 2007). In support of this, Fyn inhibition prevented phosphorylation of the NMDAR2B subunit in an animal model of AD, which was subsequently associated with rescuing of memory impairments (Kaufman et al., 2015). Thus, Fyn inhibition via AZD0530 may help to prevent pathological increases in NMDA R2A/R2B subunit phosphorylation, thereby reducing NMDA overactivity, subsequently rescuing recognition memory impairments, as observed in our study.

The effects of AZD0530 on depressive-like behaviour observed in the current study may also be attributable to actions on NMDA receptors, with several NMDA R2A/R2B antagonists exhibiting potential anti-depressant effects in PD studies, indicating that downregulation of NMDA phosphorylation may be beneficial for the treatment of depression (Vanle et al., 2018). Concurrently, Fyn has also been shown to interact with the metabotropic glutamate receptor subtype mGlu5, which is dense in both the striatum and limbic systems (Swanson et al., 2005). A variety of studies have suggested that mGlu5 is implicated in mood and anxiety symptoms, with antagonists of these receptors shown to be efficacious in preclinical models of depression (Belozertseva et al., 2007, Tallaksen-Greene et al., 1998, Tatarczynska et al., 2001, Testa et al., 1994). Recently, Fyn has been shown to form a complex with the mGlu5 receptors in striatal neurons in an animal model of depression, with Fyn kinase inhibition via PP2 significantly lowering increased expression of mGlu5 in this model (Mao and Wang, 2020). Thus, in the current study, anti-depressive effects of AZD0530 may be attributable to reduced glutamatergic signalling either via interactions with NMDAR2B subunits and/or post-synaptic mGlu5. Thus, future studies are warranted to investigate the specific mechanisms involved in interactions between Fyn and NMDA in anatomical regions associated with depression in PD.

Alternatively, another mechanism which may also account for the improvements in not only in depressive-like behaviour, but also recognition memory, seen in the current study is the 5-HT receptor 6 (5-HT₆R). 5-HT₆R has been implicated in the regulation of memory processes and, whilst its role in PD is not well understood, several studies have implicated 5-HT₆R in learning and memory disorders, depression, and cognitive impairments associated with AD (Ivachtchenko et al., 2016, King et al., 2008). Given the symptomatic parallels and the observation that, unlike most 5-HT receptors, the 5-HT₆ receptor is highly

expressed in the striatum, it is possible the 5-HT₆R may be implicated in PD. Paradoxically, 5-HT₆R antagonists and agonists have both been shown to improve recognition memory in rodents using the ORT and depression using the FST (Kendall et al., 2011, King et al., 2004, King et al., 2008, Wesółowska and Nikiforuk, 2007). Recently, evidence suggests Fyn may play an important role in the signalling pathways of the 5-HT₆R receptor, with Fyn and the 5-HT₆R receptor colocalised and similarly distributed in the rat brain in the cortex, hippocampus and hypothalamus (although striatal co-localisation was not investigated) (Yun et al., 2007). Additionally, the expression of Fyn increased 5-HT₆R expression, while 5-HT mediated activation of 5-HT₆R increased Fyn phosphorylation (Yun et al., 2007) . Thus, it is possible that Fyn inhibition reduced 5-HT₆R phosphorylation, contributing to the rescue of recognition memory and reduction of depressive-like behaviour. This may represent a future area of investigation with regards to Fyn inhibition and its effects on 5-HT₆R signalling.

5.6 CONCLUSION

Taken together, the findings of this study indicate Fyn kinase inhibition through AZD0530 may be beneficial for the treatment of non-motor symptoms of Parkinson's Disease, currently a major area of clinical need. Interestingly, however, these effects do not appear to occur through anti-inflammatory mechanisms, although a more thorough investigation of neuroinflammatory response, including microglial phenotype and cytokine production, is necessary in order to fully rule this out. Instead, it may be that Fyn inhibition led to its beneficial effects via actions on glutamatergic or serotonergic signalling, thereby potentially rescuing the loss of synaptic density in related anatomical regions. Future studies are needed

to more fully explore these effects, as this may lead to more targeted use of Fyn kinase inhibition as a treatment for non-motor symptoms of PD.

Acknowledgments

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06

Optimising Cognitive Training for the Treatment of Cognitive Dysfunction in Parkinson's Disease: Current Limitations and Future Directions

Chapter prepared in publication format by **GUGLIETTI, B.**, HOBBS., D., COLLINS-
PRAINO, L.E.

6.0 ABSTRACT

Cognitive dysfunction, primarily involving impairments in executive function, visuospatial function and memory, is one of the most common non-motor symptoms of Parkinson's disease (PD). Currently, the only pharmacological treatments available for the treatment of cognitive dysfunction in PD provide variable benefit, making the search for potential non-pharmacological therapies to improve cognitive function of significant interest. One such therapeutic strategy may be cognitive training (CT), which involves the repetition of standardised tasks with the aim of improving specific aspects of cognition. Several studies have examined the effects of CT in individuals with PD and have shown benefits in a variety of cognitive domains, but the widespread use of CT in these individuals may be limited by motor impairments and other concerns in study design. Here, we discuss the current state of the literature on the use of CT for PD and propose recommendations for future implementation. We also explore the potential use of more recent integrative, adaptive and assistive technologies, such as virtual reality, which may optimise the delivery of CT in PD.

6.1 INTRODUCTION

In addition to the well-known motor impairments, Parkinson's disease (PD) is also associated with significant cognitive dysfunction, manifesting primarily in five domains: executive function, attention, memory, speed of processing and visuospatial functioning (Williams-Gray et al., 2007, Kehagia et al., 2010). Importantly, individuals with PD carry six times the risk of dementia compared to the general population (Aarsland et al., 2001). Despite the prevalence of cognitive impairment in PD, however, pharmacological treatments have proven largely ineffective (Seppi et al., 2011). Thus, the use of non-pharmacological interventions to enhance cognitive function and to potentially prevent the emergence of dementia in PD is of significant importance.

This review will first briefly describe the prevalence and presentation of cognitive dysfunction in PD and discuss the literature regarding the effectiveness of cognitive training (CT). Although reviews and meta-analyses on CT in PD have previously been conducted (Biundo et al., 2017, Calleo et al., 2012, Hindle et al., 2013, Leung et al., 2015, Walton et al., 2017), the literature has since expanded. Additionally, significant variation in approaches to the implementation and evaluation of CT have complicated efforts to accurately assess the efficacy. This, coupled with the under-addressed need to tailor interventions for the PD population due to the unique involvement of motor symptoms, prompts the need for consideration of outcomes in the context of study design. This review will assess the evidence of current techniques to inform recommendations and provide insight into the potential utility of more recent integrative, adaptive and assistive technologies in order to optimise the delivery of CT in PD.

6.2 PD AND COGNITIVE DYSFUNCTION

Cognitive impairment in PD ranges from mild cognitive impairment (PD-MCI) to Parkinson's disease dementia (PD-D). Early on in PD, deficits are estimated to occur in 20-40% of individuals, although they are often overshadowed by motor features (Benito-Leon et al., 2011, Elgh et al., 2009, Foltynie et al., 2004, Muslimovic et al., 2005, Williams-Gray et al., 2009). These are usually subtle and detectable only with formal neuropsychological testing (Levin and Katzen, 2005) and include impairments on tests of immediate verbal recall, language production/semantic fluency, set formation, cognitive sequencing, working memory (WM), and visuomotor construction compared to healthy, non-demented controls (Cooper et al., 1991). In a population-based case-control study of individuals with early PD (<5 years duration), subjective memory complaints were present in 58.7% of PD patients, compared to 37% of controls (Benito-Leon et al., 2011). Similarly, a longitudinal population-based study of early PD patients revealed 30% were impaired in one or more cognitive domains (episodic memory, executive function and verbal function) (Elgh et al., 2009), indicating cognitive dysfunction may already be a source of considerable concern for PD patients at time of diagnosis.

Many individuals are also at increased risk of developing PD-MCI, with a meta-analysis involving over 1,346 PD patients indicating prevalence of MCI was 25.8% (Aarsland et al., 2010). At time of PD diagnosis, 15-20% of patients already meet criteria for PD-MCI (Aarsland, 2016) with this figure rising to 20%-57% of individuals by 3-5 years post-diagnosis (Caviness et al., 2007, Williams-Gray et al., 2007). MCI in PD is an early stage of cognitive decline and clinical presentation of PD-MCI can be variable, ranging from amnesic to non-amnesic and single to multiple domains (Kalbe et al., 2016). However, of all cognitive functions, deficits in executive function (e.g., impairment in the ability to plan

and to inhibit behaviours or deficits in attention and WM) are the most commonly seen in PD-MCI (Kalbe et al., 2016) and can severely impact an individual's ability to carry out activities of daily living (ADL) (Pagonabarraga and Kulisevsky, 2012). These cognitive changes may be particularly tied to dopaminergic fronto-striatal function (Owen et al., 1992).

According to the “dual syndrome hypothesis”, while fronto-striatal executive deficits, common early in the course of PD, are more stable, cognitive deficits related to posterior cortical dysfunction, such as visuospatial function and recognition memory, are linked to earlier emergence of dementia in PD (Kehagia et al., 2013, Williams-Gray et al., 2007). A recent meta-analysis found 25% of PD patients with normal cognition convert to PD-MCI and 20% of PD-MCI patients convert to dementia within 3 years (Saredakis et al., 2019). Within 20 years of diagnosis, however, over 80% of individuals with PD progress to PD-D (Hely et al., 2008). In contrast to PD-MCI, PD-D is associated with more severe and debilitating cognitive impairments in multiple domains. Furthermore, PD-D includes both cognitive features (e.g., impairments in attention, executive function, visuospatial function and memory) and behavioural features (e.g., apathy, changes in personality and mood, hallucinations, delusions and excessive daytime sleepiness) (Emre et al., 2007). These neuropsychiatric and cognitive impairments are amongst the most debilitating for PD patients as non-motor symptoms account for the biggest predictors of quality of life (QoL), mortality and caregiver burden (Duncan et al., 2014).

6.2.1 Pharmacological treatment of cognitive impairment in PD: Limitations

Despite the prevalence of cognitive impairment in PD, its treatment has remained an area of unmet clinical need, with existing treatments providing only symptomatic relief of already-established dysfunction. Treatment of PD-D with dopaminergic strategies, such as L-dopa

and dopamine agonists, has failed to produce significant improvements in cognition (Akbar and Friedman, 2015, Brusa et al., 2005, Kulisevsky et al., 1996). Treatments acting only on the dopaminergic system are likely to be ineffective, as PD-D involves alterations in a number of other neurotransmitter systems in addition to dopamine, such as noradrenaline, serotonin and acetylcholine (see (Halliday et al., 2014) for review). In support of this, the norepinephrine reuptake inhibitor atomoxetine has demonstrated mild benefits in PD patients with MCI in two small trials (Marsh et al., 2009, Weintraub et al., 2010b). Furthermore, of all neurotransmitters altered in PD-D, cholinergic changes are most striking, with cortical cholinergic function more severely affected in PD-D than in Alzheimer's disease (Bohnen and Albin, 2011, Bohnen et al., 2006, Hilker et al., 2005). In light of these abnormalities, the use of cholinesterase inhibitors is currently the preferred pharmacological treatment strategy for cognitive impairments in PD (Akbar and Friedman, 2015), and evidence from both large, randomised placebo-controlled trials and meta-analyses suggests that use of these compounds may provide at least modest benefit in the treatment of PD-D (Rolinski et al., 2012, Wang et al., 2015). A meta-analysis by Wang and colleagues (2015) found that both cholinesterase inhibitors and the NMDA receptor antagonist memantine provided a small benefit in PD-D and dementia with Lewy bodies, but only cholinesterase inhibitors led to a modest improvement in cognition as evaluated by MMSE (Wang et al., 2015).

Despite this, however, cholinesterase inhibitors have variable efficacy between patients, often not providing significant benefit (Emre et al., 2014). Furthermore, they may be associated with side-effects, such as gastrointestinal symptoms (Aarsland, 2016). Rivastigmine treatment has been linked to side-effects such as nausea (29%), vomiting (16.6%) and even tremor (10.2%) (Emre et al., 2014), leading to concerns long-term use of cholinesterase inhibitors could worsen motor symptoms in some PD patients. In support of

this, both rivastigmine and donepezil can induce tremor in response to their administration in a subset of individuals (Gurevich et al., 2006, McCain et al., 2007, Song et al., 2008), and administration of the anticholinesterase galantamine can potentially induce tremor (Collins et al., 2011). This highlights the need for a complimentary and/or alternate intervention strategy that may be adapted and targeted to address the specific needs of the individual.

6.3 COGNITIVE TRAINING IN PD

Cognitive training (CT) is defined as training programs that provide structured practice on specific cognitive tasks, designed to improve performance in one or more cognitive domains, such as memory, attention or executive function (Clare and Woods, 2004). Although studies on the neural basis of CT in PD are sparse, and have included only a handful of subjects, studies in healthy aging may also provide insight. Systematic review of various CT and MRI modalities suggest structural changes in both grey and white matter occur following CT, particularly in the hippocampus (Belleville and Bherer, 2012, Valkanova et al., 2014). A subsequent MRI study has suggested these benefits may be due to neuroplasticity, as there was an increase in cerebral blood flow and neural connectivity in the default mode network and central executive network following CT (Chapman et al., 2015).

Several studies have investigated the benefits of CT for cognitive function in PD (outlined in Table 6.1). A recent Cochrane review evaluating the effectiveness of cognitive training for PD-MCI and PD-D identified 7 studies fitting their criteria, culminating in a total of 225 participants with variable intervention lengths (Orgeta et al., 2020). The review found *‘no difference between people who received CT and people in the control groups in global cognition shortly after treatment ended and no convincing evidence of benefit in specific cognitive skills and no benefit shown in ADL or QoL’* (Orgeta et al., 2020). Whilst

comprehensive, the strict criteria limiting assessment to randomised-control trials (RCT) meant several notable studies over the last decade were excluded from evaluation. Furthermore, inclusion was restricted to studies assessing function exclusively in PD-MCI and PD-D and did not assess potential benefits in individuals with PD without cognitive impairment, a population that may arguably derive the most benefit from a CT intervention. Finally, studies utilising integrative multi-component approaches were also excluded. Whilst the results of these studies are difficult to compare to standard CT alone, they are still important to consider, as they represent intervention strategies that have potentially integrated and expanded upon current paradigms in order to optimise delivery. Given these limitations, a comprehensive review of the literature is needed to fully assess the benefits, and future potential, of CT in PD. Additionally, given differences in diagnosis, methodology and outcome measures between studies, recommendations for more uniform study design criteria are also required.

Here, we will expand on the latest findings, with search criteria incorporating studies utilising both RCT and pre-post design, as well as single and multi-component interventions targeting cognitive function in PD. Exclusion criteria include studies where cognition was not the primary outcome measure and studies that specifically excluded cognitive impairments. Due to the nature of motor impairments in PD, particular attention will be paid to the method of administration, including assessment of traditional, computerised and multi-component interventions, as well as a look towards the potential future of adaptive and assistive technology. Specifically, recommendations for the use of CT in PD will be proposed as a guide for the design of future studies.

Table 6.1: Study methodology details of Cognitive training in Parkinson's disease

Source	Sample size	Method of Administration	CT intervention(s)	Duration	Cognitive outcome measure(s)	Results
Pen and Paper CT						
(Mohlman et al., 2011)	16 participants with PD + MCI/D Mean age: 62.71, S.D.: 7.32; 10M, 6F)	<u>Worksheets + Audio CD's</u>	Attention Process Training APT-II intervention aimed to train sustained, selective, alternating and divided attention	90 minute sessions 1x/week for 4 weeks	<i>Executive Function/Attention</i> <ul style="list-style-type: none"> • Stroop Color Word Test • Controlled Oral Word Association Test (COWAT) • Digit Span Forward • TMT-B 	Patients improved on all 4 cognitive tests, and average ratings of progress were positively correlated with magnitude of change on these measures.
Pena et al. (2014)	44 participants with PD <i>CT group = 22</i> (Mean age: 67.6 (65.25-69.84); 9F, 13M) <i>Control group = 22</i> (Mean age: 68.1 (64.93-71.32); 8F, 14M)	<u>Pen and Paper</u> REHACOP program for attention, memory, language, executive function, social cognition and processing speed	REHACOP Delivered in a group setting Control group performed group occupational activities, such as drawing, reading the news, etc.	60 minute sessions: 3x/week for 3 months <i>Attention unit:</i> 4 weeks <i>Memory unit:</i> 3 weeks <i>Language unit:</i> 3 weeks <i>Executive function unit:</i> 2 weeks <i>Social cognition unit:</i> 1 week	<ul style="list-style-type: none"> • <i>Processing Speed:</i> TMT-A and Salthouse Letter Comparison Test • <i>Verbal learning and memory:</i> Hopkins Verbal Learning Test (learning and long-term recall) • <i>Visual learning and memory:</i> Brief Visual Memory Test (learning and long-term recall) • <i>Executive function:</i> Stroop test (word-colour and interference) • <i>Theory of mind:</i> Happé test • <i>Functional disability:</i> World Health Organization Disability Assessment Schedule II (WHO-DAS II), short version • <i>Depression:</i> Global Depression Scale (GDS) 	Bootstrapped analysis of variance showed significant differences in mean change scores in processing speed, visual memory, theory of mind and functional disability. Neither verbal learning and memory or executive function showed any difference between groups.
(Díez-Cirarda et al., 2018)	15 participants with PD	<u>Pen and Paper</u> REHACOP	Integrative group-based cognitive intervention (REHACOP) <ul style="list-style-type: none"> • Attention (sustained, selective, alternate, divided) 4 weeks 	1 hour sessions: 3x week for 13 weeks <i>Baseline – T0</i> <i>Post-treatment – T1</i>	Outcome Measures T1 weighted MRI <i>Motor</i> <ul style="list-style-type: none"> • UPDRS III <i>Processing Speed</i> <ul style="list-style-type: none"> • Trail Making Test-A • Salthouse Letter Comparison Test <i>Verbal Memory</i>	CT group demonstrated increased performance in VM, VIM, EF and ToM and decreased functional disability at follow-up compared to baseline testing. Increased performance in VIM and EF at follow up (T2) compared with post-testing (T1). No significant changes in PS, VM, ToM, Apathy, Depression or functional disability.

			<ul style="list-style-type: none"> • Memory (verbal, visual learning, recall, recognition) 3 weeks • Language (verbal fluency, synonyms/antonyms) 3 weeks • Executive Function (cognitive planning, verbal reasoning) 2 weeks Social Cognition (moral dilemmas, TOMS) 1 week	<i>Follow-up</i> (18 months) – T2	<ul style="list-style-type: none"> • Hopkins verbal learning test <i>Visual Memory</i> <ul style="list-style-type: none"> • Brief Visual memory test (learning and recall) <i>Executive Function</i> <ul style="list-style-type: none"> • Stroop Test • Theory of Mind (TOM) – Happe test <i>Apathy</i> <ul style="list-style-type: none"> • Lille Apathy Rating Scale <i>Depression</i> <ul style="list-style-type: none"> • Geriatric Depression Scale <i>Functional Disability</i> <ul style="list-style-type: none"> • WHO DAS II 	PD patients showed significant deterioration in UPDRS III and trend towards progression in disease on Hoehn and Yahr scale Increased brain functional connectivity and maintenance at T2 compared to T1, however, significant grey matter reduction and alterations of white matter integrity were found at T2
Computer-based CT						
(Sinforiani et al., 2004)	20 participants with PD +/- MCI (8F, 12M) Mean age: 68.9, S.D.: 7.1	TNP Software	Stimulate cognitive functions (attention, abstract reasoning, visuospatial abilities)	60 minute sessions 6 weeks (2x/week; 12 sessions total) <i>Follow-up:</i> 6mo	<i>Cognition</i> <ul style="list-style-type: none"> • MMSE <i>Visual/Spatial Memory</i> <ul style="list-style-type: none"> • Corsi's test <i>Attention</i> <ul style="list-style-type: none"> • Stroop's test and Wisconsin card sorting test (WCST) <i>Verbal Memory</i> <ul style="list-style-type: none"> • Babcocks story <i>Verbal Fluency</i> <ul style="list-style-type: none"> • Phonological word fluency (FAS) <i>Executive Function</i> <ul style="list-style-type: none"> • Raven's matrices and Digit Span 	Patients performed significantly better compared to baseline on Babcocks story, Raven's matrices and phonological word fluency. Effects were maintained at 6-months follow-up
(Edwards et al., 2013)	87 participants with PD <i>CT group</i> = 44 (Mean age: 69.4, S.D.: 7.8; 16F, 28M) <i>Control group</i> = 43 (Men age: 68.2, S.D.:	InSight Program 5 different programs designed to improve information processing in realistic visual contexts.	CT = Self-administered speed of processing training Control = no contact.	60 minute sessions: 20 hours over 3 months, with every other session on the Road Tour exercise only	<ul style="list-style-type: none"> • The Cognitive Self-Report Questionnaire • Useful field of view test (UFOV) 	CT group experienced significantly greater improvements in speed of processing than the control group. No differences between groups were seen on the Cognitive Self-Report Questionnaire.

	8.4; 17F, 26M)					
Petrelli et al. (2014)	<p>65 participants with PD</p> <p><i>NEUROvitalis (NV) group</i> = 22 (Mean age: 69.2, S.D. 4.9; 12F, 10M)</p> <p><i>Mentally fit (MF) group</i> = 22 (Mean age: 68.8, S.D.: 6.7; 7F, 15M)</p> <p><i>Waitlist Control (CG) group</i> = 21 (Mean age: 69.1, S.D.: 11.6; 9F, 12M)</p>	<p>NEUROvitalis program</p> <p>Trained attention, memory, executive function</p>	<p><u>NEUROvitalis CT group</u> - Individual tasks, group tasks and group games. Each session focused on one specific cognitive domain and started with a psychoeducational module.</p> <p><u>Mentally fit</u>: Cognitive domains were not focused on in individual sessions. Instead, individual and group tasks for training attention, memory and less specific functions (general language, creative thinking) were combined randomly over the course of the entire program. Group conversations were used in place of psychoeducational sessions. Waitlist control group received no contact.</p>	90 minute sessions: 2x/week for 6 weeks (12 sessions total)	<p><i>Attention</i>: Brief test of attention</p> <p><i>Memory</i>: Verbal short-term (DemTect, Memo), Verbal long-term (DemTect, Memo), Visual long-term (Complex figure recall)</p> <p><i>Executive functions</i>: Working memory (DemTect: digit span reverse), verbal fluency (semantic: DemTect; phonemic: FAS)</p> <p><i>Visuoconstruction</i>: Figure copy</p> <p><i>Quality of Life</i>: PDQ-39</p> <p><i>Depression</i>: Beck Depression Inventory-II</p>	NEUROvitalis group demonstrated statistically significant improvements in short term and working memory (assessed by word list learning and digit span reverse, respectively). The increase in working memory was significantly greater than that in the Mentally fit group.
(Petrelli et al., 2015)	<p>47 participants with PD</p> <p><i>NV group</i> = 16 (Mean age: 69.4, S.D. 4.2; 8F, 8M)</p> <p><i>MF group</i> = 17</p>	<p>NEUROvitalis program</p> <p>Petrelli et al. (2014) follow-up</p>	Participants received no further intervention.	1 year follow-up	<p><i>Overall cognitive function</i>:</p> <ul style="list-style-type: none"> • MMSE • DemTect <p><i>Responder</i>: Combined score of percentage change from baseline to 1-year follow-up</p> <p><i>Risk of developing MCI</i></p>	Both the NV or MF groups maintained their DemTect score at 1-year post-follow up. Individuals in the NV group also maintained their MMSE score. While only 21.4% of the CG were classified as responders, 41.2% of the MF group and 56% of the NV group were responders. Patients without MCI at baseline from the CG had a risk of 40.0% to develop MCI from baseline to 1 year follow-up, while patients who received either intervention had a risk of only 18.2%.

	(Mean age: 68.6, S.D. 7.3; 5F, 12M) CG = 14 (Mean age: 68.8, S.D. 9.2; 5F, 9M)					
Alloni et al. (2018)	31 participants with PD + MCI <i>CoRe CT</i> (17) = (12F;5M, Mean Age: 71.2;SD: 7) <i>Control Intervention</i> (14) (5F;9M, Mean age 69.5;SD 8)	CoRe System	<ul style="list-style-type: none"> • Computer-based logical-executive task <ul style="list-style-type: none"> ○ Find the category ○ Find the Intruder ○ Unscramble the Images ○ Image and Sound ○ Word Coupling ○ Logical Sequences ○ Logical analogies ○ Find the Elements Functional Planning Placebo Control intervention	45 minutes: 3x week for 4 weeks (12 sessions total) <i>Baseline – T0</i> <i>Post-treatment – T1</i> <i>Follow-up (6 months) – T2</i>	<i>Global Cognition</i> <ul style="list-style-type: none"> • MMSE • MoCA <i>Executive Function</i> <ul style="list-style-type: none"> • Raven’s Matrices 47 test (RM47) • Weigl’s Colour-For Sorting Test (WCFT) • Frontal Assessment Battery (FAB) • F-A-S Test <i>Attention</i> <ul style="list-style-type: none"> • Attentive Matrices • Trail Making Tests A and B • Stroop Test <i>Verbal Memory</i> <ul style="list-style-type: none"> • Verbal Span • Digit Span • Logical Memory Test (immediate and delayed recall) • Rey’s 15 word test (immediate and delayed recall) • Rey’s Complex figure with delayed recall - RCF-dr) • Wechsler Memory Scale WMS <i>Spatial Memory</i> <ul style="list-style-type: none"> • Corsi Block-Tapping Test • Rey-Osterrieth complex Figure Test <i>Visuo-spatial ability</i> <ul style="list-style-type: none"> • Rey-Osterrieth Complex Figure Copy Test 	After intervention (T0-T1), CT group improved significantly on MoCA compared to control interventions. Compared to baseline, CT group improved in 12/21 assessments (MoCA, R 15-word test recall, Logical Memory, Raven’s Matrices, Weigl’s,FAB,TMTa,TMTb,Stroop Tests (both), FAS, RCF-dr). Control group only improved in RCF-dr. At follow up (T1-T2), CT and control groups displayed significant worsening on MoCA and FAS, however, only the control group experienced worsening in MMSE and Logical Memory delay recall. No significant differences were observed between groups Overall, (T0-T2), CT intervention demonstrated significant improvements in Rey’s 15 word test (immediate), Weigl’s sorting test, Stroop test (time interference) compared to baseline, whilst control group only showed improvement in Rey Complex Figured (delayed recall), whilst worsening in MoCA, Digit Span, Raven’s Matrices, FAB, TMT A and Stroop test error interference. Improvements in CT were significant compared to controls in MoCA, Corsi’s, Reys 15 word test (immediate and delayed recall), Weigl’s test, TMTa and Stroop test.
Folkerts et al. (2018)	12 participants with	NEUROvitalis System	CT – Modified NEUROvitalis (modified version)	60 Minutes: 2x weekly/	<i>Global Cognition</i> <ul style="list-style-type: none"> • Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) 	

	<p>PD+D</p> <p><i>NEUROvitalis</i> CT (6) = (5M;1F, Mean Age 76.67; SD 5.58)</p> <p>Control Group (6) (5M;1F, Mean Age 76.5; SD 8.94)</p> <p>(Randomised crossover trial)</p>	(Modified)	<ul style="list-style-type: none"> • Targets executive function and visual spatial function <p>Control treatment - Usual care (includes sports, music and arts) - went on to receive CT</p>	<p>8 weeks (16 sessions in total)</p> <p>Post assessment + 6 week follow up</p>	<p><i>Verbal Fluency</i></p> <ul style="list-style-type: none"> • Word Fluency Test <p><i>Attention</i></p> <ul style="list-style-type: none"> • TMT <p><i>Activities of Daily Living</i></p> <ul style="list-style-type: none"> • Barthel Index <p><i>Quality of Life</i></p> <ul style="list-style-type: none"> • QUALIDEM Scale <p><i>Depression</i></p> <ul style="list-style-type: none"> • GDS • Cornell Scale for Depression in Dementia (CSDD) <p><i>Health Related Quality of Life</i></p> <ul style="list-style-type: none"> • EQ-5D-5L <p><i>Neuropsychiatric Symptoms</i></p> <ul style="list-style-type: none"> • Neuropsychiatric Inventory (NPI) 	<p>Group differences favoured CT, with a trend for improvement in overall CERAD score and NPI, although these did not reach statistical significance (p=0.067 and 0.075 respectively).</p> <p>Compared to baseline, CT group demonstrated a trend for improvement in CERAD and GDS, however, these also failed to reach statistical significance (p=0.06 and 0.07 respectively).</p>
(Cerasa et al., 2014)	<p>15 participants with PD</p> <p>CT group = 8 (Mean age: 61.1, S.D.: 12.4)</p> <p>Control group = 7 (Mean age: 58.3, S.D.: 9.6)</p>	<p>Rehacom Software</p> <p>Training Attention and Information Processing Tasks</p>	<p>Rehacom as in (Cerasa et al., 2013)</p> <p>Control group performed a simple visuomotor coordination tapping task</p>	<p>60 minutes sessions: 2x/week for 6 weeks (12 sessions total)</p>	<p><i>Spatial memory:</i></p> <ul style="list-style-type: none"> • ROCFT <p><i>Verbal memory:</i></p> <ul style="list-style-type: none"> • Selective reminding test (SRT) <p><i>Visuospatial processing:</i></p> <ul style="list-style-type: none"> • Judgment Line Orientation Test <p><i>Verbal fluency:</i></p> <ul style="list-style-type: none"> • Controlled Oral Word Association Test <p><i>Sustained attention and information processing:</i></p> <ul style="list-style-type: none"> • Symbol digit modality test and PASAT <p><i>Executive functions:</i></p> <ul style="list-style-type: none"> • Digit span forward/backward, Stroop word-color task and TMT A and B <p><i>Mood:</i></p> <ul style="list-style-type: none"> • Beck II, STAI-/y <p><i>General Cognition:</i></p> <ul style="list-style-type: none"> • MMSE <p><i>Quality of Life:</i></p> <ul style="list-style-type: none"> • PDQ-39 	<p>CT group showed improved cognitive performance compared to the control group on a measure of attention (Symbol-digit modality test) and executive function (digit span forward).</p> <p>These improvements were associated with significantly increased intrinsic functional activity in the left dorsolateral prefrontal cortex within the left central executive resting state network (RSN) and in the left superior parietal lobule within the attention RSN.</p>
Fellman et al. (2020)	<p>52 participants with PD +/- MCI; 54 healthy controls</p>	<p>Home training using Working Memory Tasks including:</p>	<p>Home based RCT</p> <p>PD CT group = Working memory (WM) training</p>	<p>30 Minute sessions: 3 x 30 minute sessions p/w for 5 weeks</p>	<p>WM</p> <ul style="list-style-type: none"> • N-back training task • Selective Updating of Sentences Training Task (SUST) 	<p>PD patients WM function was well-preserved, with performance comparable to healthy controls. PD patients were, however, impaired in self-assessment of WM and executive function.</p>

	<p><i>CT Group (26) = (17M;9F, Mean age 64.8; SD 6.2)</i></p> <p><i>Active Control Group (26) = (17M;9F, Mean age 66.5, SD 4.7)</i></p> <p><i>Healthy Control Group (54) = (41M;13F, Mean age 66; SD 4.1)</i></p>	<ul style="list-style-type: none"> • <i>N-back training task</i> • <i>Selective Updating of Sentences Training Task (SUST)</i> • <i>Forward Simple Spain Training Test (FSST).</i> 	<p>Active control group = received quiz training (general knowledge)</p> <p>Healthy Control group = no intervention</p>	<p>(5 stages over 8 weeks (1-3, pre-testing, 3-7 testing, 7-8 post-testing)</p>	<ul style="list-style-type: none"> • Forward Simple Spain Training Test (FSST). • Working memory questionnaire <p><i>Task specific near transfer tasks</i></p> <ul style="list-style-type: none"> • N-back with colours • Selective updating of digits (SUD) • Forward colour span <p><i>Task general near transfer</i></p> <ul style="list-style-type: none"> • Running memory task • AWM task • Minus 2 span task <p><i>Far transfer</i></p> <p><i>Verbal Memory</i></p> <ul style="list-style-type: none"> ▪ Sentence Recall and Word List Recall <p><i>Executive Function and Attention</i></p> <ul style="list-style-type: none"> ▪ Continuous performance task (CPT) and Stroop test ▪ BRIEF-A <p>Depressive Symptoms</p> <ul style="list-style-type: none"> • GDS-30 	<p>Compared to active controls, the WM CT group showed significant improvement in 2/3 WM tasks and near-transfer improvements, however, this did not translate to improvements in far transfer domains such as verbal memory, executive function and attention or self-assessed measures of WM and executive function. There was, however, a decrease in depression scores associated with WM CT.</p>
Combination – Pen & Paper + Computer-based (or unspecified)						
Nombela et al. (2011)	<p>10 participants with PD; 10 healthy controls</p> <p><i>PD group = 5 E-CT/5-C-CT (mean age: 60.5 SD 3.45, 6F, 4 F)</i></p> <p><i>Healthy Control = 10 (mean age: 59.6 SD 4.47, 6F, 4M)</i></p>	Not Specified	<p>Experimental CT = Modified Stroop Test</p> <p>Control CT = Sudoku</p> <p>Participants with tremor, dyskinesia or substantial motor impairment were not considered</p>	<p>Control CT One Sudoku table every day at home for 6 months</p>	<ul style="list-style-type: none"> • <i>Cognitive Screening</i> <ul style="list-style-type: none"> • UPDRS • MMSE • Montgomery Asberg Depression Rating Scale (MADRS) • <i>Attention</i> <ul style="list-style-type: none"> • Stroop Test 	<p>Experimental (Trained) PD patients showed significant improvement in Stroop Test Reaction time compared to CT control and HC. This corresponded with attenuated pattern of brain activation</p>

Paris et al. (2011)	<p>28 participants with PD +/- MCI <i>CT group</i> = 16 (Mean age: 64.8, S.D: 9.2; 7M, 9F) <i>Control group</i> = 12 (Mean age: 65.4, S.D: 9.6; 7M, 5F) 50% of participants in both groups met criteria for MCI.</p>	<p>Combination SmartBrain Software (Tarraga et al., 2006) Individualised program</p>	<p>Paper-based homework exercises, consisting of 20 cognitive exercises Control group received speech therapy.</p>	<p>45 minute sessions: 4 weeks (3x week; 12 sessions total) Homework exercise (1x week; 4 sessions total)</p>	<p><i>Cognitive screening:</i></p> <ul style="list-style-type: none"> MMSE and Addenbrooke Cognitive Examination <p><i>Attention and working memory:</i></p> <ul style="list-style-type: none"> Digits subtest of WAIS-III; California Verbal Learning Test (CVLT), 1st trial <p><i>Information processing speed:</i></p> <ul style="list-style-type: none"> Symbol-Digit modalities test; Trail-Making-test A; Stroop Word subtest <p><i>Verbal memory:</i></p> <ul style="list-style-type: none"> CVLT-II Short-Delay and Long-Delay Free Recall) and Logical Memory subtest (WMS-III) <p><i>Verbal fluency:</i></p> <ul style="list-style-type: none"> Phonemic-FAS; Semantic- Animals <p><i>Learning:</i></p> <ul style="list-style-type: none"> CVLT-II (List A Total) <p><i>Visual Memory and Visuoconstructive Ability:</i></p> <ul style="list-style-type: none"> Rey Osterrieth Complex Figure Test (ROCFT) <p><i>Visuospatial abilities:</i></p> <ul style="list-style-type: none"> RBANS Line orientation subtest <p><i>Executive function:</i></p> <ul style="list-style-type: none"> Tower of London (TOL); Trail-Making-Test B (TMT-B); Stroop Interference subtest <p><i>Quality of Life:</i></p> <ul style="list-style-type: none"> PDQ-39 <p><i>Cognitive difficulties in ADLs:</i></p> <ul style="list-style-type: none"> CDS 	<p>CT group significantly improved in tests of:</p> <ul style="list-style-type: none"> <i>Attention and working memory</i> (WAIS-III Digit Span Forward) <i>Processing speed</i> (Stroop Word subtest) <i>Visual memory/ visuoconstructive abilities</i> (ROFCT) <i>Visuospatial abilities</i> (RBANS Line Orientation subtest) <i>Verbal fluency</i> (Semantic-Animals) <i>Executive function</i> (TOL- total moves and total correct; TMT-B) <p>There were no significant improvements in self-rated quality of life or ADLs.</p> <p>No significant effects on overall cognitive function MMSE/ACE</p>
Costa et al. (2014)	<p>17 participants with PD + MCI and 8 healthy controls <i>Healthy Controls</i> = 8 (Mean age: 67.2, S.D.: 6.2)</p>	<p>Combination Aiming to train shifting ability in prospective memory tasks</p>	<p>Patients alternately select between stimuli (e.g., letters, numbers and shapes) belonging to different semantic categories or with different visual/spatial features □ 4 modules of increasing difficulty, with each</p>	<p>45 minute sessions: 3x/week for 4 weeks (12 sessions in total)</p>	<p><i>Prospective memory (PM)</i> (after (McDaniel et al., 2004)) and <i>Verbal Fluency/Shifting Ability</i> Alternate Fluency and TMT</p>	<p>Significant improvement in the experimental group in accuracy on the PM procedure and performance on alternate, but not phonemic fluency compared to baseline and placebo group</p>

	<p><i>CT group = 9</i> (Mean age: 66.1, S.D.: 7.1)</p> <p><i>Placebo group = 8</i> (Mean age: 70.9, S.D.: 4.8)</p>		<p>module consisting of 3 sessions</p> <p>Control participants= language exercises (dictation and reordering of sentences) that did not vary in difficult and respiratory exercises</p>			
Vlagsma et al. (2020)	<p>43 participants with PD +/- MCI , 90 healthy controls</p> <p><i>PD CT group - ReSET (24) = (14M;10F, Mean age 60.21; SD 10.42)</i></p> <p><i>PD Control CT -CogniPlus (19) = (13M;15F, Mean age 62.58, SD 8.84)</i></p> <p><i>Healthy Control (90) = (42M;48F, Mean age 58.97; SD 6.41)</i></p>	<p>Not specified</p> <p>ReSET Neurorehab sessions - involving strategy training to improve executive function</p>	<p>PD CT group = Cog Rehab using strategy training ReSET</p> <p>PD Control = computerised repetitive practice training for attention with Cogniplus</p> <p>Healthy Controls = no intervention</p>	<p>60 Minute sessions: 1-2 x week / 14 weeks (14 sessions in total)</p> <p><i>Baseline- T0</i> <i>2 weeks post-intervention- T1</i> <i>3-5 mo post-intervention – T2</i></p>	<p><i>Activities of Daily Living</i></p> <ul style="list-style-type: none"> • Role Resumption List (RRL) <p><i>Executive Function</i></p> <ul style="list-style-type: none"> • Treatment Goal Attainment (TGA) • Dysexecutive Questionnaire (DEX) • Brock Adaptive Functioning Questionnaire (BAF-Q) <p><i>Attention</i></p> <ul style="list-style-type: none"> • TMTA <p><i>Verbal Memory</i></p> <ul style="list-style-type: none"> • RAVLT (immediate and delayed recall) <p><i>Caregiver Burden</i></p> <ul style="list-style-type: none"> • Zarit Burden Interview (ZBI) <p>Quality of Life</p> <ul style="list-style-type: none"> • PDQ-39 	<p>Immediately following treatment (T0-T1), both groups reported significant improvement in executive function (TGA and DEX), with improvements greater with ReSET than Cogniplus. No differences were observed between CT groups on ADL (RRL), Quality of Life (PDQ-39), Caregiver burden (ZBI) or BAF-Q.</p> <p>At follow-up (T0-T2), both treatment groups maintained improvements in executive function (TGA and DEX) compared to baseline, however, there was no significant difference between groups.</p>
Integrated/Multi-Modal CT						

<p>Reuter et al. (2012)</p>	<p>240 participants with PD+MCI (Mean age: 64, S.D: 4)</p> <p><i>Group A:</i> CT = 71 (35F, 36M)</p> <p><i>Group B:</i> CT and transfer training = 75 (36F, 39M)</p> <p><i>Group C:</i> CT, transfer training and motor training = 76 (36F, 40M)</p>	<p>Computer-based (Individually Tailored)</p> <p>Integrated with transfer training</p> <p>Targeting executive function and memory</p>	<p><u>1. CT</u> Individually tailored based on scores on baseline tests. Set of tasks requiring executive and memory function</p> <p><u>2. Transfer training</u> 90 minute sessions: Goal: to manage daily life better and become more self-confident Composed according to baseline results and patient preferences Example tasks: go to grocery store, prepare a meal, pay a bill, look after a vegetable patch, etc.</p> <p><u>3. Motor training:</u> 60 minute sessions Goal: to improve coordination, strength, speed, perception and orientation. Composed according to individual capabilities and needs Example tasks: perform motor sequences, dual task performance, find items, remember hidden items, obstacle course completion with changing rules, mental imagery, aerobic training, etc. Caregivers received a 5-module educational training program.</p>	<p>60 minute sessions: 4 weeks in rehab center: <u>1. CT</u> = 4x/week, at least 14 sessions in total</p> <p><u>2. Transfer training</u> = 3x/week, at least 10 sessions in total</p> <p><u>3. Motor training:</u> Minimum 10, maximum 12 sessions</p> <p><u>Followed by 6 months at home:</u> <u>1. CT</u> = 3 x 45 minute sessions per week (All groups) <u>2. Transfer training</u> = 2x per week (Groups B and C) <u>3. Motor training</u> = 2x per week (Group C)</p> <p><i>Follow-up:</i> 6mo</p>	<p><i>Primary outcome measure:</i></p> <ul style="list-style-type: none"> Alzheimer's assessment scale cognition (ADAS-COG) <p><i>Secondary outcome measure:</i></p> <ul style="list-style-type: none"> Scale for Outcomes in Parkinson's disease Cognition (SCOPA-COG) <p>Information processing speed:</p> <ul style="list-style-type: none"> Paced auditory serial addition test (PASAT) <p><i>Executive function:</i></p> <ul style="list-style-type: none"> BADS 	<p>All groups improved significantly on both the primary outcome measure (ADAS-COG), and the secondary outcome measure (SCOPA-COG), with Group C having the most benefit.</p> <p>At 6-mo follow-up, 50% of Group A, 31% of Group B and 28% of Group performed worse on the ADAS-COG compared to performance at discharge. Further improvement was observed in 21% patients of Group A, 37% patients of Group B, and 50% patients of Group C. At 6-mo follow-up, 70% of Group A, 80% of Group B, and 94% of Group C maintained their discharge-level performance on the SCOPA-COG.</p> <p>On the BADS-subcales, all groups showed improvement at discharge, with Group C showing the most improvement. At 6-mo post-completion, Groups A and B had lost most of their improvement, while Group C largely maintained their scores.</p> <p>On the PASAT, group A did not improve, while both Group B and Group C showed improvement, with Group C benefitting the most from training.</p>
<p>(Naismith et al., 2013)</p>	<p>50 participants with PD</p>	<p>Computer-Based (Individually Tailored)</p>	<p>□ 1 hour: Psychoeducation,</p>	<p>2 hour group sessions</p>	<p><i>Primary outcome measure:</i></p> <ul style="list-style-type: none"> Logical Memory subtest of the Wechsler Memory Scale-III (immediate recall and memory retention) 	<p>CT was associated with improvements in learning and memory, as measured by the Logical memory test. There were no differences in secondary outcome measures or knowledge between the groups.</p>

	<p><i>CT group = 35</i> (Mean age: 68.5, S.D.: 7.1; 26F, 35M)</p> <p><i>Waitlist control group = 15</i> (Mean age: 64.9, S.D.: 6.5; 10F, 15M)</p>	<p>Neuropsychological Education Approach to Remediation (NEAR) Program</p> <p>Integrated +/- Psychoeducation</p>	<p>modified for PD (see (Naismith et al., 2011))</p> <p>□ 1 hour: CT using NEAR. individualised computer-based training program based upon their neuropsychological test results. COGPAK</p>	<p>2x/week for 7 weeks (14 sessions in total)</p>	<p><i>Secondary outcome measure:</i></p> <ul style="list-style-type: none"> psychomotor speed and mental flexibility (TMT-A and TMT-B) and verbal fluency (COWAT) <p><i>Knowledge:</i></p> <ul style="list-style-type: none"> 20-item MCQ test based on psychoeducation sessions 	
Biundo et al. (2015)	<p>24 participants with PD (16 completed follow-up at 16 weeks)</p> <p><i>CT + real tDCS = 12</i> (7 completed) (Mean age: 69.1, S.D: 7.6; 1F, 6M)</p> <p><i>CT + sham tDCS = 12</i> (9 completed) (Mean age: 72.3, S.D: 4.1; 1F, 8M)</p>	<p>Computer-based</p> <p>RehaCom software (includes Adapted specialized keyboard)</p> <p>Integrated with tDCS</p>	<p>RehaCom</p> <p>Non-invasive tDCS of the left dorsolateral prefrontal cortex (direct current = 2mA, 20min/session)</p>	<p>30 minute sessions: 4x/week for 4 weeks</p> <p><i>Follow-up:</i> 16 weeks post-intervention</p>	<p>Repeatable Battery Assessment of Neuropsychological Status (RBANS)</p> <ul style="list-style-type: none"> <i>Cognition:</i> MMSE <i>Executive Function/Attention:</i> Digit Span/Written Coding <i>Memory:</i> Immediate memory Index, Story learning test (delayed) <i>Visuospatial Function:</i> RBANS VS index <i>Language:</i> RBANS Language Index <i>Quality of Life:</i> PDQ8 <i>Depression:</i> BDI-II <i>Anxiety:</i> STAI-Y 	<p>Immediately following the intervention, there was a significant decrement in performance in the real tDCS group compared to the sham t-DCS group in attention/executive skills (Written coding test). At the follow-up in Week 16, there was a trend for better performance in the real t-DCS group in the story learning test and the immediate memory test.</p> <p>Reported decline in executive skills and improved attention and memory</p>
Lawrence et al. (2018)	<p>42 participants with PD+MCI</p> <p><i>Standard CT (5) = (3M;2F, Mean Age</i></p>	<p>Computer-based</p> <p>Smartbrain Pro</p> <p>Integrated with tDCS</p>	<p>Smartbrain Pro CT</p> <ul style="list-style-type: none"> Target's attention, working memory, psychomotor speed, executive function, visuospatial ability Implemented as 'standard' and 	<p>45 Minutes: CT= 3x weekly/ 4 weeks</p> <p>tDCS = 20min sessions, 1Xweek / 4 weeks</p>	<p><i>Global Cognition</i></p> <ul style="list-style-type: none"> MMSE Parkinson's Disease Cognitive Rating Scale <p><i>Executive Function</i></p> <ul style="list-style-type: none"> Stockings of Cambridge (SOC) test Controlled Oral Word Association Test (COWAT) <p><i>Attention</i></p>	<p>Compared to baseline, participants who underwent standard CT improved in ADL and QoL immediately post-intervention; however, this was not maintained at follow-up. Overall, at follow-up, participants improved in Verbal Memory and Visuo-spatial ability. In contrast, participants who underwent tailored CT improved in QoL and this was maintained at follow-up. Additionally, at follow-up, the tailored CT group improved in Attention (as measured by LNS).</p>

	<p>68.14; SD 8.69) <i>Tailored CT</i> (6) = (4M;2F, Mean Age 65.57; SD 5.2) <i>tDCS</i> (7) = (5M; 2F, Mean Age 72; SD 6.45) <i>Standard CT + tDCS</i> (7) = (5M; 2F, Mean Age 63.57; SD 15.68) <i>Tailored CT + tDCS</i> (7) = (5M; 2F, Mean Age 67.43; SD 6.37) <i>Control</i> (6) = (4M; 2F, Mean Age 72.29; SD 6.21)</p>		<p>‘tailored’ based on baseline testing</p> <p>Control group received no intervention</p>	<p><i>Follow-up:</i> 5 weeks (Intervention concluded in week 7; follow-up was at week 12)</p>	<ul style="list-style-type: none"> • Letter number sequencing test (LNS) • Stroop Task <p><i>Verbal Memory</i></p> <ul style="list-style-type: none"> • Hopkins Verbal Learning Test-Revised • Paragraph Recall Test <p><i>Language</i></p> <ul style="list-style-type: none"> • Boston Naming Test • Similarities Test <p><i>Visuo-spatial ability</i></p> <ul style="list-style-type: none"> • JLO test • Hooper Visual Organisation Test (HVOT) <p><i>Activities of Daily Living</i></p> <ul style="list-style-type: none"> • UPDRS Part II <p><i>Quality of Life</i></p> <ul style="list-style-type: none"> • PDQ-39 	<p>The combination of standard CT + tDCS saw improvements in executive function, language and ADLs beyond those observed with just tDCS immediately post-intervention; however, only improvements in executive function were maintained at follow-up.</p> <p>Conversely, the combination of tailored CT + tDCS saw improvements in executive function, verbal memory and language beyond those observed with just tDCS. At follow-up, improvements were observed in executive function, attention, verbal memory and language.</p> <p>Overall, the control group did not improve in any outcomes measured.</p>
<p>Bernini et al. (2019)</p>	<p>41 participants with PD+MCI</p> <p><i>G1 – Physical rehab + CT with CoRE</i> (17) = (7M;16F, Mean age 71.18; SD 7.04)</p>	<p>Computer-based CoRe System</p> <p>Integrated with physical rehabilitation</p>	<ul style="list-style-type: none"> • CT group = Computer-based logical-executive task (CoRe) <ul style="list-style-type: none"> ○ Find the category ○ Find the Intruder ○ Unscramble the Images ○ Image and Sound ○ Word Coupling ○ Logical Sequences ○ Logical analogies ○ Find the Elements ○ Functional Planning 	<p>45 minutes: 3x week / 4 weeks (12 sessions total)</p> <p><i>Baseline – T0</i> <i>Post-intervention – T1</i> <i>6-month follow up – T2</i></p>	<p><i>Primary Outcome - Global Cognitive Function</i></p> <ul style="list-style-type: none"> • MMSE • MoCA <p><i>Memory</i></p> <ul style="list-style-type: none"> • Verbal Span (Verbal Span, Digit Span) • Spatial Span (Corsi’s block-tapping test (CBTT)) • Verbal Long-term memory <ul style="list-style-type: none"> ○ (Logical Memory Test immediate and delayed recall ○ (Rey’s 15-word test immediate and delayed recall • Spatial long-term memory (Rey complex figure delayed recall – RCF-dr) 	<p>After intervention, compared to baseline (T0-T1) G1 showed significant improvements in MoCA, Verbal LTM (Reys 15 + LMT-IDR), Categorical abstract reasoning and Phonological Fluency. G2 showed no significant changes. Compared to controls, G1 performed better than G2 on MoCA, Verbal LTM, categorical abstract reasoning, simple speed processing and complex attention(A), non-verbal reasoning and selective attention/susceptibility. Both groups showed motor improvements.</p> <p>At 6-month follow up (T1-T2), G1 showing significant worsening on MoCA and phonological fluency G2 showed worsening on MoCA and FAS but also MMSE and frontal functionality. Accordingly, no significant improvements were maintained at 6-month follow-up.</p>

	<i>G2 – Physical Rehab control group = 18 (11M;7F, mean age 69.33; SD 7.72)</i>		<ul style="list-style-type: none"> Control group = standard physical rehabilitation involving warm-up, active and passive exercises to improve joints' range of motion, stretching of abdomen, strengthening, postural, balance and control exercises 		<p><i>Logical-executive functions</i></p> <ul style="list-style-type: none"> Non-verbal reasoning (Raven's Matrices 1947 – RM47) Categorical abstract reasoning (Weigl's sorting test) Frontal functionality (Frontal Assessment Battery (FAB)) Semantic Fluency (animals, fruits, car brands) Phonological fluency (FAS) <p><i>Attention</i></p> <ul style="list-style-type: none"> Visual selective attention (Attentive Matrices) Simple speed processing and complex attention (Trail Making Test part A and B – TMT) Selective Attention/susceptibility to interference (Stroop test) <p><i>Visuospatial abilities</i></p> <ul style="list-style-type: none"> Rey complex figure copy 9RCF-copy) <p><i>Functional Status</i></p> <ul style="list-style-type: none"> Activities of Daily Living + Instrumental (IADL) <p><i>Mood</i></p> <ul style="list-style-type: none"> Beck Depression Inventory <p><i>QoL</i></p> <ul style="list-style-type: none"> PDQ-8 	Overall (T0-T2), compared to baseline, G1 showed improvements in MoCA, delayed and immediate recall and Weigl's test for executive function, whilst control groups significantly deteriorated over-time in MMSE, MoCA and FAB. Compared to controls, 6-months post intervention CT appeared to significantly improve in global cognition, verbal LTM, executive function and attention. No significant changes in mood or QoL at were observed.
Adaptive/Assistive Technology						
Pompeu et al. (2012)	<p>32 participants with PD</p> <p><i>CT group = 16 (Mean age: 68.6, S.D.: 8.0)</i></p> <p><i>Control group = 16 (Mean age: 66.2, S.D.: 8.3)</i></p>	<p>Computer-based</p> <p>Wii Fit + Global Exercises (Adaptive technology)</p> <p>Cognitive demands of the games included attention, working memory and performance management.</p>	<p>CT Group=</p> <ul style="list-style-type: none"> □ 30 minutes of global exercises □ 30 minutes of playing 10 Wii Fit Games (5 per session, 2 trials per game) <p>The control group received balance exercise therapy with exercises requiring the same movements and time required by each game.</p>	60 minute sessions: 2x/ week for 7 weeks (14 sessions total)	<p><i>Primary outcome:</i></p> <ul style="list-style-type: none"> independent performance of activities of daily living (UPDRS-II) Montreal Cognitive Assessment (MoCA) 	Both groups demonstrated improvements in UPDRS-II and cognitive function. There were no statistically significant differences between the two groups.

<p>(Cerasa et al., 2014)</p>	<p>15 participants with PD</p> <p><i>CT group</i> = 8 (Mean age: 61.1, S.D.: 12.4)</p> <p><i>Control group</i> = 7 (Mean age: 58.3, S.D.: 9.6)</p>	<p>Computer-based</p> <p>Rehacom Software (Adaptive keyboard)</p> <p>Training attention and information processing tasks</p>	<p>Rehacom as in (Cerasa et al., 2013)</p> <p>Control group performed a simple visuomotor coordination tapping task</p>	<p>60 minutes sessions: 2x/week for 6 weeks (12 sessions total)</p>	<p><i>Spatial memory:</i> ROCFT <i>Verbal memory:</i> Selective reminding test (SRT) <i>Visuospatial processing:</i> Judgment Line Orientation Test <i>Verbal fluency:</i> Controlled Oral Word Association Test <i>Sustained attention and information processing:</i> Symbol digit modality test and PASAT <i>Executive functions:</i> Digit span forward/backward, Stroop word-color task and Trail Making Test (TMT) A and B <i>Mood:</i> Beck II, STAI-Y <i>Quality of Life:</i> PDQ39 <i>General Cognition:</i> MMSE</p>	<p>CT group showed improved cognitive performance compared to the control group on a measure of attention (Symbol-digit modality test) and executive function (digit span forward).</p> <p>These improvements were associated with significantly increased intrinsic functional activity in the left dorsolateral prefrontal cortex within the left central executive resting state network (RSN) and in the left superior parietal lobule within the attention RSN.</p>
<p>(Zimmerman et al., 2014)</p>	<p>39 participants with PD</p> <p><i>CT</i> (CogniPlus)= 19 (Mean age, 69.9, S.D.: 6.3; 68% male)</p> <p><i>Control</i> = 20 (Mean age: 66.3, S.D.: 66.3; 60% male)</p>	<p>Computer-based</p> <p>CogniPlus Software</p> <p>+ Wii (Adaptive technology)</p>	<p>CogniPlus = 4 modules in a fixed order for 10 min/module:</p> <ul style="list-style-type: none"> • FOCUS (attention) • NBACK (working memory) • PLAND (planning and action) • HIBIT (response inhibition) <p>Control – Game Console = 4 sports games from Wii Sports Resort: Table Tennis, Swordplay, Archery, and Air Sports Both adapt difficulty to performance</p>	<p>40 Minute sessions: 3x/week for 4 weeks (12 sessions total)</p>	<p><i>Attention and Working Memory:</i> Tests of Attentional Performance (alertness and working memory) <i>Executive function:</i> Trail Making test (B/A) <i>Visuoconstruction:</i> Block-Design test <i>Episodic memory:</i> California Verbal Learning test</p>	<p>Following intervention, individuals in the Wii group scored more highly for tests of attention than the CogniPlus group. There were also trends towards improvement in the Wii group in visuoconstruction and episodic memory.</p>

Maggio et al. (2018)	<p>20 participants with PD+MCI</p> <p><i>Experimental CT group</i> (10) = (6M; 4F, Mean Age 69.9; SD 6.3)</p> <p><i>Control Group</i> (10) = (4M; 6F, Mean Age 68.9; SD 8.2)</p>	<p>Computer-based</p> <p>BTS-Nirvana</p> <p>Virtual Reality (Adaptive technology)</p>	<ul style="list-style-type: none"> • CT Group = Semi-immersive Virtual-reality training with BTS-Nirvana • Control Group = traditional CT with face-face interaction and paper-and-pencil activities 	<p>60 minutes: 3 X week / 8 weeks (24 sessions total)</p>	<p><i>Global Cognition</i></p> <ul style="list-style-type: none"> • MMSE • Addenbrooke Cognitive Examination-Revised (ACE-R) for detecting mild CI in attention, orientation, visual-spatial cognition, language and fluency and memory <p><i>Executive Function</i></p> <ul style="list-style-type: none"> • Weigl test • Frontal Assessment Battery (FAB) <p><i>Anxiety</i></p> <ul style="list-style-type: none"> • Hamilton Rating Scale – Anxiety (HRS-A) <p><i>Depression</i></p> <p>Geriatric Depression Scale (GDS)</p>	<p>Compared to baseline, VR CT group improved in measures of cognition, executive function, attention and orientation, memory, Verbal Fluency, Language and Visual-Spatial ability, with control group only improving in 1 measure of executive function, global cognition (ACE-R) and visuo-spatial ability (ACR-VS)</p> <p>Compared to controls, VR CT demonstrated improvements in global cognition, executive function and visuo-spatial abilities, with the control group demonstrating significant deterioration over-time. No significant differences in mood (GDS and HRS-a) were observed.</p>
Van De Weijer et al. (2020)	<p>41 participants with PD+MCI</p> <p><i>CT</i> (21) = (Mean age, 64.65; SD 7.4)</p> <p><i>Waitlist Control</i> (20) = (Mean age, 64.01; SD 7.41)</p>	<p>Computer-based</p> <p>Parkin' Play (AquaSnap Program)</p> <p>Gamification (Adaptive technology)</p>	<p>Parkin'Play (AquaSnap) = an adaptive online CT gaming platform targeting attention, working memory episodic memory, psychomotor speed and executive function</p>	<p>30 Minute sessions, 3X week for 12 weeks (recommended agenda – not fixed schedule)</p> <p>Voluntary gameplay weeks 12-24</p> <p><i>Follow-up:</i> 12 weeks</p>	<p><i>Global Cognition (not reported individually)</i></p> <ul style="list-style-type: none"> • Stroop Colour and Word Test • Category fluency and Letter Fluency • Rey Auditory Verbal Learning Test • Location Learning Test • Judgement of Line Orientation • Rey-Osterrieth Complex Figure • Boston Naming Test <p>MyCQ</p>	<p>Primary objective was to study feasibility of intervention, reporting strong accessibility and motivation for the program, with compliance and technical smoothness to be improved.</p> <p>Compared to waitlist controls, the CT group improved in global cognition after 24 weeks of training, however, this was not maintained at 12 weeks follow up</p>

Table 6.2 Summary of Cognitive training in Parkinson's disease study outcomes

Study	PD Status	Cognitive Outcomes												Secondary Outcomes				FU
		GI Cog	EF	Att	ProSp	Learn	Mem	Verb Flu	Verb Mem	VS Mem	VS Abil	Lang	ToM	ADL/ Func	QoL	Dep	Anx	
Sinforiani <i>et al.</i> 2004	PD(+/-MCI)	Red	Diagonal	Red				Diagonal	Diagonal									Y ⁽⁶⁾
Sammer <i>et al.</i> 2006	PD (-CI)/2		Teal	Red												Red		N
Mohlman <i>et al.</i> 2011	PD (-CI)		Yellow	Yellow														N
Nombela <i>et al.</i> 2011	PD (+/-MCI/D)/2 + HC			Teal														N
Paris <i>et al.</i> 2011	PD (+/-MCI)/2	Red	Teal	Teal	Teal			Teal		Teal	Teal			Red	Red			N
Pompeu <i>et al.</i> 2012	PD (-D)/2	Diagonal												Diagonal				Y ⁽²⁾
Reuter <i>et al.</i> 2012	PD (+MCI)/3	Diagonal	Diagonal		Diagonal													Y ⁽⁶⁾
Edwards <i>et al.</i> 2013	PD (+/-MCI,-D)/2	Red			Teal											Red		N
Naismith <i>et al.</i> 2013	PD (+/-MCI,-D)/2		Red		Red	Teal	Teal	Red								Red		N
Cerasa <i>et al.</i> 2014	PD (+MCI)/2		Teal	Teal				Red		Red	Red				Red	Red		N
Costa <i>et al.</i> 2014	PD (+MCI)/2 + HC		Teal				Teal	Teal										N
Pena <i>et al.</i> 2014	PD (+/-MCI,-D)/2		Red		Teal				Red	Teal			Teal	Teal		Red		Y ⁽¹⁸⁾
Zimmerman <i>et al.</i> 2014	PD (-CI)/2			Yellow			Yellow				Yellow							N
Petrelli <i>et al.</i> 2014/15	PD (+/-MCI,-D)/3	Dark Blue	Diagonal	Red				Red	Teal	Red	Red				Red			Y ⁽¹²⁾
Biundo <i>et al.</i> 2015	PD (-MCI)/	Red	Red	Red			Dark Blue			Red	Red	Red	Red	Red	Red	Red	Red	Y ⁽⁴⁾
Diez-Cirarda <i>et al.</i> 2018	PD (+/-MCI,-D)/2		Diagonal		Red				Yellow	Diagonal			Yellow	Yellow				NA
Alloni <i>et al.</i> 2018	PD (+MCI)/2	Teal	Yellow	Yellow					Yellow	Yellow	Red							Y ⁽⁶⁾
Folkerts <i>et al.</i> 2018	PD (-D)/2	Dark Blue												Red	Red	Dark Blue		Y ^(6W)
Lawrence <i>et al.</i> 2018	PD (+MCI)/6	Red	Diagonal	Dark Blue					Dark Blue		Dark Blue	Diagonal	Diagonal	Red	Red	Diagonal		Y ⁽³⁾
Maggio <i>et al.</i> 2018	PD (+MCI)/2	Diagonal	Diagonal	Yellow			Yellow	Yellow		Teal	Yellow					Red	Red	N
Bernini <i>et al.</i> 2019	PD (-MCI)/2	Diagonal	Diagonal	Teal					Diagonal		Red			Red	Red	Red		Y ⁽⁶⁾
Fellman <i>et al.</i> 2020	PD (+/- MCI, -D)/2 + HC		Red	Red			Teal		Red							Teal		N
Vlagsma <i>et al.</i> 2020	PD (+/- MCI,, -D)/2 + HC		Diagonal	Red					Red					Red	Red			Y ⁽³⁻⁵⁾
van de Weijer <i>et al.</i> 2020	PD + MCI, -D/ 2	Teal																Y ⁽³⁾

Gl Cog = Global Cognition, EF = Executive Function, Att = Attention, Pro Sp = Processing Speed, Learn = Learning, Mem = Memory, Verb Flu = Verbal Fluency, Verb Mem = Verbal Memory, VS Abil = Visuospatial ability, Lang = Language, ToM = Theory of Mind, ADL =Activities of Daily Living/Function, QoL = Quality of Life, Dep = Depression, Anx = Anxiety, FU = Follow-up period (months) unless stated otherwise.

MCI = Mild Cognitive Impairment, CI = Cognitive Impairment, D = Dementia, HC = Healthy Controls. +/- indicates inclusion of participants with and without MCI/CI, - = exclusion of MCI/CI and/or D. + = specific inclusion of MCI and/or D. Categorisation of MCI/CI/D are based on varied criteria used by each study.

Yellow = Improvements compared to baseline (if no control improvements), Green = Improvements compared to PD control group, Red = No Improvements, Navy Blue= Improvements/maintenance at follow-up. Navy Stripe. = Improvements/maintenance at follow-up. Several cognitive outcomes may have more than one measure, outcome is reported based on improvement in at least 1 test.

6.4 CONSIDERATION 1: METHOD OF DELIVERY

6.4.1 *Pen and paper-based CT*

Due to the heterogenous and debilitating nature of motor impairments in PD, method of CT administration is an important factor to consider when designing and evaluating the efficacy of any cognitive rehabilitation program and, as such, addressing these considerations is particularly pertinent. Of the studies considered in this review, 2 specified the exclusive use of ‘pen and paper’ for the administration of CT. An active control group trial conducted by Pena and colleagues in 2014 utilised a structured program of paper-pencil tasks in the form of a previously validated exercise book called REHACOP (Pena et al., 2014, Sanchez et al., 2014). Made up of 300 tasks administered by psychologists in a group setting, REHACOP was originally designed for schizophrenia and adapted to elderly populations to train attention, memory, processing speed, language, executive functioning, social cognition and functional ADL. Tasks from REHACOP were administered for 13 weeks in 60-minute group training sessions 3x/week. Improvements were reported in processing speed, visual memory and theory of mind, and also appeared to generalise to everyday tasks, with significant improvement on a self-administered measure of functional disability. No improvements were noted in either measures of executive function or verbal learning/memory (Pena et al., 2014).

More recently, a 2018 follow-up study investigated the long-term effects of REHACOP at 18 months post-intervention, finding improved performance in verbal memory, visual memory and decreased functional disability were maintained at follow-up, in addition to the appearance of previously unseen improvements in executive function (Díez-Cirarda et al., 2018). Interestingly, these improvements were observed despite significant grey matter volume loss and reductions in

frontal activity, as well as significant deterioration in Unified Parkinson's Disease Rating Scale (UPDRS) III (motor score), indicating progression of disease. It is important to note, however, that these changes were compared to baseline, with no control group at follow-up for comparison. The initial 2014 study reported a 4.2% attrition rate, with positive comments collected in an unpublished focus group, indicating satisfaction with the program. Such program enjoyment is an important factor to consider in assessing the feasibility of such programs, as also highlighted by another pen-and-paper based study in this area (Mohlman et al., 2011).

Similar to the intervention using REHACOP, Mohlman and colleagues (2011) also drew upon the adaptation of a previously developed program, administering CT using an adapted version of the Attention Process Training II (APT-II), which targets attention (Mohlman et al., 2011). In particular, they investigated the feasibility and acceptance of the regime, which utilized audio CDs and written worksheets to administer and evaluate the program in both a clinic-based and home-based setting. In addition to improvements in executive function, attention and verbal fluency, researchers determined a high degree of acceptance and successful engagement in the program. In particular, self-rating of progress was positively related to post-training improvement. The study, however, excluded participants with cognitive impairment, defined by a score of <24 score on the MMSE and degree of motor impairment was not taken into consideration. This is particularly pertinent, as the study reported correlation of effort with MMSE scores, indicating those with better cognitive ability are more motivated to engage in the program. Subsequently, by excluding participants with cognitive impairment, this may be biasing the sample towards a population who are already highly motivated. Furthermore, the study reported a 14% attrition rate, with data from these participants not included in assessment of feasibility, resulting in a potentially positive skew of attitudes towards the program. Irrespective of this, these findings highlight the

importance of a patient's confidence in CT programs, with perceived progress an important predictor of motivation and subsequent success, a factor that should be taken into consideration when assessing the efficacy of different CT intervention strategies.

6.4.2 Computer-based CT

In terms of studies utilising a solely computer-based regime, Sinforiani and colleagues conducted the initial pilot study of CT in PD in 2004 (Sinforiani et al., 2004): 20 PD patients with MCI underwent 12 × 1-hour sessions of computer-based CT (TNP software) over six weeks. This regime delivered individualised exercises targeting attention, abstract reasoning and visuospatial function. At the end of the 12 sessions, participants performed significantly better on neuropsychological tests for verbal fluency, verbal memory and executive function, maintaining performance at a 6-month follow-up (Sinforiani et al., 2004). However, no improvements were observed on measures of global cognition or attention. Whilst participants reported increased self-confidence, the CT program was also combined with a motor rehabilitation regime, which may have influenced these results; additionally, there was no control group. Positively, researchers reported no attrition and commented on the employment of a mouse to ameliorate the need for fine motor activity; however, this was not directly assessed or compared. Together, these limitations make it difficult to assess the full extent of these benefits and to attribute them directly to employment of a computer-based CT program.

A number of studies have since implemented various CT programs utilising cognitive rehabilitation software previously validated in other neurological disorders, such as dementia and stroke, including Cognitive Rehabilitation System (CoRe) (Alloni et al., 2018, Bernini et al.,

2019), NEUROvitalis (Petrelli et al., 2014, Petrelli et al., 2015), Cogniplus (Vlagsma et al., 2020, Zimmermann et al., 2014) and Strategic Executive Training (ReSET) (Vlagsma et al., 2020).

The CoRE system computer-based CT program specifically targets executive function through a battery of 8 activities. A study by Alloni and colleagues (2018) assessed the efficacy in a cohort of PD patients with mild executive and/or cognitive impairment. Results were promising, revealing significant improvements in 12 out of the 21 assessments, including verbal and spatial memory, executive function, attention and global cognition, following intervention (Alloni et al., 2018). Unfortunately, a study assessing long-term benefits at 6-month follow-up noted improvements in global cognition and attention were no longer as pronounced, with significant worsening compared to immediate post-test; however, overall improvements were still maintained in measures of executive function and attention when compared to baseline. Furthermore, it is interesting to note that the losses were not as extensive as those observed in the control group, indicating preservation of function which may otherwise deteriorate with disease progression. The CoRe system underwent a usability assessment in healthy volunteers, demonstrating an overall positive score. Subsequently, this was repeated in a small cohort of PD patients (n=6), where it was reported subjects were more entertained and involved in tasks including visual-stimuli, which led to the adaptation of exercises to include 3D graphics to allow for more complex interaction and improve engagement (Alloni et al., 2014).

Another CT program validated in a comparable population is NEUROvitalis. Originally developed for training in older populations with mild mental impairments, a NEUROvitalis ‘structured’ program was adapted by Petrelli and colleagues (2014) to specifically target attention, memory and executive function, domains preferentially affected in PD (Petrelli et al., 2014) This study compared the efficacy of this ‘structured’ approach (NEUROvitalis+ psychoeducation

program) to an unstructured ‘Mentally Fit (MF)’ program. 65 PD patients were randomized either to one of the NEUROvitalis or MF groups and underwent 12 × 90-minute group sessions over 6 weeks, or to a control group, receiving no contact. Immediately following intervention, both the MF and NEUROvitalis CT group improved in short-term, and WM compared to controls, with an increase in WM significantly greater with NEUROvitalis than MF (Petrelli et al., 2014). Interestingly, a significant decrease in depression was only noted in the MF group, an effect attributed to the social interactions associated with the strategy. At one-year post-intervention, on an assessment of overall cognitive function, 56.3% of the NEUROvitalis group and 41.3% of the MF group retained or improved their cognitive performance, compared to only 21.4% of the control (Petrelli et al., 2015). Excitingly, in both intervention groups, the chance of developing MCI (18.2% each) was lower than that of the control group (40.0%) (Petrelli et al., 2015). These results indicate CT could help prevent conversion to MCI in PD; however, the benefits of a specifically structured program designed to target domains affected in PD did not appear to be superior to the non-domain specific unstructured intervention.

A 2018 study went on to assess the efficacy of the NEUROvitalis program in a cohort of PD-D patients using a modified version of the program adapted for patients with dementia living in a nursing home and designed to target executive and visuo-spatial function (Folkerts et al., 2018). Results are preliminary due to the small sample size ($n = 12$) and failure to reach statistical significance ($p = 0.067$, $r = 0.43$); however, the strong effect size indicated the intervention may have been beneficial for cognition, although this was not maintained at a 6-week follow-up. Unfortunately, specific cognitive domains were not probed individually. In terms of supplementary outcomes, PD-D participants demonstrated potential improvements in both depression and ADL compared to baseline (Folkerts et al., 2018). This is in contrast to the earlier study in PD-MCI

participants, possibly due to a ceiling effect, with milder cognitive impairments associated with reduced depression and impairments of ADL (Petrelli et al., 2014). This suggests adapting CT programs for their specific population of interest, as was done for the PD-D cohort, may help to optimise improvement and improve transferability to real-life applications.

Finally, Cogniplus is another computerised CT program shown to improve both attention and executive function in patients with MCI and schizophrenia; however, to date, its assessment in PD has been limited to use as a control intervention (Vlagsma et al., 2020, Zimmermann et al., 2014). Interestingly, Cogniplus was used as a cognitive-specific control in a study assessing the efficacy of a non-cognitive-specific, physically demanding, interactive video game (Wii Sports), which proved as, if not more, effective than Cogniplus training (Zimmermann et al., 2014). On the other hand, in a recent 2020 study by Vlagsma and colleagues, Cogniplus was utilised as a non-specific control intervention ($n = 16$) for a domain-specific psychoeducational program (ReSET) ($n = 24$) aimed at improving executive function in a cohort of PD patients with executive dysfunction. Significant improvements were observed in measures of both executive functioning and everyday life in both groups following treatment, as well as at 3-5 month follow-up, indicating no significant treatment effects and demonstrating Cogniplus to be as effective as a specifically designed program in improving executive function (Vlagsma et al., 2020).

6.4.3 Combination of pen and paper and computer-based CT

Several CT programs over the last decade have also utilised a combination of ‘pen and paper’ and computer-based delivery. Paris and colleagues (2011) were the first to conduct a study of CT in PD in which a control group received a placebo intervention (Paris et al., 2011). In this study, over the course of 4 weeks, the experimental group ($n=16$) received 12×45 min CT sessions using

interactive multimedia software (SmartBrain tool), as well as weekly paper and pencil-based homework exercises and a weekly tutoring session. The control group (n=12) received speed therapy. Following the intervention, the CT group significantly improved in several cognitive domains (See Table 6.2 for summary outcomes) (Paris et al., 2011). However, there were no significant improvements in ADL or self-rated QoL (Paris et al., 2011), suggesting that, while CT may be beneficial for improvements on specific neuropsychological tests, these skills may not generalize to improvements in everyday functioning. Similarly, in a study investigating the effects of CT specifically targeting prospective memory (PM), Costa and colleagues (2014) utilized a combination of pen and paper and computer-based tests, finding the intervention improved performance on measures of executive function; however, generalizability was not assessed across other domains. It is difficult to comment on the acceptability of combined programs in the PD population from these studies alone; however, their investigations suggest the need for future studies utilizing this combination of techniques.

Overall, it is difficult to compare the efficacy of these different methods of administration interventions based on their cognitive outcomes alone, due to the significant variability in sample selection criteria, demographics, outcome measures and study duration. Furthermore, participants with dementia and those with severe motor impairments were excluded from the majority of studies above, making it difficult to interpret the role that method of administration may have on outcomes for these individuals. To date, there are also no studies which have directly assessed pen and paper vs computerized CT programs. However, there are several factors that may speak to the potential advantages of computer-based CT. In particular, with deterioration of handwriting (i.e. micrographia) considered a diagnostic sign of PD, pen and paper methodology carries a specific

requirement of manual dexterity, which may be inherently difficult for PD patients who are severely motor impaired, thus likely to affect participation and outcomes for the population (Thomas et al., 2017). Additionally, significant advancements in technology have enabled the use of a variety of different modalities, including touch-screen, mobile-adapted, virtual reality and even interactive gaming. In addition to advantages for researchers in ease of delivery and analysis, the potential advantages of such technology-based interventions for patients include the ability to easily tailor interventions based on the individual's needs, improved accessibility and interactivity, and the ability to modify, update and provide real-time feedback (Lampit et al., 2014). These may also assist in reducing fatigue, maintaining engagement and improving interaction with the program. This is significant, given evidence that enjoyment of the CT intervention may drive more beneficial outcomes (Mohlman et al., 2011).

In support of this, a recent systematic review of the use of CT for individuals with mild cognitive impairment concluded technology-based interventions demonstrated better effects than traditional 'pen and paper' CT programs in improving function and QoL (Ge et al., 2018). Taken together, there is evidence to suggest CT may be a promising avenue for the non-pharmacological treatment of cognitive impairment in PD. In particular, computerised implementation represents a cost-effective and adaptable option and appears to now be the predominant approach.

6.5 CONSIDERATION 2: STANDARD VS TAILORED DELIVERY

The NEUROvitalis 2014 and 2018 studies assessed the efficacy of a CT program tailored to target domains dominant in cognitive dysfunction in PD and then further adapted these for specific sub-populations (PD-MCI and PD-D) (Petrelli et al., 2014, Folkerts et al., 2018). By tailoring CT

delivery and refining investigations to appropriate outcome measures, this may improve the reliability of outcomes, which may otherwise be prone to ceiling effects in milder-PD populations.

Beyond this, a growing body of research has begun to investigate the potential to tailor CT programs not just to the specific population, but to the needs of the individual. The facilitation of tailored CT has been made much less resource intensive through the use of computer technology, which is able to assess impairments whilst simultaneously adapting the difficulty level and delivery of an otherwise-standard CT program, in order to target the deficits reflected by the individual. Studies discussed above (Alloni et al., 2018, Díez-Cirarda et al., 2018, Mohlman et al., 2011, Paris et al., 2011, Pena et al., 2014, Petrelli et al., 2014, Petrelli et al., 2015, Sinforiani et al., 2004) have all utilised a standard CT program. On the other hand, Naismith and colleagues (2013) used Neuropsychological Education Approach to Remediation (NEAR) to implement an individually tailored CT regime. NEAR was originally developed to address cognitive impairment in psychiatric disorders, targeting learning as its core domain, and includes an extensive software library of activities (Medalia and Freilich, 2008). Naismith and colleagues (2013) assessed the efficacy of NEAR in a cohort of PD patients with and without cognitive impairment (n=35 intervention, n=15 wait-list control). Delivery included 2 × 2-hour sessions per week over 7 weeks, paired with psychoeducation sessions, with waitlist control participants waiting 7-weeks before participation. Due to the online delivery platform, CT exercises were easily able to be tailored to the individual participant's needs based on baseline testing (Naismith et al., 2013). Acceptability was high, with a low attrition rate of 4%, and significant improvements were noted in primary outcome measures of learning and memory; however, no changes were observed in psychomotor speed, executive function or depression, with no follow up to assess long-term benefits. This may again be due to the relatively mild cognitive impairment observed in the PD sample, representing

a ceiling effect. Similarly, a study by Cerasa and colleagues (2014) also utilized a computer-based CT program (see Table 1 for details) targeting attention and information processing, which was tailored to individuals' pretraining cognitive impairment(s). The intervention group demonstrated improvements in attention, which were also associated with increased fMRI activity in areas essential in executive function, providing additional support for a tailored approach (Cerasa et al., 2014).

Lawrence and colleagues (2018) were the first group to directly investigate the benefits of a standard vs tailored CT program in a PD population. PD participants (n=7 per group) with diagnosed MCI received either computer-based training at home via Smartbrain Pro for 3 x 45 minutes per week for 4 weeks or a control (no intervention). CT was tailored based on baseline impairments, with standard training leading to improvements in memory, ADL and QoL, whereas the tailored intervention improved attention/WM and QoL (Lawrence et al., 2018). No improvements were observed with control intervention. This is the first study to report improvements in QoL with CT, with the former Paris et al. (2011) study also utilising the Smartbrain tool finding no significant improvement, potentially due to implementation in a less severely impaired population (Paris et al., 2011). Whilst these results are preliminary given the small sample size and assessment comparison using only one program, they are cautiously indicative of the potential benefits of tailored CT. Additionally, it is worth noting that, although standard CT resulted in improvement in memory, this is a less-impaired domain in PD (Monastero et al., 2018). Improvements in attention/WM exhibited in the tailored program may in fact be more relevant for the PD and PD-MCI population, with a 2018 study identifying attention and executive function impairments affecting 39.5% and 28.5% of individuals, respectively, compared to a prevalence of 21.8% for memory (Monastero et al., 2018).

These results further support the use of computer-based technology, due to the ability to specifically tailor CT programs to the needs of the individual whilst prompting the need for future investigations into tailored CT programs. This may be particularly pertinent for further investigations of tailored CT efficacy on outcomes such as global cognition and depression, where improvements are often potentially overlooked due to the frequent exclusion of participants with moderate-severe CI and clinical depression, resulting in a ceiling effect. Taken together, tailored CT programs prompt a potential benefit in transferability due to their ability to target cognitive dysfunction in domains preferentially affected by the individual. This is perhaps most evident in the improvements observed in QoL, which have been otherwise been largely uninfluenced in standard CT programs.

6.6 CONSIDERATION 3: GROUP-BASED VS HOME-BASED ADMINISTRATION

Another variable which complicates the comparison of overall CT program efficacy are inconsistencies in administration. Many initial CT programs included administration by researchers or clinicians in a group-based clinical-setting; however, although computer-based CT may have improved adaptability and ease of use for PD patients, they have also resulted in an increased number of platforms available online and, as such, implemented in an unsupervised home-setting. This approach has been adopted, in part, as a less resource-intensive and cost effective alternative to lab-based CT (Fellman et al., 2020).

One such study conducted by Edwards and colleagues (2013) assessed the efficacy of a tailored CT program specifically targeting cognitive speed of processing training (SOPT) in a PD population using InSight software. Patients were randomised to receive 20 hours of self-

administered training over 3 months via InSight (n=44), or a control (no-contact, n=43). Results indicated greater performance in visual attention from control participants; however, both groups improved from baseline performance (Edwards et al., 2013). This, however, did not translate to previously reported improvements in secondary outcomes, such as improved cognition or depressive symptoms (Wolinsky et al., 2009). This may be due to the fact that only 69% of the intervention group completing the minimum required training hours. Importantly, a 15% attrition rate was reported in the study, which is higher than the ~4% attrition rate reported in group-based CT studies in this population (Naismith et al., 2013, Pena et al., 2014) indicating that participants may be less motivated to complete the program when it is self-directed, as opposed to administered in a more guided and supportive manner.

A more recent study utilising a home-based CT program was conducted by Fellman and colleagues (2020) to investigate the efficacy of an online CT program comprised of WM tasks aimed at improving WM and transfer to every-day. The groups received either WM training (intervention) or quiz training (control) in 3 x 30-minute sessions/week for 5 weeks. Unfortunately, the population were relatively well-preserved with regards to WM, with performance comparable to healthy controls, making it difficult to assess benefits of the program. Nevertheless, compared to controls, the intervention group did demonstrate significant gains in two of three WM tasks (Fellman et al., 2020). Despite this, no transfer of benefit was seen in domains such as verbal episodic memory, executive function or attention. Furthermore, although a decrease in depression was observed, post-test self-assessment of WM function and executive function were unaffected, suggesting limited transfer effects to noticeable improvements in everyday life. With regards to acceptability of the program, the study observed a more favourable attrition rate of 8.6% compared to Edwards et al., with otherwise positive feedback and high adherence noted; however, the

minimum number of sessions for inclusion were not specified. Overall, evidence for the efficacy of home-based delivery of CT in the PD population is currently limited, with potentially poorer translation to outcomes related to improved QoL and lower adherence to the CT protocol.

This is consistent with findings in cognitively healthy older adults, with a systematic review of computerised CT in this population concluding that group-based training was significantly more efficacious than home-based training, with home-based ineffective at improving cognitive performance. The authors attributed this to factors such as the ability to directly supervise participants in order to ensure adherence and compliance, to provide motivational support and encouragement and to problem solve IT-issues as they occur, as well as increased social interaction for participants (Lampit et al., 2014). This may be particularly advantageous for individuals with PD, who often experience disrupted social connectedness and social isolation (Soleimani et al., 2014), which is a major predictor of decreased health-related QoL in individuals with PD (Andreadou et al., 2011). Thus, group-based computerised CT may be indicated.

6.7 CONSIDERATION 4: STANDARDISATION OF ASSESSMENT BATTERIES

Another factor affecting whether or not a study may observe improvements with CT is dependent upon the assessment batteries used to evaluate outcomes. This may be best demonstrated by looking at differences in studies which have utilized an extensive assessment battery, with multiple tests used to assess a single domain. Alloni et al. (2018) and Bernini et al. (2019) are two recent examples of such studies. As discussed, Alloni and colleagues implemented a CT program and assessed outcomes using a total of 21 tests to evaluate cognition (2), verbal and spatial memory

(8), executive function (5), attention (4), visuospatial ability (2) (Alloni et al., 2018). Compared to controls, CT resulted in 1/2 of cognition tests, 1/8 of verbal and spatial memory tests, 3/5 of executive function tests, 2/5 of attention tests and 0/2 of visuospatial function tests, with similar variability compared to follow up. A similar pattern was also observed in the Bernini 2019 study (Bernini et al., 2019). Without such extensive post-intervention assessment, improvements in key domains may not have been identified.

The choice of which assessments are used to examine cognitive benefit may also be of critical importance. In support of this, improvements in cognitive function have been observed using the MoCA but not the MMSE (Alloni et al., 2018). Despite this, the MMSE is the most commonly used test to both screen for cognitive impairment in PD and to assess global cognition, as reflected in the majority of studies consulted for this review. This may represent a significant limitation, as the MMSE is considered to be less sensitive to changes and, as such, subtle improvements in cognition may have been missed. This highlights the need for further research to determine the most valid measures of outcome assessment for the relevant cognitive domains in the PD population, in order to inform the development of a standardised assessment battery. Such an effort would also allow for direct comparison of results between studies, which is currently quite complicated due to significant variability in study design and outcome assessment.

6.8 SPECIFIC RECOMMENDATIONS FOR THE DELIVERY OF CT IN THE PD POPULATION

Although the recent Cochrane review did not conclude a benefit of CT for cognitive impairment in PD (Orgeta et al., 2020), several reviews that take into consideration a larger subset of the literature have supported its potential (Díez-Cirarda et al., 2018, Leung et al., 2015). Our findings further support this; however, as discussed, there are several inconsistencies and limitations that limit the ability to directly compare the efficacy of CT programs. Despite this, there are a number of recommendations for future study design in order to improve the utility of CT programs for therapeutic use in PD. In summary, these include:

1. The use of computer-based technology to improve engagement, accessibility and CT delivery.
2. Tailoring of CT programs to suit the cognitive domains predominantly affected in the specific sub-population of PD (PD-MCI/PD-D), in addition to tailoring based on the specific cognitive impairment demonstrated by the individual.
3. Use of group-based (rather than home-based) training, in order to encourage compliance and social interaction.
4. Refinement and standardisation of assessment batteries, including the use of non-cognitive batteries, such as QoL and ADL, in order to better assess real-world transferability.

6.9 THE FUTURE OF CT FOR PD

In addition to the recommendations above, in order to further improve the delivery and efficacy of CT programs, specific consideration should also be given to developing technologies that better adapt the CT platform to the unique needs and physical limitations of the PD population. One way this may be done is via multi-modal techniques, incorporating CT with interventions targeting the neural mechanisms that underlie cognitive function. Evidence in healthy aging supports this potential, with older participants who walked on a treadmill while playing a spatial navigation game demonstrating stability of hippocampal volume over a 4-month training period, whilst volumes in the control population deteriorated (Lövdén et al., 2010). Potential techniques identified that lend themselves to integration include transcranial Direct-Current Stimulation (tDCS) and exercise/aerobic training.

6.9.1 Multimodal delivery: CT + transcranial Direct-Current Stimulation (tDCS)

Non-invasive brain stimulation via transcranial Direct-Current Stimulation (tDCS) may prove an effective technique to pair with CT due to its potential to facilitate neuronal plasticity, amongst other potential mechanisms (Mohammadi, 2016). Initial studies have reported improved cognition following tDCS in PD, including improvements in WM (Boggio et al., 2006) and executive function (Pereira et al., 2013) with tDCS of the dorsolateral prefrontal cortex (DLPFC). In a comparable study by Doruk (2014), results also appear to be maintained up to 1 month following intervention (Doruk et al., 2014). It is theorised tDCS of the prefrontal cortex may enhance declarative and long-term memory consolidation (Javadi et al., 2014). These studies were in cohorts of cognitively healthy PD patients; however, a study combining tDCS with physical rehabilitation (PR) in PD-MCI reported reduction of depressive symptoms, as well as

improvements in motor ability (PR +/- tDCS), cognition and verbal fluency (PR + tDCS only), which were all stable at 3-month follow-up (Manenti et al., 2016). Taken together, these results suggest a promising outlook for tDCS for improving cognitive outcomes in both cognitively healthy PD and PD-MCI patients; however, its efficacy in a PD-D cohort remains to be seen.

Due to these promising results, it is hypothesised that pairing tDCS with CT may synergistically boost the treatment effect of either intervention alone. The first study to do so paired a computer-based program incorporating attention and information processing tasks (RehaCom) with tDCS in a PD-MCI cohort with 30-minute sessions 4 times per week for 4 weeks. Interestingly, initially, a significant decrement in performance on attention and executive tasks was observed with tDCS compared to sham; however, at 16-week follow-up, a strong trend towards improved memory and attention performance was observed with tDCS + CT compared to CT alone, although a decline in executive skills was reported (Biundo et al., 2015). Similarly, in a recent study by Lawrence and colleagues, while no difference between standard or tailored CT was observed, participants receiving both standard or tailored CT in combination with tDCS demonstrated the most substantial benefit overall, with improvements in executive function, attention, working memory and ADL (Lawrence et al., 2018). Taken together, results suggest pairing CT with non-invasive brain stimulation via tDCS, specifically of the left dorsolateral prefrontal cortex, may improve performance compared to CT alone; however, in order to determine the ideal tDCS parameters and specific CT technique for the most effective translation to therapeutic delivery, further investigations are required (Biundo et al., 2015).

6.9.2 Multimodal delivery: CT + exercise

Exercise is a common non-pharmacological intervention for neurodegenerative diseases, particularly PD (Crotty, 2020). Aerobic training is believed to promote neural rearrangement and, as such, may complement and enhance the efficacy of cognitive rehabilitation programs. While the cellular mechanisms via which these neuroplastic effects occur are still unclear, they may involve enhanced neurogenesis/synaptogenesis (Valkanova et al., 2014) or increases in myelination (Song et al., 2005). Physical exercise leads to increased levels of neural growth factors, including BDNF, which is essential for facilitating neurogenesis, cell survival and SP (Gomez-Pinilla et al., 2008). Thus, physical exercise may promote neurogenesis/synaptogenesis and CT may promote the survival of these cells and synapses (Nuechterlein et al., 2016). In support of this, several recent studies have reported beneficial effects when combining CT with aerobic exercise in schizophrenia (Malchow et al., 2015, Nuechterlein et al., 2016, Oertel-Knochel et al., 2014).

In PD, a study by Reuter and colleagues in 2012 adopted an individually tailored multimodal cognitive rehabilitation program. The CT involved a set of well-established ‘pen and paper’ neuropsychological batteries, such as the Behavioural Assessment of Dysexecutive Syndrome (BADS) and Raven’s Progressive Matrices, which target executive and memory functions, as well as computer-based exercises. This was paired with transfer training and transfer+ psychomotor endurance training, in order to investigate the transferability of post-CT cognitive improvements into everyday life (Reuter et al., 2012). In a cohort of PD-MCI participants, immediately following treatment, all groups improved in measures of global cognition and specific domains, such as executive function. Additionally, participants who received CT paired with transfer+ psychomotor endurance training showing the most significant improvements, persisting

for up to 6-months (Reuter et al., 2012). They also reported less PD-specific impairments following intervention, indicating improved QOL. In terms of compliance, participants who received CT combined with psychomotor endurance training were also more likely to continue training at home (90%) compared to those only receiving CT (60%). Thus, a multimodal approach may be superior to ‘pen and paper’ based activities based on both compliance and outcomes, indicating integration of CT with motor training may be a promising future direction.

Another study which explored CT in combination with physical rehabilitation was Bernini and colleagues (2019), who utilised the CoRe system previously discussed. The study combined CoRe with cardiovascular activities and exercises designed to improve range of motion, balance and postural control. In a cohort of PD-MCI participants, CT+physical rehabilitation resulted in improvements in global cognition and executive function compared to baseline, both immediately following intervention and at 6-month follow-up (Bernini et al., 2019). This improvement was also significant compared to controls receiving only physical rehabilitation. The inclusion of physical rehabilitation also led to an improvement in motor performance for both groups. Overall, individuals receiving CT+physical rehabilitation also showed less cognitive decline than those who received physical rehabilitation alone, who displayed a significant worsening of cognitive function over time, signifying a potential delay in cognitive disease progression. Taken together, studies in healthy aging, schizophrenia and PD provide support for the integration of physical rehabilitation with cognitive training to improve outcomes. This is particularly apt for PD patients, given the defining accompaniment of motor dysfunctions observed in the population. Physical rehabilitation and exercise in PD patients is already a well-established non-pharmaceutical intervention for the motor impairments of PD and studies incorporating the two only provide further support for its integration to potentially target cognitive impairments (Reuter et al., 2012,

Bernini et al., 2019). Building from this, whilst a promising future direction, there may be a way to further improve efficacy in PD by incorporating the benefits of motor-training with the physical delivery of CT programs themselves through the use of technology specifically adapted for the PD population.

6.10 THE USE OF ADAPTIVE AND ASSISTIVE TECHNOLOGY TO DELIVER CT IN PD

To date, the vast majority of studies of CT in PD have typically used either a manual ‘pen and paper’ approach or a computer-based approach with standard keyboard and mouse functionality. This may represent a substantial and under-addressed barrier for the successful implementation and assessment of CT in the population. In support of this, a 2010 survey found that nearly 80% of PC-users with PD have significant and severe difficulties using a computer due to their illness (Nes Begnum, 2010). In particular, muscle stiffness, inertia and tremor were frequent problems, resulting in significant-highly severe difficulties using a standard mouse (42%) and keyboard (27%). This represents a significant barrier to the current technical delivery of CT in PD, potentially altering the successful evaluation of outcomes, as well as prospective benefits. Consequently, not only should commercially available CT programs be adapted to address the cognitive dysfunctions specific to PD patients, but technical implementation should also be approached in light of the restrictions imposed by the often-debilitating motor impairments. To address this concern, this may involve the use of currently available technologies for adaptation, or the optimisation of new assistive technologies to aid in delivery.

While still a critically under-researched area, a few studies have begun to look at CT delivery utilizing adapted hardware. For example, Cerasa and colleagues (2014) used a specialised keyboard designed for severe motor impairment, which incorporates large buttons for navigation and selection, in order to deliver the RehaCom software, a program targeting attention and information processing. Over six weeks, non-demented PD patients with attentional deficits underwent either RehaCom CT (n=8) or completed a visuomotor coordination task 2x/week. Improvements were seen in the CT group on measures of attention, which were associated with significantly increased intrinsic functional activity in the left dorsolateral prefrontal cortex within the left central executive resting state network (RSN) and in the left superior parietal lobule within the attention RSN (Cerasa et al., 2014). These brain areas are essential for executive function, particularly WM. Thus, increased activation in these areas could represent a compensatory strategy, allowing for enhanced performance in these cognitive domains. Whilst results appear promising, it is difficult to determine if the specialised keyboard played a part in the improvements observed, beyond what would otherwise be seen with the RehaCom software alone. In fact, another study utilising the RehaCom software to deliver CT in a PD cohort also reported improvements in both memory and attention, although these were less than those observed when paired with tDCS (Biundo et al., 2015). In order to fully assess potential benefits, a comparison of outcomes obtained with the adapted keyboard, compared to traditional mouse/keyboard delivery, is needed.

Another tool that may prove useful for addressing the barriers of CT in PD is the Nintendo Wii™. The Nintendo Wii™ has been proposed as a tool for balance training in the elderly and those with motor impairments (Pessoa et al., 2014). In addition to potential motor improvements, due to the complexity of tasks in already developed Wii™ Fit compatible games, it has been postulated that the platform may improve integration of motor and cognitive abilities in order to

improve ADLs (Pompeu et al., 2012). Subsequently, two studies have used Nintendo Wii™ consoles to deliver CT in PD. In the first of these, the experimental group (n = 16) used a Nintendo Wii™ for one-hour training sessions 2x/week for seven weeks, while the control group (n = 16) received balance exercise therapy (Pompeu et al., 2012). Following intervention, both the CT and the control group demonstrated improvements in cognitive function and ADL from baseline; however, there were no statistically significant differences between the two groups in terms of cognition, indicating comparable outcomes for both the Nintendo Wii™ and balance exercise therapy (Pompeu et al., 2012). Conversely, in another study using the Wii™ console, Zimmermann et al. (2014) randomised PD patients to either a computer program specifically designed to improve cognition (CogniPlus, n = 19) or a Nintendo Wii™ game console (n = 20) (Zimmermann et al., 2014) Participants utilised their respective CT devices for 40 minutes, 3x/week for four weeks. Following intervention, the only statistically significant difference between the groups was that individuals in the Wii™ group scored higher on tests of attention than the CogniPlus group (Zimmermann et al., 2014) indicating commercially available gaming consoles may be as effective as specifically designed computer interventions for attention; however, larger scale studies, long-term follow-up assessments, and comparisons with other CT programs are necessary in order to fully evaluate this.

Incorporating virtual reality (VR) technology into the delivery of CT is another up-and-coming area of research. Several studies have established the efficacy of VR training for the rehabilitation of motor function in PD; however, the effects on cognitive outcomes are not well studied (Cikajlo and Peterlin Potisk, 2019, Mirelman et al., 2013). BTS Nirvana is a VR system that delivers a 3-dimensional multisensory simulation that can be used for interactive training and that has been designed to specifically target executive function, attention and visuospatial skills

(Maggio et al., 2018). Using this system, a cohort of PD-MCI patients participated in 60-minute sessions 3x/week for 8 weeks, with the control group taking part in a traditional pen and paper CT program for the same amount of time. The authors noted a greater improvement in executive and visuospatial abilities in those using the BTS Nirvana system compared to controls, concluding VR may represent an innovative direction to improve cognitive outcomes for PD patients (Maggio et al., 2018). Despite this, given the small sample size and lack of long-term follow up, as well as the lack of supporting studies, it is difficult to draw any definitive conclusions, although this represents an exciting area for future research.

As established previously, compliance and acceptability are important factors when considering the effectiveness of CT programs. VR is a delivery method that may promote engagement due to its immersive nature; however, gamification may be another way in which enhanced interactivity could potentially improve confidence, engagement, and compliance. Whilst several computer-based CT programs incorporate elements of gameplay, such as real-time feedback, they do not necessarily include key features often attributed to traditional video games. Potential benefits of gamification include high-score and reward incentives, personalisation, self-directed challenge, exploration and free-play (Nagle et al., 2015). These are particularly important for the PD population, where patients have a decreased reward sensitivity in an off-dopaminergic medication state, as well as increased apathy (Muhammed et al., 2016). Therefore, enhancement of the rewarding elements of game play may improve perceived self-efficacy, motivation and adherence, subsequently improving outcomes (Van De Weijer et al., 2019). Whilst this area of CT adaptation is in its infancy, one recent 2020 study investigated the efficacy of a gamified CT program called 'Parkin'Play' in a cohort of PD-MCI patients (n=21) against a no-intervention waitlist control (n=20). The program consisted of an online CT game (called 'AquaSnap') that was

required to be played by participants at home for 12 weeks ad hoc, followed by a supplementary voluntary phase (weeks 12-24). In terms of feasibility, the study demonstrated moderate compliance, with an average of 98.3%; however, the compliance rate reduced to 68.3% when sessions included were individually capped at 36. The authors also reported successful accessibility (100%), as well as successful motivation, with 87.5% reporting a positive acceptability score. After 24 weeks of training, the intervention group improved in global cognition compared to controls; however, this was not stable at 12 weeks follow-up (Van De Weijer et al., 2020). Although preliminary, these results suggest a need to further investigate the value of gamification for the implementation of CT in PD. Furthermore, gamification may also be integrated with other multi-modal interventions, such as exercise or assistive technology, to further enhance its potential to target cognitive outcomes in PD patients.

While interpretation should be cautious given the small number of studies and participants and significant variations in methodologies, the literature appears to support the use of CT in PD. In a recent meta-analysis on the use of CT in PD, of seven studies included in the final analysis (n = 272 participants across all studies), the overall effect on cognitive function was small, but statistically significant, and there were a number of significant improvements in several specific cognitive domains, including WM, executive functions and processing speed (Leung et al., 2015). Global cognition, memory, visuospatial skills and attention, however, were not significantly improved (Leung et al., 2015). Additionally, there were no significant improvements in either Independent ADLs or QOL measures (Leung et al., 2015). This suggests that more targeted CT approaches for areas such as memory, visuospatial skills and attention may be needed, and that further focus needs to be placed on enhancing the transferability and length of benefit of CT.

6.11 CONCLUSIONS

A number of considerations have been put forward in this review regarding study design, with the overarching goal of identifying the most effective CT technique for clinical translation. Efficacy may potentially be enhanced through combination with other evidence-based non-pharmacological strategies, such as exercise and tDCS, which may further compound the alterations in neural mechanisms that underlie CT benefits. Additionally, given patients' impairments in manual dexterity, which may significantly hamper ability to use standard equipment involved in CT, focus should be given to developing CT delivery equipment appropriate for use in this population. For example, adding a sensory feedback component to CT delivery equipment may also prove beneficial, as the coupling of hand position, sensory feedback and controlled hand movements with cognitive stimulation has been shown to heighten hand-brain connectivity in a variety of neurological conditions (Borstad et al., 2013). With refinement of delivery mechanism and standardisation of study protocols, CT may lead to notable improvements in cognitive function, or even delay the onset of PD-MCI or PD-D, an outcome that would be particularly critical given the limitations of current pharmacological approaches to improve declines in cognitive performance in PD.

07

Development and Co-design of OrbIT: A Novel ‘Serious Gaming’ System Targeting Cognitive Impairment in Parkinson’s Disease

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7.0 ABSTRACT

Whilst Parkinson's disease (PD) is typically thought of as a motor disease, a significant number of individuals also experience cognitive impairment (CI), ranging from mild-CI to dementia. Unfortunately, CI is progressive in nature, becoming increasingly worse over time and at present, treatment to prevent or reduce the onset of are limited, representing a significant unmet clinical need. One technique that may prove effective for improving CI in PD is cognitive training (CT), with many studies investigating its use demonstrating benefits, however, evidence is variable. This may be due to the implementation of such CT programs, with the severe motor impairment in PD potentially hampering the ability to use standard equipment such as pen and paper or computer mouse or keyboard, promoting negative attitudes which have been correlated with poorer outcomes. Consequently, optimising delivery for the PD population may improve the accessibility, validity and subsequent outcomes of CT for the treatment of CI in PD. To this end, the use of a novel controller specifically designed for use in people with motor impairments was coupled with software specifically developed to targeted cognitive domains impaired in PD via delivery in a 'serious gaming' style. This pilot study aimed to investigate the feasibility of this novel CT program, OrbIT, in a cohort of PD patients in a co-design. After implementation of feedback, attitudes towards enjoyment and usability of the OrbIT system were positive, providing rationale for future implementation of a larger trial to assess outcomes.

7.1 INTRODUCTION

Whilst Parkinson's disease (PD) is primarily characterised as a motor disorder, non-motor symptoms represent a significant burden to the PD population. In addition to anxiety, depression and fatigue, cognitive impairment (CI) represents the single biggest predictor of quality of life, mortality and caregiver burden for PD patients, and tends to be degenerative in nature (Duncan et al., 2014). Due to the ageing population, incidence of CI is expected to increase with the growing prevalence of PD (Dorsey et al., 2007). Whilst impairments in one or more cognitive domains are observed even in early PD (<5 years) (Goldman et al., 2015), many individuals are at risk of progression to mild CI (PD-MCI) and dementia (PD-D) (Litvan et al., 2012). By 3-5 years post-diagnosis, an estimated 20-57% of individuals qualify for diagnosis of PD-MCI (Caviness et al., 2007), with 20% converting to PD-D within 3 years (Saredakis et al., 2019). After 8 years, approximately 80% of individuals with PD develop PD-D (Aarsland et al., 2003).

CI in PD manifests in multiple domains, including higher-order executive functions (reasoning, problem solving, planning), core executive functions (response inhibition, attention, working memory, cognitive flexibility/set-shifting), processing speed, reinforcement learning, visuospatial function, memory and verbal fluency (Kehagia et al., 2010). Not all domains are equally affected, particularly early in the course of the disease. For example, impairments in visuospatial function, memory and verbal fluency may be mild or absent in PD-MCI and more pronounced in PD-D (Kehagia et al., 2010). Executive dysfunctions also appear to become more pronounced in PD-D (Kehagia et al., 2010). Furthermore, patients suffering from specific motor subtypes of PD are also more at risk for dementia than others, with patients of the akinetic-rigid subtype at increased risk of cognitive and neuropsychiatric impairments (Alves et al., 2006).

Despite the prevalence and significant burden of CI in PD, interventions are limited. Whilst dopamine (DA) replacement therapies, such as the current ‘gold-standard’ treatment for PD, Levodopa, are effective at providing symptomatic relief for motor impairments, evidence for the efficacy of treatment of CI is mixed, with some studies noting intervention may paradoxically worsen cognition in certain domains (Schneider et al., 2013, Poston et al., 2016). The only currently approved treatment for CI in PD are cholinesterase inhibitors. These are, however, associated with prominent side effects (Ravina et al., 2005), variable efficacy between patients (Emre et al., 2014) and may even exacerbate the motor-symptoms of the disease (Collins et al., 2011). Furthermore, such therapies only address symptomatic presentation of established CI, unable to prevent or slow the development of cognitive dysfunction in PD (Cerasa and Quattrone, 2015). Consequently, current pharmacological interventions fall short in addressing CI in PD and, as such, interest has grown in non-pharmacological interventions, such as cognitive training (CT).

CT is defined as training programs providing cognitive stimulation that offer structured practice on specific cognitive tasks, designed to improve performance (Clare and Woods, 2004). Multiple studies have established the efficacy of CT in improving or maintaining cognitive function in various neurological patient populations in areas such as global cognition, executive functions, learning, visuospatial abilities and memory (Folkerts et al., 2018, Mohlman et al., 2011, Naismith et al., 2013, Petrelli et al., 2015, Sinforiani et al., 2004). CT has also been shown to be effective both in cognitively healthy PD patients (Glizer and MacDonald, 2016) as well as those with PD-MCI (Reuter et al., 2012, Maggio et al., 2018), and the benefits may be maintained long-term up to 12 months (Bernini et al., 2019, Petrelli et al., 2015). Despite this, however, recent reviews of the literature as a whole have reported mixed results on the benefits of CT in PD (Walton et al., 2021, van Balkom et al., 2019), with a Cochrane review evaluating the effectiveness

of CT for PD-MCI and PD-D reporting no difference between CT intervention and control groups in measures of global or specific cognitive skills (Orgeta et al., 2020). It is important to note, however, that this review had strict inclusion criteria, capturing only randomised control trials.

The variability in outcomes for CT in the PD population may potentially be linked to differences in implementation strategies between programs, with many not optimised for use in individuals with PD. In particular, the motor impairments observed in PD may represent a significant barrier to traditional CT modalities, with activities such as the use of pen and paper challenging due to the specific requirement of manual dexterity proving difficult for PD patients with dyskinesia/akinesia (Thomas et al., 2017). Whilst the move from traditional pen and paper techniques to more computer-based programs may have addressed some of these barriers, a 2010 survey found that nearly 80% of PC-users with PD have significant and severe difficulties using a computer due to their illness (Nes Begnum, 2010). In particular, muscle stiffness, inertia and tremor were frequent problems, resulting in significant-highly severe difficulties using a standard mouse (42%) and keyboard (27%) (Nes Begnum, 2010). Similarly, previous studies have shown one-third to half of computer users' time is spent dragging a cursor via the mouse, which is considered a complex motor operation (Johnson, 1993) and represents a major obstacle to successful computer use for people with motor difficulties (Kouroupetroglou, 2014, Trewin and Pain, 1999). This could represent a significant barrier to the delivery of CT in PD, potentially confounding the evaluation of outcomes. In order to address such barriers, assistive technologies and appropriate hardware adapted for the PD population are needed.

Engagement with the CT paradigm itself may also be key to the ultimate success of the intervention. In support of this, CT in a cohort of psychiatric patients determined engagement during training was a significant independent predictor of cognitive gains, irrespective of simple

exposure (Harvey et al., 2020). One strategy which may improve engagement is the addition of game-like features (gamification) into CT programs (Lumsden et al., 2016, Van De Weijer et al., 2019). This can be attributed to the incorporation of features such as high-score and reward incentives, narrative, personalisation, self-directed challenge, exploration, free-play, competition and graphics into the training platform (Nagle et al., 2015). Gamification may also have benefits beyond improvement of engagement alone, with a systematic review of the literature of gamification in CT highlighting seven reasons researchers opted to gamify CT programs, including increased usability/intuitiveness for target age groups, increased ecological validity, increased suitability for the target disorder, and increased brain stimulation (Lumsden et al., 2016). The review concluded that gamified training is highly engaging and motivational and found evidence that gamification may be effective at enhancing cognitive training in the elderly and ADHD populations (Lumsden et al., 2016). However, improvement in outcomes overall was variable, likely due to heterogeneity in study design and small sample size. The features of gamification that lead to improvements in engagement are particularly important for the PD population, where patients have a decreased reward sensitivity in an off-dopaminergic medication state, as well as increased apathy (Muhammed et al., 2016). Furthermore, anxiety is a common comorbidity in 20-50% of PD patients and game-like tasks may also reduce feelings of anxiety towards tests (Chen and Marsh, 2014, Lumsden et al., 2016). Accordingly, utilisation of elements of game play may improve the efficacy of CT in PD by improving engagement and motivation, leading to increased adherence and reduced anxiety, subsequently improving outcomes.

To date, a system that targets the motor impairments that limit the use of traditional CT delivery methods in PD, while also incorporating elements of gamification that may improve engagement and treatment adherence, is lacking. In order to address this, we aimed to develop a

novel “Serious Gaming” system, OrbIT, that would incorporate both assistive hardware and a custom gaming suite, designed to target the cognitive domains most affected in PD. Taken together, these novel developments in both hardware and software may improve the delivery of CT in PD patients, ultimately leading to an effective non-pharmacological strategy to slow or even prevent the onset of CI in PD, currently a major unmet clinical need. In order to improve the likelihood of this, we engaged with individuals with PD in a reiterative co-design process in order to develop and optimise the OrbIT system for the delivery of CT in this population.

7.2 PARTICIPANTS AND METHODS

7.2.1 *Participants*

Participants were recruited from the community via Parkinson’s SA. Inclusion criteria were a prior diagnosis of PD by a registered neurologist and fluency in English. Exclusion criteria included significant hearing impairments and visual impairments not corrected by glasses/contacts, a brain disorder other than PD, such as AD/dementia, stroke, tumour, infection, epilepsy, multiple sclerosis, or head injury (unconscious >5 minutes), or previous diagnosis of a learning disability. Eligible participants ($n=14$) were invited to take part in the study, with one participant withdrawing after one session, resulting in a final $n=13$. All participants provided written informed consent prior to testing and the research conducted was approved by the Human Research Ethics Committee of the University of Adelaide (H-2020-214).

7.2.2 *The OrbIT System*

The OrbIT serious gaming system involves two main components. The first is the use of assistive hardware via the ‘Orby’ controller (Figure 1). Orby is an innovative novel controller which has been custom designed to address barriers associated with motor dysfunction, such as reduced fine motor control and tremor. A review of assistive technologies and computer access for motor disabilities identified 3 ‘R’s, which represent particular barriers for computer use in motor impaired populations: (1) ‘Rapidly’, referring to a generalised slower speed of movement, or increased speed with reduced accuracy; (2) ‘Reliably’, referring to variation in movements, and (3) ‘Repeatedly’, with propensity for fatigue, degrading performance and ability to accomplish task (Kouroupetroglou, 2014). These were taken into account when designing the Orby controller (Figure 1), which includes several features specifically adapted for motor dysfunction. Firstly, the rounded shape allows for bimanual control, with grip pads on either side to indicate ideal hand placement. Importantly, the sensitivity of responsiveness can be adjusted for the individual, with the movement recognised as ‘purposeful’ altered to take into account extent of motor impairment. This is particularly important for PD patients with tremor, as unintentional tremors can be ignored, leaving only intentional movements to direct the controller during CT. The controller also includes vibration, which allows for haptic feedback triggered by actions within each game. Finally, the large red button for selection minimises the requirement of fine motor control that may be required with devices such as iPad or keyboard keys, which may otherwise be a concern for PD patients. Orby has previously been trialled successfully in individuals with hand impairments due to disability, such as cerebral palsy and in adults post-stroke (Hobbs, 2019), but has not previously been trialled in individuals with PD.



Figure 7.1. Orby controller and set up.

Secondly, to improve user engagement, we designed a custom serious gaming suite to target the cognitive domains most affected in PD, including executive function (working memory, attention, cognitive flexibility, problem solving), visuospatial function and learning (Watson and Leverenz, 2010). Further information of the cognitive domains targeted by each game is summarised in Table 1. This gamification of the CT paradigm introduces elements of high-user control, self-directed challenge, exploration and free-play, which have previously been shown to improve outcomes in home-based CT compared to more automated task delivery (Nagle et al., 2015).

7.2.3 CT Games Catalogue

The CT game catalogue was developed to incorporate training in a range of cognitive domains commonly affected in PD (Das et al., 2016). Due to the nature of gameplay, several of the games encompass training in multiple domains (Table 1).

Table 7.1 Overview of primary and supplementary cognitive domains trained in gaming suite

Game	Primary Rationale	Supp 1	Supp 2	Supp 3
A Bridge Too Far	Working Memory	Cognitive Flexibility/ Set-Shifting	Visuospatial	
Farm Quest	Problem Solving/ Abstract Reasoning	Planning	Attention	
Snake	Visuospatial Function	Avoidance Learning	Attention	
Squirrel	Attention	Working Memory	Cognitive Flexibility/ Set Shifting	Visuospatial
Sunday Driver	Attention	Working Memory	Spatial Navigation	Cognitive Flexibility
Marine Life	Attention/ Working Memory	Cognitive Flexibility/ Set-Shifting	Avoidance Learning	Spatial Navigation
Driving Maniac	Visuospatial	Attention		
Swimma	Attention	Working Memory	Problem Solving/Abstract Reasoning	Cognitive Flexibility/ Set Shifting
Whack-A-Mole	Attention	Working Memory	Sequence Learning	
Who's the Boss?	Problem Solving/ Abstract Reasoning	Working Memory	Risk-Taking Behaviour	
Munchkinis	Abstract Reasoning	Cognitive Flexibility/Set Shifting		
Sushi Train	Response Inhibition	Attention	Working Memory	

7.2.3.1 A Bridge Too Far

A Bridge Too Far is a running game incorporating navigation of quick turns and avoidance of obstacles with the collection of objects, involving visuospatial function. The primary objective is to collect the correct coloured gem as dictated by a sign, with instructions about which gem to collect becoming less obvious as the game progresses, in order to increase difficulty. The prompt

disappears and remembering the correct coloured gem while running is a measure of working memory. Additionally, the colour of the required gem changes throughout the game as the player progresses, with ability to shift sorting criteria considered a measure of cognitive flexibility/set-shifting. Collection of the incorrect gem will result in a loss of health. Supplementary elements of game-play include the collection of ‘soda cans’ for energy and coins for a high score to promote engagement.



Figure 7.2. A Bridge Too Far

7.2.3.2 Farm Quest

Farm Quest is a puzzle game requiring problem solving/abstract reasoning skills, planning and attention to build a fence to a flag on the other side of a vegetable patch. Each level has instructions regarding a new sorting criterion by which you must separate and/or pair the produce within their own sections and increases in difficulty as the player progresses. The changing of sorting criteria also introduces elements of cognitive flexibility.



Figure 7.3 Farm Quest.

7.2.3.3 Snake

Snake is a game which primarily involves training of visuospatial function. The player must navigate a snake within the arena, avoiding obstacles, edges and its body, whilst collecting 'orbs'. Collection of the orbs involves different nominated point values (gold = 100, purple = 50), an element of gameplay. In addition, an eagle is intermittently introduced as an element of danger, where the player must decide if they will risk lives to collect the orbs, or seek safety in a nearby lake, introducing an element of avoidance learning and risk-taking behaviour.



Figure 7.4. Snake.

7.2.3.4 Squirrel

Squirrel requires forced navigation around a constantly moving environment (tree), avoiding branches, with speed and number of obstacles increasing as the player progresses. Players are shown instructions with coloured berries, and a number they are required to collect before progressing, which also increases in difficulty. They must avoid all other coloured berries not specified for collection. The sign will disappear, requiring attention and working memory to collect the correct colour/number of berries, whilst the changing of collection criteria requires cognitive flexibility/set-shifting. High scores are based on the level the player reaches and the collection of various acorns scattered throughout to further incentivise game play.

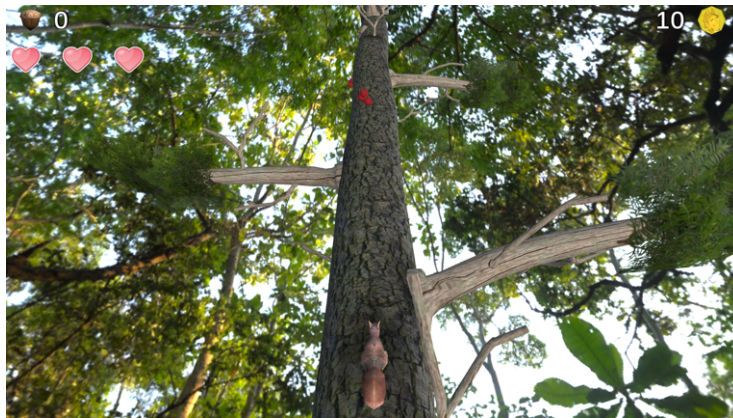


Figure 7.5. Squirrel.

7.2.3.5 Sunday Driver

Sunday Driver is a game in which the player must navigate around a large area to collect characters whilst avoiding obstacles, incorporating spatial navigation. A chest in the central tent will specify the character to be collected (e.g. cat) and the player must navigate to the colour-coded tower which contains the correct character. The player cannot see the contents of the chest until it is opened and, therefore, they must remember the correct location from previous encounters when

required to collect the new character. This involves the use of attention and working memory, as well as visuospatial memory. The arena also involves several obstacles, including ‘mystery cubes,’ which contain both positive (e.g. increased health) or negative (e.g. slowed travel) elements as elements of game-play.



Figure 7.6. Sunday Driver.

7.2.3.6 Marine Life

In Marine Life, the player is a sea creature which must navigate the underwater environment to eat a required number of sea creatures before progressing to the next level. Each level includes a ‘danger’ creature, which the player must avoid. The player's character (sea creature) changes with each level, as does the danger creature they are told to avoid. The game requires attention and working memory, in addition to cognitive flexibility/set-shifting in order to recognise, remember and adjust to eat the required number of correct creatures to progress.



Figure 7.7. Marine Life.

7.2.3.7 Driving Maniac

Driving Maniac involves navigation of a car around a forced, constantly moving environment (road). The player must avoid obstacles such as other cars, oil slicks and roadblocks, whilst collecting extra lives and fuel-tanks for a high score. The speed increases with distance travelled to progress difficulty level and requires attention and visuospatial function to navigate the environment.



Figure 7.8. Driving Maniac.

7.2.3.8 *Swimma*

Swimma involves navigation of a character in a forced, constantly moving environment (underwater), whilst avoiding moving obstacles, which involves spatial navigation. During each level, the player must navigate to the end whilst collecting floating gems; however, they are required to avoid a particular colour which will cost them lives. They are not advised of this colour and must therefore work out the pattern through trial and error, which changes each level. This is a measure of attention and working memory, as well as problem solving and cognitive flexibility/set-shifting. The player must also collect bubbles for air along the way to avoid losing lives, adding an additional element of gameplay.



Figure 7.9. *Swimma.*

7.2.3.9 *Whack-A-Mole*

Whack-A-Mole is a game in which moles will appear and then disappear from a grid of burrows in a sequence. The player must remember that sequence and, under a timer, use the hammer to select the correct burrows in the order in which the moles appeared. The number of burrows

increases with each stage (e.g., from 4-6-12 etc.) and the number of moles appearing in a sequence also increases (e.g. 3-4-5). This is a measure of sequence learning, attention and working memory. Incorrect selections will cost lives and too many incorrect selections will force the player to begin again.



Figure 7.10. Whack-A-Mole.

7.2.3.10 *Munchkinis*

Munchkinis is a puzzle game requiring guidance of 'Munchkini' characters through a series of gates in order to guide them home. Each level involves a series of gates (2 or 4) that allow entry based on a particular trait (e.g., glasses vs no glasses, hat vs hair etc.). The player must use trial and error to determine the sorting criteria and allocate each Munchkini through their respective gate. The final level requires the player to build the 'perfect spaceship', where they must use trial and error to determine the correct features the spaceship requires in order to fly (e.g., window/no window, boosters/no boosters). The game requires abstract reasoning to identify patterns and sort features, in addition to cognitive flexibility/set-shifting to adjust criteria.



Figure 7.11. Munchkinis.

7.2.3.11 Who Stole the Coin/Who's the Boss?

This game underwent considerable changes throughout the consultation process; however, in the finalised version, 'Who's the Boss' is a puzzle game which presents the user with a set of 2 characters (e.g., Cat/Sheep). Initially, the player must select who they believe is the 'boss'. They will then be shown another 2 characters (e.g. Penguin/Chicken) and, through a series of exposures of different combinations of the same characters, they must determine the correct hierarchy (e.g., Cat beats Sheep and Penguin beats Chicken, but Cat beats Penguin and Sheep beats Chicken and Penguin - the hierarchy is Cat, Sheep, Penguin, Chicken). Players must make their selections and sort in the correct order, receiving coins depending on the number of paired exposures they required to make their guess. The player also chooses the number of coins they bet, based on their confidence in their decision. As the game progresses, more characters are introduced and the hierarchy changes. This game involves training of abstract reasoning, learning, attention and working memory, and also allows measurement of risk-taking behaviour.



Figure 7.12. Who Stole the Coin?/Who's the Boss?

7.2.3.12 Chow Time

In Chow Time, players are presented with a moving conveyer belt with various foods/items. The player must sort the edible food (e.g. melon) from the items (e.g., trash bag). In order to make their selection, players must make an intentional selection of the edible food by moving the controller towards the second conveyer belt to collect; however, no movement is required when faced with inedible food. The conveyer belt increases in speed with each level. This game aims to train and assess response inhibition and processing speed.



Figure 7.13. Chow Time.

7.2.4 Procedure

Eligible participants ($n=13$) attended the Brain and Body Fitness Studio (BBFS) at Parkinson's SA and completed the program in three 50-minute sessions over the course of a week whilst supervised. A pre-test battery was administered on day 1, followed by a 50-minute session. Day 2 involved 50-minutes of supervised gameplay and day 3 involved 50-minutes of gameplay, followed by a post-test battery. Participants were provided with a brief controller demonstration and written instructions for each game and assistance was provided if requested during the first session, in order to help familiarise individuals with the controller and game objectives. Play for each game was restricted to 15 minutes over the course of the week to ensure an even spread of training across multiple domains. Feedback was collected regarding ergonomics and game usability via both written surveys and verbal communication across the three sessions and adjustments to the gaming suite and controller setup were made based on this feedback. Participants were invited back 1-month later for an additional 50-minute session with the adjusted setup, in order provide feedback on the implementation of suggested changes.

7.2.5 Outcome Measures

Participants were first administered the MMSE to assess baseline cognitive function (Folstein et al., 1975). Following this, participants completed the Parkinson's Disease Questionnaire-39 (PDQ-39) to assess disease-specific quality of life (QoL; (Jenkinson et al., 1997). On an iPad, participants then completed the World Health Organisation QOL (WHO-QOL-BREF) (Harper, 1998) to assess health-related QoL and the Geriatric Depression Scale (GDS) as a self-report measure to assess depression in older adults (Pocklington et al., 2016). Although the trial is considered a pilot study, with the primary outcome to assess usability, acceptability and feasibility and optimise the

program, measures of QoL and depression were repeated post-CT. Following engagement with the OrbIT system, participants completed a series of custom questionnaires on previous game experience (session 1 – Appendix 1.1), initial system feedback (session 1 - Appendix 1.2, individual game feedback (session 3 - Appendix 1.3, and follow-up feedback (follow-up session - Appendix 1.4). Additionally, an adapted version of the System Usability Scale (SUS) was used to assess overall acceptability of the OrbIT gaming system (session 3 – Appendix 1.5) (Peres et al., 2013). Observations were also made throughout the sessions and verbal feedback was noted.

7.2.6 Outcome Measure Interpretation and Statistics

PDQ-39 results were calculated based on interpretation guidelines (Jenkinson et al., 1997); briefly, scores for each dimension were summed, divided by the maximum possible score and then multiplied by 100. To calculate the overall PDQ (PDQ-39 SI), dimension scores were summed and then divided by the total number of dimensions (8), higher scores represent indicate more severe PD. For the WHO-QOL BREF, scores across each domain were transformed to a score from 0-100 based on WHO guidelines (Harper, 1998), in addition to an overall transformed score, with a higher score indicating better QoL. The GDS and the MMSE scores were derived by summing scores for each item. Calculation of the SUS involves summing each odd numbered question (e.g., 3, 5, 7 and 9) and subtracting 5 from the total. The score of each even numbered question (e.g., 2, 4, 6 and 8) was subtracted from 5 and then summed. The sum of these two values is then multiplied by 2.5 to achieve a score out of 100. For all other surveys, scores were averaged, and the mean and SD reported. For outcomes repeated pre and post intervention (PDQ-39, WHO-QOL BREF and GDS), calculated scores were compared via paired t-test with significance set at $p<0.05$.

7.3 RESULTS

Thirteen participants were included in the pilot (6M/7F, Mean Age 68.15 ± 8.54) years), with an average disease duration of 8 ± 5.43 years. Demographics are summarised in Table 7.2. Twelve of the 13 participants completed the 3 sessions of the CT training period, with 1 completing 2/3 due to time constraints. Eleven of the final 12 participants attended the final follow-up evaluation. Participants did not appear to be cognitively impaired, with MMSE scores all >27 (Mean 29 ± 0.82). Despite not scoring in the cognitive impairment range on the MMSE, a subjective survey revealed participants commonly reported cognitive concerns, including remembering events (30.1%), remembering information (38.5%), paying attention (15.4%), learning new tasks (15.4%), remembering words (15.4%) and managing day-to-day tasks (15.4%). In addition, 38.5% self-reported experiencing motor difficulties.

Table 7.2 Demographic and patient data ($n=13$). Mean \pm SD

Patient Data	Mean \pm SD	Range/ Proportion
Age (Years)	68.15 ± 8.54	48-81
Gender (M/F)	-	6/7
Years Since Diagnosis	8 ± 5.43	1-19
MMSE	29 ± 0.82	27-30/30
Affected Side (Right/Left/Equal)	-	2/6/5
Dominant Hand Affected	-	7/13
DBS Surgery	-	2/13
Time Since Medication (hours)	1.85 ± 1.11	-

7.3.1 Quality of Life and Depression

At baseline, PD participants did not appear to report an overall reduced QoL, as measured by the WHO-QOL-BREF, compared with normative data (Hendred and Foster, 2016), nor did they report a decrease in health-related QoL in any of the domains measured by the PDQ-39 compared with normative data (Jenkinson et al., 1997). (Table 7.3). Furthermore, participants mean GDS (3.42 ± 4.08) did not indicate depression, with scores >11 indicating depression on this measure (Mondolo et al., 2006). As expected, due to the short nature of the CT intervention, no significant differences were observed between baseline and session 3 in the WHO-QOL BREF (98.58 ± 8.92 vs 98.83 ± 10.32 , $t(11) = 0.23$, $p = 0.82$), GDS (3.42 ± 4.08 vs 2.83 ± 3.71 , $t(11) = 2.03$, $p = 0.07$) or PDQ-39 SI (143.9 ± 83.02 vs 136.6 ± 87.3 , $t(11) = 0.68$, $p = 0.51$), as summarised in Table 3.

Table 7.3 WHO-QOL-BREF, GDS and PDQ-39 ($n=12$). Mean \pm SD

	PRE	POST	Mean Difference
WHO-QOL BREF			
Physical	97.33 ± 12.57	95.92 ± 13.99	
Psychological	87.67 ± 10.71	87.92 ± 12.74	
Social	48 ± 5.12	49.08 ± 9.55	
Environment	131 ± 11.20	130 ± 13.59	
Overall	98.58 ± 8.92	98.83 ± 10.32	$0.25 (p = 0.82)$
GDS	3.42 ± 4.08	2.83 ± 3.71	$-0.58 (p = 0.07)$
PDQ-39			
Mobility	24.17 ± 23.53	25 ± 23.86	
Activities of Daily Living	26.04 ± 20.03	24.65 ± 19.09	
Emotional Well-Being	22.22 ± 17.71	19.44 ± 15.21	
Stigma	13.02 ± 15.64	9.38 ± 9.42	
Social Support	6.25 ± 10.73	7.64 ± 9.03	$-1.29 (p = 0.51)$
Cognition	25 ± 15.54	22.4 ± 15.18	
Communication	22.92 ± 17.09	24.31 ± 24.48	
Bodily Discomfort	34.03 ± 18.28	30.56 ± 20.52	
Summary Index (SI)	21 ± 11.25	20.42 ± 12.04	

7.3.2 Game Experience

With relation to previous game experience, 92.3% of participants reported playing games, with 53.8% reporting a frequency of 1 or more times daily. The preferred gaming formats reported were card games (76.9%), word and number games (76.9%) and puzzle/tile/board games (38.5%). In terms of computerised games, 30.8% reported playing games in an online format, whilst only 1 participant (7.7%) reported a preference for video games, indicating the sample group had minimal experience with computerised video games prior to engaging in the co-design process.

7.3.3 System Feedback

7.3.3.1 Session 1 - Initial feedback

After one session, 91% of participants reported that they enjoyed the OrbIT gaming system, with an average rating of 7.58/10 (Table 7.4). 100% of participants reported finding the games challenging, with an average difficulty level of the games rated as 6.25/10, and 5.83/10 for controller use. Participants reported a high degree of confidence in the ability of the OrbIT system to improve/maintain cognitive function (7.92/10). One participant reported they would not use the system at home, 1 reported occasional use, 6 reported use several times a week and 5 reported likelihood of daily use.

Table 7.4. Session 1 – Initial feedback. \pm = SD

Initial Feedback		Mean \pm SD
	% Yes	91.7%
Initial Enjoyment	Games	7.75 \pm 2.18
	OrbIT System	7.58 \pm 2.19
Challenging	% Yes	100%
Difficulty	Games	6.25 \pm 1.29
	Controller	5.83 \pm 1.85
Confidence	Likelihood of improvement/ maintenance of cognitive function	7.92 \pm 1.78

7.3.4 Individual Game Feedback

Following completion of the third session of play, feedback was collected on the individual games. Data was separated into two categories: content (enjoyment, interest, challenge, difficulty and features) and usability (instructions, ease of play and controls). An overall system rating was also obtained. For each game, the number of participant responses received depended on whether the game was played during their sessions. Overall ratings for each game are summarised in Table 5.

Table 7.5 Individual Game Feedback

	CONTENT						USABILITY			OVERALL RATING
	RES.	ENJOYED (% YES)	ENJOYMENT RATING (AV INTEREST + ENJOYMENT) / 10	CHALLENGE (% YES)	DIFFICULTY RATING / 10	FEATURES (COLOR/ ANIMATION/ SOUND) / 3	CLEAR INSTRUCTION (% YES)	EASE OF PLAY (% YES)	CONTROLS / 3	
A Bridge Too Far	13	100%	7.42	84.6%	7	2.81	69.2%	100%	2.62	7.69
Farm Quest	11	36.4%	5.73	100%	7.36	2.77	70%	54.6%	2.7	5.5
Squirrel	12	91.7%	7.17	81.8%	6.33	2.73	100%	81.8%	2.64	7.17
Snake	12	41.7%	4.5	75%	6.67	2.64	70%	33.3	1.82	4.83
Sunday Driver	9	66.67%	6.44	100%	7.44	2.59	22.2%	22.2%	2.62	6.33
Marine Life	11	90.9%	7	90%	6.73	2.83	88.9%	72.7%	2.5	7.27
Swimma	7	85.7%	6.71	85.7%	6	2.56	66.7%	57.1%	2.33	6.43
Driving Maniac	12	100%	7.71	100%	7.33	2.52	85.7%	75%	2.46	7.83
Whack-A-Mole	10	80%	7.55	80%	7.2	2.73	100%	88.9%	2.33	7.7
Munchkinis	7	85.7%	7.64	100%	8	3	100%	57.1%	3	7.57
Who's the Boss?	10	70%	5.85	100%	7.3	2.67	66.7%	80%	2.67	6.3
Chow Time	10	80%	6.3	30%	4.2	2.63	100%	100%	2.75	6.4

Res = Respondents, % indicates percent of participants who answered 'yes'. Ratings are on a scale of 1-10 (1=very poor/easy – 10=very challenging/enjoyable). Features and controls are on a scale of 1-3 (1=Not Good, 2 = Average, 3 = Good).

7.3.5 Overall Usability

Following the third session, participants were asked to complete the SUS to assess overall usability of the OrbIT System. Results are summarised in Table 6. Interpretation of usability based on standard SUS guidelines resulted in an overall score of 66 / 100.

According to the SUS grading system, this is considered below the average score of 68 (Gomes and Ratwani, 2019).

Table 7.6. Results of the System Usability Scale Questionnaire. Scores are based on a scale (1=Strongly Disagree, 5 = Strongly Agree). \pm = SD

System Usability Scale - Questions	Mean \pm SD
I think that I would like to use the OrbIT System frequently	3.23 \pm 0.6
I found the OrbIT System unnecessarily complex	1.92 \pm 0.86
I thought the OrbIT System was easy to use	3.46 \pm 0.78
I think that I would need the support of a technical person to be able to use the OrbIT System	2.08 \pm 0.95
I found the various functions of the OrbIT System were well integrated	3.46 \pm 0.88
I thought there was too much inconsistency in the OrbIT System	2.39 \pm 1.04
I would image that most people would learn to use the OrbIT System very quickly	3.77 \pm 0.93
I found the OrbIT System very cumbersome to use	2.38 \pm 0.96
I felt very confident using the OrbIT System	3.39 \pm 0.96
I needed to learn a lot of things before I could get going with the OrbIT System	2.31 \pm 0.95
OVERALL SCORE	66 / 100

7.3.6 Follow-Up

Following integration of feedback from sessions 1-3, substantial changes were made both to the individual games, as well as the deployment of the Orby system itself. Subsequently, a follow-up session was held about a month later for participants to trial the changes. A post-session survey revealed a positive response to the changes made, with 100% of participants reporting they felt their comments were addressed (Table 6). Specifically, changes made to the sensitivity and ergonomics of the controller were rated 7.64/10 and changes to the games 7.91/10. Confidence in the ability of repeated use of the OrbIT gaming system to be beneficial for cognitive function was reported by all participants (100%) and an overall enjoyment rating of the system was 8.18/10, which represents an 8% improvement upon the initial system rating in the first session.

Table 7.7. Results of a Follow-Up Survey.

Follow-Up Survey		Mean \pm SD (% Change)
	% Comments Addressed	100%
Feedback of changes	Controller Changes	7.64 \pm 1.75
	Overall Rating of Changes to Games	7.91 \pm 1.04
Confidence	Do you feel repeated use of gaming system could be beneficial?	100%
Enjoyment	Overall	8.18 \pm 1.08 (+8%)

% = percent of participants who answered 'yes'. Scores are based on a scale of 1-10 (1=much worse, 10 = much better). n=11, Mean \pm SD

7.4 DISCUSSION

This study used a reiterative co-design process in order to develop a novel serious gaming system, OrbIT, for the delivery of CT in individuals with PD. Feasibility was assessed by evaluating a combination of outcomes, including enjoyment, accessibility and acceptability of both the software and hardware. Overall, the OrbIT system demonstrated positive feedback in all areas assessed, with integration of feedback resulting in high ratings of enjoyment and confidence in the benefits of the CT program.

7.4.1 *Sample population*

With regards to the sample cohort, participants appeared to be cognitively unimpaired, with an average MMSE score of 29. Evidence suggests a score of 27.5 may be indicative of PD-MCI, whilst a score of <26.5 indicating possible dementia (Lucza et al., 2015). Participants also did not report significant impairments in either overall or disease related QoL across domains, suggesting that, not only was the cohort cognitively intact, but motor symptoms of the disease were either mild and/or well controlled with medication, so as not to negatively impact upon everyday life. This is surprising, as the cohort included a broad range of disease duration, ranging from 1-19 years (average 8 years +/- 5.43). Finally, mean scores did not indicate the presence of depression based on self-report measures. There were also no significant differences observed in either disease-specific or health related measures of QoL or self-reported depression between baseline and the final session; however, this was anticipated given the limited duration of the training program. Overall, the cohort in the current study was high functioning, with no evidence of cognitive impairment, depression or motor impairment significant enough to interfere with daily activities. While this may represent a selection bias, and may make it difficult to extrapolate the results from this small sample to the wider PD population, including those with more severe motor and cognitive

impairments, this is also likely to represent the target demographic who may derive the most benefit from CT. In support of this, recent research supports that older adults with higher baseline cognitive function are more likely to benefit from CT than those who are already impaired (the so-called magnification effect) (Fu et al., 2020, Mohlman et al., 2011). Thus, this represents an appropriate sample for participation in the co-design process.

7.4.2 Initial feedback on the OrbIT system

Initial impressions of the gaming system as a whole were positive, with 91.7% of participants reporting they enjoyed the system and an average rating of 7.58/10. Indications of acceptability for the implementation of the CT program were also considered high, with an average rating of 7.92/10 for confidence for the likelihood of the OrbIT system to improve or maintain cognitive function. These are important positive predictors, as game enjoyment and perceptions of cognitive benefit towards gamified CT in an older population have been correlated with motivation (Boot et al., 2016). A recent study found individuals who chose to engage in self-motivated CT for short periods and discontinued use rated it lower in usefulness than those who sustained or had never used CT (Goghari et al., 2020). Accordingly, high levels of initial enjoyment and confidence reported in the OrbIT system are likely to reflect a high degree of motivation to engage with the system. This is particularly relevant in PD, given that diseases of DA dysfunction are particularly associated with motivational disorders, with approximately ~40% of individuals with PD experiencing disorders of motivation (den Brok et al., 2015). This is likely due to the role of DA in motivating individuals to invest effort in return for reward (Mcguigan et al., 2019). In support of this, 77% (10/13) of the participants in the current study reported that they would use the OrbIT system several times per week or even daily if it were available commercially.

This may translate to improvements in adherence to a long-term CT regime, enhancing the efficacy of the CT program.

For accessibility of the gaming suite, initial feedback was considered moderate. On a scale of 1 (very easy) to 10 (very challenging), participants rated the difficulty of the games as 6.25/10. Although moderate, this may be considered a positive response with regards to difficulty of the games, as it is important to find a balance to maintain compliance with the regime. To this effect, self-rating of progress has been shown to be positively related to post-training improvements (Mohlman et al., 2011). If the games were considered too easy initially, this could lead to participants becoming bored and limit their ability to notice progression. This could affect their confidence in the ability of the system to improve cognitive function, subsequently decreasing motivation to engage further. On the other hand, if the gaming suite was deemed too difficult, this may affect participants' confidence in their performance and ability to engage with the games, leading to increased apathy and decreased motivation to complete the program. Collectively, these factors may influence the efficacy of the program for the individual. Importantly, users seemed to enjoy the games, as indicated by their initial rating of 7.75/10, suggesting that they would be motivated to engage with the system further.

With regards to accessibility of the Orby controller itself, this also received a moderate rating (5.83/10) after the first exposure to the system. Given the challenges associated with motor function in the PD population (Mazzoni et al., 2012), it is important for the controller to be considered accessible, and initial accessibility feedback was not as positive as hoped. This rating could be related to several factors; for example, it may indicate issues with handling of the controller, sensitivity and responsiveness of the controller to specific games, or ergonomics of the overall set-up. Furthermore, this rating may be reflective of the minimal exposure of the participants to computerised games (30.8%), and

specifically video games (7.7%). Minimal experience with handling similar technologies suggests a steeper learning curve, which may also have impacted initial accessibility impressions of the controller. Accordingly, it was important to probe this further following additional exposures to the controller, as well as following the implementation of several suggested changes to both the controller and the gaming suite.

7.4.3 Feedback on the OrbIT system following one week of exposure

7.4.3.1 The Orby Controller

Observationally, participants appeared to become much more comfortable with controller use by the second exposure and proficient by the third session. Although survey data was not collected after the initial pilot period regarding controller use, there was an opportunity for participants to make comments. One participant noted *‘the gaming platform was not too awkward to operate once I became used to the tension of the OrbIT i.e., not to be too forceful gripping the item’*. This is encouraging, given that the system has been custom-designed to take into account the three ‘R’s suggested by Kouroupetroglou and colleagues (2014) to represent particular barriers for computer use in motor impaired populations: ‘Rapidly,’ ‘Reliably’ and ‘Repeatedly’ (Kouroupetroglou, 2014). For example, the selection field of the Orby controller has been expanded, demanding less accuracy compared to conventional pointer cursors, which have a very narrow pixel ‘hot-spot’ and thus require a high degree of accuracy (Worden et al., 1997). This, in turn, directly impacts upon the ability of the participant to engage with the games; for example, a larger field of collection of gems in *Swimma* results in reduced accuracy demands and an improved margin of error.

Encouragingly, with repeated use, participants were also able to optimise their own use of the Orby controller. Despite being shown a traditional grip (one hand over each grip pad) in the initial session, throughout the trial and across different games, participants

adopted several different techniques to control the device, including one handed (A), upper hold (B) or lower hold (C), fingertips (D) and bear grip (E) for those with more prominent motor dysfunction (Figure 7.14). This versatility exemplifies a positive adaptive feature of the Orby controller, allowing it to cater for the heterogeneity of motor impairments in the PD population (Greenland et al., 2019).

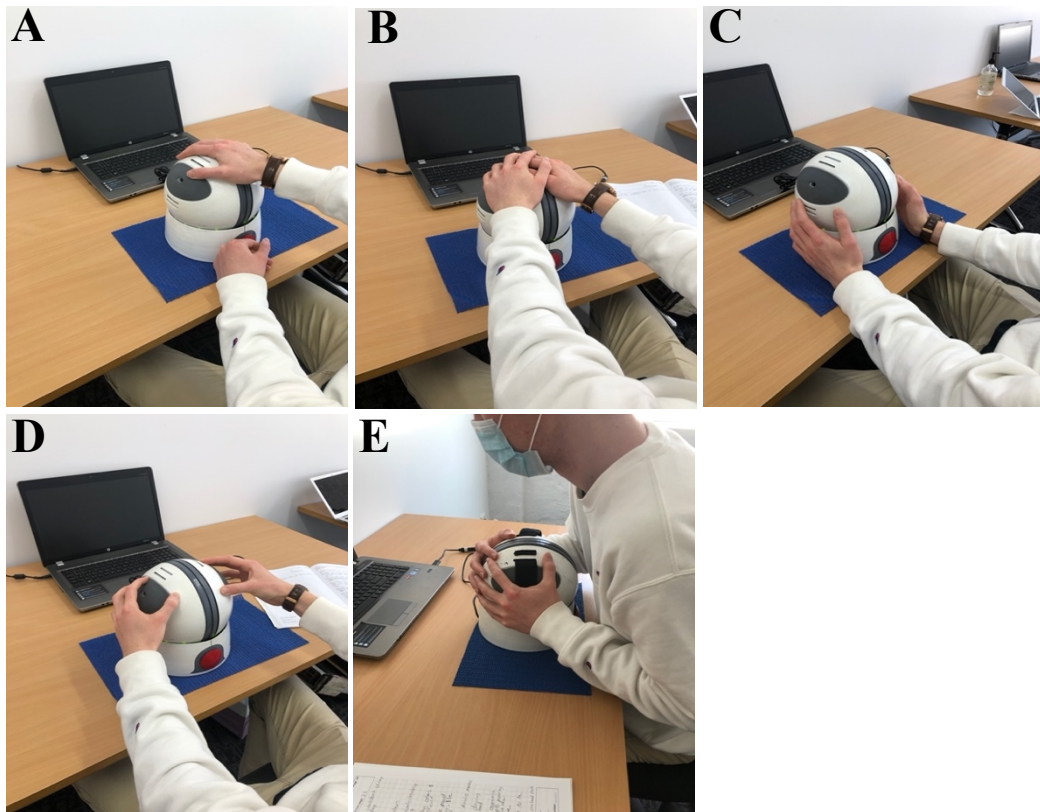


Figure 7.14 – Images depicting variations in controller grip (A) one handed, (B) upper holder, (C) lower hold, (D) fingertip and (E) bear grip.

Although patients did appear to become more comfortable with the system with repeated exposure, some problems persisted, with an overall below-average SUS of 66/100 after three sessions, indicating compromised usability. Based on participant feedback, this may, at least in part, be due to general setup/handling issues, with several participants noting comments such as, ‘*I think the device needs to be anchored to a base, so it doesn’t move about the table*’. Fatigue/discomfort may also have played a role, with two participants

commenting that their shoulders/arms became tired or sore during the 50-minute sessions. Additionally, one participant ended 2/3 of their sessions 5 minutes early due to fatigue. Fatigue is a commonly reported symptom in PD, with a reported prevalence of 50% (Siciliano et al., 2018) and is one of the three Rs (i.e. 'Repeatedly') suggested by Kouroupetroglou and colleagues (2014) to represent particular barriers for computer use in motor impaired populations. As such, it may have affected the ability of participants to engage throughout the session and, in turn, affected their perception of the usability of the system overall. It is also possible, however, that difficulties with the games themselves negatively impacted on overall system usability, making it critical to interpret overall usability in the context of ratings of the gaming suite.

7.4.3.2 Gaming Suite Software

Overall, the response to the games was positive, with all games (except Snake) receiving an above average (>5) overall rating. These results were probed further based on feedback regarding content (encompassing enjoyment, challenge/difficulty level and game features) and usability (encompassing ease of play, clarity of instructions and controller responsiveness).

Content

In terms of enjoyment, the majority of games were considered enjoyable, with >90% of participants reporting enjoyment of A Bridge Too Far (7.42/10), Squirrel (7.17/10), Marine Life (7/10) and Driving Maniac (7.71/1-) and >50% of participants reporting enjoyment of Swimma (6.71/10), Sunday Driver (6.44/10), Whack-A-Mole (7.55/10), Munchkinis (7.64/10), Who's the Boss? (5.85/10) And Chow Time (6.3/10). As mentioned, this is important as enjoyment is a strong motivator and positively associated with effortful engagement (Cacioppo et al., 1996). Games with below average (<50%) number of

participants reporting enjoyment included Farm Quest (5.73/10) and Snake (4.54/10). Lower participant enjoyment of these games may be reflective of a number of factors, such as inappropriate difficulty or issues with game features. As such, these results were probed further with corresponding questions for each game to determine the reason and implement appropriate changes.

The relationship between perceived task difficulty and performance is not well understood for CT in PD; however, studies suggest a balance is important. Previous literature suggests that increases in task difficulty via adjustments in complexity correspond to lower levels of performance and higher levels of stress in university students (Robinson, 2001). In older adults specifically, Selective Engagement Theory (Hess, 2014) proposes that increased ‘cognitive costs’ associated with activities later in life results in a reduction in the cost/benefit ratio, reducing the willingness of older adults to engage in demanding activities (Buchman 2014). It is critical to balance this, however, against the theoretical framework proposed by Lövdén and colleagues for achieving cognitive plasticity in adults (Lövdén et al., 2010). According to this model, the transfer of gains from cognitive training across multiple cognitive domains or to real-world contexts depends on the difficulty of the training task, with sustained cognitive challenges required in order to induce lasting neural changes. This necessitates a continual mismatch between the demand of the task (i.e. the cognitive load) and the cognitive capacity of the individual. In support of this, adaptive training of working memory (i.e. where task demands are continually increased based on performance) resulted in far transfer to an untrained episodic memory task, as well as accompanying neural changes (Flegal et al., 2019).

In light of these considerations, in the design of the gaming suite, we aimed to achieve a difficulty level of between 6 and 8 on a ten-point scale, in order to achieve an effective challenge, while also reducing the ‘cognitive cost.’ All games were within this

range, with the exception of Chow Time (4.2/10), with only 30% of participants reporting this game to be challenging. This was corroborated with observations and verbal feedback, with participants reporting the speed of the conveyer belt started off '*too slow*' and did not become challenging until at least level 5. Conversely, although still within an acceptable range, Munchkinis was considered the most difficult game included in the suite (8/10). Based on observations during the first three sessions, this also appeared to be related to progression. For example, the first level of Munchkinis involved the sorting of features based on 2 criteria (e.g., with hat (left) or without hat (right)), before level 2 progressed to sorting based on multiple characteristics (e.g., hats (left) - glasses + hat (upper left)/no glasses + hat (lower left) and no hats (right) - glasses + no hat (upper right)/ no glasses + no hat (lower right)). This progression was reported to be '*too steep*', with only one participant observed to successfully complete the second stage.

In terms of game features, including the use of colour, animations and sound, all games were rated >2.5 on a three-point scale, with no notable comments or observations made with regards to the visual features of the games' design. This is important, as basic stimuli (images and texts) associated with traditional pen and paper CT can make therapy boring for patients (Alloni 2015). The inclusion of 3D graphics in computerised training such as this is considered beneficial, due to increased entertainment and involvement of the patient, as well as the introduction of new elements (such as spatial perception) into training, ultimately improving direct interaction compared to more abstract 2D counterparts (Alloni 2015). Interestingly, one participant did comment on the reliance on colour for sorting in many of the games, particularly Squirrel, as this would be a potential barrier for implementation in those with colour blindness. Whilst this feedback was not directly addressed in the initial round of changes, future adaptations could include the use of shape, rather than colour, to overcome this particular concern.

Usability

Usability of the games was assessed based on the ease of play, clarity of instructions provided and controller responsiveness for each game. A Bridge Too Far and Chow Time were considered easy to play by 100% of participants. Whilst Chow Time's ease is likely attributed to the slow progression of the game discussed above, A Bridge Too Far appeared to be quite challenging for participants based on observations. This reported ease may potentially be attributed to two reasons. Firstly, the game is the first listed in the suite and, as such, all participants chose to begin with the game. This meant that, when observers were guiding participants through the features and use of the controller for the first time, it was via this game. This may have led users to feel particularly supported in how to play the game, raising their confidence level and inflating their perception of the ease of the game.

Conversely, games which scored poorly (<60%) for ease of play include Farm Quest, Snake, Sunday Driver, Swimma and Munchkinis. Based on participant comments, this was attributable to poor clarity of the instructions and poor responsiveness of the controls. For example, participants specifically commented '*instructions not clear*' or '*not easy to follow*' for Farm Quest. For Sunday Driver participants commented it was '*not clear had to go to centre tent first*'. Whereas comments for Snake included '*didn't seem to respond to controls*' and '*controlling the snake was difficult, needs refining*'. In light of this feedback, significant alterations were made to these games, as well as to the setup of the Orby controller itself

7.4.4 Implementation of participant feedback to alter the OrbIT system

Following review of all participant feedback, several key changes were made to the OrbIT system. These included changes to the ergonomics of the controller setup itself, modulation of task difficulty within specific games, improved clarity of instructions, alterations of game

objectives and improvements to controller responsiveness. Specifics of these changes, as well as their outcomes, are discussed in further detail below.

7.4.4.1 Ergonomics of the controller setup

Based upon participant feedback, several improvements were made to the ergonomics of the controller setup, in order to improve user experience and decrease fatigue associated with extended use. Firstly, a grip mat was introduced, allowing the controller to remain in place on the table (Figure 7.15). Optional straps were also introduced, which allowed participants to place their hands inside the straps, providing additional grip/handling of the controller. The straps also enabled the participants to ‘rest’ their hands to reduce fatigue and shoulder strain whilst still maintaining control.



Figure 7.15– Orby controller set-up - Featuring addition of a grip mat and straps.

Finally, participants were also provided with ergonomic chairs that allowed for height adjustment and arm rests, which could also be utilised to provide support, thereby relieving shoulder/arm discomfort and reducing fatigue.

Overall, changes to the ergonomics of the set-up and controller were well received in verbal/written feedback, as demonstrated by comments such as '*mat beneath controller helps*;' '*straps help when moving object on game i.e., are great help when having to just select*' and '*using straps and rubber mat was good*'. Most encouragingly, one participant noted, '*the controller was much easier than a mouse and keyboard*' and mentioned that, although they hadn't taken their medication yet and were feeling '*Parky, it was still easy to navigate on the OrbIT*'. This was also reflected in the follow-up survey where, on a scale of 1 (much worse) to 10 (much better), changes to the controller itself were rated 7.64.

7.4.4.2 Modulation of task difficulty

In order to ensure that all games fell in our ideal range of 6-8/10, allowing for a balance between effective challenge and 'cognitive cost,' modulation of task difficulty was made for both Chow-Time (rated 4.2/10) and Munchkinis (rated 8/10). For Chow-Time, the initial speed of the conveyer belt, and accordingly the required processing speed, was increased. This was well received, as reflected in verbal feedback in the follow-up session, with participants commenting '*starting speed is better,*' which '*made it more interesting*'. For Munchkinis, conversely, an additional level comparable to the first was added, in order to allow the participants more time to identify and familiarise themselves with the sorting criteria. Although performance on games was not an outcome assessed at this stage of the study, observers noted an increase in the number of participants reaching the final level at follow-up. While formal rating of game difficulty was not assessed following alterations, in order to avoid the influence of learning and familiarity effects, these initial observations are encouraging that concerns with regards to task difficulty have now been resolved and that all games will now fall within the optimal 6-8/10 range of difficulty.

7.4.4.3 Improved clarity of instructions

Concerningly, only half of all games received an endorsement of >85% for clarity of instructions. For five of the remaining games (A Bridge Too Far, Farm Quest, Snake, Swimma and Who's the Boss), a more moderate percentage of participants (65-70%) reported that the instructions were clear. Overall, participants reported that *'having instructions built into the program would be ideal.'* This discrepancy was also noticed in observations, with considerable guidance required initially to assist participants in identifying the objectives and features of the games. This was particularly noted for A Bridge Too Far. To this effect, one of the main changes implemented across all games was the incorporation of instructions into each game's menu, rather than in a separate written booklet. Additionally, images were included in the instructions to assist in familiarising the participants with elements they encounter during game play (Figure 7.16).



Figure 7.16 – A Bridge Too Far . Before and after incorporation of built-in instructions and incorporation of image cues.

These overall changes to the instructions were well-received at follow-up sessions. For example, participants commented they had not previously recognised elements of gameplay prior to reading the new on-screen instructions, such as acorns providing extra points in

Squirrel. The addition of the explanatory pictures seemed to be a key driver of this, with one participant reporting, *‘I love the pictures in the instructions so I knew what to look for’*.

Of all games, Sunday Driver received the poorest rating of instruction clarity (22.2%), a finding corroborated by observers who needed to provide considerable guidance for players. Accordingly, for this game, instructions were incorporated to appear during game play itself, rather than solely all at the beginning. This allows the player to be guided by instructions based on their game play (e.g. if they stayed stationary, a prompt would appear instructing them of their next goal) (Figure 7.17). This leads to enhanced clarity about what is required at each stage of game play, and appeared to result in better engagement with the game during a follow-up session.



Figure 7.17– Sunday Driver. Implementation of instructions triggered by activity during gameplay in Sunday Driver.

Changes were also made to the presentation of instructions for Farm Quest. In particular, instructions were rephrased to better reflect the objectives of each level. For this game, the inability to progress through the levels was a source of significant frustration for some participants, with one commenting *‘goal seems simple enough but unable to attain it’*. Interestingly, this was the only game where participants comments attributed their difficulty in understanding what was required not to the instructions but instead to their perceived ability, with one stating, *‘problem was mainly me I feel’* and going on to state that their

dissatisfaction with the game was *'just due to my comprehending or 'not understanding the rules'*. Given that the main focus of Farm Quest is problem solving and abstract reasoning, skills not tapped into by the other games in their original iteration, this may reflect particular challenges with this cognitive domain in individuals with PD (Cronin-Golomb et al., 1994, Beatty and Monson, 1990, Young et al., 2010). As such, in order to minimise frustrations, a time-activated hint trigger was added, offering the player the option of a hint for the next move if required (Figure 7.18).

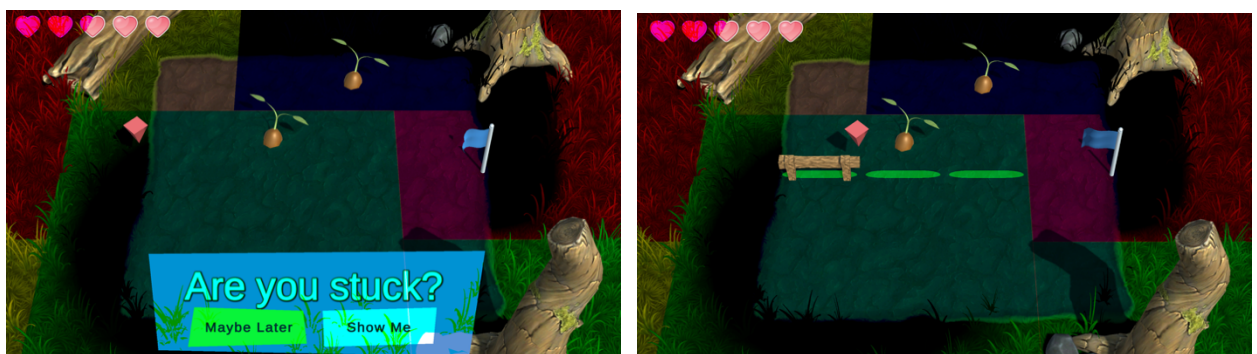


Figure 7.18 – Farm Quest. Implementation of a voluntary ‘hint’ feature in Farm Quest

For other games receiving moderate feedback on clarity of instructions (i.e. Snake, Swimma and Who’s the Boss), it was determined upon further analysis that elements of the game play itself, rather than the presentation of instructions, were likely to be driving participant confusion. In light of this, changes were made to the objectives of the game itself (Who’s the Boss) or to the controller sensitivity/play area (Snake, Swimma).

7.4.4.4 Change to game objectives

With regard to Who’s the Boss (formally Who Stole the Coin?), this was the least successful of all games in the catalogue, receiving an average enjoyment score of just 5.85/10 and a difficulty rating of 7.3/10. It received the most negative comments of all games, with participants reporting the game to be *'frustrating'*, *'hard to find a pattern'* and

'just a bit of random guesswork most of the time'. Observers also noted the game required extensive verbal instructions for all participants. Accordingly, the game received the most extensive changes based upon feedback.

Originally, the game involved continuous pairings of characters (up to 100), with assigned probabilities of being correct (e.g. 80% vs 20%) based on reinforcement learning training principles (Shiner et al., 2012). Unfortunately, participants appeared to struggle with understanding the task and, given the games are designed to be played without assistance, it was difficult to overcome this with written instructions alone. Furthermore, in order to keep the game entertaining for 6 weeks, pairings up to 100 were required; however, this proved monotonous for participants, with several reporting they were *'just guessing'*. To overcome this, the game was instead given a hierarchical structure that requires problem solving/abstract reasoning for individuals to solve, where participants must identify 'the boss' based on several exposures during the acquisition phase. The game was divided into 'stages' to incorporate an element of progression, with the number of new characters increasing by 2 with each stage (e.g., 4, 6, 8 etc.). The aim for the participant was to determine the hierarchy based on the lowest number of exposed pairings, with more coins rewarded for faster guesses. These changes appeared to be well received during follow-up, with participants verbally reporting the game was a *'better format'* and *'much better than last time'*. An additional advantage of these changes is that it allows for further training of problem solving/logical deduction, which is significant as, formerly, the only other game in the catalogue to train these abilities was perceived by participants as being one of the most challenging (100% BA; 7.36/10 difficulty rating). Problem solving/logical deduction is a commonly reported cognitive difficulties in individuals with PD (Cronin-Golomb et al., 1994), which may impact upon everyday reasoning, and, subsequently, on ability to carry out instrumental ADLs (Young et al., 2010).

7.4.4.5 Improved controller sensitivity/responsiveness

In terms of usability related to specific controls within the game, Snake was the only game to receive a below average (<2) rating (1.82/3), an attitude that was also reflected in the verbal feedback and observations of the game. This translated to poor measures of enjoyment, with only 41.7% of participants reporting that the game was enjoyable and an overall rating of just 4.83/10, the lowest for any game. Participants were not able to pass the first level and did not attempt to play the game after the first exposure. Accordingly, the controller sensitivity to movement and directional changes was optimised. Additionally, the speed of the snake was reduced, the arena was made larger, and obstacles were removed from the first 3 levels, in order to give the player a larger margin of error to make directional decisions and more time to adjust to controls. These changes were also well received, with participants reporting they '*feel it responding better*' and that the '*speed was more appropriate*'. Such refinement of controller sensitivity/responsiveness is critical, as it increases the usability of the controls to navigate gameplay in CT, a factor which is particularly relevant when considering the unique challenges posed by the motor impairments in the PD population (Mazzoni et al., 2012). As a whole, attitudes towards the changes to the gaming suite were positive, with an overall rating of changes of the games of 7.91/10.

Encouragingly, when taking participant feedback into account in our alterations to the OrbIT system, we seemed to successfully address all points raised, with 100% of respondents stating that they felt as though their feedback was addressed in the changes made. Following implementation of these changes, participants rated changes to both the controller (7.64/10) and the games (7.91/10) extremely highly. Furthermore, overall enjoyment of the system as a whole increased from 7.58/10 after session 1 to 8.18/10 at follow-up. This represents an 8% improvement in the overall enjoyment rating after

integration of feedback. As it was not possible to assess changes in ratings of individual games in terms of either the content or usability based on the data collected in this study, future co-design work could seek to assess the impact of system re-design on these measures but will need to be careful to control for learning effects and familiarity bias. Additionally, future work should assess whether these changes impact upon rating of SUS as whole, as this was not re-assessed at follow-up. Based on improvements reported in enjoyment and accessibility of the system following changes, it would be reasonable to expect that SUS would be similarly increased, inducing improved usability of the system as a whole.

Taken together, the current study has resulted in a customised “Serious Games” approach to CT, the OrbIT, optimised for use in individuals with PD and ready to deploy in subsequent trials. A significant advantage of the current work was its co-design format. This allowed us to not only to identify any potential bugs or inconsistencies in the platform, but the format allowed us to receive extensive feedback on optimal parameters of the system directly from the key stakeholders who may most benefit from its eventual use to deliver CT. This is an improvement upon current techniques in the CT field, which often use commercially available software packages or non-validated paradigms, without consultation from key stakeholders (Thompson et al., 2020).

Despite these advantages, however, there were several limitations in the current co-design trial that necessitate further research in this area. In particular, this study relied on custom-designed surveys as the main outcome measure, rather than thematic analysis of qualitative feedback received from participants. Thematic analysis could have provided a more flexible approach to the analysis of the verbal feedback received from participants, allowing for better identification of key themes for improvement (Braun 2006). This was further limited by the lack of follow-up surveys on individual game changes or alterations

in system usability, which may have led subtle persistent issues with either the hardware or software to be masked by overall improvements in the system. Finally, the small number of participants included in the current co-design may not be representative of the wider PD population. In support of this, the current cohort did not display any cognitive impairment, as detectable using the MMSE, or impaired QoL.

Thus, it is possible that the changes needed to optimise the system for use in those with established cognitive impairment, including PD-MCI or PD-D, may vary from those identified and implemented in this trial. Nevertheless, however, based on outcomes of participants' ratings of the OrbIT's content, usability and controller accessibility, the results of this co-design pilot trial provide promising feasibility to warrant use of the OrbIT gaming system in a larger trial to investigate its potential for the improvement/maintenance of cognitive function in PD.

08

General Discussion

8.0 INTRODUCTION

The premise for this thesis has highlighted the significant unmet clinical need that is cognitive impairment in PD, which represents a frequent and burdensome comorbidity for the PD population. Estimates of cognitive impairment in PD vary widely, depending on several factors, including age of the individual, motor subtype of PD, assessment method and diagnostic criteria used (Litvan et al., 2012, Monchi et al., 2016, Tremblay et al., 2013). At time of diagnosis, 15-20% of individuals with PD already meet criteria for PD-MCI (Aarsland, 2016). Concerningly, cognitive decline in PD can occur relatively quickly, with half of patients showing significant decline within three years (Muslimovic et al., 2009). In line with this, the prevalence of PD-MCI has been found to increase to 20-57% within three to five years following diagnosis (Caviness et al., 2007, Williams-Gray et al., 2007). A significant number of individuals also decline further to develop PD-D. The prevalence of dementia in PD has been found to range between 17% and 31% (Aarsland et al., 2005), and individuals with PD are up to six times more likely to develop dementia than the general population (Hobson and Meara, 2015). In fact, within 8 years of diagnosis, up to 78% of individuals may qualify for a diagnosis of PD-D (Aarsland et al., 2003).

Cognitive impairment in PD can also be a labile state. A recent systematic review and meta-analysis of over 4,000 patients across 39 studies reported that 21% of individuals with PD-MCI converted to dementia across a three-year period; however, 28% reverted back to normal cognitive function (Saredakis et al., 2019). Given this, the early targeting of cognitive impairment in PD may be able to restore individuals back to a cognitively unimpaired state. This is particularly significant, as cognitive impairment in PD is known to be a major predictor of quality of life for both the individual (Duncan et al., 2014, Leroi et al., 2012) and their caregiver (Kudlicka et al., 2014, Jones et al., 2017). Cognitive impairment is also

a risk factor for early mortality in PD (Oosterveld et al., 2015). These effects appear to be severity dependent, with those with more severe cognitive impairment exhibiting poorer quality of life as measured by the PDQ-39 (Lawson et al., 2014a). Interestingly, however, these effects appear to be independent of the nature of the cognitive impairment itself, with similar reductions in quality-of-life scores seen across all subtypes of PD-MCI (Lawson et al., 2014b).

8.1 PHARMACOLOGICAL INTERVENTION

Given the prevalence and significant burden of cognitive symptoms for individuals with PD and their caregivers, treatment options are critically needed. In order to assess the current therapeutic landscape, a critical review of the literature was conducted, with its results presented in **Chapter 1**. Concerningly, from that review, it was clear that treatment options for cognitive impairment in PD remain limited both in their ability to address pathological disease progression and their efficacy to treat the diverse range of cognitive symptoms seen in the disease. Whilst levodopa is considered the ‘gold-standard’ treatment for PD, there are side effects associated with its long-term use, in addition to a potential paradoxical worsening of cognitive function (Schneider et al., 2013, Poston et al., 2016). The only currently approved therapeutic strategy for cognitive impairments in PD are cholinesterase inhibitors, which demonstrate mixed efficacy and may paradoxically worsen motor symptoms (Collins-Praino et al., 2011). This may, in large part, be due to the fact that cognitive impairment goes beyond alterations to the dopaminergic or cholinergic systems alone (Halliday et al., 2014). While some strides have been made in recent years to develop treatment strategies targeting the other neurotransmitter systems affected in PD, as discussed in **Chapter 1** of this thesis, these have also fallen short. Of those trialled to date, the most

promising appears to be selective reuptake inhibitors of norepinephrine, such as atomoxetine, but results have of these trials have been mixed, with some reporting positive outcomes (Kehagia et al., 2014, Marsh et al., 2009) while others failed to find any benefit (Ghosh et al., 2020, Hinson et al., 2017, Marsh et al., 2009, Weintraub et al., 2010b). Furthermore, significant adverse events have been reported with use (Hinson et al., 2017, Marsh et al., 2009).

Beyond even these significant limitations, however, the most significant disadvantage of pharmacological treatment options developed to date is the fact that they aim to treat the symptoms themselves, rather than attempting to modify the underlying pathophysiology leading to cognitive decline. Memantine, an NMDA antagonist, was originally proposed to represent a potential disease-modifying therapeutic based on results from preclinical work (Ferro et al., 2007). However, despite some beneficial effects for the treatment of cognitive impairment in PD (Aarsland et al., 2009, Litvinenko et al., 2010, Wesnes et al., 2015, Meng et al., 2019), deterioration in global cognitive function has been shown to occur if discontinued (Leroi et al., 2009), suggesting that memantine is not capable of modifying underlying disease pathology. While new therapeutics targeting alternative pathways, such as the adenosine A2A antagonist istradefylline, have been proposed to have potential benefits for cognitive dysfunction in PD (Torti et al., 2018), these are still unlikely to be disease modifying. Other classes of drugs, such as glycogen synthase kinase-3 inhibitors, which have been shown to ameliorate cognitive deficits in multiple animal models of CNS disorders, including PD, are still in their infancy (King et al., 2014) and it is unclear if these will proceed further to clinical trials or modify underlying pathophysiology. Accordingly, the aim of this thesis was to address this significant gap in the literature by developing and assessing both a novel pharmacological and a non-pharmacological approach to the treatment of cognitive decline in PD.

8.1.1 Fyn in Parkinson's Disease: Potential implications for cognitive impairment

Following a critical review of the literature, Fyn kinase was selected as a potentially disease-modifying novel therapeutic strategy for PD. To provide justification for this decision, **Chapter 3** reviewed the role of Fyn kinase in CNS processes, specifically the function Fyn may play in the pathological mechanisms associated with neurodegenerative diseases. This highlighted the role of Fyn in myelination and the evidence of Fyn dysfunction in influencing pathological processes associated with multiple sclerosis, such as demyelination and T-cell signalling (Zhang et al., 2008, Yang et al., 2016, Ueda et al., 2012). The potential of Fyn kinase inhibition for the treatment of AD was also explored, given the role of Fyn in pathways associated with the hallmark pathological proteins of AD, A β (Poulsen et al., 2017) and Tau (Larson et al., 2012, Li and Götz, 2017), in addition to the role of Fyn in NMDA dysfunction and excitotoxicity (Um et al., 2012). Most pertinent to this study, we reviewed the potential role of Fyn kinase in the pathophysiology of PD. Specifically, Fyn kinase has recently been shown to play a key role in inducing the neuroinflammatory processes observed in PD.

Seminal studies by Panicker and colleagues identified Fyn was required for pro-inflammatory cytokine release in cell culture and animal models of PD via the PKC δ signalling pathway and activation of microglia (Panicker et al., 2015). Subsequently, Fyn was also shown to play a role in priming and activating the NLRP3 inflammasome in response to the binding of α -syn to microglia, leading to upregulation of the inflammatory response (Panicker et al., 2019). As neuroinflammation has been shown to play a potentially key role in the pathogenesis of PD, leading to the death of DA neurons (see (Tansey and Goldberg, 2010) for review), kinase inhibition may therefore represent a novel, disease-modifying therapeutic strategy for PD. What is less clear, however, is the role that Fyn kinase may play in the emergence of cognitive impairment in PD.

Overexpression of Fyn has been shown to be sufficient to induce both synaptic and cognitive deficits in the hAPP transgenic model of AD (Chin et al., 2005). This may be because, as outlined in **Chapter 3**, Fyn plays a critical role in processes known to be implicated in the pathophysiology of cognitive dysfunction in AD, including NMDA dysfunction (Um et al., 2012) and binding of prion protein to pathological oligomers of proteins, such as A β (De Mario et al., 2019, Nygaard, 2018). In recent years, Fyn inhibition has been shown to improve cognitive function in multiple preclinical models of AD (Tang et al., 2020, Kaufman et al., 2015), as well as following status epilepticus (Luo et al., 2021), interventricular haemorrhage (Liu et al., 2017) or traumatic brain injury (Liu et al., 2014). Palmitoylated Fyn expression was also recently found to be increased in the frontal cortex of aged mice, with these increases associated with impairments in reference memory/executive function, suggesting that Fyn may play a critical role in age-related cognitive decline more broadly (Zamzow et al., 2019). Thus, it is reasonable to hypothesise that Fyn may also play a role in the cognitive impairment observed in PD. To date, to the best of our knowledge, the relationship between Fyn and cognitive impairment in PD has not been assessed. In spite of this gap in the literature, given the role that inflammation plays in cognitive dysfunction in PD (Zhang et al., 2021, Magistrelli et al., 2020, Sanjari Moghaddam et al., 2018), coupled with the key role that Fyn plays in the regulation of the inflammatory response in PD (Panicker et al., 2015, Panicker et al., 2019) it is reasonable to posit that any beneficial effects on cognition obtained through targeting Fyn would occur due to reductions in the inflammatory response. These hypotheses formed the basis of the investigations outlined in **Chapters 4 and 5**.

Investigations in **Chapter 4** assessed the distribution and activation state of Fyn in the post-mortem human PD brain. Specifically, Fyn distribution in areas commonly associated with neurodegeneration and cognitive dysfunction in PD were chosen for

assessment, including not only the striatum and SN, but also the prefrontal cortex and hippocampus, two regions of particular relevance for cognitive function. This study observed marked increases in Fyn phosphorylation compared to total Fyn levels in the striatum and SN of post-mortem PD brains compared to age-matched controls. The findings corroborate and build upon observations of Panicker and colleagues (2019), who reported similar upregulation in the SN in the brains of individuals with PD compared to controls (Panicker et al., 2019). Interestingly, however, this pattern was not observed in either the prefrontal cortex or the hippocampus, with no significant differences found between PD and controls.

This may potentially be related to differences in anatomical/pathological disease progression with relation to disease stage. In particular, PD is primarily associated with loss of DA neurons in the SNpc, which has extensive efferent projections to the striatum, forming the nigrostriatal pathway (Freund et al., 1984, Pollanen et al., 1993). This progression is supported by accompanying pathologies, with aggregated α -syn (Lewy Body) and microglial-mediated neurodegeneration noted to follow a similar pattern of degeneration (Braak et al., 2004) (reference for microglia). Together, these are typical of early-mid PD, corresponding with stages II-III of the Braak staging hypothesis. Conversely, pathologies and disruptions in the prefrontal and hippocampus are more typical of later stages (IV/V and VII), where cortical spread is evident (Braak et al., 2004). It is not until these later stages of LB spread that cognitive impairment emerges (Braak et al., 2005). As all cases included in the current analysis had a pathologist confirmed diagnosis of PD, based upon dopaminergic cell loss in the SNpc, it can be inferred that all cases would be classified as at least stages II-IV in the Braak model. However, information on Braak staging model was unfortunately not available from existing pathology reports. Consequently, this may indicate a skewing of the sample population towards the earlier stage of disease at time of death, without significant

involvement of either the hippocampus or prefrontal cortex. In order to probe this further, future work should assess α -syn phosphorylation levels, as well as the presence of Lewy body pathology, within these regions. Nevertheless, the findings of the current work do support the pathological upregulation of Fyn activation in PD within at least some key brain regions, although whether this is a driver or consequence of disease pathology more broadly remains to be investigated.

Interestingly, despite evidence of the role of Fyn kinase in the upregulation of the inflammatory response in pre-clinical PD models (Panicker et al., 2015) there was no increase in markers of neuroinflammation, namely astrocyte or microglial activity, in any of the regions assessed. This held true even in the striatum and SNpc, despite upregulation of Fyn kinase activity in these regions. Whilst there is support for this in the literature (Tong et al., 2015, Mirza et al., 2000, Kouli et al., 2020), the lack of neuroinflammation may also be related to limitations associated with post-mortem tissue, as well as the semi-quantitative techniques used to assess levels of astrogliosis and microglial activation in this study. Furthermore, although the study used age-matched controls, given the nature and difficulties associated with obtaining ‘neurologically healthy’ post-mortem tissue, the control samples available may have exhibited increased levels of neuroinflammation and may therefore not truly be representative of the control population. Given these limitations, neuroinflammation cannot be ruled out entirely as the driver of increased Fyn phosphorylation in the striatum and SN. In order to assess this further, future work should investigate other markers of inflammation not included in the current study, such as IL-1 β , which has been shown to be involved in both the Fyn/PKC δ and Fyn/NLRP3 inflammasome signalling pathways (Panicker et al., 2015, Panicker et al., 2019). Other alternative pathways, including interactions between Fyn, prion protein and NMDA receptor phosphorylation, which will be discussed in more detail below, should also be assessed. Encouragingly, however, despite

remaining questions involving potential mechanism, this study further supported the potential utility of Fyn kinase as a novel therapeutic target for PD. Accordingly, **Chapter 5** investigated the potential of a Fyn kinase inhibitor to lead to improvements in both motor and non-motor symptoms in the striatal 6-hydroxydopamine (6-OHDA) model of PD, as well as the mechanisms via which such improvements may occur.

8.1.2 AZD0530 (Saracatinib) as a novel therapeutic strategy for PD

AZD0530 is a novel Fyn kinase inhibitor which has previously been shown to lead to significant improvements in a mouse model of AD, rescuing spatial memory impairments and synapse loss (Kaufman et al., 2015). These disease-modifying improvements persisted, even following drug washout, an effect that was not seen for memantine (Smith et al., 2018) and that was replicated in a subsequent study (Toyonaga et al., 2019). More recently, AZD0530 has also been shown to improve cognitive function following status epilepticus in mice (Luo et al., 2021). Excitingly, preliminary results suggest improvements with AZD0530 in human AD clinical trials (Clinical Trial Identifier: NCT01872598). Despite these positive findings, however, to date the efficacy of Fyn kinase inhibition had not been investigated in a preclinical model of PD.

To address this gap, the striatal 6-OHDA rodent model of PD was utilised to investigate whether four weeks of AZD0530 treatment would lead to improvements in motor, cognitive or neuropsychiatric outcomes. As the focus of our study was primarily on understanding the effects of Fyn kinase inhibition on non-motor impairments in PD, we purposely chose a model and 6-OHDA dose that would result in a mild dopamine depletion (20-40%), in order to not confound cognitive and neuropsychiatric assessment with motor impairment. Thus, as expected, there were no impairments observed in motor function at earlier time points, given that, from the clinical literature, a loss of approximately 60-80%

of the dopamine content of the striatum is necessary before motor symptoms become apparent (Cheng et al., 2010). Nevertheless, progressive loss is a feature of the striatal 6-OHDA model and a significant decrease in involuntary locomotion was observed between controls and vehicle-treated animals by one-month post-injection. This was improved with AZD0530 treatment (6mg/kg), indicating that progressive motor dysfunction may be treatable with Fyn inhibition. In order to fully assess the effects of AZD0530 on motor function, however, future investigations in models designed to highlight motor dysfunction, such as medial forebrain injection of 6-OHDA, are necessary.

Excitingly, with regards to cognitive and neuropsychiatric impairment, animals receiving 6mg/kg and 12mg/kg showed significant improvement in recognition memory and a reduction in depressive-like behaviour. This suggests that AZD0530 may have particular utility for treating the non-motor symptoms of PD, currently a significant clinical need. Given that AZD has been shown to be safe and well-tolerated in a phase 1b multiple ascending dose study in AD (Nygaard et al., 2015), this may allow for a pathway to rapid translation to the clinic. Prior to this, however, a number of confirmatory additional investigations are warranted, including in alternate models of PD and with a cognitive battery more targeted to detect the types of impairment commonly seen in PD, such as executive dysfunction. Greater insight into the mechanisms of action that may lead to improvements in non-motor function may also help to further refine therapeutic targeting.

Pathologically, a significant decrease in DAergic innervation, as measured by TH-staining, and an increase in inflammatory response, as measured by microglial activation, was observed within the striatum following 6-OHDA injection. Similar increases in microglial activation were not observed within the striatum within the human PD brain in that study, which used similar techniques. This may reflect a divergence between the neuroinflammatory response in rodent models of PD compared to idiopathic PD. It may also,

however, be a reflection of the limitations associated with the investigation of post-mortem tissue outlined above, or a response to injection of the neurotoxin itself. Advances in neuroimaging, as well as in the preclinical modelling of PD, may help to answer some of these questions. For example, the TSPO ligand, [18F]-GE180, is making it increasingly possible to measure microglial activation *in vivo* using PET imaging in both pre-clinical models (Liu et al., 2015, Brendel et al., 2016, Nack et al., 2019) and patient populations (Fan et al., 2016). This may make it possible to overcome current limitations associated with post-mortem assessment of neuroinflammation, although concerns about the use of [18F]-GE180 have been expressed (Zanotti-Fregonara et al., 2019). Similarly, the development of new animal models of PD, such as the preformed fibril model, which more closely replicate the pathology of the disease, may be more translationally relevant, allowing for more appropriate comparisons to individuals with PD (Carta et al., 2020, Chung et al., 2020).

Surprisingly, in contrast to our hypothesis, Fyn kinase inhibition did not lead to reductions in either astrocytic reactivity or microglial activation in the striatum. This seems to suggest that changes in Fyn activation may occur without necessarily altering neuroinflammation. This is in line with our findings in **Chapter 4**, where there was significant up-regulation of Fyn activation in the striatum, without a concomitant increase in markers in neuroinflammation. This is, however, in contrast to the existing literature, which suggests that Fyn activation is a key upstream regulator of neuroinflammation (Panicker et al., 2015). In order to investigate this further, future work will seek to characterise Fyn kinase activation and its anatomical relation to markers of neuroinflammation in the rodent brain, both under baseline conditions, as well as in pre-clinical models of PD. It does, however, raise the possibility that the beneficial effects of Fyn kinase inhibition for cognitive and neuropsychiatric impairment observed in this study may be occurring through an alternative mechanism to neuroinflammation. This would be

in line with our critical review of the literature presented in **Chapter 3**, which highlighted the diverse role of Fyn in the CNS. Of the potential candidates raised in that chapter, the ones with the most relevance for the cognitive and neuropsychiatric benefits seen with Fyn inhibition are likely the interaction between Fyn and cellular prion protein and the subsequent relevance of this for NMDA receptor phosphorylation. In support of this, extracellular α -syn oligomers have recently been shown to form a complex with cellular prion protein, leading to phosphorylation of Fyn kinase (Ferreira et al., 2017a, Brás et al., 2018). This, in turn, leads to hyperphosphorylation of the NMDA receptor, altering calcium homeostasis and leading to synaptic dysfunction and impairments in LTP. Importantly, blocking phosphorylation of the NMDA receptor in an α -syn transgenic mouse model rescued both synaptic and cognitive deficits (Ferreira et al., 2017b). This suggests that Fyn inhibition may likewise act through these mechanisms, supporting the use of Fyn kinase inhibitors, like AZD0530, as disease-modifying agents to slow or even prevent the onset of non-motor symptoms in PD, including cognitive impairment.

Taken together, the investigations in **Chapters 3-5** proposed and investigated the potential of Fyn as a novel intervention for cognitive impairment in PD, which may target an aspect of the underlying disease pathophysiology. In spite of preliminary beneficial effects in our early-stage rodent model of PD, however, it is important to keep in mind that idiopathic PD may be quite different. Unfortunately, by the time of diagnosis, PD patients have already lost ~80% of their striatal DA and ~60% of their SNpc DA neurons (Dauer and Przedborski, 2003), suggesting that significant neurodegeneration and subsequent anatomical changes have already taken place. Accordingly, even disease-modifying therapies may have limited benefit at this stage of pathology. Thus, in order to maximise therapeutic potential, it will be necessary to identify biomarkers and/or better indicators of prodromal signs of PD in order to enable early identification of risk, allowing people to begin

treatment prior to catastrophic levels of neurodegeneration. Unfortunately, research suggests that this may be significantly hampered by the heterogeneity of the clinical and biochemical presentation of the disease (Gramotnev et al., 2019). This highlights the need for non-pharmacological interventions which may be able to delay the onset of cognitive impairment in PD and/or reduce the rate of progression of MCI to dementia. For example, associations have been made between the rate and severity of decline and multiple individual factors, including healthy lifestyle, weight, nutrition, metabolic syndromes and cardiovascular risk factors (Martín-Fernández et al., 2015). This raises the possibility that targeting these factors may be able to slow or even prevent the onset of dementia in PD. In line with this, a recent report on modifiable risk factors associated with the development of dementia, including lower education, hypertension hearing impairments, smoking, obesity, depression, physical inactivity, diabetes, low social contact, alcohol consumption, traumatic brain injury and air pollution, suggested that targeting such factors could prevent a stunning 40% of cases of dementia worldwide (Livingston et al., 2020). Thus, in addition to exploring a novel pharmacological strategy for the treatment of cognitive impairment in PD, the second part of the thesis worked to scope and develop a novel non-pharmacological “Serious Games” approach.

8.2 NON-PHARMACOLOGICAL INTERVENTION

In order to identify the most appropriate approach to target, it was first necessary to critically review the literature on non-pharmacological strategies designed to target cognitive impairment in PD. We identify several such strategies in **Chapter 1**, including diet, exercise, non-invasive brain stimulation and cognitive training (CT). With regards to diet, evidence for the potential of diet manipulation and supplementation to improve cognitive function is

still progressing and there is a significant lack of studies investigating their efficacy in PD-MCI and PD-D populations specifically. Additionally, implementation and adherence to the strict diets likely needed to achieve maximal benefits for cognitive dysfunction remain a significant barrier to this approach. This was a similar case for aerobic exercise, with efficacy for the motor symptoms of PD well-established (Ascherio and Schwarzschild, 2016); but investigations assessing cognitive improvements limited (Nocera et al., 2010, Ridgel et al., 2011, Tanaka et al., 2009, Cruise et al., 2011). Despite this, investigations to date do provide at least preliminary evidence for efficacy, which may persist long-term (David et al., 2015); however, assessment in PD-MCI and PD-D specifically are lacking, as these individuals are often excluded from clinical trials (Inskip et al., 2016), making it difficult to fully assess potential benefits of exercise for cognitive function. Finally, while non-invasive brain stimulation techniques also appear to be efficacious (Khedr 2003,2006; Boggio 2006; Pereira et al 2013; Mally 2017) there is significant variability with regards to stimulation techniques, location targets and parameters, such as frequency and voltage, which makes it difficult to ascertain the full extent of benefits. In light of these limitations, CT may be one of the most promising non-pharmacological approaches to target cognitive impairment in PD.

8.2.1 Cognitive Training in PD

Accordingly, **Chapter 6** involved a detailed review to assess the potential of CT to treat cognitive dysfunction in PD. CT offers the significant advantage of being able to target cognitive processes directly through structured practice on specific tasks, rather than attempting to influence cognitive function indirectly through exercise or diet. Overall, while interpretation should be cautious given the small number of studies conducted to date, as well as the small number of participants and heterogeneity of design between studies, we

conclude that the literature appears to support the use of CT in PD. In fact, of the 25 studies included in our analysis, significant improvements were reported on at least one outcome measure in 23/25 studies (92%), with trends towards improvement, which did not reach statistical significance, in the remaining two studies (Pompeu et al., 2012, Fellman et al., 2020). This is in line with a previous meta-analysis of the literature, which found a small, but statistically significant, benefit of CT for cognitive impairment in PD, with particular improvements noted in several specific cognitive domains, including working memory, executive function and processing speed (Leung et al., 2015).

It is in contrast, however, with a recent Cochrane review, which found no evidence of benefit of CT either for global cognitive function or on measures of specific domains (Orgeta et al., 2020). It is important to note, however, that the inclusion criteria for the Cochrane review were very strict, encompassing only 7 studies and representing a total sample size of just 225 participants. This in contrast to our review, which cast a wider net and ultimately included 25 studies, conducted between 2004 and 2020. representing a total sample size of 1,046 individuals with PD and 162 healthy controls. Additionally, in contrast to the Cochrane review, which included only individuals with PD-MCI or PD-D, our review looked at the benefits of CT for the PD population as a whole, with only 9/25 (36%) focusing exclusively on individuals with either PD-MCI or PD-D (although 13/25 studies (52%) included individuals with either PD-MCI or PD-D). This is significant, as cognitively normal individuals may be most likely to derive benefit from CT, with the aim being to prevent the development of cognitive dysfunction, rather than restoring what has already been lost.

Despite these promising findings, however, key questions remain. In particular, it is still unclear whether long-term benefits for cognitive function can be obtained through the use of CT. Of the studies included in the current analysis, only 6/25 (24%) included a long-term (>6 months post-intervention) follow-up testing point. Of these, the majority, 4/6 (67%)

had a follow-up at 6-months post-intervention (Alloni et al., 2018, Bernini et al., 2019, Reuter et al., 2012, Sinforiani et al., 2004). Three of these (Alloni et al., 2018, Reuter et al., 2012, Sinforiani et al., 2004) reported that improvements were maintained at this 6-month follow-up, although not consistently across all measures, and one study reported no maintenance of benefits (Bernini et al., 2019). An additional study investigating the NEUROvitalis program looked one-year post-intervention and found not only that improvements in cognitive function had been maintained at this long-term follow-up, but also that individuals who had engaged with NEUROvitalis had a reduced risk of developing MCI (Petrelli et al., 2015). Similarly, the study with the longest follow-up period to date (18 months post-intervention) actually reported improvements in visual memory and executive function compared to immediately post-intervention, although no changes were seen in the other domains assessed (Díez-Cirarda et al., 2018). While the results of these studies do seem to suggest that CT can have lasting benefits for cognitive function in PD, further assessment, particularly at longer timepoints post-intervention are warranted. Additionally, as practice effects can be observed following repeated exposure to the same neuropsychological tests, this needs to be taken into account when designing follow-up assessment of cognitive function, in order to prevent unfairly biasing results.

In addition to evaluating the long-term utility of CT in PD, it is also necessary to assess whether there is transfer of benefits to other domains of cognitive function or, more notably, to improvements in everyday life. To date, this literature has been limited, with many studies not including measurements of transfer, quality of life (QoL) or functional disability. Of the 25 studies reviewed for this thesis, only five (20%) included a measure of functional disability. Of these, four (16%) reported reduced functional disability with CT (Díez-Cirarda et al., 2018, Lawrence et al., 2018, Pena et al., 2014, Pompeu et al., 2012), with one further reporting that they were not maintained at 18-month follow-up (Díez-

Cirarda et al., 2018). In contrast, one study reported no benefit on ability to complete ADLs with ReSET neurorehabilitation sessions (Vlagsma et al., 2020). With regards to QoL measures, 8/25 (32%) studies included a specific measurement, with the majority reporting no effect on QoL following CT (Bernini et al., 2019, Biundo et al., 2015, Cerasa et al., 2014, Folkerts et al., 2018, París et al., 2011, Petrelli et al., 2014, Vlagsma et al., 2020). Only one study reported improvements in QoL following both standard and tailored CT, with tailored CT resulting in the maintenance of these benefits at follow-up five weeks post-intervention (Lawrence et al., 2018). Taken together, this seems to suggest that CT may lead to increased ability to complete everyday tasks, at least in the immediate aftermath of the intervention, but that these benefits do not translate to significantly improving QoL for individuals. With regards to transfer of benefit, to the best of our knowledge, only one study has investigated this to date, reporting near transfer of skills to similar cognitive tests, but not far transfer to other cognitive domains (Fellman et al., 2020).

Given the paucity of the literature, particularly with regards to long-term benefits and transfer of skills, we have put forth a number of specific recommendations that should be considered when designing CT for the PD population, in order to maximise the potential for the individual to derive a benefit from CT. The first of these is the use of computer-based CT over traditional paper-and-pencil based methods, in order to reduce reliance on the fine motor skills associated with handwriting. This is a particularly important consideration for the PD population, given the impairments in handwriting associated with the disease. In support of this, one case control study reported that micrographia and other related impairments are present in at least half of all individuals with PD and correlate with severity of cognitive dysfunction (Wagle Shukla et al., 2012).

In addition to being more accessible to those with motor impairment, computer technology also has the ability to tailor delivery for the individual patient, another of our

recommendations for the use of CT in individuals with PD. For example, programs such as NEAR (Naismith et al., 2013) and Smartbrain Pro (Lawrence et al., 2018) are able to assess cognitive function, identify impairments and tailor programs accordingly for an individual. This means that difficulty level can be adjusted based on the individual's progression. These are vital considerations given the significant heterogeneity observed in the presentation and progression of CI in PD. Furthermore, recent evidence has suggested that adaptability, where cognitive challenge can be sustained for the individual, is necessary in order to derive maximal benefit from CT (Lövdén et al., 2010).

Other recommendations derived from our review of the literature include the use of group-based, rather than individual or home-based training, in order to increase compliance with the program (Lampit et al., 2014, Naismith et al., 2013, Pena et al., 2014), as well as refinement and standardisation of testing batteries, in order to maximise the likelihood of detecting cognitive benefit and allowing for direct comparisons between studies. Finally, one of the major benefits of CT is the ability to integrate the program with other interventions. For example, patients can maintain normal medication regimes, in addition to diet and aerobic exercise program, while participating in a CT program. Additionally, CT can be paired with non-invasive brain stimulation in order to potentially obtain synergistic benefits. In support of this, combining CT with exercise have demonstrated improvements beyond those associated with exercise alone (Bernini et al., 2019), with similar results for CT with non-invasive brain stimulation (Lawrence et al., 2018). Nevertheless, even when taking into account the specific recommendations outlined above, significant barriers to the uptake of CT in individuals with PD may remain.

Of these, one of the most significant may be accessibility of the system itself, given the motor impairments associated with the disease. Whilst the move to more computer-based programs, in line with one of our recommendations above, may address some of these

barriers, nearly 80% of PC-users with PD still struggle to use a computer due to the motor impairments of the disease (Nes Begnum, 2010). This may be further complicated by a lack of engagement with the CT system itself, due either to boredom with or dislike of the CT paradigm itself or to the apathy and decreased sensitivity to reward that is a common part of PD (Muhammed et al., 2016). In fact, a meta-analysis across 23 studies reported that the prevalence of apathy in PD is nearly 40% (den Brok et al., 2015). This is particularly significant, as engagement may be a key predictor of the success of a cognitive intervention (Harvey et al., 2020). From our critical review of the literature, it was clear that the majority of studies to date have assessed the efficacy of programs designed for CT in general, rather than modifying these programs to meet the unique needs of individuals with PD.

8.2.2 OrbIT Development

To address these limitations, we set out to solve a two-pronged problem – a cognitive training system that is both more accessible and more engaging. In order to achieve this, we drew upon principles from “serious games,” incorporating practical targeting of the cognitive domains frequently affected in PD along with entertaining elements, designed to keep the user invested in each game (Laamarti et al., 2014, Alvarez, 2008). Such gamification may be associated both with increased motivation to engage with CT, as well as with improved adherence to the CT regimen (Lumsden et al., 2016). Serious games have previously been proposed as a novel method to deliver CT in Alzheimer’s disease (Imbeault et al., 2011, McCallum and Boletsis, 2013). Additionally, they may have utility for assessing cognitive function in Alzheimer’s disease (Leduc-Mcniven et al., 2018, Manera et al., 2015); with one study indicating that having participants complete a series of virtual-reality based daily living tasks can be used to distinguish between patients with Alzheimer’s disease and healthy controls (Vallejo et al., 2017). Similar benefits have also been proposed for PD. In

support of this, a systematic review of the literature identified six studies that had used the Wii in order to deliver exergaming in PD, with all reporting beneficial effects for balance and motor symptoms (Barry et al., 2014). Serious games may also have utility beyond improving motor function, however. For example, a music-based serious game, Rhythm Workers, was able to be successfully used for at-home training of rhythm skill in individuals (Dauvergne et al., 2018). Similarly, recently, the Parkin'Play study showed enhanced global cognition scores after 24-weeks of participating in a home-based, gamified CT intervention (Van De Weijer et al., 2019).

The development of our novel serious gaming system, OrbIT, is outlined in **Chapter 7**. The OrbIT system is comprised of several features designed and integrated specifically for use with individuals with PD. In terms of features of the hardware which help to minimise the confounding motor impairments of PD, the controller itself is rounded, allowing for bimanual control; it has grip pads on either side to enhance ease of use; and selections can be made using a large red button, eliminating the need to navigate or double click using a mouse, which requires a high degree of fine motor control. Importantly, in order to account for tremor, the sensitivity of the controller can be adjusted for the individual to recognise only purposeful movements. Finally, the controller includes sensory haptic feedback triggered by actions during game play, which may enhance hand-to-brain coupling. While Orby has previously been trialled successfully in individuals with hand impairments due to disability, such as cerebral palsy and in adults post-stroke (Hobbs, 2019), this was the first study to trial the controller in individuals with PD. In addition to optimising the controller, we custom-designed a novel gaming suite of 12 video games, developed in order to target the most common cognitive impairments reported in PD. In line with this, the majority of games targeted aspects of either executive function, including attention, working memory, cognitive flexibility/set-shifting, problem solving/abstract reasoning and response

inhibition, or visuospatial function. We believe that this coupling of novel elements to both the hardware and software represent a significant improvement on other CT systems developed to date, allowing us to target the areas of cognitive function that present the most concern for individuals in an accessible and highly engaging way. This makes us well-placed to obtain maximal benefit from use of the system to deliver CT in individuals with PD.

While well-grounded in the scientific literature, as well as in design principles, however, there may be elements required for accessibility and engagement that would only be apparent to someone actually living with PD. In light of this and in order to maximise the potential benefits of the system for those who will actually use it, we decided to engage in a reiterative co-design process, in collaboration with key stakeholders at Parkinson's SA. This approach is in line with current best practice guidelines for the design of interventions for use in patient populations, with such a cooperative approach anecdotally reported to lead to more effective services and better outcomes for patients (although more rigorous assessment of outcomes and cost benefit analysis is needed) (Clarke et al., 2017). Co-design has been successfully used in many healthcare indications. In PD specifically, co-design has been used to design eHealth services (Revenas et al., 2018), collaborative care (Kessler et al., 2019), and even smart home technology (Bourazeri and Stumpf, 2018). Most recently, and highly relevant to the current work, co-design has been used to put forth recommendations for the design of a personalised gaming suite for use by individuals with PD (Dias et al., 2020) Within our own co-design process, participants completed 3 x 55-minute supervised sessions with the OrbIT program, with survey outcomes collected for initial feedback, as well as detailed feedback regarding controller, gaming and overall system after completion. These data were assessed in conjunction with verbal feedback noted by observers throughout each session.

Overall, while the OrbIT system was viewed favourably by participants (with nearly a 92% initial enjoyment rating), accessibility of the system was rated as only moderate, as evidenced by a score of 6.25/10 for difficulty of the games and only 5.83 for usability of the controller. In probing this further, several key themes for improvement emerged throughout the co-design process. First, was the *ergonomics of the controller* itself, in order to reduce fatigue when using the system. This is particularly relevant in PD, where fatigue has a reported prevalence of 50% in the population (Siciliano et al., 2018). Fatigue may also be a barrier for computer use in motor impaired populations (Kouroupetroglou, 2014). Secondly, the *difficulty level of several tasks needed to be re-calibrated*, in order to bring all tasks within the ideal difficulty range of 6-8 on a ten-point scale. This allows for a balance between keeping the task difficult enough to remain cognitively challenging, thereby leading to more significant gains (Lövdén et al., 2010) without becoming so challenging that individuals choose to not engage with it, given that the effort cost outweighs the benefits (Buchman et al., 2014). The *clarity of the instructions* and the *responsiveness of the controller* also needed to be adjusted, in order to improve user experience of the system. Finally, in the case of one game (Who's the Boss?), a *change in game objective* was required, as participants found the game confusing, overly difficult and not enjoyable to play. This re-pitch of the game had the added benefit of allowing us to further train problem solving and abstract reasoning, a domain that was not previously extensively covered by the gaming suite, but which represents a significant area of concern for individuals with PD (Cronin-Golomb et al., 1994).

Given the issues identified above, we were able to make significant alterations to the system. The effect of these changes was evaluated in a final 55-minute session of gameplay, followed by the opportunity for additional feedback. Overall, participants were pleased with these changes, with changes to both the controller (7.64/10) and gaming suite (7.91) rated

highly. Significantly, all participants reported feeling as if their comments were addressed and overall enjoyment of the system increased 8% compared to initial ratings. Without consultation with key stakeholders in a reiterative co-design process, many of the issues identified would have been missed. This could have had a catastrophic impact on any clinical trials, as issues with the games themselves (e.g. understanding what the objective is) or with the hardware (e.g. navigating the controller or avoiding fatigue) could have negatively impacted affected engagement with the system or even successful completion of the trial. This, in turn, may have confounded the assessment of effects on cognitive function, potentially masking any benefits derived from the OrbIT. Instead, we are now well-placed to proceed to a large-scale clinical trial using OrbIT to deliver customised CT in individuals with PD. While it ultimately remains to be determined if OrbIT will result in cognitive benefits for individuals with PD, or whether such benefits will last or transfer to everyday tasks of daily living, this process nevertheless illustrates the importance of co-design and appropriate consultation of key stakeholders when designing future therapeutic benefits.

Taken together, **Chapter 6 and 7** provide preliminary support for the use of CT as a non-pharmacological treatment approach for PD, and raises the possibility that serious gaming may play a key role in the design of a system optimised to deliver maximal benefits, which may be long-lasting and lead to improvements in cognitive function for individuals with PD.

8.3 CONCLUSION

More broadly, this thesis as a whole has explored the current treatment landscape for cognitive impairment in PD, and has highlighted both a novel pharmacological and non-pharmacological treatment strategy for this debilitating non-motor symptom of PD. Current

treatment strategies focus mainly on the use of dopamine replacement therapy, which has mixed effects for cognitive function, and cholinesterase inhibitors, which do little to improve cognitive symptoms and may actually worsen motor symptoms associated with the disease. With improvements in neuroimaging and other techniques, it may become possible to mitigate some of these risks and predict who is most likely to derive benefit from a given therapeutic. In support of this, the integrity of the locus coeruleus, the location of noradrenergic cell bodies within the CNS, was recently demonstrated to predict benefit of atomoxetine (O'Callaghan et al., 2021). It is important to keep in mind, however, that such a strategy will still require significant development and validation before it will have clinical utility. Additionally, neither the treatment strategies in use, nor those currently in clinical trials, do anything to modify the underlying pathophysiology that leads to the emergence of cognitive impairment in PD. Thus, a new approach is needed.

Given its diverse actions in neurological function, and its purported role in neurodegenerative disease, including PD, Fyn kinase may represent an ideal treatment target. This thesis has provided a rationale for the targeting of Fyn and has explored the distribution of Fyn within the parkinsonian brain. In line with previous literature (Panicker et al., 2019), Fyn was upregulated in both the striatum and substantia nigra in human post-mortem tissue. While similar increases weren't seen in either the PFC or hippocampus, areas that are key for cognitive function, this nevertheless provided a justification for further exploring the effects of Fyn kinase inhibition in an early-stage model of PD. Fyn kinase inhibition using AstraZeneca's AZD0530 (Saracatinib). Treatment with AZD0530 for one month was able to significantly improve progressive impairment in volitional locomotion, as well as reference memory and depressive-like behaviour, in our model. Interestingly, however, contrary to existing literature (Panicker et al., 2015), this did not seem to occur through actions on neuroinflammation. While the mechanisms that may explain these effects are

unclear, it may instead be through activation of Fyn by interactions with cellular prion protein and subsequent hyperphosphorylation of NMDA-receptor subunits (Ferreira et al., 2017b). Future work will seek to further clarify these mechanisms. Nevertheless, these findings are exciting, given that AZD is safe and well-tolerated in individuals with AD (Nygaard et al., 2015) and thus may be able to be translated rapidly to the clinic for assessment of benefit in individuals with PD with cognitive impairment in PD.

Despite these positive effects, however, given current limitations in the diagnosis of PD, it is also necessary to investigate strategies that may prevent the emergence of cognitive impairment in PD. Lifestyle interventions, such as diet, exercise or cognitive training, are likely to be particularly successful for achieving this, with a recent report by Livingston and colleagues (2020) suggesting that up to 40% of cases of dementia could be prevented with lifestyle modification. This thesis has reviewed the current evidence supporting the use of each of these as a non-pharmacological approach to the treatment of PD and argued particularly for the utility of PD to directly target the cognitive domains affected in the disease. The literature in this area has then been critically reviewed, concluding that, while CT appears to be beneficial for targeting cognitive dysfunction in PD, further investigations are needed, given methodological limitations to date. Specifically, well-controlled, randomised clinical trials, with larger sample sizes, are needed to fully assess the benefit of such interventions. Additionally, further focus needs to be placed on understanding whether such benefits are long-lasting or transient, as well as whether they have capacity to lead to improvements in everyday functioning. Finally, the accessibility of CT paradigms needs to be carefully considered in light of the motor impairments, apathy and reduced reward sensitivity commonly seen in PD.

In line with this, the final chapter of this thesis focused on the design of a serious games-based approach to deliver CT in PD (the Orbit system). This system was custom

designed to address a two-pronged problem, with the end goal of creating a cognitive training system that is both accessible and engaging for individuals with PD. Such a system allows for over-coming common barriers to computer use due to motor impairments in PD, while also increasing motivation to engage with the system and adhere to the treatment regimen. In order to ensure that benefits would be maximised for individuals with PD, and in line with current best practice guidelines when developing an intervention, we carried out a reiterative co-design trial in collaboration with key stakeholders from Parkinson's SA. In the course of this, it became clear that there were several issues with the system, including ergonomics, game difficulty, system responsiveness and instruction clarity, that needed to be addressed prior to moving forward with a large-scale clinical trial to assess the cognitive benefits of the OrbIT system. This necessitated refinement of the system, as well as complete re-design of one game and significant alterations to several others. Such improvements were critical, as issues raised may otherwise have masked potential cognitive benefits of the system. Following these changes, however, and re-evaluation by key stakeholders, we are now poised to successfully launch our trial.

In conclusion, given the heterogeneous nature of cognitive impairment in PD, a heterogeneous approach, involving both pharmacological and non-pharmacological strategies, is likely to yield the best outcomes. It is also possible that different treatment strategies may be necessary in order to adequately address the different presentations of cognitive impairment in PD. By investigating the underlying pathophysiology of cognitive dysfunction in PD, and considering critically how to target this impairment, it is hoped that we can move closer to tailored, personalised medicine approaches for the effective treatment of this major area of unmet clinical need.

09

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10

Appendices

Appendix 1.1 Previous Game Experience

A1. Do you play games?

- Yes (go to A2)
- No (go to A3)

A2. If YES

2a. How often? (How many times per day, week, etc)

2b. What games are your favourite? (You can select more than one response)

- Puzzle/Tile/Board Games (Rummikub, Chess, Backgammon)
- Online Games (Bejewelled, Candy Crush, Mah-jongg)
- Video Games (Playstation, Nintendo Wii)
- Card Games (Gin Rummy, Bridge, Solitaire)
- Word and Number Games (Crossword Puzzles, Word Search, Sudoku, Scrabble)
- Outdoor Games (Lawn Bowls, Bocce, Croquet, Badminton)
- Creative (Drawing/Painting)
- Other _____

A3. If NO

3a. Why not? (you can select more than one option)

- Limited Access
- Haven't tried before
- Difficulty due to impairments
- Not interested/Do not enjoy
- Other _____

A4. Do you currently have any particular concerns with regards to your physical and cognitive health?

- | | |
|--|---|
| <input type="radio"/> Motor difficulties | <input type="radio"/> Managing day-to-day tasks (taking medication, finances) |
| <input type="radio"/> Learning a new task or skill | <input type="radio"/> Decision making |
| <input type="radio"/> Remembering events | <input type="radio"/> Keeping appointments/meetings |
| <input type="radio"/> Remembering information | <input type="radio"/> Learning a new task or skill |
| <input type="radio"/> Language (remembering words) | <input type="radio"/> Paying attention |
| <input type="radio"/> Language (understanding what is said to you) | <input type="radio"/> Motivation |
| <input type="radio"/> Language (pronunciation) | <input type="radio"/> Other _____ |

Appendix 1.2 Initial Game Feedback

C1. Did you enjoy using the Serious Gaming system?

- Yes No

C2. Did you find the games challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of using the controller?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. Using a scale from 1-10, how would you rate the difficulty level of the games?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C5. Using a scale from 1-10, how would you rate your enjoyment of the games?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of the gaming system as a whole?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C7. Using a scale from 1-10, how likely do you think it is that the gaming system would improve/maintain your cognitive function?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Not at Very
all likely likely

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Game name: Squirrel (running up the trunk as a squirrel, collecting berries)

CONTENT

C1. Did you enjoy playing this particular game?

- Yes No

C2. Did you find the game challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of the game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. For this particular game, what do you think about: (please tick your responses below)

Feature ↓ →	Rating	Not good	Average	Good
a. The colours used				
b. The animations				
c. The sounds				
d. The controls				

C5. Using a scale from 1-10, how would you rate your interest level in this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Game name: Swimma (swimming around, collecting jewels)

CONTENT

C1. Did you enjoy playing this particular game?

- Yes No

C2. Did you find the game challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of the game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. For this particular game, what do you think about: (please tick your responses below)

Feature ↓	Rating	Not good	Average	Good
→				
a. The colours used				
b. The animations				
c. The sounds				
d. The controls				

C5. Using a scale from 1-10, how would you rate your interest level in this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Game name: Driving Maniac (driving on road, avoiding obstacles)

CONTENT

C1. Did you enjoy playing this particular game?

- Yes No

C2. Did you find the game challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of the game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. For this particular game, what do you think about: (please tick your responses below)

Feature ↓	Rating	Not good	Average	Good
→				
a. The colours used				
b. The animations				
c. The sounds				
d. The controls				

C5. Using a scale from 1-10, how would you rate your interest level in this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Game name: Whack a Mole (tapping sequence of holes that moles appeared in)

CONTENT

C1. Did you enjoy playing this particular game?

- Yes No

C2. Did you find the game challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of the game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. For this particular game, what do you think about: (please tick your responses below)

Feature ↓	Rating	Not good	Average	Good
→				
a. The colours used				
b. The animations				
c. The sounds				
d. The controls				

C5. Using a scale from 1-10, how would you rate your interest level in this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Game name: Who stole the coin? (guessing which animal has the coin)

CONTENT

C1. Did you enjoy playing this particular game?

- Yes No

C2. Did you find the game challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of the game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. For this particular game, what do you think about: (please tick your responses below)

Feature ↓	Rating	Not good	Average	Good
→				
a. The colours used				
b. The animations				
c. The sounds				
d. The controls				

C5. Using a scale from 1-10, how would you rate your interest level in this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Game name: Chow Time! (Plating food from the conveyer belt)

CONTENT

C1. Did you enjoy playing this particular game?

- Yes No

C2. Did you find the game challenging?

- Yes No

C3. Using a scale from 1-10, how would you rate the difficulty level of the game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Easy Challenging

C4. For this particular game, what do you think about: (please tick your responses below)

Feature ↓	Rating	Not good	Average	Good
→				
a. The colours used				
b. The animations				
c. The sounds				
d. The controls				

C5. Using a scale from 1-10, how would you rate your interest level in this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

C6. Using a scale from 1-10, how would you rate your enjoyment of this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
Very Very
Poor Interesting

USABILITY

C7. Did you need to read the instructions before playing this game?

- Yes No

C8. Did the game instructions make sense? (If NO, any in particular that were confusing?)

- Yes No

C9. Was the game easy to play? (if NO, what elements did you find difficult?)

- Yes No

OVERALL

C10. Are there any additional features that you would like to be included in this game?

- Yes No

If YES, what are they?

C11. Are there any parts of the game you did not like?

- Yes No

If YES, what are they?

C12. Using a scale from 1-10, what overall score would you give this game?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Very Poor Brilliant

C13. Do you have any further comments about this game?

Appendix 1.4 Final Feedback

E1. Do you feel that most of your comments were actioned?

- Yes No

E2. Was there anything you'd hoped would be changed that wasn't?

- Yes (If 'Yes' what was this? / which particular game?) No

E3. A) How would you rate the changes to the games overall? (specifically, the games)

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Much Much
Worse Better

B) Any further comments about enjoyment/difficulty of any of the games in particular?

E4. A) How do you feel about changes to the usability of the Orby controller system (ergonomics)

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Much Much
Worse Better

B) Any further comments about usability of the controller in any particular games?

E5. A) How would you rate your enjoyment of the games and gaming system overall?

- ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩
- Much Much
Worse Better

E6. What was your favourite game?

E7. Do you think repeated use of the games and gaming system could be beneficial for your cognitive function?

- Yes No

Appendix 1.5 System Usability Scale

PARTICIPANT NAME: _____

DATE: _____

System Usability Scale

For each of the following statements, please mark one box that best describes your reactions to the OrbIT Serious Gaming Platform today.

	Strongly disagree				Strongly agree
1. I think that I would like to use the OrbIT Serious Gaming Platform frequently.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
2. I found the OrbIT Serious Gaming Platform unnecessarily complex.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
3. I thought the OrbIT Serious Gaming Platform was easy to use.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
4. I think that I would need the support of a technical person to be able to use the OrbIT Serious Gaming Platform.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
5. I found the various functions in the OrbIT Serious Gaming Platform were well integrated.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
6. I thought there was too much inconsistency in the OrbIT Serious Gaming Platform.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
7. I would imagine that most people would learn to use the OrbIT Serious Gaming Platform very quickly.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
8. I found the OrbIT Serious Gaming Platform very cumbersome (awkward) to use.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
9. I felt very confident using the OrbIT Serious Gaming Platform.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
10. I needed to learn a lot of things before I could get going with the OrbIT Serious Gaming Platform.	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

Comments (optional):