Comparisons Between Parkinson's Disease Patients and At-Risk Individuals: Can Olfactory Deficits, Cognitive Measures, and Brain Network Connectivity Serve as Preclinical Markers?

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia,
July 2021

Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

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ABSTRACT

Olfactory deficits (hyposmia) are one of the most common symptoms of Parkinson's disease (PD). These often appear before PD has been clinically diagnosed, making hyposmia a potential early, pre-clinical marker of the disease. However, as hyposmia is not specific to PD, additional markers are needed in order to identify PD in its early stages. This study examined whether specific cognitive deficits and/or abnormalities in resting-state functional connectivity (FC) within the default mode network (DMN) may serve as additional markers of PD for at-risk individuals. Patients with PD, healthy controls, and an at-risk group (AR) including hyposmic first-degree relatives of PD patients and unrelated hyposmic individuals were compared on FC of the DMN and tests of processing speed, working memory, and executive function. It was found that patients and the at-risk group show significant deficits in verbal working memory compared to controls. PD patients also exhibited processing speed deficits. AR individuals showed increased FC between the anterior medial prefrontal cortex and the right middle temporal gyrus of the DMN compared to controls and PD patients. It was also found that cognitive deficits are not associated with abnormal FC. This study demonstrated that impaired verbal working memory and increases in DMN FC in addition to hyposmia could indicate the progression towards PD. AR individuals may show early alterations in DMN FC that may either indicate compensatory processes in response to commencing neuronal loss as an attempt to maintain cognitive performance, or a sign of disease-related changes independent from cognitive processes. Future research is needed to confirm the results and to determine their clinical applicability.

LIST OF ABBREVIATIONS USED

α	Alpha Coefficient
AD	Alzheimer's Disease
AFNI	Analysis of Functional Neuroimages
amPFC	Anterior Medial Prefrontal Cortex
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANTs	Advanced Normalization Tools
AR	At-Risk Group
aTL-L	Left Anterior Temporal Lobe
aTL-R	Right Anterior Temporal Lobe
AUC	Area Under the Curve
BOLD	Blood Oxygen Level Dependent
CO_2	Carbon Dioxide
CSF	Cerebrospinal Fluid
DAT	Dopamine Transporter
DCM2NIIX	DICOM to NIFTI Converter
DICOM	Digital Imaging and Communications in Medicine
DKEFS	Delis Kaplan Executive Function System
DMN	Default Mode Network
dmPFC	Dorsal Medial Prefrontal Cortex
DTI	Diffusion Tensor Imaging
DVARS	Derivative of the Variance

DWI	Diffusion-Weighted Imaging
EPI	Echo Planar Imaging
F	F-value
FAST	FMRIB's Automated Segmentation Tool
FC	Functional Connectivity
FD	Framewise Displacement
FDR	False Discovery Rate
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FOV	Field of View
FSL	FMRIB Software Library
FSPGR-BRAVO	Fast Spoiled Gradient-Recalled-Echo Brain Volume
FWHM	Full-Width Half-Maximum
GM	Gray Matter
H1	First Hypothesis
H2	Second Hypothesis
H3	Third Hypothesis
HC	Healthy Control
HF-L	Left Hippocampal Formation
HF-R	Right Hippocampal Formation
hFDRs	Hyposmic First-Degree Relatives
hNoFDRs	Hyposmic Non-Relatives
Hz	Hertz

ICA	Independent Component Analysis
IFG-L	Left Inferior Frontal Gyrus
IFG-R	Right Inferior Frontal Gyrus
IWK	Izaak Walton Killam Hospital
LNS	Letter-Number Sequencing
LRRK2	Leucine-Rich Repeat Kinase 2
M	Mean
M_{adj}	Adjusted Mean
MCFLIRT	FMRIB's Motion Correction Tool
MCI	Mild Cognitive Impairment
MDS	Movement Disorder Society
min	Minutes
MNI	Montreal Neurological Institute
MNI152	MNI152 Stereotaxic Brain Template
MRI	Magnetic Resonance Imaging
MTG-L	Left Middle Temporal Gyrus
MTG-R	Right Middle Temporal Gyrus
rsfMRI	Resting-State Functional Magnetic Resonance Imaging
n	Group Size
N	Total Sample Size
NEX	Number of Excitations
NIFTI	Neuroimaging Informatics Technology Initiative
NSHA	Nova Scotia Health Authority

p	p-value
PARS	Parkinson Associated Risk Syndrome Study
pCC	Posterior Cingulate Cortex
PCu	Precuneus
PET	Positron Emission Tomography
PD	Parkinson's Disease
PD-MCI	Parkinson's Disease with Mild Cognitive Impairment
PDD	Parkinson's Disease with Dementia
pIPL-L	Left Posterior Inferior Parietal Lobule
pIPL-R	Right Posterior Inferior Parietal Lobule
PPMI	Parkinson Progression Markers Initiative
PSMR	Parkinson Society Maritime Region
QEII	Queen Elizabeth II Hospital
r	Correlation Coefficient
RBD	Rapid Eye Movement Sleep Behaviour Disorder
ROC	Receiver-Operating Characteristic Analysis
ROI	Region of Interest
SD	Standard Deviation
SDMT	Symbol Digit Modalities Test
SE	Standard Error
SEM	Standard Error of the Mean
SFG-L	Left Superior Frontal Gyrus
SNCA	Synuclein Alpha

SPECT	Single-Photon Emission Computed Tomography
t	t-value
T	Tesla
T1w	T1-weighted
TE	Echo Time
TI	Inversion Time
TMT-4	Trail Making Test Condition 4
TMT-5	Trail Making Test Condition 5
TMT4-5	Trail Making Test Condition 4 Controlled for Motor Speed
TPJ-L	Left Temporal Parietal Junction
TPJ-R	Right Temporal Parietal Junction
TR	Repetition Time
UPDRS-III	Unified Parkinson Disease Rating Scale Part Three
UPSIT	University of Pennsylvania Smell Identification Test
vmPFC	Ventromedial Prefrontal Cortex
WM	White Matter
WMS-III	Wechsler Memory Scale Third Edition
η_p^2	Partial Eta Squared

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor, Dr. Kimberley Good for her excellent guidance and support throughout the past two years. Her willingness to give her time so generously has been very much appreciated. I would also like to show gratitude to my committee members, Dr. Javeria Hashmi and Dr. Beverly Butler, who have given me invaluable advice on areas of cognition and functional brain imaging. Their insights were indispensable for the completion of this project. I am particularly grateful for the help of Carl Helmick. Without his work on the functional connectivity analysis script, this project would not have been feasible. His dedication and enthusiasm for the topic has been wonderful. I would also like to thank Denise Lewis for her dedicated work on recruiting, coordinating, and conducting participant data collection and I appreciate the valuable contributions from Dr. Harold Robertson, Dr. Ron Leslie, Dr. Kerrie Schoffer, Dr. Tyler Rolheiser, Dr. Roger McKelvey, Dr. John Fisk, Dr. Gail Eskes, Dr. Gosia Phillips, Dr. Naeem Khan, and Dr. Ben Rusak that were made to the Predict Parkinson's project of which this study was a part. I also appreciate the time and efforts made by participants that took part in this study. Finally, I would like to thank my family for the continuous encouragement and support throughout my studies. This accomplishment would not have been possible without them.

Chapter 1: Introduction

1.1 Parkinson's Disease

Parkinson's disease (PD) is a progressive, neurodegenerative movement disorder that affects one to two individuals out of 1000 within the general population (Tysnes & Storstein, 2017). Typically, the age of onset of PD is over age 50 years and the prevalence in people over 60 years of age is one percent, with the prevalence increasing with advancing age (de Lau & Breteler, 2006). After Alzheimer's disease (AD), PD is the second most common neurodegenerative disease and its occurrence in the general population seems to be rising since the turn of the century, possibly due to the increased number of older individuals within the population and the increase of other risk factors of PD, such as traffic-related air pollution (Tysnes & Storstein, 2017). PD is characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra and the nigrostriatal pathway (Khan et al., 2017). Common motor signs and symptoms of PD include tremors, bradykinesia (slowness of movement) and rigidity; however, non-motor signs and symptoms are also common and may be more debilitating than the motor abnormalities. Non-motor symptoms include, for example, sleep disturbances, autonomic dysfunction, cognitive impairments, and olfactory deficits (Jankovic, 2008).

The hallmark of PD's neuropathology is the presence of abnormal alpha-synuclein depositions (Tysnes & Storstein, 2017). Alpha-synuclein is a misfolded presynaptic protein that uncharacteristically accumulates in the form of Lewy bodies within affected neurons (Braak et al., 2004). The progression of neuropathology has been described to occur in six stages by Braak et al. (2003). In the first two stages, Lewy bodies appear in the dorsal motor nucleus of the vagus nerve, the anterior olfactory structures and in the brain stem. As the disease progresses, the

pathology spreads to the midbrain (e.g., substantia nigra and dopaminergic pathways; Stage 3-4) and eventually to the limbic system and cortical brain regions (Stage 5-6), resulting in severe motor and cognitive symptoms (Braak et al., 2003, 2004). Typically, PD is clinically diagnosed with the appearance of motor symptoms after there has already been substantial neuronal loss (between Stage 3 and Stage 4). However, neurodegenerative processes and associated non-motor symptoms develop several years before the onset of motor symptoms (Braak et al., 2004; Liu et al., 2018). Given that the olfactory structures are affected in the earliest stages of the disease, the onset of olfactory deficits could help to identify PD in its preclinical stages (Liu et al., 2018). Motor and non-motor symptoms decrease patients' quality of life (Munhoz et al., 2015), which makes PD a burdensome condition and highlights the importance of discovering early diagnostic markers in order to develop interventions to stop PD from progressing.

1.2 Risk Factors of PD: Olfaction Deficits and Genetic Factors

In order to find early diagnostic markers, at-risk populations and etiological factors for PD should be considered. A deficit in the sense of smell (hyposmia) has been classified as an early marker of PD as part of the Movement Disorder Society (MDS) criteria for prodromal PD. These criteria intend to guide future research regarding the prodromal stage of PD, a stage that is characterized by the presence of symptoms and signs (e.g., non-motor symptoms) that are still insufficient for a definite disease diagnosis (Berg et al., 2015). Hyposmia is one of the earliest non-motor symptoms that is reported by PD patients and it is experienced by about 96% of patients (Haehner et al., 2009). Olfactory deficits that are seen in PD patients are also in line with early structural brain changes that were mentioned by Braak et al. (2003; i.e., alpha synuclein deposition in the anterior olfactory nucleus). Similarly, with the use of diffusion-weighted imaging (DWI), Scherfler et al. (2006) described that early-stage PD patients show increased

measures of diffusivity and therefore abnormal white matter integrity in frontal olfactory brain regions. DWI abnormalities in the anterior olfactory regions correctly classified PD patients from control participants. Similarly, Rolheiser et al. (2011) used diffusion tensor imaging (DTI) to examine white matter tract connectivity in anterior olfactory structures and the substantia nigra in early-stage PD patients. This study also examined olfactory function, an omission that was notable in the Scherfler et al. (2006) study. Early PD patients demonstrated significant impairments in olfactory function and white matter integrity abnormalities in olfactory structures of the brain and in the substantia nigra (Rolheiser et al., 2011). These studies of early PD patients suggest that microstructural abnormalities in olfactory structures and the presence of hyposmia could be early markers of PD. Moreover, a systematic review of seven studies examining the association between hyposmia and PD indicated that unaffected hyposmic individuals have a 2.12−6.95-fold increased risk of eventually developing PD and that olfactory deficits, therefore, may be an early, preclinical biomarker of PD with a relatively high sensitivity (Sui et al., 2019). In comparison with other early non-motor symptoms such as constipation, excessive daytime sleepiness, and symptomatic hypotension, the presence of olfactory deficits has a higher disease probability (Berg et al., 2015) and idiopathic hyposmic individuals therefore represent a population that is at increased risk of future PD.

Although the etiology of PD is largely unknown, several genes, such as the parkin gene, the SNCA gene, and the LRRK2 gene have also been identified to play a role in the onset of PD, as their mutations have been related to neuronal loss in PD patients (Houlden & Singleton, 2012). Studies on genetic risk factors also suggest that unaffected first-degree relatives of affected patients are one population that is at higher risk of developing PD (Rocca et al., 2004). In a meta-analysis by Thacker and Ascherio (2008), the risk of developing PD was three times

higher for first-degree relatives of PD patients compared to non-relatives. Having a first-degree relative with early-onset PD or being a sibling of an affected person increases the risk further, possibly due to a greater effect of genetic factors for early-onset compared to late-onset PD, and shared familial environmental exposures at similar young ages, respectively (Thacker & Ascherio, 2008).

First-degree relatives who are at an elevated risk of developing PD but who do not yet show motor symptoms may show preclinical non-motor symptoms as early indicator for PD. Liu et al. (2019) reported that compared to controls, first-degree relatives show a higher frequency of non-motor symptoms, which included cognitive impairments, anxiety, depression, sleep impairments, and constipation. In addition, individuals at familial risk of developing PD have been shown to experience olfactory deficits that cannot be explained by other physical conditions and that may indicate the progression towards PD (Berendse et al., 2001). In a prospective cohort study, first-degree relatives of PD patients had a higher risk of developing PD within five years when they performed poorly on olfactory tasks, including odour detection, discrimination, and identification (Ponsen et al., 2009). The same group had suggested this in a previous study as well, demonstrating that unaffected first-degree relatives of PD patients with hyposmia have a 10% higher risk of developing PD in the future (Ponsen et al., 2004). The degree of olfactory dysfunction in hyposmic relatives was also similar to those of early PD patients (Ponsen et al., 2004). Another study assessed olfactory deficits along with possible molecular brain changes in first-degree relatives by using single-photon emission computed tomography (SPECT) and found that this two-step approach (behavioural measures and brain imaging) can be useful in determining that olfactory deficits are related to a higher risk for first-degree relatives to develop PD (Ponsen et al., 2010). These studies also add to the notion that olfactory deficits serve as

early markers of PD and that hyposmic first-degree relatives of PD patients may be in the prodromal stage of PD.

Specifically screening for hyposmia in a first-degree relative population and studying this particular subgroup in addition to hyposmic non-relatives could be useful in determining whether the presence of hyposmia increases the risk of developing PD in the future compared to healthy normosmic (normal sense of smell) non-relatives. However, olfactory deficits can also be a marker for other neurodegenerative diseases, such as Lewy body dementia or AD (McShane et al., 2001, Seligman et al., 2013). McShane et al. (2001) investigated individuals with dementia and demonstrated that dementia due to Lewy body pathology is linked to more severe olfactory impairments than dementia linked to AD, although patients with AD pathology also show decreased olfactory function when compared to healthy individuals. Olfactory deficits are experienced by about 90% of patients with AD (Ruan et al., 2012) and have been linked to AD neuropathology (i.e., neuritic plaques, and neurofibrillary tangles) as well as to prodromal symptoms of AD such as mild cognitive impairment (MCI) and poor performance in episodic memory (Wilson et al., 2009). Moreover, a longitudinal, population-based study, the Epidemiology of Hearing Loss study (Schubert et al., 2011) examined risk factors for the development of hyposmia other than early neurodegenerative disease stages. It was found that a history of nasal polyps or heavy alcohol use in older adults increased the risk of developing hyposmia. Given that hyposmia is also a risk factor for dementia and may occur due to disorders that are not related to neurodegenerative processes, it is important to consider additional measures that can accurately predict PD. Although several studies have shown that olfactory testing together with brain imaging can predict progression towards PD (e.g., Ponsen et al., 2010), fewer studies have examined whether it could be useful to assess other non-motor

symptoms, such as specific cognitive deficits, that could further predict the onset of PD in hyposmic individuals.

1.3 Cognition as Preclinical Marker for Parkinson's Disease

Compared to individuals without PD, the prevalence of dementia is three-times higher in PD patients. Approximately 78% of PD patients develop dementia over the course of their illness, adding further burden to their disability (Aarsland et al., 2003). Nevertheless, cognitive deficits are also reported in some PD patients without dementia (Aarsland et al., 2009). A meta-analysis has shown that executive function, verbal memory, and visuospatial function are cognitive domains that are affected most often in non-demented PD patients (Curtis et al., 2019). Compared to healthy individuals, non-demented PD patients with a disease duration of about six years show impaired executive functioning, including deficits in verbal fluency and in tasks involving attention and visual scanning (e.g., trail making). In addition, greater variability in performance on a spatial judgment task, but relatively preserved working memory, were also reported (Salazar et al., 2019). Working memory requires the ability to keep in mind and manipulate information (Bublak et al., 2002). In contrast, another study showed that performance on working memory tasks, especially on tasks that mainly require the manipulation of information (i.e., reordering a sequence of digits), is reduced in PD patients compared to healthy controls (Bublak et al., 2002). In the same study, a higher task demand on attentional working memory resources also affected working memory performance in PD patients as PD patients' initiation times for task responses were even slower. Bublak and colleagues (2002) reported these findings in patients who had a relatively low disease duration, suggesting that working memory deficits are present in early phases of PD when measured with a task that requires a high working memory capacity. The notion that cognitive impairment can also occur in the earliest

stages of illness is supported by Aarsland et al. (2009), who demonstrated that early PD patients show cognitive deficits in the domains of executive function and psychomotor processing speed (Aarsland et al., 2009).

Between 15-43% of early-stage PD patients meet criteria for the diagnosis of mild cognitive impairment (PD-MCI), a cognitive phase that represents an intermediate stage between normal cognition and dementia (Pedersen et al., 2017). While Braak and colleagues have stated that neurodegeneration in underlying brain structures that affect cognition appear later in the course of PD (Braak et al., 2003), others have suggested that early cognitive deficits could occur because of functional alterations in frontal-striatal and temporoparietal brain systems (Nombela et al., 2014), and/or abnormalities in other non-dopaminergic neurotransmitter pathways which may be present before motor symptoms appear (Chahine et al., 2016). If cognitive deficits are observed early in the course of illness, it is also possible that those in the pre-motor stage may present with abnormalities on cognitive tasks.

1.3.1 The Link between Olfactory Deficits and Cognition in Parkinson's Disease

Many studies have described associations between cognitive performance and olfactory functioning in PD. Gjerde et al. (2018) found that the decline in cognitive function (in the domains of global cognition, verbal memory, and processing speed) was greater in patients who were hyposmic at study entry than those who had a normal sense of smell (were normosmic). In a further study that compared hyposmic PD patients to PD patients with better olfactory functioning, patients with severe hyposmia had deficits in the domains of verbal memory, executive function, working memory, and global cognition (Morley et al., 2011). Finally, olfactory deficits were specifically related to deficits in executive functions but not to verbal

memory in another study (Leonhardt et al., 2019), suggesting that executive dysfunction appears to be an important component of the cognitive deficits found in hyposmic patients.

It is still unknown why olfactory and cognitive functions may be related to each other in PD. However, it was suggested that PD-related changes in the frontal cortex and in temporolimbic areas of the brain, such as a decrease in dopaminergic and cholinergic innervation, may be underlying the relationship between olfaction and cognition in PD (Bohnen et al., 2008; Sunwoo et al., 2015). Reduced dopamine transporter (DAT) binding in the hippocampus has been related to odour identification deficits in early-stage PD patients (Bohnen et al., 2008). The hippocampus is commonly involved in cognitive functioning, especially in memory (Das et al., 2019), and dopaminergic denervation may therefore affect the encoding of olfactory information, resulting in poor performance in olfactory identification tasks (Bohnen et al., 2008). Bohnen et al. (2010) further reported that abnormal acetylcholine activity in the limbic system, and more specifically in the hippocampus, is linked to cognitive as well as olfactory deficits in PD. Thus, neurotransmitter system alterations in the limbic system may be underlying the link between hyposmia and cognitive impairment in PD (Bohnen et al, 2010).

1.3.2 Potential Cognitive Deficits in Hyposmic At-Risk Individuals

The relationship between olfactory deficits and cognitive function has been examined in a limited number of studies of at-risk individuals. One study has employed data from the Parkinson Progression Markers Initiative (PPMI), a multicenter longitudinal cohort study which recruited newly diagnosed PD patients and three groups of individuals at higher risk of developing PD (Chahine et al., 2018). The first group of at-risk individuals included participants with a risk gene mutation, while a second group included participants diagnosed with Rapid Eye Movement Sleep Behaviour Disorder (RBD). Many participants in both of these groups and in

the PD group also exhibited olfactory deficits. A third group of at-risk participants were individuals with hyposmia only. Except for the gene mutation carrier group, the majority of participants in each group had to demonstrate DAT binding deficits to ensure that at-risk participants were in the prodromal PD stage. The groups were compared on a variety of cognitive tests, and it was found that the RBD group performed significantly worse on processing speed and visuospatial function measures compared to the other at-risk groups. In addition, the hyposmic group did not significantly differ in cognitive performance compared to newly diagnosed PD patients or mutation carriers (Chahine et al., 2018). Furthermore, another study employed the Parkinson Associated Risk Syndrome (PARS) data and compared hyposmic individuals who also demonstrated reduced DAT binding with normosmic and hyposmic individuals with normal DAT binding on a battery of cognitive tests (Chahine et al., 2016). Compared to all other participants, the hyposmic group with reduced DAT binding showed deficits in executive functioning, working memory, and global cognition (Chahine et al., 2016). Thus, hyposmic individuals who are at higher risk of developing PD may display early cognitive deficits that could be related to the progression towards PD. Nevertheless, Chahine et al. (2016) did not include PD participants so that potential similarities in cognitive functioning between PD patients and hyposmic individuals at increased risk of PD could not be assessed. The examination of PPMI data may complement the findings from the PARS study as no differences between a similar hyposmic group and early PD patients were reported (Chahine et al., 2018). However, a normosmic control group including individuals that are not at risk of developing PD was not included in the PPMI study so that it remains unknown whether both the hyposmic group and the PD group are cognitively impaired or not. Therefore, there is a need for studies

that compare idiopathic hyposmic individuals to PD patients and healthy controls on cognitive measures in order to examine whether cognitive deficits could represent PD progression.

Other studies that have examined cognition in people at risk for PD have not produced consistent results. In one study, first-degree relatives were compared to healthy control subjects on a battery of neuropsychological tests. Although only one of the comparisons yielded a significant difference (a motor task), the authors used a discriminant function analysis and observed a subgroup of relatives with a global executive dysfunction syndrome similar to that demonstrated by PD patients. The authors suggested that this subgroup of PD relatives may be experiencing a dysexecutive syndrome due to nigrostriatal degeneration (Dujardin et al., 1999). Thaler and colleagues (2012) extended this finding by demonstrating executive functioning deficits in first-degree relatives who were carriers of the PD risk LRRK2 gene mutation. However, in a study that prospectively followed at-risk individuals (i.e., hyposmic first-degree relatives of PD patients who later transitioned to PD), the at-risk subjects were found to have normal executive function at baseline (Ponsen et al., 2009). However, this latter study only included two executive function tasks, one that measured perseveration of motor behaviour generation and another which examined sequential visuospatial memory span. Working memory has also been examined in genetic high-risk samples. No impairment was observed on working memory (using N-back and digit span tasks) in healthy relatives of PD patients who were LRRK2 gene mutation carriers (Thaler et al., 2016). From these studies, further research is clearly needed, employing more comprehensive measures of cognitive function. In particular, more cognitively demanding working memory tasks are necessary (Bublak et al., 2002) along with a greater range of executive tasks. More extensive neuropsychological testing, combined with olfactory assessment may be useful for predicting PD in at-risk samples.

Behavioural measures of olfaction and cognition could be useful in detecting prodromal PD. Furthermore, other studies have noted that brain imaging together with behavioural measures of early PD symptoms also have been valuable in assessing the risk of PD (Ponsen et al., 2010; Rolheiser et al., 2011). Given that cognitive deficits have been found to be associated with functional brain changes in different brain regions in PD patients (Nombela et al., 2014), examining behavioural measures of cognition together with functional brain imaging measures that have been associated with cognition may be helpful in assessing whether individuals at higher risk of developing PD may show additional preclinical markers of this disease.

1.4 Resting-State Functional MRI and the Default Mode Network (DMN)

An emerging technique to examine brain functioning has shown promise for examining abnormalities in patients with brain disorders (Hohenfeld et al., 2018). Resting-state fMRI (rsfMRI) measures low frequency spontaneous fluctuations of the blood oxygen level dependent (BOLD) signal within the brain when individuals are not engaging in any task, in order to examine neural activity at rest (Fox & Raichle, 2007). To identify spatial patterns of neural activity across brain regions, rsfMRI measures functional connectivity (FC). Functional connectivity analyses detect correlation patterns of neural activity time courses of different areas of the brain to determine to what extent they are functionally connected with one another (i.e., synchronized brain activity; Fox & Raichle, 2007). Additionally, FC can be detected within specific networks where multiple brain regions can be observed that are functionally connected to one another (Fox & Raichle, 2007). One of the known networks is the default mode network (DMN), which is involved in internally directed mental activity and which could be altered by neurological illnesses such as PD.

The DMN consists of several brain regions which are anatomically and functionally connected with one another, including the medial prefrontal cortex, the posterior cingulate cortex, the inferior parietal lobule, the lateral temporal cortex, and the hippocampal formation (Buckner et al., 2008). The DMN is active during rest and during internally-directed tasks or events such as mind-wandering. The network's activity decreases while doing a task or while attending to externally-oriented events (Buckner et al., 2008). Specifically, the DMN has implications for autobiographical memory tasks, social inference (Buckner & DiNicola, 2019), and in the allocation of attentional resources during the resting-state (Gusnard & Raichle, 2001). Moreover, the DMN is negatively correlated to regions of a network that has implications in cognition (i.e., dorsal attention network), which is active during externally-driven tasks that require attentional resources (Buckner & DiNicola, 2019). It has been proposed that the deactivation of the DMN during task performance is essential for directing attentional processes to a goal-directed task (van Eimeren et al., 2009).

1.4.1 The Default Mode Network and Parkinson's Disease

Several studies have reported reduced functional connectivity in the DMN of PD patients at rest (Amboni et al., 2015; Hou et al., 2017; Lucas-Jiménez et al., 2016; Tessitore et al., 2012), suggesting that alterations of the DMN could identify PD patients from healthy individuals. Although some inconsistencies exist across previous studies, reduced FC was mainly found between temporal regions and the posterior cingulate cortex as well as between the posterior cingulate or temporal regions with the inferior parietal cortex (Amboni et al., 2015; Hou et al., 2017; Lucas-Jiménez et al., 2016; Tessitore et al., 2012). In a study by Tessitore and colleagues (2012), connectivity within the DMN was reduced between the medial temporal and the inferior parietal regions in non-demented, cognitively unimpaired PD patients, but no significant gray

matter structural alterations in cortical or subcortical regions were found. This group concluded that functional DMN abnormalities can be independent of certain structural brain alterations; however, a relationship between reduced FC and cognitive function in the domains of memory, executive and visuospatial function suggest that FC alterations have implications in the development of cognitive decline and may precede associated gray matter loss (Tessitore et al., 2012). Moreover, the DMN was shown to have reduced connectivity in a non-demented, cognitively unimpaired group of PD patients, showing that alterations of the DMN are related to PD even in the absence of cognitive impairments (i.e., attention, processing speed, working memory, and motor function; Disbrow et al., 2014). However, a reduced connectivity within the DMN was associated with decreased processing speed, acknowledging a potential role for this network in this cognitive domain (Disbrow et al., 2014). Furthermore, Lucas-Jiménez et al. (2016) found low DMN connectivity in PD patients, especially between regions of the posterior cingulate and the temporal lobe. Reduced connectivity was related to poor verbal and visual memory performances, and in visual abilities, supporting the role of the DMN in specific cognitive functions and suggesting that functional alterations within the DMN are linked to cognitive decline (Lucas-Jiménez et al., 2016). In a further study, PD patients who exhibited cognitive impairments in at least two cognitive domains, including executive functions, memory, language, and visuospatial abilities, were also more likely to show decreased DMN connectivity between the midline cores of the DMN (i.e., the posterior cingulate and the medial prefrontal cortex) and the hippocampus; however, FC values between these regions were not associated with cognitive test scores (Gorges et al., 2015). In the same study, PD patients with intact cognition demonstrated increased DMN FC compared to controls, suggesting a compensatory response of PD-related pathology in cognitively unimpaired patients. Gorges et al. (2015)

concluded that reduced DMN FC is related to cognitive decline and PD progression. Overall, studies that have assessed the resting-state FC of the DMN in PD patients indicate that an aberrant FC may identify PD patients from healthy controls. Abnormalities in resting-state DMN connectivity can characterize patients with PD independently of structural brain atrophy, and may be related to specific cognitive impairments, such as processing speed, memory, and visuospatial abilities.

1.4.2 The Default Mode Network as Early PD Marker for Hyposmic Individuals?

Whether changes in the DMN can be an early preclinical marker for people who are progressing towards PD is still unclear. Few studies have examined the DMN in hyposmic individuals who may be progressing towards PD. However, Sunwoo et al. (2015) reported that hyposmic PD patients showed decreased FC between the posterior cingulate cortex and right superior parietal area as well as right frontal areas compared to PD patients with good olfactory functions, suggesting that the presence of hyposmia is related to a greater degree of FC alterations. In the same study, hyposmic patients also showed more deficits in visuospatial abilities, language, and executive function, which suggests a relationship between hyposmia, the presence of cognitive deficits, as well as aberrant FC between regions of the DMN. Additionally, patients with good olfactory functioning did not differ from controls on FC (Sunwoo et al., 2015), and although this finding may have been the result of a Type II error (i.e., may represent a false negative due to a low sample size and thus, reduced power to detect a significant result), this finding indicates that olfactory dysfunction may be an important factor that contributes to FC alterations. In another study, compared to healthy controls, PD patients with severe hyposmia were also observed to demonstrate decreased FC between the precuneus and the right inferior parietal lobule (Yoneyama et al., 2018), regions which have been commonly associated with the

DMN. Therefore, there might be a link between olfactory dysfunction and specific regions of the DMN in PD patients. PD-related functional connectivity alterations within the DMN seem to be related to hyposmia and occur in cognitively impaired patients. Although further research is warranted, individuals with hyposmia that progress towards PD may potentially show early DMN FC abnormalities and related cognitive deficits as well.

Furthermore, there is very limited research on first-degree relatives of PD patients and functional connectivity within the DMN. Yet, one study has compared functional connectivity measures of the DMN between non-manifesting first-degree relatives that carried a PD-related mutation in the LRRK2 gene, and relatives that were non-carriers of that mutation (Jacob et al., 2019). Mutation carriers, who are at greater risk of developing PD, had a decreased DMN connectivity, specifically between the right inferior temporal cortex and the posterior cingulate cortex. In addition, both groups did not significantly differ in cognitive test scores. The findings of this study suggest that rsfMRI of the DMN might help to identify individuals at risk of PD. It also suggests that cognition is unaffected between carriers and non-carriers of the risk gene mutation. However, Jacob et al. (2019) did not include unaffected non-relatives of PD patients or a group of PD patients as controls. Consequently, it cannot be concluded that cognition is generally unaffected in subgroups of first-degree relatives of PD patients. Moreover, whether first-degree relatives at a greater risk of PD show a decreased DMN connectivity that is similar to that of PD patients is also unanswered. It is also unclear whether first-degree relatives who are at greater risk of developing PD due to the presence of olfactory deficits also display a reduced DMN connectivity compared to healthy non-relatives, and whether specific cognitive functions are related to the DMN connectivity in this group.

As mentioned above, an association between olfactory dysfunction and cognitive deficits could exist due to dopaminergic and acetylcholine denervation in limbic areas (Bohnen et al., 2008; Bohnen et al., 2010). In support for this idea, Nagano-Saito et al. (2009) have suggested that the DMN in human participants is linked to dopamine function. Using Positron Emission Tomography (PET), cerebral blood flow was examined in PD patients and healthy controls before and after being treated with apomorphine, a dopamine agonist. During performance of an executive function task, the Tower of London, deactivation in ventromedial prefrontal cortex (vmPFC) and posterior cingulate cortex (pCC) was observed in both the patient and the control group. These two brain regions are thought to be part of the DMN and should be deactivated during active task completion. Treatment with apomorphine also increased association between DMN deactivation and task complexity, showing that the neurotransmitter may enhance cognitive functions through anterior regions of the DMN (Nagano-Saito et al., 2009). An animal model demonstrated that the DMN-like network in mice is modulated by acetylcholine and serotonin activity, as acetylcholine and serotonin receptor antagonists decrease network FC. This finding could have implications for understanding deficits in the DMN in neurodegenerative diseases (Shah et al., 2016). Specific neurotransmitter systems seem to play a part in human DMN FC and activation patterns that have been related to cognition. Because abnormalities in the same neurotransmitter systems have been linked to olfactory deficits as well (Bohnen et al., 2008), deficits in olfaction, cognition, and DMN FC that may coincide in PD patients could be due to underlying abnormalities in one or more neurotransmitter systems. Furthermore, the hippocampus and the vmPFC are part of the DMN and also have implications in olfactory function (Eiler II et al., 2012; Gottfried & Zald, 2005). Additionally, both regions play important roles in cognition. The hippocampus is associated with memory (Das et al., 2019) and the

vmPFC with decision making (Schneider & Koenigs, 2017). Therefore, altered activity in these regions may be related to the presence of hyposmia in PD patients and individuals at higher risk of PD as well as to changes in DMN FC and cognitive function.

1.5 The Present Study

Research on individuals who do not have a relative with PD has shown that olfactory dysfunction increases the risk for developing the disease (Ross et al., 2008). Being a first-degree relative of a PD patient in addition to having hyposmia also increases the risk. Therefore, in the current study, a combined 'at-higher risk' group included both hyposmic first-degree relatives and hyposmic non-relatives. These two groups were assessed with a battery of neuropsychological tests and rsfMRI to examine DMN connectivity to determine whether cognition and FC could be possible additional preclinical markers of the disease.

Executive function, verbal and spatial working memory, and processing speed were assessed in the present study to extend previous research on cognitive impairments in non-manifesting hyposmic individuals. Deficits in the mentioned cognitive domains have been reported in early PD patients and executive dysfunction was reported in healthy first-degree relatives as well. Thus, the first goal of the present study was to examine whether unaffected individuals who are at higher risk of developing PD show specific patterns of cognitive deficits compared to healthy normosmic controls, and to PD patients, and to see whether certain cognitive deficits could be markers for the progression towards PD. Our first hypothesis (H1) was that compared to controls, hyposmic at-higher risk individuals would show cognitive deficits within the mentioned domains and that PD patients would also show cognitive deficits which would be more pronounced than those of at-higher risk individuals.

Furthermore, because early PD patients show a reduced resting-state DMN functional connectivity which could be related to cognitive impairments in memory, executive function, and processing speed, and because there is a lack of evidence about DMN functional connectivity in hyposmic individuals in relation to PD, the second goal of the present study was to examine whether hyposmic at-higher risk individuals show alterations in DMN functional connectivity compared to controls, and whether those changes are approaching the level of DMN functional connectivity of PD patients. Moreover, it was examined whether functional connectivity of the DMN is related to potential cognitive deficits in those individuals. Our second hypothesis (H2) posited that hyposmic at-higher risk individuals would show reduced functional connectivity within the DMN compared to healthy controls. At-higher risk individuals' DMN functional connectivity will be more similar to the DMN connectivity that is expected to be found in PD patients. It was also hypothesized that a reduced DMN connectivity would be related to cognitive deficits (H3). Furthermore, this study assessed the diagnostic accuracy of potential cognitive deficits and DMN FC abnormalities as an additional step to see how well these measures can distinguish between healthy individuals and individuals at-risk of PD.

Chapter 2: Methods

2.1 Participants

Early-stage Parkinson's patients (PD) were recruited from the Movement Disorders Clinic, Division of Neurology, QEII Health Sciences Centre in Halifax, Nova Scotia. PD patients were diagnosed by a qualified neurologist and were required to score below 3 on the Hoehn and Yahr scale. The Hoehn and Yahr scale assesses the severity of illness of PD patients and ranges from 1 (low level of disability) to 5 (high level of disability; Hoehn & Yahr, 1967). A clinician also administered the Unified Parkinson Disease Rating Scale Part III (UPDRS-III), which examines the progression of motor impairments in PD patients and ranges from 0 (no impairment) to 56 (extremely impaired; Fahn et al., 1987). All PD patients included in this study had been medicated with antiparkinson medications (e.g., levodopa, dopamine receptor agonists, etc.). The second group included healthy controls who do not have relatives who have been diagnosed with PD (HCs). HCs included in this study were age- and sex-matched to the PD group from a total of 61 HC participants (for every PD patient, an HC participant was chosen from the dataset that most closely resembled the demographic of the index patient), and they were recruited via word of mouth, through online advertising on websites such as Kijiji.com and PredictParkinsons.com, and through advertisement board notices that are placed in local hospitals and universities. HCs were included in the study if they scored over 30 out of 40 in the University of Pennsylvania Smell Identification Test (UPSIT; see below) in order to rule out pre-clinical neurodegenerative disorders (Driver-Dunckley et al., 2014). The third group of participants were individuals at higher risk of developing PD (at-risk group; AR). Subgroups of the at-risk group included idiopathic hyposmic first-degree relatives (hFDRs) of an individual with a diagnosis of PD (e.g., siblings or children) and idiopathic hyposmic individuals who do

not have a first-degree relative with PD (hNoFDRs). The groups were combined in order to increase power through a higher group sample size. Participants in the at-risk group were recruited though the Parkinson Society Maritime Region (PSMR) and through online advertisements and advertisement board notices.

Hyposmic at-risk participants were included if they met criteria for severe olfactory deficits defined by scoring under the 10^{th} percentile of the UPSIT which was based on norms that take the age and sex of the participant into account. Furthermore, participants in both the PD and the HC group had to be between the ages 45 and 75 years old and participants in the at-risk group had to be between the ages of 40 and 65 years old. Because preclinical stages of PD begin several years before being diagnosed, at-risk participants' age range was lower. Participants were excluded from the study if other medical causes of olfactory impairments, for example nasal or facial trauma, rhinitis, or chronic allergies, were present. Additionally, all participants were required to have normal or corrected-to-normal vision and hearing, as well as no contraindications to MRI scanning, such as claustrophobia, metal fragments inside the body, or artificial heart valves (see Appendix A for MRI screening questionnaire). Besides a diagnosis of PD in the PD group, participants with other serious neurological or psychiatric disorders that require ongoing treatment were also excluded from the study.

Because previous research did not investigate seed-based DMN functional connectivity and cognition in hyposmic individuals at higher risk of developing PD together with PD patients and healthy controls (three groups), an appropriate and accurate a priori sample size determination could not be conducted. Nevertheless, the required sample size was estimated by assuming an effect size of Cohen's $f = 0.4$ for the differences between FC values across participant groups, and an alpha level of 0.05 in order to obtain a power of 0.90. This resulted in

a required total sample size of 84, with 28 participants included in each group, which we intended to recruit. Previous literature on DMN functional connectivity commonly report sample sizes of about 20 participants in each group as well (e.g., Amboni et al., 2015; Gorges et al., 2015).

2.2 Measures

2.2.1 University of Pennsylvania Smell Identification Test (UPSIT)

The UPSIT is a widely used test for smell identification and is highly internally consistent and reliable with a split-half reliability of $r = .93$ to $r = .96$ along with high internal validity with an estimated test-retest reliability of $r = .95$ (Doty et al., 1985). The UPSIT is commonly used as a measure for olfactory function in PD patients and correctly identifies PD with a sensitivity of 84% (Morley et al., 2018). The test consists of four booklets with each containing 10 pages. Each page includes a patch which encapsulates a specific odour. Participants are required to scratch the patch to release the odour and to smell it afterwards. Then, participants had to choose the option that best describes the odour out of four provided answers, even if no odour was detected. The test contains 40 odours in total. The dependent measure of this test is the total number correct out of 40. This score was used to identify the hyposmic participant group (hNoFDRs and hFDRs).

2.2.2 DKEFS Trail Making Task Condition 4 (TMT-4) and Condition 5 (TMT-5)

This cognitive task is part of the Delis Kaplan Executive Function System (DKEFS; Delis et al., 2001) and is a measure of executive functioning. Adequate performance on the fourth condition of the trail making test (TMT-4) requires visuospatial attention, mental flexibility, planning and motor speed. For this task, participants were provided with a sheet of paper which contains randomly distributed circles. Each circle includes a different number or a

letter. Participants were required to connect the numbers with the letters in order: First a number, starting with the number one, then a letter, starting with the letter A, and alternating with numbers (e.g., number two) and letters (letter B; etc.) until the participant reached the last circle that is marked with the word “End”. The goal of this task was to connect the circles as quickly and as accurately as possible. For the fifth condition of the trail making test (TMT-5), participants had to connect circles as quickly and accurately as possible in a given order without missing the circles. Circles are blank and lines between the circles indicate which ones have to be connected until the circle marked with “End” is reached. The TMT-5 measures psychomotor speed. The outcome measures of the two conditions (TMT-4 and TMT-5) were the time in seconds it took to complete each and the number of errors made. The pure motor task time of TMT-5 was subtracted from the completion time of TMT-4 in order to control for possible motor deficits that PD patients might exhibit while fulfilling the task on paper. The difference between completion times (TMT4-5) was used to compare the participant groups in executive functioning performance, where a higher score indicated worse performance. The TMT has been shown to be valid and to be a possible predictor of daily functioning and executive dysfunction (Mitchell & Miller, 2008). Additionally, internal consistency (split-half reliability) measures of the trail making tests have been found to be relatively high (.57 to .81; Shunk et al., 2006).

2.2.3 DKEFS Verbal Fluency

The verbal fluency task is also a part of the Delis Kaplan Executive Function System (Delis et al., 2001) and measures the ability to generate words as fast as possible (fluency of verbal responses). The test consists of two conditions, letter (phonemic) fluency and category (semantic) fluency. In the letter fluency condition, on each of three 60-second trials, participants were asked to provide as many words that are not normally capitalized as they could, beginning

with one specified letter (i.e., 'F', 'S', and 'A'). In the category fluency condition, participants were asked to provide as many words as they could that belong to a specified semantic category (i.e., animals and boys' names) within 60 seconds. The primary outcome measure of this task is the total number of acceptable words produced in each of the conditions. Relatively high split-half reliability has been reported for the phonemic fluency condition (.68 to .90). as well as good test-retest reliabilities for both conditions (phonemic fluency: .80, semantic fluency: .79; Homack et al., 2005)

2.2.4 Letter-Number Sequencing - Wechsler Memory Scale Third Edition (WMS-III)

The letter-number sequencing (LNS; Wechsler, 1997) task is a subtest of the WMS-III and assesses verbal working memory with a good internal consistency reliability (r = .88; Lovato et al., 2013). Participants were asked to re-order strings of numbers and letters that were verbally provided to them in pseudo-random sequences. To re-order the sequence, participants had to repeat the numbers first in numerical order, followed by the letters in alphabetical order. The digit-letter strings increased in length until the participants could no longer accurately re-order them. For each length, three trials were administered, and the task continued until incorrect responses were given for all trials at one length. The outcome measure of this task was the total number of trials that are correct.

2.2.5 Digit Span (Backwards) - WMS-III

The digit span task is also a measure of verbal working memory and a subtest of the WMS-III (Wechsler, 1997). It was found to have a relatively high internal consistency reliability (α = .82; Gignac et al., 2019). For this task, participants were required to listen to a string of numbers from the administrator and then repeat the same numbers in the reverse order that they were presented. The number of digits increased in length for each trial until the participants can

no longer accurately re-order them. There were two trials at each length and the task continued until incorrect responses were given for all trials at one length. The outcome measure of this task was the total number of trials that are correct.

2.2.6 Spatial Span (Backwards) - WMS-III

The spatial span task is a measure of spatial working memory and it is another subtest of the WMS-III (Wechsler, 1997). Participants were shown a board with cubes that is placed in front of them on a table. The administrator then tapped on the cubes in a specific order and the participant was required to tap the same cubes in the reverse order. The number of cubes tapped increased at each trial until participants could no longer accurately reconstruct the sequence. For each length, two trials were administered, and the task continued until all trials at one length were completed incorrectly. The dependent measure of this task was the total number of trials that are correct. An acceptable split-half reliability has been reported for a comparable spatial working memory task (.73; DeDe et al., 2014). Both the digit span and spatial span tests were included in this study to be able to examine possible differences between the verbal and spatial working memory of participants.

2.2.7 Symbol Digit Modalities Test (SDMT)

The SDMT is sensitive to impairments in psychomotor processing speed and has been found to be valid and reliable in measuring cognitive deficits in neurodegenerative disorders (Strober et al., 2018). The task required participants to pair numbers to a sequence of given symbols as quickly as possible within 90 seconds. Nine different symbols are presented repeatedly on paper in a pseudo-randomized sequence. Each symbol corresponds to a specific number (1 through 9) as shown in a symbol-number key at the top of the page. Participants wrote the number corresponding to each symbol in the sequence in a box under the symbol. The total

number of correct responses within 90 seconds was the outcome measure in this task. A reasonable alternate form reliability has been reported for the SDMT (r = .74; Hinton-Bayre & Geffen, 2005).

2.3 Resting State Functional MRI

2.3.1 Image Acquisition

As part of a larger study, MRI data was collected on a 1.5T GE Signa HDx scanner using an 8-channel head coil. During each scan session, a high-resolution anatomical and a resting-state functional sequences were collected. The axial 3D T1-weighted (T1w) FSPGR-BRAVO anatomical sequence had the following parameters: TR = 11.8 ms, TE = 4.7 ms, TI = 450 ms, acquisition matrix = 224 x 224, FOV = 240 mm, reconstruction matrix = 512 x 512, voxel size = 0.44 x 0.44 x 1 mm, slice thickness = 1.0 mm, slices = 186, NEX = 1, flip angle = 12°, total acquisition time = 6.0 min. The axial 2D Gradient Echo Planar Imaging (EPI) resting-state functional sequence had the following parameters: TR = 2.0 sec, TE = 25 ms, matrix = 64 x 64, FOV = 240 mm, voxel size = 3.75 mm, slice thickness = 3.7 mm, gap = 0 mm, slices = 34, volumes = 305, NEX = 1, flip angle = 90°, acceleration = ASSET2, total acquisition time = 6:10 min.

2.3.2 Resting-State fMRI Preprocessing and Functional Connectivity Analysis

Anatomical and functional imaging data was converted from DICOM to NIFTI file format with DCM2NIIX (Li et al., 2016) to remove identifying (clinical) information from each participant which is associated with the DICOM format. The NIFTI file format was used for further analyses. MRIQC was used to calculate image quality metrics and summary images of each participant's T1w and functional images (Esteban et al., 2017). Summary images for all

datasets were visually reviewed, and datasets with image quality metrics 2-3 standard deviations above or below the mean across all datasets were investigated for potential issues.

Preprocessing was conducted with fMRIPrep version 20.2.1 (Esteban et al., 2019). Anatomical T1w images were corrected for intensity non-uniformity with N4BiasFieldCorrection (Tustison et al., 2010) distributed with ANTs 2.3.3 (Avants et al., 2008) and used as T1w-reference throughout the workflow. This reference was skull-stripped to remove non-brain tissue with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs) and brain tissue was segmented using FAST (FSL 5.0.9; Zhang et al., 2001) distributed with FMRIB Software Library (FSL; Smith et al., 2004) to facilitate spatial normalization. Spatial normalization to the standard MNI152 template space was performed through linear and nonlinear registration with antsRegistration (ANTs 2.3.3), using the brain-extracted version of the T1w reference (Fonov et al., 2009). Spatially normalizing each participant's brain image to a standard space through linear registration (i.e., matching the brain's anatomy to the template through translation, rotation, scaling, and shearing) and nonlinear registration (i.e., using mathematical nonlinear functions to improve normalization) facilitates between-subject and group comparisons of imaging data as participants' brains typically differ in size. Brain tissue segmentation masks for cerebrospinal fluid (CSF), gray matter (GM), and white matter (WM) were generated with FAST. Tissue masks were used to generate confound signals (see below).

Preprocessing of the functional data included calculating framewise head-motion correction with MCFLIRT (FSL 5.0.9; Jenkinson et al., 2002) and saving the rigid-body (6-DOF) framewise motion parameter estimates into a motion parameter file. A BOLD reference image was generated as the temporal average across motion-corrected volumes, followed by

skull-stripping the BOLD reference image using a custom methodology of fMRIPrep. This BOLD reference was then co-registered to the T1w reference using bbregister (FreeSurfer) which implements boundary-based registration (i.e., mapping the white matter boundary of the structural image to the functional image; Greve & Fischl, 2009). This registration step is required to accurately map the regions of the DMN which were defined within structural images onto the functional image so that functional time-series of each region can be calculated and used for the FC analysis (see below). Functional runs were slice-time corrected using 3dTshift from AFNI 20160207 (Cox & Hyde, 1997). Preprocessed functional time-series images were resampled into both original native space by applying the transforms to correct for head-motion (referred to as preprocessed BOLD), and into MNI152 space by combining head-motion correction transform, BOLD to T1w transform, and nonlinear warp of T1w to MNI152 template and applying a single resampling interpolation (output referred to as preprocessed BOLD in MNI152-space). Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS, WM signal, CSF signal, and global signal. FD was computed using two formulations following Power (absolute sum of relative motions; Power et al., 2014) and Jenkinson (relative root mean square displacement between affines; Jenkinson et al., 2002). Average time course signals were extracted within the masks for CSF, WM, and whole-brain masks. Head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. Time points with motion outliers or 'spikes' were censored out of the analysis. When more than 20% of time points showed motion within one participant's data set, the entire data for the participant was excluded.

To analyse functional connectivity between DMN regions, region of interest (ROI) masks of 18 DMN ROIs were created by using the MNI-coordinates from Spreng et al. (2013). The ROIs were the following: Anterior medial prefrontal cortex (amPFC), bilateral anterior temporal lobe (aTL-R and aTL-L), dorsal medial prefrontal cortex (dmPFC), bilateral hippocampal formation (HF-R and HF-L), bilateral inferior frontal gyrus (IFG-R and IFG-L), posterior cingulate cortex (PCC), bilateral posterior inferior parietal lobule (pIPL-R and pIPL-L), precuneus (PCu), the left superior frontal gyrus (SFG-L), bilateral middle temporal gyrus (MTG-R and MTG-L), bilateral temporal parietal junction (TPJ-R and TPJ-L), and the ventral medial prefrontal cortex (vmPFC). This ROI Network template has shown FC alterations of PD patients in several studies (e.g., Baggio et al., 2015; Hou et al., 2017) and the included coordinates of the DMN have been shown to overlap with the DMN established through independent component analysis (ICA; Baggio et al., 2015). Additionally, this template included the hippocampal formation, a region that is hypothesized to be related to hyposmia and cognitive deficits.

A custom python script using Nilearn (Abraham et al., 2014) was created to complete functional connectivity analysis of the DMN using the 18 ROIs described above (see Appendix B for the script). In addition to the preprocessing steps included in fMRIPrep, low-pass and high-pass temporal filtering at 0.08 Hz and 0.008 Hz, respectively, spatial smoothing at 7 mm FWHM, and detrending were performed. A regression analysis was performed with all confound regressors (6 motion parameters, FD, white matter and CSF signal, and global signal regressors) that were created with fMRIPrep (see above). Temporal filtering, spatial smoothing, and confound regression were performed to remove noise and to increase the signal-to-noise ratio.

Functional time-series for all 18 DMN ROIs were extracted by averaging across voxels within 6mm-radius sphere centered on the ROI coordinate. Correlations between all ROI's were

calculated with Pearson's correlational analysis. ROI pairs were excluded if the mean of the correlation ± the standard error of the mean was between -0.1 and 0.1 for all groups. These would represent non-sense correlations signifying an absence of connectivity between specific ROIs. The researcher conducting the preprocessing and first-level analyses was blinded to the group to which the participants belonged.

2.4 Design and Procedure

This study was a between-group design with the dependent variables being the cognitive and rsfMRI outcome measures. Participants underwent a telephone screening before being invited to participate. The screening verified that the participants meet the inclusion criteria in order to be eligible to participate. Once the inclusion/exclusion criteria were met, participants signed the required consent materials and were able to ask questions and discuss the consent with the administrator of the study. Participants signed two consent forms: the first one informed about the MRI scan, the olfactory testing and questionnaires, while the second consent informed about the cognitive testing (see Appendix C). To complete the UPSIT and a demographics questionnaire (see Appendix D), participants could come into the laboratory at the Nova Scotia Health Authority (NSHA; Abbie J. Lane Building of the QEII) in Halifax, Nova Scotia, or have a package that included the UPSIT and the questionnaire mailed to them.

First-degree relatives and non-relatives who fell below the 10^{th} percentile on the UPSIT (AR group), PD patients, and HCs came in for an appointment at the MRI suite in the IWK hospital in Halifax. During the resting-state fMRI, all participants were instructed to keep their eyes closed and not to engage in any specific mental or motor activity to reduce noise and artifacts on the MRI output images. Additionally, padding was placed between the participants' arms and the MRI scanner to reduce motion. Earphones were provided to reduce the effect of

noise from the scanner. The MRI session took approximately 45 minutes to complete. Structural, resting-state functional MRI, and diffusion tensor imaging measures were taken, but for the purpose of this paper, the analysis and the discussion focuses on rsfMRI measures. High resolution structural scans were necessary for the analysis of the rsfMRI data.

After the MRI session, participants were scheduled for cognitive testing on a separate day. The cognitive tests were administered by a member of the research team and included the digit span and spatial span tests, LNS, SDMT, and the DKEFS trail making and verbal fluency tasks, in that order (see Appendix G for cognitive test instructions). Other tests were also administered as part of a larger study which will not be discussed within this report. The cognitive testing took approximately two hours to complete. All participants received monetary compensation for taking part in the study. This study was approved by the NSHA Research Ethics Board (NSHA 2007-224, NSHA 2010-349, and NSHA 2010-369).

The author of this thesis was involved in data collection (rsfMRI and cognitive test data) and data entry for a sub-sample of participants. She took part in the decision of how rsfMRI data would be preprocessed and analysed (e.g., inclusion of confound regressors, choosing the seed-based FC approach and the DMN ROI coordinates for this study). Statistical analyses of both the cognitive test data and the rsfMRI data were conducted by the author.

2.5 Statistical Analyses

Demographic data was compared across groups using one-way analysis of variance (ANOVA) procedures with Tukey's post hoc tests for continuous variables. A Pearson Chi-Square test was used to examine sex differences in the frequency of females and males among the participant groups.

In order to compare cognitive test data across the groups, separate one-way Analysis of Covariance (ANCOVA) procedures with age and education as the covariates were performed and post hoc analyses of group comparisons were conducted with a Bonferroni correction. This analysis was used to assess H1. Assumptions for ANCOVAs were tested. Resulting p-values from each ANCOVA were corrected for multiple comparisons with Bonferroni adjustments as well. A follow-up independent-samples t-test was used in order to determine whether the hFDRs and hNoFDRs subgroups differed from one another.

To assess whether the at-risk group show FC abnormalities within the DMN and to examine H2, functional connectivity values of all DMN ROI-to-ROI connections (i.e., edges) were first compared between the at-risk group and the HC group with an independent-samples t-test. False discovery rate (FDR) multiple comparison correction with the Benjamini-Hochberg method (Benjamini & Hochberg, 1995) was applied to all resulting p-values in order to decrease the Type I error rate. Edges whose FC values were significantly different between the groups after correction were followed up with an independent-samples t-test comparing the at-risk group with the PD group in order to determine whether the at-risk group's FC abnormalities are similar to expected alterations in PD. Furthermore, in a post hoc analysis, a t-test was used in order to determine whether the hFDRs and hNoFDRs subgroups differ from one another and whether one drove the results more than the other. An independent-samples t-test was also conducted between PD patients and HCs in order to confirm whether FC values in the PD group were abnormal. These follow-up steps and post hoc tests were also performed for significant differences in FC values between the at-risk and HC groups that did not survive correction for multiple comparisons (i.e., trends) and would be discussed as secondary results. Assumptions that are

required to conduct t-tests were tested and appropriate changes would be made to the statistical analysis if violations existed.

Effect sizes (i.e., Cohen's *d* for equal sample sizes or Hedges' *g* for unequal sample sizes) were calculated for each significant group-to-group difference in cognitive test scores and in functional connectivity. To assess the relationship between potential aberrant functional connectivity of the DMN and potential cognitive deficits and to test H3, Pearson correlational methods were used, and multiple testing was accounted for. As primary analyses, correlation coefficients were calculated between abnormal FC values (based on the comparison of the at-risk and HC groups after FDR correction) and cognitive tests scores which showed significant deficits in the at-risk or PD groups after Bonferroni correction. Exploratory secondary correlational analyses included assessing the relationship between abnormal FC values and abnormal cognitive test scores before FDR and Bonferroni correction, respectively.

Receiver-operating characteristics (ROC) analyses were used, and ROC curves were created to assess the diagnostic accuracy (sensitivity and specificity) of potential cognitive deficits and FC changes (that survived multiple comparison correction) for the AR group. AR individuals were considered as cases and the value of the area under the curve (AUC) was examined to determine the level of which these measures can correctly classify AR individuals compared to HCs. The significance level was set at $p < .05$ and two-tailed tests were conducted for each variable. All statistical analyses were performed in SPSS version 25 and R software version 4.0.4 was used to correct p-values from separate statistical tests for multiple comparisons.

Chapter 3: Results

3.1 Demographic Data and Olfactory Test Scores

A total of 82 participants were included in this study. Early-stage PD participants (n = 26, M_{age} = 62.4, SD_{age} = 6.4, 11 females) had a mean Hoehn & Yahr score of 1.8 (*SD* = 0.6), a UPDRS-III mean score of 22.5 (*SD* = 11.2), and a mean disease duration from diagnosis of 3.0 years (*SD* = 3.2). Four PD patients were left-handed and 22 were right-handed. The healthy control group (HC) included 26 participants (M_{age} = 61.2, SD_{age} = 5.4, 11 females) and the at-risk group (AR) consisted of 30 participants (M_{age} = 58.9, SD_{age} = 5.9, 15 females). One participant in the HC group and 4 participants in the AR group were left-handed. All other participants in those groups were right-handed. Participants did not significantly differ in age ($F(2, 79) = 2.45$, $p = .09$) or education ($F(2, 79) = 0.56$, $p = .58$). There were no significant sex differences across groups, $\chi 2(2) = .46$, $p = .80$ (see Table 1 for descriptive statistics of the demographic data).

There was a significant difference in UPSIT scores across the groups ($F(2, 79) = 59.25$, $p < .001$, $\eta_p^2 = .60$). In accordance with the inclusion criteria, at-risk individuals had significantly lower UPSIT scores (*M* = 23.07, *SD* = 5.69) than HCs (*M* = 36.73, *SD* = 2.25, $p < .001$), as determined with Tukey's test. This finding was expected as hFDRs and hNoFDRs were identified based on olfactory performance. PD patients scored lower on UPSIT scores (*M* = 22.46, *SD* = 7.04) compared to HCs as well ($p < .001$); however, PD patients and the AR group did not significantly differ in olfactory functioning ($p = .908$; see Table 1 and Figure 1).

The AR group consisted of 14 hFDRs (M_{age} = 58.1, SD_{age} = 6.7, 4 females) and 16 hNoFDRs (M_{age} = 59.6, SD_{age} = 5.2, 11 females). The subgroups did not significantly differ in demographic variables such as age, education, and UPSIT scores ($p > .05$; see Table 2).

However, there was a significant sex difference between the AR subgroups ($\chi 2(1) = 4.82$, $p = .028$). The hFDR group included significantly more males than the hNoFDR group.

3.2 Cognitive Assessments

Table 3 shows summary data of the test scores from each cognitive test across the three groups. One participant from the PD group had missing data for the SDMT and the LNS task and was therefore excluded from the analysis for these specific tasks. ANCOVAs were run to determine whether cognitive tasks performance differed among the PD, AR, and HC groups after controlling for age and education. Assumptions pertaining to ANCOVAs were met for all variables. There was homogeneity of regression slopes and homoscedasticity. There were no outliers in the data, as assessed by no cases with standardized residuals greater than ±3 standard deviations. Homogeneity of variances was met for all test scores (Levene's test for equality of variances, $p > .05$). Standardized residuals for scores from each cognitive test were normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$) and visual inspection of histograms.

Age as a covariate had a significant effect on scores from the LNS task ($F(1, 76) = 12.84$, $p = .001$), the semantic fluency task ($F(1, 77) = 7.57$, $p = .007$), the spatial span backwards task ($F(1, 77) = 8.78$, $p = .004$), and the TMT4-5 ($F(1, 77) = 11.36$, $p = .001$). Education as covariate had a significant effect on scores from the TMT4-5 ($F(1, 77) = 4.56$, $p = .036$). While controlling for age and education, the one-way ANCOVA revealed that the groups significantly differed, after Bonferroni multiple comparison correction, on LNS scores ($F(2, 76) = 6.89$, $p = .002$, $\eta_p^2 = .15$, corrected $p = .014$) and SDMT scores ($F(2, 76) = 9.42$, $p < .001$, $\eta_p^2 = .20$, corrected $p = .002$). There were significant differences in semantic fluency scores ($F(2, 77) = 3.68$, $p = .03$, $\eta_p^2 = .09$) as well; however, the difference did not survive correction and was therefore considered a trend (corrected $p = .21$). There were no significant differences in spatial span test scores ($F(2,$

77) = 1.44, p = .242, η_p^2 = .04), digit span test scores, F(2, 77) = 2.42, p = .096, η_p^2 = .06, TMT4-5 scores (F(2, 77) = 2.41, p = .097, η_p^2 = .06) or phonemic fluency scores (F(2, 77) = 0.78, p = .463, η_p^2 = .02) when age and education was controlled for.

The post hoc analyses showed that HCs had significantly higher scores on the LNS verbal working memory test than PD patients (p = .039, Hedges' g = 0.89) and AR individuals (p = .002, Hedges' g = 0.84; see Figure 2A). PD patients and AR participants did not differ on LNS scores (p = 1.00). SDMT scores were significantly lower in PD patients compared to HCs (p < .001, Hedges' g = 1.35) and AR participants (p = .002, Hedges' g = 1.05; see Figure 2G). HCs and the AR group did not significantly differ from one another in SDMT scores (p = 1.00). Additionally, the analysis revealed a trend towards significant group differences in semantic fluency when age and education was controlled for. Follow-up comparisons disclosed that the trend towards significant differences existed between the PD group and the HC group. PD patients scored significantly worse compared to HCs (p = .033, Cohen's d = 0.77). AR individuals did not significantly differ from the PD group (p = .142) or the HC group (p = 1.00) in semantic fluency scores (see Figure 2E). Independent-samples t-tests indicated that the subgroups of the AR group, hFDRs and hNoFDRs, did not significantly differ on any cognitive test that was included in this study (p > .05; see Table 2).

3.3 Resting-State Functional Connectivity Analysis

Due to excessive head movement (defined above), 6 participants (3 PD patients, 1 HC participant, and 2 AR participants from the hFDR subgroup) were excluded from analysis of the resting-state FC within the DMN. Out of the 150 ROI-to-ROI correlations, 13 were excluded as non-sense correlations (absence of correlation), leaving 137 edges for analysis. Figure E1 and

Figure E2 in Appendix E show correlation matrices and the strength of each ROI-to-ROI correlation for each participant group, respectively.

The first step to analyse functional connectivity values was to run an independent-samples t-test to determine if there were differences in FC between the AR group and the HC group. Assumptions pertaining to t-tests were examined for violations and it was established that there were no influential outliers in the data. For the majority of edges, FC values (correlation coefficients) were also normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$), and there was homogeneity of variances, as assessed by Levene's test for equality of variances ($p > .05$). Edges that violated the normality assumption or the homogeneity of variances assumption were assessed with the nonparametric Mann-Whitney U test and the Welch t-test, respectively. All p-values were corrected with the Benjamini-Hochberg method to account for the FDR. Independent-samples t-tests revealed that the AR group significantly differed from the control group in FC values for six edges. Compared to HCs, the AR group had significantly higher values between the amPFC and MTG-L ($t(51) = -3.32$, $p = .002$, Hedges' $g = 0.91$; see Figure 3), the amPFC and MTG-R ($t(51) = -4.64$, $p < .001$, Hedges' $g = 1.28$), the pCC and vmPFC ($t(51) = -2.58$, $p = .013$, Hedges' $g = 0.71$), the Pcu and MTG-L ($t(51) = -2.16$, $p = .036$, Hedges' $g = 0.59$), and between the MTG-L and vmPFC ($t(51) = -2.36$, $p = .022$, Hedges' $g = 0.65$). Significantly lower values were seen in the at-risk group for FC values of the IFG-R and MTG-L connection ($t(51) = 3.01$, $p = .004$, Hedges' $g = 0.83$; see Table 4 for descriptive statistics). Nevertheless, only the connection between the amPFC and the MTG-R survived the multiple comparison correction (FDR-corrected $p = 0.004$; see Table 5 for details). Table F1 in Appendix F describes analysis outcomes between the AR group and HC group for all edges. FC values of

edges that were significantly different between the AR and control groups but did not survive correction were considered trends (secondary findings, see Figure 4).

The aberrant FC values of the AR group between the amPFC and the MTG-R were followed up with a t-test between the AR and PD groups as part of the primary FC statistical analysis. Significant differences in FC values were present ($t(49) = -2.527$, $p = .015$, Hedges' $g = 0.71$). The AR group had significantly higher FC between these ROIs compared to PD patients (see Table 4 for descriptive statistics). Moreover, post hoc t-tests of FC values from the amPFC and MTG-R connection revealed that hFDRs and hNoFDRs did not significantly differ from one another ($t(26) = 0.16$, $p = .874$, see Table 6) and there were also no significant differences between PD patients and HCs ($t(46) = 1.56$, $p = .0125$, see Appendix F: Table F2).

As secondary analyses, significant differences in FC values between the AR and HC groups of edges that did not survive FDR correction (amPFC to MTG-L, vmPFC to pCC, Pcu to MTG-L, MTG-L to vmPFC, and IFG-R to MTG-L) were also followed up with a t-test comparing the AR group with the PD group; no significant differences were found for any FC values. Post hoc tests revealed that the hFDR group significantly differed from the hNoFDR group in FC values between the IFG-R and the MTG-R ($t(26) = 2.89$, $p = .008$, Hedges' $g = 1.11$), whereby hyposmic first-degree relatives had a higher FC compared to hyposmic non-relatives (see Table 6). PD patients did not significantly differ from controls in this connection ($t(46) = -0.71$, $p = 0.482$). None of the other edges showed significant different FC values between hFDRs and hNoFDRs. PD patients showed significant higher FC compared to controls between the amPFC and MTG-L ($t(46) = 2.79$, $p = .008$), the pCC and vmPFC ($t(46) = 2.72$, $p = .009$), and the MTG-L and vmPFC ($t(46) = 2.93$, $p = .005$).

3.4 Correlational Analyses

Table 7 shows the results of the Pearson's correlational analyses between abnormal FC values of edges that survived FDR correction and cognitive tests that showed abnormalities in the AR group and PD group compared to HCs. There were no significant correlations between the FC of the amPFC and the MTG-R and LNS scores in the AR group. There were also no significant correlations between FC of these ROIs and cognitive test scores that were abnormal in PD patients (i.e., SDMT scores and LNS scores).

As part of a secondary analysis, the assessment of correlations between FC of edges that were considered trends and abnormal cognitive test scores (significant p-value before Bonferroni correction) showed that semantic fluency scores were significantly related to FC values between the IFG-R and the MTG-L in the PD group ($r = .41$, $p = .049$). However, this correlation did not survive Bonferroni correction. No other edges that were considered trends showed significant correlations to cognitive test scores (see Table 7).

3.5 Analyses of Diagnostic Accuracy

A ROC analysis of the FC from the connection that was significantly different between AR and HC groups, and which survived multiple comparison correction (FC between the amPFC and the MTG-R) was conducted to assess the sensitivity and specificity of this measure to identify AR individuals. The results show that FC measurements between these regions have a good discriminatory power in differentiating AR individuals from HCs with a significant AUC value of .82. Figure 5 displays the ROC curve of the FC between the amPFC and the MTG-R.

In addition, a ROC analysis of scores from the LNS test (which were significantly lower in the AR group compared to the HC group) was also conducted. The results demonstrated that lower LNS test scores have a fair discriminatory power in differentiating AR individuals from

HCs with a significant AUC value of .76. The ROC curve of LNS test scores is shown in Figure 6.

Chapter 4: Discussion

Although impaired olfaction is a potential marker for the development of PD in otherwise not at-risk individuals (Berg et al., 2015; Ross et al., 2008), its specificity is low and may indicate the development of any number of neurological diseases. Olfaction may be seen as a screening tool, while other, more specific markers could be combined in order to better predict the onset of PD early in the course of illness. Cognitive deficits in the domains of processing speed, executive function, and working memory have been reported in relatively early phases of the disease (Aarsland et al., 2009; Bublak et al., 2002, Nombela et al., 2014). Furthermore, several studies have described that PD patients have abnormal FC between regions of the DMN (Amboni et al., 2015; Hou et al., 2017; Tessitore et al., 2012), and that DMN FC was associated with cognitive function (Disbrow et al., 2014; Lucas-Jiménez et al., 2016). Therefore, it may be possible that both cognitive deficits and aberrant DMN FC can be additional markers of prodromal-stage PD. The purpose of this study was to identify a group of individuals who were at higher risk of developing PD (hFDR and hNoFDR) and to examine cognitive function and resting state functional connectivity in the default mode network. We wished to determine whether the AR group performed more like PD patients than control subjects on cognitive testing and on functional connectivity. We also wished to determine the degree of diagnostic accuracy of these measures and whether there were associations between cognition and DMN FC in at-risk individuals. PD patients included in this study were in early stages of the disease which was determined by relatively short disease durations (approximately three years on average) and mild motor impairments based on scores of the Hoehn and Yahr scale (scores less than 3) and the UPDRS-III.

4.1 Cardinal Findings

The cardinal findings of this study are:

1. AR individuals had deficits in verbal working memory when assessed with a cognitively demanding task (i.e., LNS), but not when a less cognitively demanding task was used (i.e., digit span backwards).
2. Similar to the AR group, PD patients showed deficits in verbal working memory performance only when task demand was high, partially supporting our first hypothesis (H1) that AR individuals and early-stage PD patients would show similar cognitive deficits in the domains of executive function, working memory, and processing speed. PD patients also demonstrated deficits in the domain of psychomotor processing speed and a trend towards lower scores in a semantic fluency task.
3. Compared to controls, neither PD or AR individuals had reduced scores on tests that measure phonemic fluency and executive function. Also, groups did not differ on verbal and spatial working memory tests that had a lower cognitive demand.
4. With regard to DMN FC measures, the primary findings were that, compared to controls and PD patients, the AR group showed higher DMN FC between the amPFC and the MTG-R. These findings do not support H2 because we expected that, compared to HCs, the AR group would show reduced FC within the DMN similar to PD patients.
5. Secondary FC results of this study were that, compared to controls, the AR group showed trends of increased FC for multiple DMN connections (between the pCC and the vmPFC, the amPFC and MTG-L, the PCu and MTG-L, the MTG-L and vmPFC) and a FC decrease for one connection (IFG-R and MTG-L). FC values were similar to FC values of PD patients.

6. H3 was not supported because no significant correlations between cognitive performance and FC alterations in the DMN were found.
7. The accuracy of increased FC values to discriminate between AR individuals and HCs was good and the accuracy of working memory deficits as measured with the LNS test to discriminate between AR individuals and HCs was fair.

4.2 Cognitive Function in PD Patients and Hyposmic Individuals At-Higher Risk of PD

According to Braak's staging of disease progression, cognitive impairment is likely to appear in later stages when neuronal loss occurs in cortical brain regions that are associated with cognition (Braak et al., 2003). However, there have been reports of reduced cognitive functioning in newly diagnosed PD patients as well, such as poor performance in processing speed, attention, and executive function (Aarsland et al., 2009; Nombela et al., 2014). Our study adds to the existing literature on the cognitive profile of early-stage PD patients because patients exhibited deficits compared to controls in processing speed and in a high-demand verbal working memory task. Performance on verbal and spatial working memory tasks that are less cognitively demanding seems to be intact in early PD. Our findings are consistent with Bublak et al. (2002), who demonstrated that PD patients show deficits in working memory capacity that are associated with increasingly slowed initiation time in a more difficult working memory task condition compared to less demanding conditions. A reduced working memory capacity has implications for the ability to manipulate complex cognitive processes because less attentional resources are available to manage the processing of given information (Bublak et al., 2002). Consistent with the study by Bublak et al. (2002), PD patients from the current study were in early disease stages with a relatively short mean disease duration (i.e., approximately three years). Early-stage PD was also confirmed by the mild disease severity with respect to motor impairments in the current

study and mild to moderate disease severity in Bublak et al. (2002). In a study by Liozidou and colleagues (2012), non-demented PD patients with a longer disease duration (i.e., 10 years) but also with mild to moderate motor impairments (Hoehn and Yahr scale score of 3 or less) were shown to score lower than controls on a working memory task (digit span backwards) that has a relatively low complexity or cognitive demand. Consequently, working memory capacity may decrease with longer disease duration and deficits on working memory tasks with lower cognitive demand may only appear as the disease progresses beyond the early stage.

In the current study, early PD patients did not show deficits in executive function and verbal fluency tests compared to healthy controls, a finding which is inconsistent with previous reports of early PD patients' cognitive profile (Nombela et al., 2014; Parrao et al., 2012). However, in our study, a trend towards lower semantic fluency scores compared to controls was observed, which suggests that deficits may be present in some patients. A previous study has demonstrated that executive function scores are related to UPDRS-III motor impairment, implying that poorer performance in executive function is linked to motor progression (Riggeal et al., 2007). Thus, deficits in the domain of executive function in patients from our sample may appear in later disease stages and with progressive changes in neurodegeneration, which is in accordance with the staging system of Braak et al. (2003). The discrepancies between the current and past studies with regard to executive dysfunction may also be explained by participants' education level. Higher years of education have been shown to influence executive function test scores (Miranda et al., 2020). The mean education of participants was relatively high for all groups in the current study and although education was controlled for in the analysis of group differences, it did not seem to have a significant effect on test scores of verbal fluency but it significantly affected mean scores of the executive function task (TMT4-5). In contrast,

education was not controlled for and lower mean years of education were reported for participants in Parrao et al. (2012), who found executive function deficits in early PD patients. Better performance on tests of executive function was also linked to more years of education in Nombela et al. (2014), supporting the idea that higher education serves as cognitive reserve, a factor that preserves cognitive processes in the presence of neuropathology (Barulli & Stern, 2013).

One of the main findings of the current study is that the AR group demonstrated the same pattern of working memory deficits as PD patients. The effect sizes of the working memory deficit on the LNS task were large and similar for the AR group (Hedges' $g = 0.84$) and the PD group (Hedges' $g = 0.89$). Our finding suggests that working memory deficits in addition to hyposmia may be an early marker of the progression towards PD. Furthermore, our analysis of diagnostic accuracy demonstrated that lower LNS scores can reliably classify AR individuals of PD compared to HCs and may therefore be useful in clinical and research settings where individuals at-risk of neurodegeneration are assessed. Past studies have reported that olfactory deficits are related to working memory dysfunctions in PD patients (Morley et al., 2011), and the present finding points out that shortcomings in working memory performance may occur in hyposmic individuals even before PD is clinically diagnosed. Chahine et al. (2016) reported that idiopathic hyposmic individuals perform worse on tasks of working memory, executive function, and global cognition compared to normosmic controls, but only if they exhibited DAT binding deficits. It is possible that having both DAT binding abnormalities and hyposmia predicts the development of PD. In the current study, DAT binding was not examined, but working memory deficits were observed in the AR group. Not knowing whether hyposmic participants have a reduced DAT binding lowers confidence in the conclusion that diminished working memory

performance of hyposmic individuals is linked to the development of PD. Instead, it may also represent the progression towards other neurodegenerative diseases, such as AD (Kessels et al., 2011). Both hyposmia (Hagemeier et al., 2016) and working memory deficits have been reported to be present in AD patients and idiopathic MCI patients, who are thought to be in the prodromal stage of AD/dementia (Kessels et al., 2011). It would be beneficial for future research to assess DAT binding deficits in AR individuals to determine whether they are in the prodromal stage of PD rather than in the prodromal stage of dementia.

Nevertheless, about half of the participants in the AR group were first-degree relatives of PD patients, who, due to genetic vulnerabilities, have an increased risk of developing PD rather than other neurodegenerative diseases (Mickel et al., 1997). Because working memory performance did not differ between hyposmic relatives and hyposmic non-relatives, it remains possible that working memory deficits in the included AR group may be linked to early PD-related pathological processes. It has been reported that 10% of idiopathic hyposmic individuals that do not have a first-degree relative with PD develop PD (Haehner et al., 2019), which is comparable with the 10% increased risk of unaffected first-degree relatives of PD patients to develop PD due to the presence of olfactory deficits (Ponsen et al., 2004). In addition, hyposmic individuals without PD have an odds ratio of 3.1 - 5.2 for developing PD within four years compared to individuals with mild or no olfactory dysfunction (Ross et al., 2008). In contrast, the odds ratio for individuals with olfactory dysfunction to develop dementia within five years was lower (odds ratio = 2.13; Adams et al., 2018), suggesting that individuals with idiopathic hyposmia that were included in the AR group of this study may be more likely to develop PD than they are to develop dementia.

Hyposmic first-degree relatives and hyposmic non-relatives did not differ on any of the cognitive tests examined in our study, suggesting that being a first-degree relative of a PD patient does not differentially affect cognitive performance when hyposmia is present. This finding also supports our decision to combine both hyposmic subgroups into one at-risk group. Previous research has reported that first-degree relatives who do not have olfactory impairments and who have an increased genetic risk of developing PD do not show working memory deficits compared to individuals with a lower genetic risk (Thaler et al., 2016). Moreover, as the presence of hyposmia in first-degree relatives of PD patients increases the risk of developing PD (Ponsen et al., 2004), olfactory deficits seem to play an important role in the progression towards PD. The presence of hyposmia has also been related to working memory deficits of PD in the past (Morley et al., 2011). Therefore, hyposmia may be a key element that is associated with working memory deficits in the prodromal stage of PD.

The link between abnormal cognitive functions and hyposmia is proposed to be related to neurotransmitter system alterations in PD, including the dopaminergic and cholinergic systems (Bohnen et al., 2008; 2010). Specifically, Bohnen et al. (2008) described that DAT binding in the hippocampus is linked to odour identification abilities in early-stage PD patients, indicating that PD-related dopaminergic denervation has an influence on olfactory function. As the hippocampus also plays a role in working memory processing (Leszczynski, 2011), dopaminergic denervation may affect cognitive functioning as well. Thus, the observed working memory deficits in the AR group are perhaps linked to reduced dopaminergic function in the hippocampus. Furthermore, Bohnen et al. (2010) report that cholinergic denervation within the hippocampus also plays a part in olfactory functioning of PD patients and cognitive function. Thus, the findings of working memory deficits in the hyposmic at-risk group support the notion

that AR individuals may be in prodromal stages of PD and exhibiting early PD-related neurotransmitter system alterations. Furthermore, it has been proposed that adequate olfactory identification requires the recruitment of frontal and prefrontal brain areas (Bohnen et al., 2008; Wang et al., 2005). PD patients who have hyposmia demonstrate alterations in connectivity patterns with frontal areas compared to patients with good olfactory functioning and healthy controls, suggesting that alterations within frontal regions may be related to olfactory deficits in PD (Sunwoo et al., 2015). In addition, separate meta-analyses reported that activations in both prefrontal and frontal brain areas are typically seen during working memory processing (Owen et al., 2005; Wang et al., 2019). Thus, it could be possible that alterations in frontal brain activity, which may be due to PD, could also be underlying the relationship between olfactory deficits and poor working memory performance.

4.3 DMN Functional Connectivity as Potential Early Marker for Neurodegeneration

In addition to working memory deficits, this study found that the AR group also demonstrated alterations in resting-state FC between the amPFC and the MTG-R, suggesting that early neurodegenerative processes have an effect on the DMN. The increased FC between the amPFC and the MTG-R has a good diagnostic accuracy as the AUC value was relatively high (i.e., .82), meaning that FC between these regions can help to correctly identify AR individuals, and that the chance of random classification into the AR group or the HC group is low. PD patients do not share the abnormal FC between the amPFC and the MTG-R with AR individuals. Yet, the secondary findings showed that PD patients have similar FC increases as the AR group between multiple ROIs, and although these findings represent trends and are more exploratory in nature, they are lending confidence that PD-related FC alterations exist.

Studies that have assessed the DMN at rest in PD show high heterogeneity of results (Hohenfeld et al., 2018). Specifically, there is no consensus on which DMN edges show abnormal FC, as different studies report that different edges are affected. Additionally, although most studies report reduced DMN FC between certain edges (e.g., Amboni et al., 2015; Hou et al., 2017; Lucas-Jiménez et al., 2016; Tessitore et al., 2012), some studies describe increased FC between some ROIs along with reduced FC between others (Baggio et al., 2015; Campbell et al., 2015; Gorges et al., 2015). The majority of studies that have assessed the DMN during the resting state have examined patients that have similar degrees of motor impairment (i.e., Hoehn and Yahr or UPDSR-III scores); as such, inconsistencies in findings cannot be explained by varying disease severity. PD participants with mild to moderate disease are typically recruited for MRI studies so that MRI measurements as well as additional cognitive testing are not affected by motor symptoms (Disbrow et al., 2014). Compared to the current study, most of the previous research recruited PD patients with a longer mean disease duration (i.e., over five years; e.g., Amboni et al., 2015; Baggio et al., 2015; Tessitore et al., 2012). Therefore, the finding of reduced FC for most DMN edges may be related to increased disease duration. In contrast, Campbell et al. (2015) reported increases in the average connectivity of the DMN in patients with a relatively short disease duration (i.e., under 5 years), supporting the idea that a different pattern of FC alterations exists in early PD patients compared to patients who are living with the disease for a longer time. The current study supports findings of increased DMN FC in early PD patients and expands previous literature on the notion that alterations could be present in preclinical stages of the disease. Moreover, the current study primarily assessed FC abnormalities in individuals that are expected to be in the prodromal stage of PD, and thus may be demonstrating early FC changes that are different from subsequent disease stages. However, it

cannot be ruled out that some AR participants may be progressing towards another neurodegenerative disease.

Inconsistencies in study outcomes may be related to methodological differences, such as differences in MRI acquisition parameters or analysis approaches for functional connectivity data as well. Several studies have used independent component analysis (ICA) to define the DMN (e.g., Amboni et al., 2015; Tessitore et al., 2012). ICA is a data-driven approach which decomposes signals into spatially independent components. Although ICA has the advantage of enabling the automatic removal of noise components (e.g., physiologic processes that may bias the functional signal), it is challenging to obtain the same functional network components for each participant, making group comparisons difficult (Rosazza et al., 2012). Moreover, it is possible that different edges show FC alterations across studies because different ICA components were included to define the DMN. In contrast, the current study used an ROI-to-ROI analysis approach which assesses correlations of temporal activity patterns between two or more a priori defined regions (Rosazza et al., 2012). DMN ROIs were based on the coordinates from Spreng et al. (2013). In a further study that employed these coordinates (Baggio et al., 2015), PD patients with and without MCI were compared to HCs. This group reported ordered reductions in FC between medial prefrontal regions (i.e., vmPFC, amPFC, and dmPFC) and bilateral aTL, the HF-L, and the pIPL, as well as between the pCC and the HF-L, with HCs having the highest FC followed by PD without MCI. PD-MCI patients had the lowest connectivity values. FC increases were only observed between the MTG-R and the TPJ-R, with PD-MCI patients having the highest FC followed by PD patients without MCI and HCs (Baggio et al., 2015). Hou et al. (2017) also used this ROI approach and described that cognitively unimpaired early-stage PD patients with a rigidity-dominant subtype displayed reduced FC between the aTL-L and the HF-

L, the MTL-R and the pIPL-R, the aTL-R and the IFG-R, the MTG-R and the pCC, and lastly, the MTG-R and the precuneus. The affected edges from both studies do not overlap with the current study's findings. This could be due to the inclusion of comorbid conditions of PD patients such as PD-MCI (Baggio et al., 2015) or the inclusion of the rigidity-dominant subtype of PD (Hou et al., 2017) which may affect certain connections of the DMN in different ways. In addition, variations in the preprocessing of the rsfMRI data such as the inclusion of additional confound regressors to increase the signal-to-noise ratio may have affected the results. For example, the present study controlled for the global signal, which is an omission from Hou et al. (2017). The global signal is an fMRI timeseries component that is shared among all brain voxels and is thought to include non-neuronal confounds of cardiac activity, respiratory cycles, or arterial CO_2 concentration among others (Murphy & Fox, 2017). Murphy & Fox (2017) described that not controlling for global signal can lead to different results across studies. Removing the global signal has the advantage of enhancing the detection of true ROI-to-ROI correlations and can reduce artefacts. Nevertheless, the global signal can also introduce spurious anticorrelations, which are negative ROI-to-ROI correlations that are difficult to interpret (Murphy & Fox, 2017).

4.3.1 Increased Functional Connectivity as a Result of Compensatory Processes

Our primary findings show that FC is increased between the amPFC and the right MTG of the DMN in the AR group compared to controls. Increased activity and FC within brain networks have been related to compensatory processes in neurodegenerative diseases in the past (De Marco et al., 2017; Poston et al., 2015; Yang et al., 2013). This enhanced activity/connectivity is posited to occur in response to neuronal loss and is related to the maintenance of behavioural or cognitive performance (Gregory et al., 2017). A model of FC

changes has been proposed describing that FC within brain networks is elevated during disease onset. As the disease progresses, FC declines and reductions will be shown at higher disease levels, which are associated with reductions in cognitive performance (Gregory et al., 2017). Increased FC in hyposmic individuals may therefore be indicative of compensatory processes in response to commencing neuronal loss and early neurodegenerative changes in order to maintain behavioural and cognitive performance. Although the amPFC and the MTG-R are part of the DMN and are functionally connected to other DMN ROIs, in this study, these two brain regions are not positively connected to one another in healthy controls. Increased resting-state FC between two network regions has been proposed to be linked to past simultaneous activation during cognitive processes, to a prediction and expectations about which regions will be used together in the future, or a combination of both (Fox & Raichle, 2007; Jolles et al., 2011). Thus, an indirect relationship is thought to be present between cognitive processes and resting-state FC. Therefore, the elevated FC in hyposmic individuals may also have occurred as the amPFC and the MTG-R have been synchronously recruited during cognitive processes in the past and are expected to be in the future. The amPFC has been associated with performance on a semantic fluency task and as part of the DMN shows a change in activation during task performance (Shapira-Lichter et al., 2013). Adequate functioning of this region has implications for semantic fluency. The increased connectivity between these ROIs in the AR group may therefore represent the recruitment of additional neural resources to delay oncoming deficits in semantic fluency. The analysis of cognitive test scores supports this notion because AR individuals' semantic fluency is intact.

AR individuals also show higher FC between the amPFC and the MTG-R compared to early PD patients. PD patients had a functional connectivity value close to zero, indicating that

these ROIs are not functionally connected to one another in PD (i.e., they show differences in spontaneous activation patterns over time). As it is expected that PD patients already have significant neuronal loss after diagnosis (Braak et al., 2003), compensation should no longer be sustainable. Thus, greater deficits in cognitive performance should emerge with increased disease progression, and this notion is supported by the trend towards reduced semantic fluency scores in the PD group. Similarly, the loss of compensatory processes has also been related to poor cognitive performance of PD patients in the past (Poston et al., 2015) and reduced FC within the DMN has been demonstrated in PD patients with PD-MCI compared to patients with less cognitive impairments for some edges, including connections with the amPFC (Baggio et al., 2015). Gorges et al. (2015) also reported increases in DMN FC that include a network expansion in cognitively unimpaired patients compared to controls, indicating elevated FC and the recruitment of additional neuronal resources may be a compensatory response to cognitive impairment. Compared to healthy controls and PD patients without MCI, the presence of MCI decreased the DMN FC of PD patients (Gorges et al., 2015). Similarly, as patients included in our study show deficits in multiple domains (i.e., working memory and processing speed), FC may be decreased compared to hyposmic individuals who exhibit better cognitive function. Olde Dubbelink and colleagues (2014) conducted a longitudinal study to assess the relationship between cognitive decline and alterations in resting-state FC of the brain. PD patients with moderate motor symptoms and cognitive deficits at baseline demonstrated progressive reductions in whole-brain FC (including regions of the DMN) over a three-year period. These reductions were also associated with decline in global cognitive performance (Olde Dubbelink et al., 2014). Overall, these findings also demonstrate that FC decreases, while cognitive impairments increase with the progression of PD, but that FC changes in PD are not restricted to the DMN.

In addition to the primary findings, secondary findings, that are exploratory, support that hyposmic first-degree relatives and non-relatives show abnormal DMN FC, as there are trends of increased FC for multiple edges that may be explained by compensatory processes. Also, the AR group did not differ from PD patients on FC measures of these edges. This suggests that early-stage PD patients may also show signs of compensatory processes, and it supports the notion that increased FC may be a prodromal marker of PD. Consistent with the primary findings, FC with medial prefrontal regions and with the middle temporal gyrus was elevated, highlighting the importance of these DMN regions in early disease stages. For example, the findings show a trend of increased FC between the vmPFC and pCC, which may be a compensatory response to PD-related pathological changes affecting working memory. The connection between these main DMN ROIs has been associated with working memory during task-based fMRI scans previously (Sambataro et al., 2010), with higher connectivity being linked to better performance in healthy individuals. Alterations in DMN function of healthy individuals in the form of enhanced deactivations, have also been associated with an increase in working memory task demand (McKiernan et al., 2003). Therefore, possible PD-related pathological changes that increase FC in these regions may improve working memory performance. With respect to the present study, increased FC between the vmPFC and the pCC in both the AR and early PD groups may therefore be related to the maintenance of working memory performance on tasks that require a lower WM demand (i.e., spatial span backwards and digit span backwards). However, as task demand increases, compensatory processes are not sufficient to maintain performance, and deficits appear on a high-demand working memory task. Nevertheless, it is unclear how resting-state FC can be compared to previous research in task-based FC because both measure different aspects of brain activity. Typically, task-based FC evaluates the correlation between changes in

two regions of brain activation in response to a cognitive task while taking into account baseline brain activity (i.e., resting-state spontaneous activations). In contrast, resting-state FC assesses the correlation of the spontaneous activation between two regions to identify intrinsic brain networks (Greicius et al., 2003). However, it has been proposed that resting-state networks represent the organization of specific regions that are recruited together during task performance (Buckner et al., 2013), suggesting that disease-related changes in FC at rest may appear in FC during task-based activation as well.

Furthermore, the vmPFC has been shown to be implicated in olfactory processing as it is activated when odours are present (Eiler II et al., 2012). The presence of hyposmia in both the AR group and the PD group may indicate abnormalities in brain areas that are related to olfactory processing. With respect to the notion of compensatory processes, an elevated FC with the vmPFC may represent the recruitment of additional neural resources to counteract underlying causes of olfactory impairment. However, these compensatory processes are incomplete as olfactory impairments are present (Gregory et al., 2017). PD patients with hyposmia have demonstrated decreased FC between the pCC and areas of the limbic system, and increased FC between the pCC and the left inferior parietal lobule compared to patients with no olfactory deficits or mild hyposmia (Su et al., 2015). The vmPFC was not assessed by Su et al. (2015) but their findings show that PD-related olfactory impairment is linked to elevated FC changes that involve the pCC and can therefore support the current proposition that increased FC between DMN ROIs may be an early marker of increased risk of developing PD in AR individuals.

The secondary findings are based on trends because they did not survive multiple comparison correction and because of this, they have to be viewed with caution. Nevertheless, they are supportive of the primary findings and prompt further research to investigate possible

increased connectivity with prefrontal, middle temporal, and posterior cingulate regions of the DMN in individuals who are considered to be in the prodromal stage of PD. Interestingly, the secondary findings also propose a trend towards lower FC between the IFG-R and the MTG-L in the AR group compared to controls which warrants further explorations. In contrast to edges that showed increased FC in AR individuals, this finding seems to be driven by hyposmic individuals who do not have a relative with PD, since this subgroup had significantly lower connectivity values compared to hyposmic relatives. The hyposmic groups did not differ on any of the cognitive test scores, so the difference in FC does not seem to be related to differences in cognition. The only difference found between these groups based on the assessments of this study was that the hNoFDR group included more females that the hFDR group. Yet, it was reported that there is no sex difference in resting-state DMN connectivity in older adults, so it is unlikely that the imbalance of males and females in the groups would have influenced the results (Bluhm et al., 2008). FC between these regions may not be PD related but may be linked to olfactory deficits due to other diseases that some hyposmic non-relatives may develop. As mentioned above, olfactory deficits were reported to be present in both MCI and AD (Hagemeier et al., 2016). In addition, individuals with MCI also show decreased FC within the DMN (Das et al., 2013). This supports the idea that some hyposmic non-relatives included in the current study may be progressing towards MCI, which predicts the development of AD. The sample sizes of the hyposmic relative and hyposmic non-relative groups were relatively small, which may have influenced these results. However, a large effect size between these groups for this DMN connection was found (Hedges' $g = 1.11$), increasing the confidence of the differences between hyposmic relatives and non-relatives on FC measures between the IFG-R and the MTG-L and suggests that some hyposmic non-relatives may be progressing towards MCI and/or AD.

Nevertheless, we did not find a difference in FC values between hFDRs and hNoFDRs for any other DMN edge that showed increases in FC. Similar to the cognitive test results, this supports the notion that some individuals in both subsamples may be in the prodromal stages of PD although other participants in the AR groups may be progressing towards MCI and dementia.

4.3.2 Increased Functional Connectivity as a Sign of Disease-Related Changes

As mentioned above, it is possible that increased FC within the DMN is related to compensatory processes as an attempt to maintain cognitive performance. Nevertheless, as this study assessed the DMN during rest, a direct relationship between cognitive functioning and FC measures within this study cannot be established. Compared to task-based fMRI, cognitive assessments and FC values were obtained independent from each other. Therefore, it remains unclear whether increased FC is the result of compensatory processes leading to the maintenance of cognitive performance. FC within the DMN in the AR group may instead be a sign of alterations related to olfactory deficits or neurodegenerative disease onset separate from cognitive function. The nature of correlational analyses also makes it difficult to interpret the results. An increased FC may represent elevated spontaneous fluctuations in the BOLD signal over time that is consistent across two regions, which is consistent with the idea of compensation in prodromal PD. However, it could also mean a synchronous reduction in spontaneous BOLD signal across two regions, representing possible disease-related pathological processes.

Amboni et al. (2015) examined the resting-state FC of the DMN in early PD patients that either had PD-MCI or that were cognitively unimpaired. Similar alterations in FC were reported for PD patients with MCI and without MCI, suggesting that DMN FC does not affect patients' cognitive status (Amboni et al., 2015). The correlation analysis of the current study supports this notion because reduced cognitive scores observed in the AR group and the PD group were not

associated with abnormalities in DMN FC. Nevertheless, there was a trend of a significant correlation between semantic fluency deficits and reduced FC between the IFG-R and the MTG-L in PD patients. As this correlation did not survive multiple comparison correction, the result is not robust. Abnormal DMN FC in AR individuals and PD patients seems to be independent from the observed cognitive deficits. As a result, it is reasonable that increased connectivity between DMN ROIs in AR individuals and PD patients is a sign of disease-related alterations or incomplete compensation as an attempt to maintain olfactory function rather than compensatory processes to maintain cognitive function. The absence of a relationship between DMN FC and reduced cognitive test scores is supported by Amboni et al. (2015) and Gorges et al. (2015), who described that DMN FC and cognitive test scores are not related in PD patients, despite patients being cognitively impaired. Moreover, the lack of an association between FC values and working memory deficits in both AR individuals and PD patients may suggest that working memory deficits are related to abnormalities within other intrinsic brain networks, such as the frontoparietal network, whose connectivity is abnormal in PD patients as well (Caminiti et al., 2015).

4.4 Limitations, Strengths, and Future Directions

While this study offers valuable insights into additional markers of the development towards PD in non-manifesting hyposmic individuals at higher risk, several limitations need to be addressed. First, a cross-sectional design was used for this study, which is insufficient for determining whether individuals in the AR group will develop PD in the future. It is possible that some AR individuals are progressing towards other neurodegenerative diseases. As mentioned above, hyposmia has also been identified as a sign of dementia (McShane et al., 2001; Seligman et al., 2013) and is linked to MCI, which has been considered in some individuals as a preclinical

stage of dementia (Hagemeier et al., 2016). Working memory deficits (Kessels et al., 2011) and abnormal FC within the DMN exist in patients with MCI (Das et al., 2013; Yang et al., 2017), and as such, some individuals from the AR group may be developing MCI as a sign of future dementia. However, PD-MCI has been reported in 15% to 43% of newly diagnosed PD patients (Pedersen et al., 2017), up to 80% of PD patients will develop dementia over the course of their illness (Aarsland et al., 2003; Yarnall et al., 2014). Hyposmic first-degree relatives of patients and hyposmic non-relatives may therefore be progressing towards a subtype of PD that is at increased risk of developing dementia (Parkinson's Disease with Dementia; PDD). The probability of idiopathic hNoFDRs and hFDRs developing other neurodegenerative diseases should also be investigated, particularly with respect to dementia (Kessels et al., 2011; Seligman et al., 2013). Future studies should use a longitudinal design to confirm whether the combination of hyposmia, working memory deficits, and DMN FC alterations in non-manifesting individuals is useful for discriminating future PD, with or without MCI, from other neurodegenerative disorders. However, one strength of the current study lies in the inclusion of hFDRs within the AR group. This subsample enhances the likelihood that the reported abnormalities found in the AR group are related to PD because of relatives' genetic vulnerabilities that increase the risk of developing PD compared to other diseases. Our findings also represent strong evidence in favour of combining the two AR subgroups because no differences between hyposmic relatives and non-relatives were noted in any of the examined measures. One exception was the difference between FC values between the IFG-R and the MTG-L; however, this finding was not robust and may have been a false positive result.

Another limitation of this study was the small sample size within both AR subgroups, which decreased the power to detect a significant effect between the groups. Therefore, it is

possible that there are additional differences between the hyposmic first-degree relatives and hyposmic non-relatives that could not be detected in this study, which affects the generalizability of the findings. Future studies should further investigate whether there are additional differences between these AR subgroups with larger sample sizes. Moreover, the generalizability of this study may have been affected by a self-selection bias, as the study required participation in a long testing session while following the instructions from the researcher, and thus, required a certain level of independence and overall function. Therefore, PD patients and AR individuals included in this study may represent a well-functioning subset of their respective populations which may have influenced the results. The relatively high mean level of education of participant groups supports this suggestion. As the analyses in this study controlled for education, however, the potential influence of this bias is reduced.

The current study is limited in how observed cognitive deficits in both the AR and PD group translate into clinically meaningful and interpretable findings. Scaled scores, which standardize raw test scores based on normative data of healthy controls would be beneficial for quantifying the level of clinical deficits. This study examined raw test scores because scaled scores would result in a loss a variability within the data. Although the observed differences between the HC group and the AR and PD groups may not be clinically significant, large effect sizes that range from 0.77 to 1.35, and the measure of diagnostic accuracy (ROC analysis) which determined that lower working memory scores can reliably discriminate between AR and HC individuals indicate that the observed cognitive deficits in the AR and PD groups are meaningful and endorse further investigations.

As part of a larger study, this research included the SDMT as a measure of processing speed. The SDMT can be administered by instructing the participant to write the answers directly

into the designated boxes or saying the answers out loud so that the administrator can write them down. For this study, the former approach was used which may have affected test outcomes for PD participants as motor symptoms cannot be controlled for (Jaywant et al., 2018). Nevertheless, patients that were included in this study were in the early stages of PD and have relatively mild levels of disability as assessed with the Hoehn and Yahr scale as well as with the UPDRS-III scale. Thus, it is less likely that lower scores on the SDMT are predominantly explained by motor impairments.

Furthermore, the analysis approach to assess the FC within the DMN has its strengths and weaknesses. The present study applied an ROI-to-ROI approach which typically relies on the use of previously established atlases or coordinates (as in the current study) to identify ROIs of resting-state networks a priori (Rosazza et al., 2012). As ROIs of the DMN have been well-defined in the past, this aspect represents a strength, rather than a limitation of the analysis approach and would facilitate the reproducibility of this study (Fox & Greicius, 2010). Compared to a data-driven method such as ICA, the ROI approach is advantageous because each ROI is the same in each participant so that they can easily be combined for group analyses. Moreover, the ROI approach leads to results that can be interpreted without many difficulties. With ICA, components that represent noise can be automatically isolated from components that represent the resting-state network. However, this introduces the problem of having to decide which components are part of the desired network and which are noise and should be excluded (Rosazza et al., 2012). The spontaneous BOLD fluctuations that are measured with rsfMRI within each ROI can also be biased by non-neuronal noise (i.e., physiological fluctuations) which can in part be accounted for by global signal correction (see above; Fox & Raichle, 2007). Yet, doing this can result in spurious anticorrelations between two ROIs and may also affect ROI-to-

ROI correlations as global signal can also include neural signals. Therefore, it is suggested that the impact of global signal correction should be assessed for each study (Murphy & Fox, 2017). Although the inclusion of the global signal correction within the current study reduced ROI-to-ROI correlations by a moderate amount, it was selected because the exclusion would have resulted in increased connections between mostly peripheral ROIs that are likely driven by artifacts. There is substantial variability between findings across studies that assessed the DMN with respect to PD, likely due to methodological differences. In order to reduce this variability, future studies should try to use similar methods or account for differences so that results are comparable more easily. For example, the inclusion of common DMN coordinates and similar data processing steps should be considered.

A strength of using rsfMRI is that it is a non-invasive technique that does not require the execution of a task. Therefore, it is applicable for a wide range of clinical disorders including patients who may not have the physical or cognitive abilities needed to perform a task in the MRI scanner (Fox & Greicius, 2010). Moreover, rsfMRI focuses on spontaneous fluctuations in the BOLD signal and requires less scanner time compared to task-based fMRI, increasing the compliance of clinical patients or research participants for this examination. Task-based fMRI focuses on changes in BOLD signal based on the task used and considers spontaneous activity as noise that needs to be accounted for in order to detect true task-related modulations. Therefore, the signal-to-noise ratio is lower compared to rsfMRI and many trials of the task are required to acquire a signal that represents task-based activation (Fox & Greicius, 2010). However, an important limitation of rsfMRI and FC analyses is that it is difficult to interpret resting-state data with regard to cognitive function. Although the resting-state DMN has implications in passive cognitive processes such as mental explorations and anticipation of future events or tasks

(Buckner et al., 2008) it is not possible to assess a direct relationship between DMN FC and task-based cognitive domains because both examinations are conducted at different time points (Gregory et al., 2017). This limitation makes the interpretation of the relationship between cognitive test scores and DMN FC challenging. Although the current study reported that cognitive deficits are likely to be independent from resting-state DMN FC alterations in both AR individuals and PD patients, future research should investigate this further as other studies have reported that an association between these variables exists (Disbrow et al., 2014; Hou et al., 2016; Lucas-Jiménez et al., 2016). This study examined domains of working memory, processing speed, and executive function. However, it is possible that the resting-state DMN may be independent from these domains but may show associations with others such as language, or short- and long-term memory, which were not assessed in this study. For example, Lucas-Jiménez et al. (2016) stated that low FC between the pCC and the temporal lobe was associated with verbal and visual memory in PD patients.

Additionally, it might be more advantageous for future research to assess the DMN in AR individuals during task-based fMRI as it can give better insights into the relationship between FC and cognition. In healthy older individuals, a higher DMN deactivation during task-based assessments in the medial prefrontal cortex, left posterior parietal cortex, and the pCC has been related to better working memory performance (Sambataro et al., 2010). Better performance was also related to increased FC between the pCC and medial prefrontal cortex, suggesting that sufficient suppression within the DMN is important for cognitive performance as it helps to allocate required cognitive resources (Sambataro et al., 2010). Early PD patients and individuals at-risk for PD that have deficits in working memory may therefore demonstrate potential decreases in DMN activation for this task or increased deactivation in the DMN due to

compensatory processes, which would support the current study's finding. However, due to the lack of studies investigating the association between the resting state and task-based DMN FC in early PD stages, this idea remains hypothetical and needs to be explored further.

Moreover, the explanation that increased FC in the AR group represents compensatory processes needs to be viewed with caution. Typically, compensation occurs with the presence of neuronal loss or other neuropathological changes (Gregory et al., 2017); yet it is unknown whether individuals included in the AR group show early neuronal changes because we did not analyse quantitative structural brain measurements in this study. Therefore, the notion of compensatory processes in preclinical stages of PD remains inconclusive and warrants further research. Multi-method approaches that include structural MRI scans in addition to functional scans may help to determine whether both structural and functional changes exist. When early PD patients have been assessed with both gray matter volume and rsfMRI FC measures, compared to controls, PD patients showed reduced gray matter volumes in the pCC, the anterior cingulate cortex, the precuneus, the left middle temporal lobe, and the bilateral inferior parietal cortex (Lucas-Jiménez et al., 2016). The reduced gray matter volume of the pCC and the precuneus correlated with FC values between the pCC and the left middle temporal lobe, suggesting that structural changes (i.e., gray matter atrophy) are measurable in early PD and that they are linked to abnormalities in DMN FC (Lucas-Jiménez et al., 2016). Moreover, the use of SPECT scans to assess deficits in DAT bindings can also be a useful method to determine pathological neuronal processes in AR individuals (Berendse et al., 2001). Specifically, SPECT scans have shown that first-degree relatives with hyposmia and DAT binding deficits have an increased risk of 12.5% to develop PD during a 5-year period (Ponsen et al., 2010). Compared to first-degree relatives, the hNoFDR group is more ambiguous with respect to the risk of

developing PD, although idiopathic hyposmia has been identified as a prodromal marker of PD by the Movement Disorder Society (Berg et al., 2015). The assessment of DAT binding deficits may reduce this ambiguity as well.

The inclusion of participants who are left-handed constitute a further limitation for this study. Left-handed individuals are often excluded from neuroimaging studies because they show more variable neural substrates of specific cognitive processes (e.g., spatial location and intensity of brain activation) which may create spatial noise within a dataset and therefore, influences the results of a study (Bailey et al., 2020). The differences between right- and left-handed individuals is mostly pronounced in the hemispheric dominance of language processes, as left-handed individuals are more likely to show bilateral or right-sided laterality for language processes, compared to right-handed individuals, who predominantly demonstrate left-sided laterality (Pujol et al., 1999). Handedness has also been reported to influence DMN FC patterns. Saenger et al. (2012) reported that an increased DMN FC in the MTG, the SFG, the middle frontal gyrus, and the inferior parietal of the right hemisphere was more noticeable in right-handed individuals compared to left-handed individuals, indicating that handedness may affect DMN FC measures. Thus, the inclusion of both left- and right-handed participants the current study may have influenced the FC results. Nevertheless, research studies that assessed potential differences in DMN FC between right-and left-handed individuals with and without neurodegenerative diseases are scarce. Thus, future research should further assess whether handedness affects the DMN in healthy individuals and in patients with a neurodegenerative disease such as PD. The inclusion of both right- and left-handed individuals, however, may increase the generalisability of the current study on a population level (Bailey et al., 2020). Approximately 10% of people in the general population are left-handed (de Kovel et al., 2019),

which is represented by a similar proportion of left-handed participants in our study sample. It has also been proposed that excluding participants based on handedness could restrict the scientific understanding of the brain and that the inclusion of left-handed individuals in neuroimaging research should be encouraged, unless there are strong justifications for the exclusion of such participants (Bailey et al., 2020).

4.5 Conclusion

This study is the first to assess both cognitive function and FC measures of the DMN in hyposmic individuals who are at increased risk of developing PD in comparison with healthy controls and early-stage PD patients. We found that verbal working memory deficits as well as increased DMN FC may be early markers of neurodegeneration in idiopathic hyposmic individuals, as similar cognitive deficits and FC alterations were found in PD patients. The diagnostic accuracy of working memory deficits and DMN FC abnormalities for individuals at increased risk of developing PD ranged from fair to good, respectively. In addition to working memory deficits, PD patients demonstrated cognitive deficits in processing speed and a trend towards lower semantic fluency performance. The results from our cognitive test analysis suggest that individuals in the prodromal stage of PD and early-stage PD patients may show deficits in specific cognitive domains. This pattern of cognitive deficits in early disease stages may differ from later disease stages because past research has indicated that PD patients with longer disease durations show performance deficits in a greater variety of cognitive tests (Liozidou et al., 2012) compared to the current study. Working memory deficits may be a marker of early PD but only when assessed with a task that requires a high level of cognitive resources. Early cognitive deficits in hyposmic first-degree relatives and hyposmic non-relatives may also indicate the progression towards a subtype of PD, PD-MCI, or the progression towards another

neurodegenerative disorder. With regard to our FC findings, increased FC in the AR group could suggest compensatory processes to maintain some aspects of cognitive function in response to early neurodegeneration. Alternatively, these alterations may be a sign of early disease onset independent of cognitive impairment. Further research is needed to delineate these concepts. Hyposmic first-degree relatives of PD patients and hyposmic non-relatives did not differ on cognitive test scores or DMN connectivity between edges that showed increased FC values. This indicates that the presence of hyposmia rather than genetic vulnerabilities may be an important element that is related to cognitive impairment and FC abnormalities in PD. The findings have implications for future research to investigate idiopathic hyposmic individuals with regard to neurodegenerative diseases, and to provide a foundation for future research to investigate whether the combination of hyposmia, working memory deficits, and increased FC measures between specific DMN ROIs can be used clinically to define the prodromal stage of PD.

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Table 1

Demographic Data for Participant Groups

	PD	AR	HCs	*F* (2,78)	*p*
N	26	30	26	-	-
Sex (male/female)	15/11	15/15	15/11	-	.797*
Handedness (right/left)	22/4	26/4	22/1	-	-
Age (years)	62.4 (6.4)	58.9 (5.9)	61.2 (5.4)	2.45	.093
Education (years)	14.8 (3.6)	15.5 (3.4)	15.8 (3.4)	0.56	.576
UPSIT score	22.5 (7.0)	23.1 (5.7)	36.7 (2.3)	59.25	**<.001**
Disease duration (from diagnosis)	3.0 (3.2)	-	-	-	-
Disease duration (from symptom onset)	4.0 (2.9)**	-	-	-	-
Hoehn &Yahr stage	1.8 (0.6)	-	-	-	-
UPDRS-III score	22.5 (11.2)***	-	-	-	-

Note. Means (standard deviations) are shown for continuous variables. UPSIT = University of Pennsylvania Smell Identification Test. UPDRS-III = Unified Parkinson Disease Rating Scale Part III. *p-value from chi-squared test, $\chi 2(2) = .46$. ** n = 24. *** n = 22.

Table 2

Comparison of Demographic and Cognitive Variables Between Hyposmic First-Degree Relatives and Hyposmic Non-Relatives

	Group			
	hFDR	hNoFDR	*t*(28)	*p*
Demographic variables				
N	14	16	-	-
Age	58.1 (6.7)	59.6 (5.2)	-0.68	.501
Sex (male/female)	10/4	4/11	-	**.028***
Handedness (right/left)	12/2	14/2	-	-
Education (years)	16.8 (2.4)	14.4 (3.8)	2.01	.054
UPSIT scores	24.3 (5.5)	22.0 (5.8)	1.10	.280
Cognitive variables				
TMT4-5	60.9 (29.6)	53.2 (36.1)	0.64	.524
Phonemic fluency	37.9 (7.9)	44.3 (12.1)	-1.70	.100
Semantic fluency	42.8 (7.7)	45.1 (6.2)	-1.20	.241
LNS	9.4 (2.5)	10.4 (2.9)	-1.02	.315
Digit span (backwards)	6.1 (2.5)	6.9 (2.5)	-0.81	.423
Spatial span (backwards)	7.4 (2.0)	7.0 (1.9)	0.503	.619
SDMT	47.6 (7.7)	53.3 (10.6)	-1.66	.108

Note. Means (standard deviations) are shown for continuous variables. UPSIT = University of Pennsylvania Smell Identification Test. TMT4-5 = Trail Making Test Condition 4 controlled for motor speed. LNS = Letter-Number Sequencing task. SDMT = Symbol Digit Modalities Test.

*p-value from chi-squared test, $\chi 2(1) = 4.82$.

Table 3

Summary Data and Analysis Outcomes of Cognitive Test Scores

Test		Group			F (2,77)	p
		PD	AR	HCs		
TMT4-5	M(SD)	67.8 (37.4)	56.8 (32.9)	47.0 (21.1)	2.41	.097
	M_{adj} (SE)	63.7 (5.8)	60.4 (5.4)	47.0 (5.71)		
	N	26	30	26		
Phonemic fluency	M(SD)	41.9 (12.1)	41.3 (10.7)	44.9 (12.8)	0.78	.463
	M_{adj} (SE)	42.6 (2.4)	40.8 (2.2)	44.8 (2.3)		
	N	26	30	26		
Semantic fluency	M(SD)	38.2 (8.8)	43.7 (7.0)	44.2 (6.4)	3.68	.030*
	M_{adj} (SE)	39.0 (1.4)	43.0 (1.3)	44.2 (1.4)		
	N	26	30	26		
LNS	M(SD)	9.8 (2.8)	10.0 (2.7)	11.9 (1.5)	6.89***	**.002**[a]
	M_{adj} (SE)**	10.2 (0.5)	9.7 (0.4)	11.9 (0.4)		
	N	25	30	26		
Digit span (backwards)	M(SD)	6.9 (2.2)	6.5 (2.5)	7.8 (2.3)	2.42	.096
	M_{adj} (SE)	7.1 (0.5)	6.4 (0.4)	7.7 (0.5)		
	N	26	30	26		
Spatial span (backwards)	M(SD)	7.4 (1.8)	7.2 (1.9)	7.8 (2.3)	1.44	.242
	M_{adj} (SE)	7.6 (0.4)	7.0 (0.4)	7.8 (0.4)		
	N	26	30	26		
SDMT	M(SD)	41.2 (8.2)	50.6 (9.6)	51.3 (6.7)	9.42***	**<.001**[b]
	M_{adj} (SE)**	41.8 (1.7)	50.1 (1.6)	51.2 (1.6)		
	N	25	30	26		

Note. M_{adj} = Estimated marginal means adjusted for age (M = 60.73) and years of education (M = 15.37). TMT4-5 = Trail Making Test Condition 4 controlled for motor speed. LNS = Letter-Number Sequencing task. SDMT = Symbol Digit Modalities Test. * Group differences did not survive multiple comparison correction (Bonferroni). ** M_{adj} adjusted for age with M = 60.86 and years of education with M = 15.38. *** F(2,76). [a] In a post hoc analysis, significant

differences were found between the AR and HC groups (p = .002), and between the PD and HC groups (p = .039) after Bonferroni correction. [b] Significant differences were found between the PD and HC groups (p = .001) and between the PD and AR groups (p = .002) after Bonferroni correction.

Table 4

Descriptive Statistic from All Participant Groups of Edges Showing Significant FC Differences between the At-Risk and Healthy Control Group

Edges	HCs (n = 25)			AR (n = 28)			PD (n = 23)		
	M	SD	SEM	M	SD	SEM	M	SD	SEM
amPFC – MTG-L	0.042	0.182	0.036	0.222	0.210	0.040	0.189	0.184	0.038
amPFC – MTG-R	-0.093	0.156	0.031	0.115	0.169	0.032	-0.014	0.195	0.041
IFG-R – MTG-L	0.208	0.168	0.034	0.068	0.171	0.032	0.166	0.241	0.050
pCC – vmPFC	0.110	0.253	0.051	0.272	0.205	0.039	0.291	0.201	0.042
PCu – MTG-L	-0.024	0.191	0.038	0.083	0.172	0.032	0.016	0.271	0.057
MTG-L – vmPFC	-0.041	0.221	0.044	0.088	0.178	0.034	0.142	0.212	0.044

Note. MTG-L = left middle temporal gyrus. MTG-R = right middle temporal gyrus. IFG-R = right inferior frontal gyrus. pCC = posterior cingulate gyrus. vmPFC = ventromedial prefrontal cortex. PCu = precuneus.

Table 5

Analysis Outcomes of Edges with Abnormal FC in the At-Risk group (Compared to HCs)

Edges	*t*(51)	*p* (uncorrected)	*p* (FDR-corrected)	Effect size (Hedges' *g*)
amPFC – MTG-L	-3.32	**.002**	.114	0.91
amPFC – MTG-R	-4.64	**.000**	**.003**	1.28
IFG-R – MTG-L	3.01	**.004**	.184	0.83
pCC – vmPFC	-2.58	**.013**	.443	0.71
PCu – MTG-L	-2.16	**.036**	.812	0.59
MTG-L – vmPFC	-2.36	**.022**	.603	0.65

Note. T-values and p-values were extracted from a t-test between at-risk and healthy control groups. amPFC = anterior medial prefrontal cortex. MTG-L = left middle temporal gyrus. MTG-R = right middle temporal gyrus. IFG-R = right inferior frontal gyrus. pCC = posterior cingulate gyrus. vmPFC = ventromedial prefrontal cortex. PCu = precuneus.

Table 6

Comparison between Hyposmic First-Degree Relatives and Hyposmic Non-Relatives on Functional Connectivity of the DMN

Edge	hFDR (n = 12)			hNoFDR (n = 16)				
	M	SD	SEM	M	SD	SEM	*t*(26)	*p*
amPFC – MTG-L	0.23	0.21	0.06	0.22	0.22	0.05	-0.08	.940
amPFC – MTG-R	0.12	0.21	0.06	0.11	0.14	0.04	0.16	.874
IFG-R – MTG-L	0.16	0.11	0.03	-0.004	0.18	0.04	2.89	**.008**
pCC – vmPFC	0.21	0.23	0.07	0.32	0.17	0.04	-1.53	.138
PCu – MTG-L	0.15	0.20	0.06	0.03	0.14	0.03	1.93	.065
MTG-L – vmPFC	0.11	0.18	0.05	0.07	0.18	0.05	0.53	.602

Note. Only edges that showed abnormal functional connectivity (before FDR correction) between the at-risk and control groups are shown. hFDR = hyposmic first-degree relatives. hNoFDR = hyposmic non-relatives. amPFC = anterior medial prefrontal cortex. MTG-L = left middle temporal gyrus. MTG-R = right middle temporal gyrus. IFG-R = right inferior frontal gyrus. pCC = posterior cingulate gyrus. vmPFC = ventromedial prefrontal cortex. PCu = precuneus.

Table 7

Correlational Results

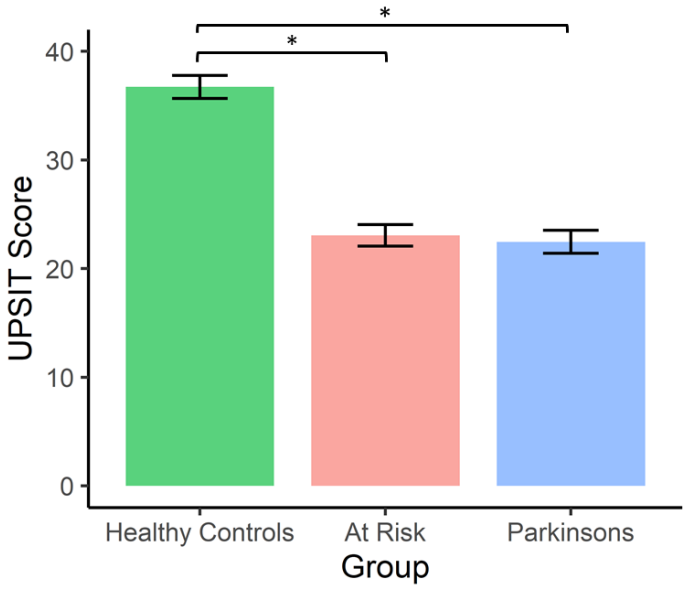
Variable	LNS score (AR group)	LNS score (PD group)	SDMT score (PD group)	Semantic fluency score (PD group)
amPFC – MFG-R	**-0.144**	**0.106**	**-0.143**	0.203
amPFC – MFG-L	0.137	0.132	-0.034	-0.123
IFG-R – MFG-L	-0.113	0.205	0.174	0.414*
pCC – vmPFC	-0.08	-0.097	-0.015	-0.098
PCu – MFG-L	0.081	0.174	0.080	-0.133
MFG-L - vmPFC	0.097	-0.023	0.117	-0.324

Note. Pearson correlation coefficients are given. Bolded values are part of the primary analysis.

*$p < .05$ (uncorrected).

Figure 1

Group Comparison of University of Pennsylvania Smell Identification Test (UPSIT) Scores

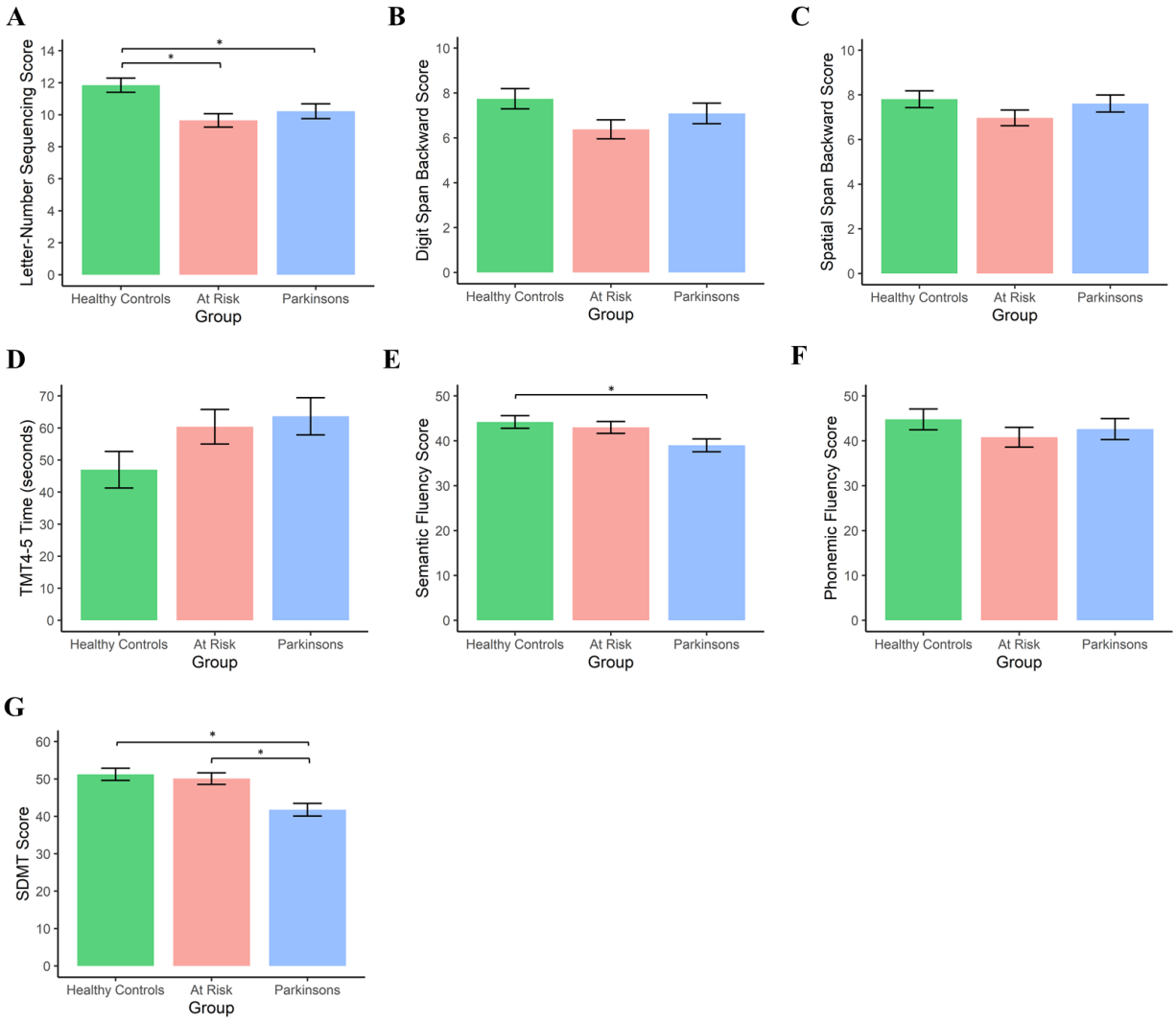


Note. Error bars represent ± 1 SEM. The UPSIT measures odour identification abilities with higher scores indicating better performance.

*$p < .05$

Figure 2

Group Comparison of Cognitive Test Scores



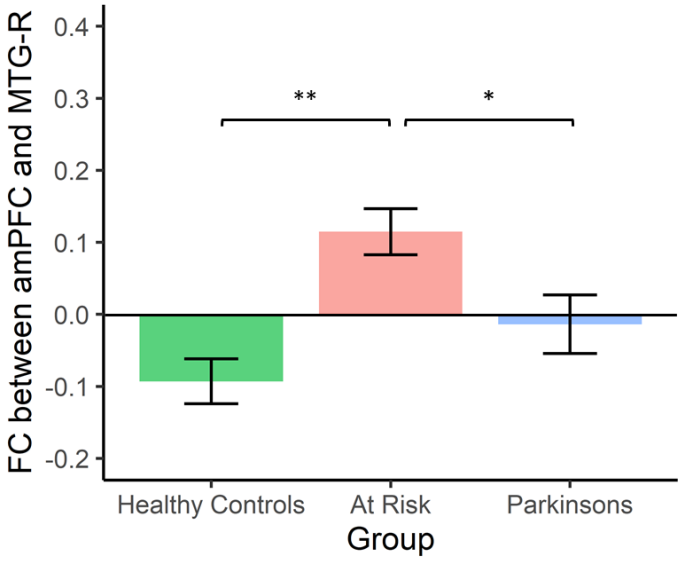
Note. Adjusted means and error bars representing ± 1 SEM are given. (A) Scores from the Letter-Number Sequencing test which assesses verbal working memory and which is more cognitively demanding that the digit span backwards test. (B) Digit span backwards test scores. The digit span measures verbal working memory. (C) Scores from the spatial span backwards measuring spatial working memory. (D) Scores from the DKEFS trail making test condition 4 controlled for motor speed (condition 5; TMT4-5). This test measures executive function. (E)

Semantic fluency test scores. Group differences for this test did not survive multiple comparison correction (Bonferroni). (F) Phonemic fluency test scores. (G) Scores from the Symbol Digit Modalities Test (SDMT) measuring psychomotor processing speed. Except for the TMT4-5, higher test scores indicate better performance. Higher test scores on the TMT4-5 indicate worse performance.

*$p < .05$

Figure 3

Functional Connectivity (FC) between the Anterior Medial Prefrontal Cortex (amPFC) and the Right Middle Temporal Gyrus (MTG-R)



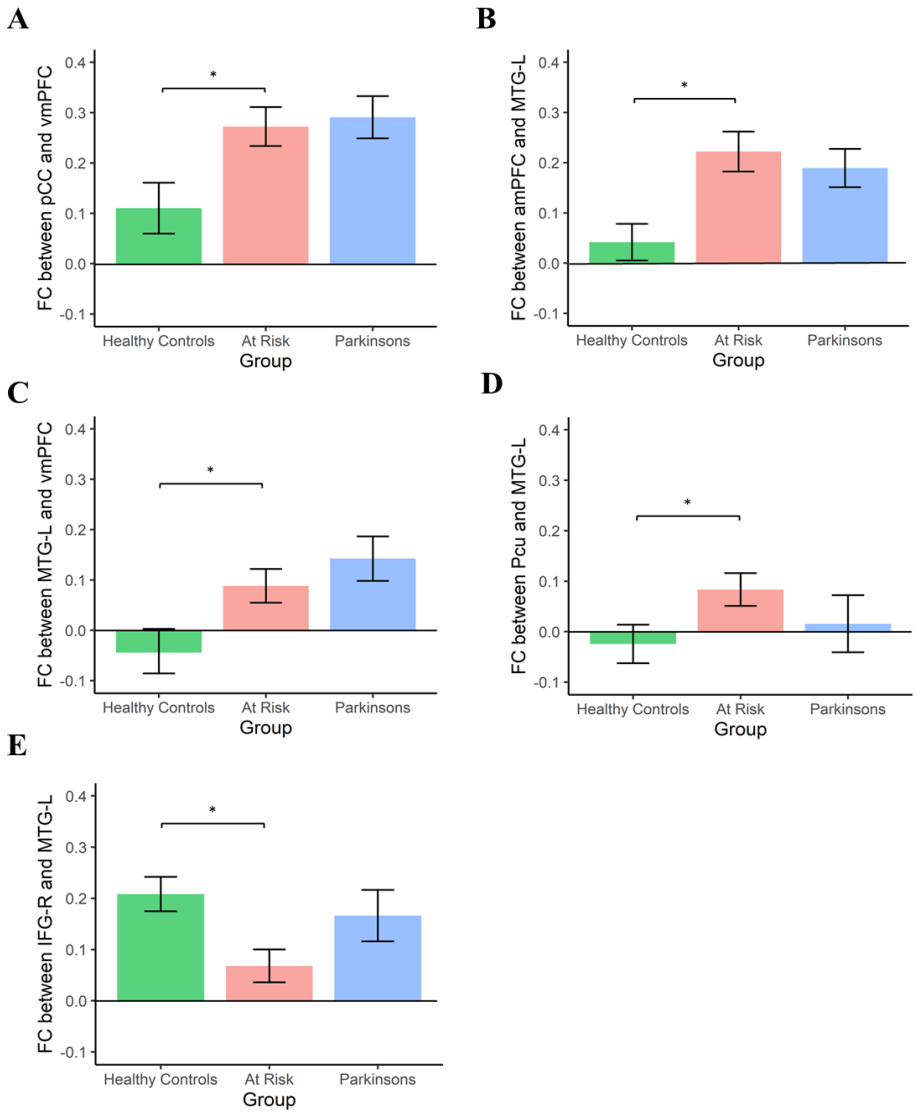
Note. Error bars represent ± 1 SEM.

*$p < .05$ (uncorrected)

**$p < .05$ (FDR-corrected)

Figure 4

Significant Functional Connectivity (FC) Differences between the At-Risk Group and Healthy Controls and between the At-Risk Group and Parkinson's Patients (Secondary Results)

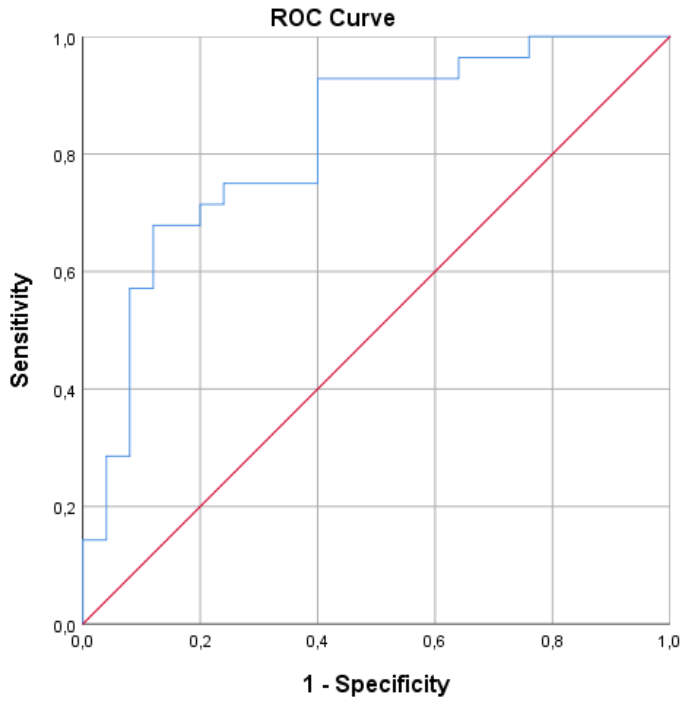


Note. Error bars represent ± 1 SEM. (A) FC between the posterior cingulate cortex (pCC) and the ventromedial prefrontal cortex (vmPFC). (B) FC between the anterior medial prefrontal cortex (amPFC) and the left middle temporal gyrus (MTG-L). (C) FC between the MTG-L and the vmPFC. (D) FC between the precuneus (PCu) and the MTG-L. (E) FC between the right inferior frontal gyrus (IFG-R) and the MTG-L.

*$p < .05$ (uncorrected)

Figure 5

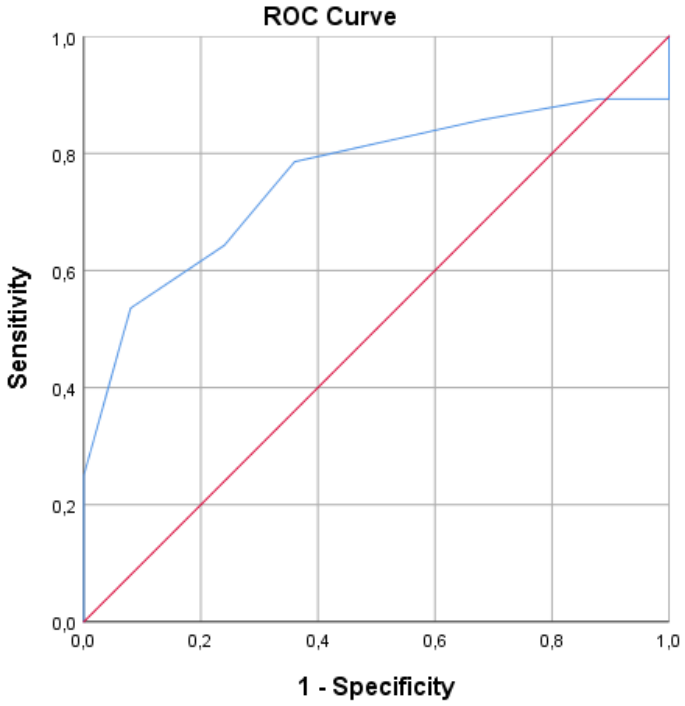
Receiver-Operating Characteristic Curve Analysis Determining the Accuracy of amPFC-to-MTG-R Functional Connectivity (FC) to Discriminate between At-Risk Individuals and Healthy Controls



Note. The receiver-operating characteristic (ROC) curve is represented in blue with an area under the curve (AUC) value of .82. At-risk individuals were considered as cases. The red line represents the reference line which indicates that the diagnostic test classifies at-risk individuals by chance. Because ROC curve of the FC between the amPFC and the MTG-R is above the reference line, the measure has good diagnostic accuracy.

Figure 6

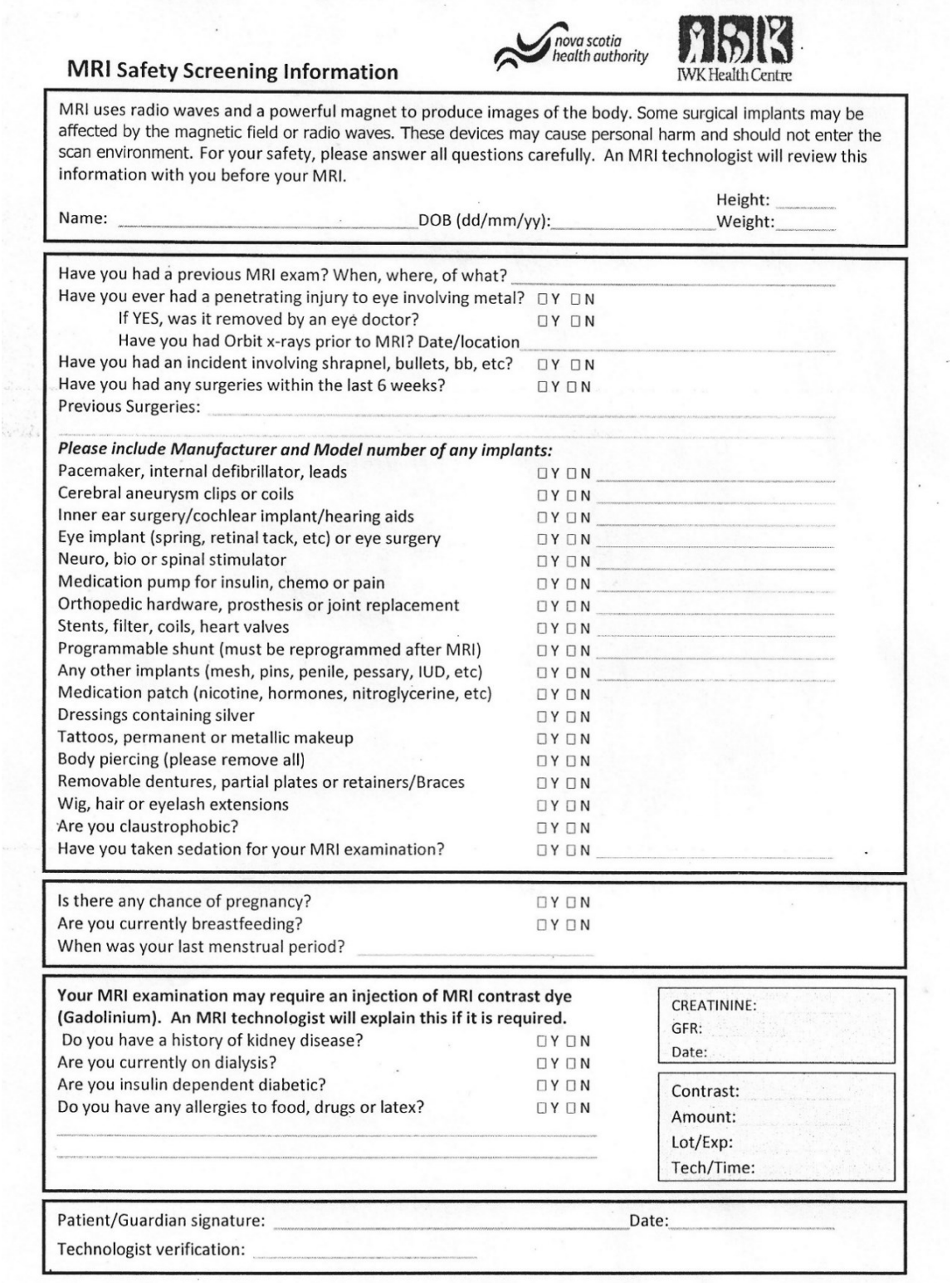
Receiver-Operating Characteristic Curve Analysis Determining the Accuracy of Letter-Number Sequencing Test Scores to Discriminate between At-Risk Individuals and Healthy Controls



Note. The receiver-operating characteristic (ROC) curve is represented in blue with an area under the curve (AUC) value of .76. At-risk individuals were considered as cases. The red line represents the reference line which indicates that the diagnostic test classifies at-risk individuals by chance. Because the ROC curve of Letter-Number Sequencing test scores is above the reference line, the measure can correctly identify at-risk individuals.

Appendix A

MRI Screening Questionnaire



MRI Safety Screening Information

nova scotia health authority

IWK Health Centre

MRI uses radio waves and a powerful magnet to produce images of the body. Some surgical implants may be affected by the magnetic field or radio waves. These devices may cause personal harm and should not enter the scan environment. For your safety, please answer all questions carefully. An MRI technologist will review this information with you before your MRI.

Name: _____ DOB (dd/mm/yy): _____ Height: _____ Weight: _____

Have you had a previous MRI exam? When, where, of what? _____

Have you ever had a penetrating injury to eye involving metal? ☐Y ☐N

If YES, was it removed by an eye doctor? ☐Y ☐N

Have you had Orbit x-rays prior to MRI? Date/location _____

Have you had an incident involving shrapnel, bullets, bb, etc? ☐Y ☐N

Have you had any surgeries within the last 6 weeks? ☐Y ☐N

Previous Surgeries: _____

Please include Manufacturer and Model number of any implants:

Pacemaker, internal defibrillator, leads ☐Y ☐N _____

Cerebral aneurysm clips or coils ☐Y ☐N _____

Inner ear surgery/cochlear implant/hearing aids ☐Y ☐N _____

Eye implant (spring, retinal tack, etc) or eye surgery ☐Y ☐N _____

Neuro, bio or spinal stimulator ☐Y ☐N _____

Medication pump for insulin, chemo or pain ☐Y ☐N _____

Orthopedic hardware, prosthesis or joint replacement ☐Y ☐N _____

Stents, filter, coils, heart valves ☐Y ☐N _____

Programmable shunt (must be reprogrammed after MRI) ☐Y ☐N _____

Any other implants (mesh, pins, penile, pessary, IUD, etc) ☐Y ☐N _____

Medication patch (nicotine, hormones, nitroglycerine, etc) ☐Y ☐N

Dressings containing silver ☐Y ☐N

Tattoos, permanent or metallic makeup ☐Y ☐N

Body piercing (please remove all) ☐Y ☐N

Removable dentures, partial plates or retainers/Braces ☐Y ☐N

Wig, hair or eyelash extensions ☐Y ☐N

Are you claustrophobic? ☐Y ☐N

Have you taken sedation for your MRI examination? ☐Y ☐N _____

Is there any chance of pregnancy? ☐Y ☐N

Are you currently breastfeeding? ☐Y ☐N

When was your last menstrual period? _____

Your MRI examination may require an injection of MRI contrast dye (Gadolinium). An MRI technologist will explain this if it is required.

Do you have a history of kidney disease? ☐Y ☐N

Are you currently on dialysis? ☐Y ☐N

Are you insulin dependent diabetic? ☐Y ☐N

Do you have any allergies to food, drugs or latex? ☐Y ☐N

_____

_____

CREATININE:
GFR:
Date:

Contrast:
Amount:
Lot/Exp:
Tech/Time:

Patient/Guardian signature: _____ Date: _____

Technologist verification: _____

Appendix B

Custom Python Script for the Functional Connectivity Analysis

```
#!/usr/bin/env python
#
# nilearn_conn_group_analysis.py
#
# This script computes functional-connectivity between all every specified
ROI seed, given coordinates,labels.
# It assumes data has been processed with FMRIprep and is located in
../derivatives/fmriprep/ (relative path).
# It also requires a participants.tsv or phenotypes.tsv style file to filter
subjects into groups.
#
# There are many processing options that need to be selected, but here are
the important details:
#
#
# ### CONFOUNDS ###########
#  Recommend using FD + 24-HMP + aCompCor + 4GS + Cosine_XX + SpikeReg(fd>0.5
| dvars>1.5).
#
#     From: https://neurostars.org/t/fmriprep-outputs-very-high-number-of-
acompcors-up-to-1000/5451/10
#   '''Since CompCor is run after high-pass filtering with Discrete Cosine
Functions, when using a/tCompCor
#   regressors you should also include the cosine_XX regressors in your
design.
#   Related to this, you probably should not include any of the global
signals (global_signal, white_matter, csf).'''
#
#  1) aCompCor: Use either the first 5-6 aCompCor WM and 5-6 CSF components
for each subject. Or use first N-components of each
#     that explain a fixed amount of cumulative variance (ie, 25%).
#     Cosines:  Use aCompCor components, should also use these DCT Cosine
regressors instead of high-pass TF (aCompCor done w/DCT @.008 Hz).
#       24HMP:    24 total head-motion-parameters =
([rotX,rotY,rotZ,transX,transY,transZ]+1derivatives)+squares
#       4GS:      Global_signal + 1deriv + squares of each.
#     FD:       Framewise_displacement.
#     REFERENCE = https://fmriprep.org/en/stable/outputs.html#confounds
#
https://github.com/nipreps/fmriprep/blob/674124ee80b3e2a8affddf005e910e4ca1c9
7cc0/fmriprep/workflows/bold/confounds.py
#
#  2) SpikeReg: Debatable. Done by building a censor matrix with one column
per SpikeTR (zeros except row @ BadTR).
#     If doing SpikeReg, recommend using FD>0.5 OR std.DVARS>1.5, as
described in discussion here:
#        https://neurostars.org/t/fmriprep-20-2-0-produces-motion-outliers-
in-confounds-timeseries-file-by-default-how-are-they-derived/18550/4
#     optionally, could censor -1 TR before Spike and +1,+2 TRs after each
excessive motion spike, see here:
```

```
#     Problems with using SpikeReg is loss of DOF for individuals wwhere more
Spikes are censored.
#     Important to set an exclude limit to drop subjects entirely when >20%
of TRs are censored.
#
#  3) SpatialSmoothing, optional, but not that fMRIprep does not do any
smoothing. (recommend 1.5-2xVoxelSize?)
#
https://fmriprep.org/en/stable/outputs.html?highlight=smoothing#outputs-of-
fmriprep
#
#  4) Detrend: detrends signal before applying TF/confounds, generally a good
idea.
#      - do not use detrend or high-pass when using aCompCor and Cosines.
#        see here: https://github.com/SIMEXP/load_confounds
#
#  5) TF low_pass/high_pass: recommend use Cosine_XX regressors with aCompCor
PCs above rather than high_pass/low_pass.
#      https://fmriprep.org/en/stable/outputs.html?highlight=FD#confound-
regressors-description
#
https://www.brainvoyager.com/bvqx/doc/UsersGuide/Preprocessing/TemporalHighPa
ssFiltering.html
#
#  6) Standardize: good idea to return z-scores, optional 'psc' Percent
Signal Change, or None.
#
# References:
#    https://nilearn.github.io/modules/generated/nilearn.signal.clean.html
#    https://fmriprep.org/en/stable/outputs.html#confounds
#
# #### Testing Confounds on PDvsHCvsAR #################
#   Tangent Plot results - EdgeThreshold=85%
#  1) aCompCor+Cosines+24HMP+FD+SpikeReg -GS
#         PD=.27, AR=.31, HC=.31
#  2) aCompCor+Cosines+24HMP+SpikeReg -GS,-FD
#         PD=.27, AR=.31, HC=.31          (conclusion: FD makes little diff)
#  3)
#
#
# ### Connectivity Measure ########
#   choices = ['correlation','partial correlation','tangent','covariance']
#   See article Dadi etal, 2019:
https://doi.org/10.1016/j.neuroimage.2019.02.062
#   Examples:
#
https://nilearn.github.io/auto_examples/03_connectivity/plot_group_level_conn
ectivity.html#sphx-glr-auto-examples-03-connectivity-plot-group-level-
connectivity-py
#
#
## MORE LEARNING:
#  + differences in correlation models (see Note #2 above)
#  + GraphLasso? node-centricity==connectedness, assume after thresholding?
#  + GraphLasso? path-length?
#  + Lag-0/Lag-1 --> Granger Causality (Learn more!)
#
```

```
# Graph Topological Metrics for node-degree centrality (centricty?), average
path-length, clustering-coefficients:
#   see:
#        https://sites.google.com/site/bctnet/measures/list
#       Varoquaux and Craddock, 2013:
#                 https://arxiv.org/pdf/1304.3880.pdf
#           Rubinov and Sporns, 2010:
#
      https://www.sciencedirect.com/science/article/pii/S105381190901074X?via
%3Dihub#bib45
#
#
#------------------------------------------------------------------------------
-----------------------------------
import os
import sys
import json
import datetime
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
from nilearn import input_data
from nilearn.connectome import ConnectivityMeasure
from nilearn import plotting
##------------------------------------------------------------------------------
-----------------------------------
## PROJECT_VARIABLES
##------------------------------------------------------------------------------
-----------------------------------
SCRIPT = os.path.abspath(sys.argv[0])
SCRIPTSDIR = os.path.dirname(SCRIPT)
PROJECT_DIR = os.path.dirname(SCRIPTSDIR)
FMRIPREP_DIR = os.path.join(PROJECT_DIR, 'derivatives','fmriprep')
RESULTS_DIR = os.path.join(PROJECT_DIR, 'results')
DOCS_DIR = os.path.join(PROJECT_DIR, 'docs')
DATE_STR = datetime.datetime.now().strftime('%Y%m%d-%H%M')
GROUP_STR = 'PDvsHCvsAR'
NETWORK_STR = 'DMNSpreng18'
CONF_STR = '6HMP1GS2PhysSpikeRegSS7'
GRP_RESULTS_DIR =
os.path.join(RESULTS_DIR,'%s_nilearn_%s_%s_%s'%(DATE_STR,GROUP_STR,NETWORK_ST
R,CONF_STR))
if not os.path.exists(GRP_RESULTS_DIR):
      os.mkdir(GRP_RESULTS_DIR)
GRP_RESULTS_FN_PREFIX = '%s_%s_%s'%(DATE_STR,GROUP_STR,NETWORK_STR)
GRP_RESULTS_FN = os.path.join(GRP_RESULTS_DIR,
'%s.csv'%(GRP_RESULTS_FN_PREFIX))
CORR_PLOTS_FN = os.path.join(GRP_RESULTS_DIR, '%s'%(GRP_RESULTS_FN_PREFIX))
PARTICIPANTS_FN = os.path.join(DOCS_DIR, 'participants.tsv')
if os.uname().nodename == 'Aoraki.local':
      PARTICIPANTS_FN = os.path.join(DOCS_DIR, 'participants_aoraki.tsv')
##------------------------------------------------------------------------------
-----------------------------------
## Global Variables
##------------------------------------------------------------------------------
-----------------------------------
DEBUG_VERBOSE=False           # bool, controls output printing
```

```
## data info -------------------------
FUNC_TR_TOTAL=180             # total TRs to expect in each func
FUNC_TR_SEC=2.0                     # sec, Repetition-Time of input func data
## sphere-masking options ------------------------
TF_LOW_PASS=0.08                    # Hz, freq cutoff for low-pass temporal
filter, standard=0.08 (default=0.08)
TF_HIGH_PASS=0.008                  # Hz, freq cutoff for high-pass temporal
filter, standard=0.008 (default=None)
DETREND_OPTION=True                 # bool, force-additional detrending - not
recommended. (default=False)
STANDARDIZE_OPTION='zscore'   # bool, option to return z-score, or Percent-
Signal-Change or raw. (default=True or 'zscore')
SMOOTH_FWHM_MM=7              # mm, full-width half maximum of spatial
smoothing to apply (default=0)
FUNC_SPHERE_RADIUS=6          # mm, radius for sphere around roi seed coord
(default=0)
## SpikeReg & censoring index pre-post high-motion -----------
DO_SPIKE_REG=True             # bool, do spikereg/censoring for each spike
(default=True)
FD_LIMIT=0.5                        # mm, framewise-displacement movement
limit (default=0.5)
DVARS_LIMIT=1.5                     # mm, std.dvars movement limit
            (default=1.5)
SPIKE_REG_ANDOR='OR'          # AND|OR, choice to filter TRs by above
FD_LIMIT,DVARS_LIMIT (default='OR')
CENSOR_TR_i_MINUS_1=False     # bool, censors TR at i-1 excessive_motion TR,
(default=True)
CENSOR_TR_i_PLUS_1=False      # bool, censors TR at i+1 excessive_motion TR,
(default=True)
CENSOR_TR_i_PLUS_2=False      # bool, censors TR at i+2 excessive_motion TR,
(default=False)
##
EXCLUDE_MOVERS_LIM_PERC=0.2 #percent limit total TRs being censored to
exclude from group (ie, 180 total TRs, limit=0.2(20%); then 180*.2 = 36 vols
CensoredTR_EXCLUDE_LIMIT = int(EXCLUDE_MOVERS_LIM_PERC*FUNC_TR_TOTAL) #int,
limit of total TRs being censored to exclude subject from group
##
CONF_USE_N_aCompCorWM=0       # int, first N aCompCor-WM PCs to use in
denoising. (default=5)
CONF_USE_N_aCompCorCSF=0      # int, first N aCompCor-CSF PCs to use in
denoising. (default=5)
##
NILEARN_CACHE_NAME='nilearn_cache_%s_%s_%s'%(GROUP_STR,NETWORK_STR,CONF_STR)
# string name for cache folder in /derivatives/ (default='')
NILEARN_CACHE_MEM_LEVEL=1     # int, cache memory level higher number caches
more info (default=1)
NILEARN_VERBOSITY=1                 # int, verbosity level (default=0)
##
CONF_SUFFIX = '_regressors'  ## filename suffix to append to confounds-
filename to save selected confounds
##
CREATE_CARPET_PLOTS=True      ##make carpet plots by group with subjects x
ROI-to-ROI-features.
Run_GraphicalLassoCV=False    # run GraphicalLassoCV to estimate Covariance
as a SparseInverseMatrix
## DMN_ROIs ---------------------------------------------------------------
-----------------------------------
```

```
## Spreng_etal_2013
##
SPRENG_DMN_ROIS=dict()
SPRENG_DMN_ROIS['long_labels'] = [
      'AnteriorMedialPrefrontalCortex',
      'AnteriorTemporalLobeLeft', 'AnteriorTemporalLobeRight',
      'DorsalMedialPrefrontalCortex',
      'HippocampalFormationLeft', 'HippocampalFormationRight',
      'InferiorFrontalGryusLeft', 'InferiorFrontalGryusRight',
      'PosteriorCingulateCortex',
      'PosteriorInferiorParietalLobuleLeft',
'PosteriorInferiorParietalLobuleRight',
      'Precuneus',
      'SuperiorFrontalGyrusLeft', #'SuperiorFrontalGryusRight',
      'MedialTemporalGyrusLeft', 'MedialTemporalGyrusRight',
#'SuperiorTemporalSulcusLeft', 'SuperiorTemporalSulcusRight',
      'TemoralParietalJunctionLeft', 'TemoralParietalJunctionRight',
      'VentralMedialPreFrontalCortex']
SPRENG_DMN_ROIS['short_labels'] = [
      'amPFC',
      'aTL_L', 'aTL_R',
      'dmPFC',
      'HF_L', 'HF_R',
      'IFG_L', 'IFG_R',
      'pCC',
      'pIPL_L', 'pIPL_R',
      'PCu',
      'SFG_L', #'SFG_R',
      'MTG_L', 'MTG_R', #'STS_L', 'STS_R',
      'TPJ_L', 'TPJ_R',
      'vmPFC']
SPRENG_DMN_ROIS['coords'] = [
      (-8,56,14),
      (-52,-10,-20), (52,-4,-16),
      (-8,50,34),
      (-26,-8,-24), (24,-14,-22),
      (-42,26,-14), (50,32,-6),
      (-2,-48,28),
      (-50,-60,28), (58,-60,28),
      (-2,-60,50),
      (-8,20,62), #(12,18,62),
      (-60,-28,-4), (50,-36,4),
      (-44,-52,22), (44,-58,18),
      (-2,44,-12)]
## MSDL-atlas
## from:
https://nilearn.github.io/auto_examples/03_connectivity/plot_group_level_conn
ectivity.html
##>>> from nilearn import datasets
##>>> msdl_data = datasets.fetch_atlas_msdl()
##>>> msdl_data.keys()
##  dict_keys(['maps', 'labels', 'region_coords', 'networks', 'description'])
##>>> for i in range(3,7):
##
print('network:',msdl_data['networks'][i],'label:',msdl_data['labels'][i],'co
ords:',msdl_data['region_coords'][i])
##  network: b'DMN' label: Med DMN coords: (-0.2, -55.21, 29.87)
```

```
##  network: b'DMN' label: L DMN coords: (-45.8, -64.78, 31.84)
##  network: b'DMN' label: R DMN coords: (51.66, -59.34, 28.88)
##  network: b'DMN' label: Front DMN coords: (-0.15, 51.42, 7.58)
##
## DMN coords from nilearn example:
##
https://nilearn.github.io/auto_examples/03_connectivity/plot_sphere_based_con
nectome.html#sphx-glr-auto-examples-03-connectivity-plot-sphere-based-
connectome-py
#DMN4_ROI_COORDS = [(0, -52, 18), (-46, -68, 32), (46, -68, 32), (1, 50, -5)]
#DMN4_ROI_LABELS_LONG =
['PosteriorCingulateCortex','LeftTemporoparietalJunction','RightTemporoparietal
alJunction','MedialPrefrontalCortex']
#DMN4_ROI_LABELS_SHORT = ['PCC','LTPj','RTPj','MPFC']
## -- choose ROI set
FUNC_ROI_COORDS = SPRENG_DMN_ROIS['coords']
FUNC_ROI_LABELS_LONG = SPRENG_DMN_ROIS['long_labels']
FUNC_ROI_LABELS_SHORT = SPRENG_DMN_ROIS['short_labels']
## SPHERE-MASKER ------------------------------------------------------------
----------------------------------------
masker = input_data.NiftiSpheresMasker(FUNC_ROI_COORDS,
radius=FUNC_SPHERE_RADIUS,
                                       low_pass=TF_LOW_PASS,
high_pass=TF_HIGH_PASS,
                                       detrend=DETREND_OPTION,
standardize=STANDARDIZE_OPTION,
                                           smoothing_fwhm=SMOOTH_FWHM_MM,
t_r=FUNC_TR_SEC, memory=NILEARN_CACHE_NAME,

memory_level=NILEARN_CACHE_MEM_LEVEL, verbose=NILEARN_VERBOSITY).fit()
##------------------------------------------------------------------------
-------------------------------------
# Source: https://neurostars.org/t/getting-started-using-fmripreps-ica-aroma-
outputs/16541
def process_confounds(confounds_file, a_comp_cor=True,
a_comp_cor_N_to_include=8):
      """
      scrubbing for TASK
      remove TRs where FD>.5, stdDVARS (that relates to DVARS>.5)
      regressors to use
      ['X','Y','Z','RotX','RotY','RotY','<-
firsttemporalderivative','stdDVARs','FD']
      junk regressor: errors, ommissions, maybe very fast RTs (less than 50
ms)
      """
      conf_df = pd.read_csv(confounds_file, sep = '\t',
na_values=['n/a']).fillna(0)
      excessive_movement = (conf_df.framewise_displacement>.5) &
(conf_df.std_dvars>1.2)
      excessive_movement_TRs = excessive_movement[excessive_movement].index
      excessive_movement_regressors = np.zeros([conf_df.shape[0],
np.sum(excessive_movement)])
      for i,TR in enumerate(excessive_movement_TRs):
            excessive_movement_regressors[TR,i] = 1
      excessive_movement_regressor_names = ['rejectTR_%d' % i for i in
excessive_movement_TRs]
      # get movement regressors
```

```
      movement_regressor_names =
['trans_x','trans_y','trans_z','rot_x','rot_y','rot_z']
      movement_regressors = conf_df.loc[:,movement_regressor_names]
      movement_regressor_names += [i+'td' for i in movement_regressor_names]
      movement_regressors = np.hstack((movement_regressors,
np.gradient(movement_regressors,axis=0)))
      # add square
      movement_regressor_names += [i+'_sq' for i in movement_regressor_names]
      movement_regressors = np.hstack((movement_regressors,
movement_regressors**2))
      # add additional relevant regressors
      add_regressor_names = ['framewise_displacement']
      if a_comp_cor:
            add_regressor_names += [i for i in conf_df.columns if
'a_comp_cor' in i][:8]
      additional_regressors = conf_df.loc[:,add_regressor_names].values
      regressors =
np.hstack((movement_regressors,additional_regressors,excessive_movement_regre
ssors))
      # concatenate regressor names
      regressor_names = movement_regressor_names + add_regressor_names +
excessive_movement_regressor_names
      return regressors, regressor_names
##-------------------------------------------------------------------------
-------------------------------------
## build-volume censor matrix with one column per bad-TR (1=remove,0=keep),
as per:
##   Parkes L, Fulcher B, Yücel M, Fornito A, An evaluation of the efficacy,
reliability, and sensitivity of motion
##   correction strategies for resting-state functional MRI. NeuroImage.
2018. doi:10.1016/j.neuroimage.2017.12.073
##
##  NOTE1: algorithm = find bad TRs exceeding limits, include 1TR before and
2TR after excessive-motion TR
##
##  NOTE2: CensorExclusionLimit > (20% of total_TRs; (500*.2) >= 100 TRs then
exclude subject)
##
## NOTE3: should we use OR or AND when thresholding FD and std_DVARS? (AND is
less aggressive)
##
## Good discussion on FD and DVARS limits and SpikeReg:
## https://neurostars.org/t/fmriprep-20-2-0-produces-motion-outliers-in-
confounds-timeseries-file-by-default-how-are-they-derived/18550/4
##  (Recommend using (FD>0.5 OR std_DVARS>1.5)
def censor_excessive_movement_spikes(conf_df,
fd_lim=FD_LIMIT,dvars_lim=DVARS_LIMIT):
      '''
      Given confounds as DataFrame and limits for FD and STD.DVARS,
         1. find excessive-motion-TRs
         2. expand list of censor TRs to include 1xTR before and 2xTR after
each (potentially)
         3. build a censor array with one column per each censor,
0=keep,1=ignore
         4. return (1) list of original em_locs, (2) censor_arr, (3)
censor_arr_col_names
      '''
```

```
      if SPIKE_REG_ANDOR.upper() == 'AND':
            em = (conf_df.framewise_displacement > fd_lim) &
(conf_df.std_dvars > dvars_lim)     ##excessive-movements
      elif SPIKE_REG_ANDOR.upper() == 'OR':
            em = (conf_df.framewise_displacement > fd_lim) |
(conf_df.std_dvars > dvars_lim)     ##excessive-movements
      else:
            print('*** ERROR: could not determine ANDOR
variable:',SPIKE_REG_ANDOR)
            sys.exit(3)
      em_vol_ind  = em[em].index
      em_vol_locs = em_vol_ind.values
      num_spikes  = len(em_vol_locs)
      if num_spikes > 0:
            print(' -- WARNING: found %d excessive movement
spikes:'%(num_spikes))

      print(conf_df[['framewise_displacement','std_dvars']].iloc[em_vol_ind])
            ## include 1TR before each spike, and 2TRs after each spike
            for i in em_vol_locs:
                  if i-1 >= 0 and CENSOR_TR_i_MINUS_1 == True:
                        em[i-1]=True
                  if i+1 < len(em) and CENSOR_TR_i_PLUS_1 == True:
                        em[i+1]=True
                  if i+2 < len(em) and CENSOR_TR_i_PLUS_2 == True:
                        em[i+2] = True
            em_vol_ind = em[em].index
      ## build [num_rows x nSpikeTR] matrix of regressors with one column per
bad-TR (1=remove,0=keep)
      em_vol_reg_arr = np.zeros([conf_df.shape[0], np.sum(em)])
      for i,TR in enumerate(em_vol_ind):
            em_vol_reg_arr[TR,i] = 1
      em_reg_names = ['reject_TR_%d' % i for i in em_vol_ind]
      return num_spikes,em_vol_reg_arr,em_reg_names
##---------------------------------------------------------------------------
-----------------------------------
##
## Good discussion on aCompCor PCs, and selection for denoising.
##  note recommendation near bottom of discussion to use CosineXX confounds
with aCompCor.
## https://neurostars.org/t/fmriprep-outputs-very-high-number-of-acompcors-
up-to-1000/5451/7
##
## Recommendation to include top5 CSF and WM A_Comp_Cor Principle Components
(PCs).
## 1. Parkes L, Fulcher B, Yücel M, Fornito A, An evaluation of the efficacy,
reliability, and sensitivity of motion
##    correction strategies for resting-state functional MRI. NeuroImage.
2018. doi:10.1016/j.neuroimage.2017.12.073
## 2. Muschelli J, Nebel MB, Caffo BS, Barber AD, Pekar JJ, Mostofsky SH,
Reduction of motion-related artifacts in resting state fMRI using aCompCor.
##    NeuroImage. 2014. doi:10.1016/j.neuroimage.2014.03.028
##
## NOTE: To get the top 5 of each CSF and WM aCompCor PCs, the confounds.json
file must be filtered.
##
```

```
## Using algo similar to '24P+aCompCor+4GSR' here:
https://github.com/sjburwell/fmriprep_denoising
##
def select_confounds(conf_fn, suffix='_regressors'):
      conf_df = pd.read_csv(conf_fn, sep='\t', na_values=['n/a']).fillna(0)
      ### load subjects confounds.json and locate first 5csf+5wm a_comp_cor
columns
      #json_fn = conf_fn[:-4]+'.json'
      #with open(json_fn,'r') as j:
      #     json_df = pd.DataFrame(json.load(j)).transpose()
      ### ------ a_comp_cor_XX -------
      #acc_csf_names =
json_df[(json_df['Mask']=='CSF')&(json_df['Retained']==True)].head(CONF_USE_N
_aCompCorWM).index.to_list()
      #acc_wm_names =
json_df[(json_df['Mask']=='WM')&(json_df['Retained']==True)].head(CONF_USE_N_
aCompCorCSF).index.to_list()
      #acc_names = acc_csf_names+acc_wm_names
      ### ------ Cosines_XX ----------
      #cos_names =
conf_df.loc[:,conf_df.columns.str.contains('cosine')].columns.tolist()
      ### ------ 24 HMP = 6HMP + 1stDerivs(6HMP) +12Squares(6HMP,1stDerivs)
      #mp_names =
['trans_x','trans_x_derivative1','trans_x_derivative1_power2','trans_x_power2
',
      #
'trans_y','trans_y_derivative1','trans_y_derivative1_power2','trans_y_power2'
,
      #
'trans_z','trans_z_derivative1','trans_z_derivative1_power2','trans_z_power2'
,
      #
'rot_x','rot_x_derivative1','rot_x_derivative1_power2','rot_x_power2',
      #
'rot_y','rot_y_derivative1','rot_y_derivative1_power2','rot_y_power2',
      #
'rot_z','rot_z_derivative1','rot_z_derivative1_power2','rot_z_power2']
      ### ----- 4GSR = Parkes,et al, 2018 -------------
      #gsr_names =
['global_signal','global_signal_derivative1','global_signal_derivative1_power
2','global_signal_power2']
      #wm_names =
['white_matter','white_matter_derivative1','white_matter_derivative1_power2',
'white_matter_power2']
      #csf_names =
['csf','csf_derivative1','csf_derivative1_power2','csf_power2']
      ### concatenate all regressor names
      #reg_names = mp_names+gsr_names+wm_names+csf_names
      reg_names =
['trans_x','trans_y','trans_z','rot_x','rot_y','rot_z','white_matter','csf','
global_signal']
                  #['framewise_displacement','std_dvars','dvars']
      ## confirm columns exist in confounds.tsv
      for c in reg_names:
            if not c in conf_df.columns.values:
                  print('\n*** ERROR: missing column=%s from confounds file =
%s\n' %(c,conf_fn))
```

```
                  sys.exit(7)
                  ## adjust later to throw an exception
      ## ------ Spike Regression -----------
      #reg_df = conf_df.loc[:,reg_names]                    ## returns df
      #reg_arr = conf_df.loc[:,reg_names].values      ##returns np.array()
      nSpikes,censor_TR_names = 0,[]
      if DO_SPIKE_REG:
            ## do SpikeReg here:
            #nSpikes, censor_arr,censor_names =
censor_excessive_movement_spikes(conf_df, FD_LIMIT, DVARS_LIMIT)
            nSpikes,censor_TR_arr,censor_TR_names =
censor_excessive_movement_spikes(conf_df,FD_LIMIT,DVARS_LIMIT)
            ## merge selected conf_arr + censor_arr
            conf_saved_arr = np.concatenate((conf_df[reg_names].values,
censor_TR_arr),axis=1)
            conf_saved_names = reg_names+censor_TR_names
      else:
            conf_saved_arr = conf_df[reg_names].values
            conf_saved_names = reg_names
      conf_sel_df =
pd.DataFrame(data=conf_saved_arr,columns=conf_saved_names)
      conf_sel_arr_fn = conf_fn[:-15]+suffix+'.csv'   ## try adding headers
here later
      conf_sel_df.to_csv(conf_sel_arr_fn,sep=',',index=False)
      print(' ++ selected confound values saved to file =', conf_sel_arr_fn)
      return nSpikes,len(censor_TR_names),conf_sel_arr_fn
##---------------------------------------------------------------------------
-----------------------------------
##    gen_label_combinations(['A','B','C']) returns "A_to_B,A_to_C,B_to_C"
def gen_label_combinations(v,incl_self=False):
      if len(v) < 2:
            return v
      if incl_self == True:
            return ['%s_to_%s'%(v[i],v[j]) for i in range(len(v)) for j in
range(i,len(v))]
      else:
            return ['%s_to_%s'%(v[i],v[j]) for i in range(len(v)) for j in
range(i+1,len(v))]
##---------------------------------------------------------------------------
-----------------------------------
## Given a numpy MxN array, return the upper-triangle as a vector.
##    Example:
##   M=[[A, B, C],
##      [D, E, F],
##      [G, H, I]]
##  flatten_matrix_triu(M) returns [B,C,F]
##  flatten_matrix_triu(M,True) returns [A,B,C,E,F,I]
def flatten_matrix_triu(arr,incl_diag=False):
      if incl_diag == True:
            return [arr[m][n] for m in range(arr.shape[0]) for n in
range(m,arr.shape[1])]
      else:
            return [arr[m][n] for m in range(arr.shape[0]) for n in
range(m+1,arr.shape[1])]
##---------------------------------------------------------------------------
-----------------------------------
```

```
##------------------------------------------------------------------------
-----------------------------------
###-----------------------------------------------------------------------
-----------------------------------
##                                  BEGIN PROGRAM
##------------------------------------------------------------------------
-----------------------------------
##------------------------------------------------------------------------
-----------------------------------
## need to create participants list by selecting all potential datasets for
each group
subj_df = pd.read_csv(PARTICIPANTS_FN, sep='\t')
subj_df.head(3)
print(' + found %d HC-
subjects:'%(subj_df[subj_df.group=='hc'].shape[0]),subj_df[subj_df.group=='hc
'])
print(' + found %d PD-
subjects:'%(subj_df[subj_df.group=='pd'].shape[0]),subj_df[subj_df.group=='pd
'])
print(' + found %d AR-
subjects:'%(subj_df[subj_df.group=='ar'].shape[0]),subj_df[subj_df.group=='ar
'])
##------------------------------------------------------------------------
-----------------------------------
## filter subjlist to only those with useable data AND apply the masker-
transform (regression)
subj_df[['func','conf','reg','nSpikes','nCensorTRs','excluded','ts']] =
object()
drop_indices = list()
mot_exclude_list=list()
for i in subj_df.index:
      S=subj_df['participant_id'][i]
      SDIR = os.path.join(FMRIPREP_DIR,S)
      C = os.path.join(SDIR,'func',S+'_task-rest_desc-
confounds_timeseries.tsv')
      F = os.path.join(SDIR,'func',S+'_task-rest_space-
MNI152NLin2009cAsym_desc-preproc_bold.nii.gz')
      if not os.path.exists(C):
            print('*** missing confounds file = %s'%(C))
            drop_indices.append(i)
            continue
      if not os.path.exists(F):
            print('*** missing func file = %s'%(F))
            drop_indices.append(i)
            continue
      subj_df['conf'][i] = C
      subj_df['func'][i] = F
      ## filter confounds
      print(' + reading and selecting confounds from file =',C)
      nSpikes,nCensorTRs,conf_sel_fn = select_confounds(C, CONF_SUFFIX)
      subj_df['nSpikes'][i] = nSpikes                 ## total TRs with
excessive-motion
      subj_df['nCensorTRs'][i] = nCensorTRs     ## total TRs being censored =
spikes+1Pre+2Post?
      subj_df['reg'][i] = conf_sel_fn
      subj_df['ts'][i] = masker.fit_transform(F, confounds=[conf_sel_fn])
```

```
      ## NOTE: could save each ts as pickled file in a separate path for easy
reuse in other analyses?
      subj_df['excluded'][i] = False
      if subj_df['nCensorTRs'][i] > CensoredTR_EXCLUDE_LIMIT:
            mot_exclude_list.append(i)
            subj_df['excluded'][i] = True
##-------------------------------------------------------------------------
-----------------------------------
## drop rows with missing files
if len(drop_indices) > 0:
      print(' + dropping MissingData
=',subj_df['participant_id'][drop_indices])
      subj_df.drop(drop_indices, inplace=True)
      if DEBUG_VERBOSE:
            print(type(subj_df))
            print(subj_df.dtypes)
            print(subj_df.shape)
            print(subj_df)
##-------------------------------------------------------------------------
-----------------------------------
## run subject-level ROI-to-ROI correlation and save as vector into output
group file.
print(' + calculating subject ROI-to-ROI correlations')
## set subject-level connectivity measure
CONN_MEASURES=['correlation','partial correlation','precision']
for C in CONN_MEASURES:
      #conn_meas = ConnectivityMeasure(kind='correlation')
      Cstr = C
      if C=='partial correlation':
            Cstr = 'partialCorr'
      conn_meas = ConnectivityMeasure(kind=C)
      labelStr = gen_label_combinations(FUNC_ROI_LABELS_SHORT)
      hdrStr = 'ssid,group,nSpikes,nCensorTRs,Excluded,'+','.join(labelStr)
      rfile = GRP_RESULTS_FN[:-4]+'_%s.csv'%(Cstr)
      rFP = open(rfile, 'w')
      rFP.write('%s\n'%(hdrStr))
      for i in subj_df.index:
            ssid = subj_df['participant_id'][i]
            group = subj_df['group'][i]
            nSpikes = subj_df['nSpikes'][i]
            nCensTRs = subj_df['nCensorTRs'][i]
            exclBool = subj_df['excluded'][i]
            ## calc and report correlations on individual subject
            cm = conn_meas.fit_transform([subj_df['ts'][i]])[0]
            if DEBUG_VERBOSE:
                  print('%s, %s, %d, %d, %s,
corr_mat:\n'%(ssid,group,nSpikes,nCensTRs,exclBool), cm)
            cmStr=['%.9f'%cm[m][n] for m in range(cm.shape[0]) for n in
range(m+1,cm.shape[1])]

      rFP.write('%s,%s,%d,%d,%s,%s\n'%(ssid,group,nSpikes,nCensTRs,exclBool,'
,'.join(cmStr)))
      rFP.close()
      print(' + completed script=%s' % (sys.argv[0]))
      print('\n + Correlation results saved to file = %s\n'%(rfile))
##-------------------------------------------------------------------------
-----------------------------------
```

```
## drop rows where subjects nSpikes >CensorTR_Limit files
if len(mot_exclude_list) > 0 and EXCLUDE_MOVERS_LIM_PERC > 0:
      print(' + dropping BigMovers
=',subj_df['participant_id'][mot_exclude_list])
      subj_df.drop(mot_exclude_list, inplace=True)
      if DEBUG_VERBOSE:
            print(type(subj_df))
            print(subj_df.dtypes)
            print(subj_df.shape)
            print(subj_df)
##----------- Explanation of Connectivity Measures ---------------------->
##
https://nilearn.github.io/auto_examples/03_connectivity/plot_group_level_conn
ectivity.html#sphx-glr-auto-examples-03-connectivity-plot-group-level-
connectivity-py
##
## [correlation] The simpler and most commonly used kind of connectivity is
correlation. It models the full (marginal) connectivity between pairwise
ROIs.
##
## [partial correlation] We can also study direct connections, revealed by
partial correlation coefficients.
##
## [tangent] We can use both correlations and partial correlations to capture
reproducible connectivity patterns at the group-level.
##      tangent_matrices model individual connectivities as perturbations of
the group connectivity matrix tangent_measure.mean_.
##      Keep in mind that these subjects-to-group variability matrices do not
directly reflect individual brain connections. For
##      instance negative coefficients can not be interpreted as
anticorrelated regions.
##      In practice such comparisons need to be performed on much larger
cohorts and several datasets. Dadi et al 2019 Showed
##      that across many cohorts and clinical questions, the tangent kind
should be preferred.
##-------------------------------------------------------------------------
-----------------------------------
## compute group connectivity correlations
## experimental stuff ----->
EDGE_THRESH="90%"
EDGE_VMIN=-0.5
EDGE_VMAX=0.5
PLOT_VMIN=-0.5    ## Matrix Plot axis
PLOT_VMAX=0.5
CONN_MEASURES=['correlation','partial correlation','precision','tangent']
if Run_GraphicalLassoCV == True:
      from sklearn.covariance import GraphicalLassoCV
      glCV = GraphicalLassoCV(verbose=2)

print(' + calculating group DMN ROI-to-ROI Correlations')
for G in list(subj_df.group.unique()):
      #grp_usable=subj_df[(subj_df.group==G)&(subj_df.nCensorTRs <
CensoredTR_EXCLUDE_LIMIT)].index
      grp_ssid_list = subj_df.loc[subj_df.group==G,'participant_id'].values
      grp_ts_list = subj_df.loc[subj_df.group==G,'ts'].values
      if len(grp_ssid_list) > 1:
            for C in CONN_MEASURES:
```

```
                Cstr = C
                if C=='partial correlation':
                      Cstr = 'partialCorr'
                #grp_ts_list = []
                #for func, conf in
zip(subj_df.loc[subj_df.group==G,'func'].values,
subj_df.loc[subj_df.group==G,'reg'].values):
                #     grp_ts_list.append(masker.fit_transform(func,
confounds=conf))
                conn_meas = ConnectivityMeasure(kind=C)
                print(' + connectome_measure.fit_transform(%s) on %d
subjects for group=%s:'%(C,len(grp_ssid_list),G.upper()))
                print(grp_ssid_list)
                grp_cm = conn_meas.fit_transform(grp_ts_list)[0]
                print(' + group=%s,subject[0]-id=%s, meas-%s
mat:\n'%(G.upper(),grp_ssid_list[0],Cstr), grp_cm[0])
                grp_cm_mean = conn_meas.mean_
                #print(' + group=%s,n=%d, mean-%s
mat:\n'%(G.upper(),len(grp_ts_list), Cstr), grp_cm_mean)
                ##
                t = 'DMN Connectivity [%s], %s'%(G.upper(),Cstr)
                #p =
plotting.plot_connectome(grp_cm_mean,FUNC_ROI_COORDS,title=t,display_mode='ly
rz',colorbar=True)
                #if os.uname().nodename == 'Aoraki.local' and
DEBUG_VERBOSE:
                #     plotting.show()
                #plot_fn = CORR_PLOTS_FN+'_grp-
%s_mean_%s_plot.pdf'%(G.upper(),Cstr)
                #p.savefig(plot_fn, dpi=1200)
                ## -- create a plot with fixed axis
                p =
plotting.plot_connectome(grp_cm_mean,FUNC_ROI_COORDS,title=t,edge_vmin=EDGE_V
MIN,edge_vmax=EDGE_VMAX,display_mode='lyrz',colorbar=True)
                if os.uname().nodename == 'Aoraki.local' and DEBUG_VERBOSE:
                      plotting.show()
                plot_fn = CORR_PLOTS_FN+'_grp-
%s_mean_%s_plot_fixed.pdf'%(G.upper(),Cstr)
                p.savefig(plot_fn, dpi=1200)
                ## create plot with 90% edge_thresh and max/min set on
values
                if isinstance(EDGE_THRESH, str):
                      t = 'DMN Connectivity [%s], %s@edge-
thresh=%s'%(G.upper(),Cstr,EDGE_THRESH)
                      plot_fn = CORR_PLOTS_FN+'_grp-
%s_mean_%s_p%s_plot.pdf'%(G.upper(),Cstr,EDGE_THRESH)
                else:
                      t = 'DMN Connectivity [%s], %s@edge-
thresh=%.2f'%(G.upper(),Cstr,EDGE_THRESH)
                      plot_fn = CORR_PLOTS_FN+'_grp-
%s_mean_%s_p%.2f_plot.pdf'%(G.upper(),Cstr,EDGE_THRESH)
                p =
plotting.plot_connectome(grp_cm_mean,FUNC_ROI_COORDS,edge_threshold=EDGE_THRE
SH,title=t,display_mode='lyrz',colorbar=True)
                if os.uname().nodename == 'Aoraki.local' and DEBUG_VERBOSE:
                      plotting.show()
                p.savefig(plot_fn, dpi=1200)
```

```
                print(' + group=%s Mean DMN Connectivity plot saved to file
= %s'%(G.upper(),plot_fn))
                ## ---- save the matrix to file
                mat_fn = CORR_PLOTS_FN+'_grp-
%s_mean_%s_matrix.tsv'%(G.upper(),Cstr)
                np.savetxt(mat_fn,grp_cm_mean, fmt='%.6f',delimiter='\t')
                ## ---- save group_mean-meas_matrix as vector
(upper_triangle)
                hdr_vals =
['nSubj']+gen_label_combinations(FUNC_ROI_LABELS_SHORT,True)
                mat_vals =
[len(grp_ssid_list)]+flatten_matrix_triu(grp_cm_mean,True)
                mat_fn   = CORR_PLOTS_FN+'_grp-
%s_mean_%s_vector.csv'%(G.upper(),Cstr)
                np.savetxt(mat_fn,
np.array(mat_vals).reshape(1,len(mat_vals)),
delimiter=',',fmt='%.9f',header=','.join(hdr_vals),comments='')
                # Mask the main diagonal for visualization:
                #np.fill_diagonal(grp_cm_mean, 0)
                t = 'DMN Correlation Matrix [%s] %s'%(G.upper(),Cstr)
                mp = plotting.plot_matrix(grp_cm_mean,
vmin=PLOT_VMIN,vmax=PLOT_VMAX, colorbar=True,title=t,
labels=FUNC_ROI_LABELS_SHORT)
                if os.uname().nodename == 'Aoraki.local' and DEBUG_VERBOSE:
                      plotting.show()
                mp_fn = CORR_PLOTS_FN+'_grp-
%s_mean_%s_matrix.pdf'%(G.upper(),Cstr)
                mp.figure.savefig(mp_fn, dpi=1200)
                print(' + group=%s Mean DMN Connectivity Matrix saved to
file = %s'%(G.upper(),mp_fn))
                ## -- build carpet plot [n_features * n_subjects] ---------
-------------------------------------------------
                if C != 'tangent' and CREATE_CARPET_PLOTS == True:
                      print(' ++ calculating individual subject corr-meas-
vectorized for carpet plot')
                      corr_meas_vec = ConnectivityMeasure(kind=C,
vectorize=True,discard_diagonal=True)
                      subj_x_features_list = []
                      for i,ts in enumerate(grp_ts_list):

      subj_x_features_list.append(corr_meas_vec.fit_transform([ts])[0])
                            print(' + finished %s of
%s'%(i+1,len(grp_ts_list)))
                      subj_x_features_arr = np.array(subj_x_features_list)
                      print(' + subj_x_features_arr.shape
=',subj_x_features_arr.shape)
                      print(' + type(subj_x_features_arr) =',
type(subj_x_features_arr))
                      plt.clf()
                      plt.imshow(subj_x_features_arr,aspect='auto')
                      plt.colorbar()
                      plt.title('[%s] - %s feature
matrix'%(G.upper(),Cstr))
                      plt.xlabel('features')
                      plt.ylabel('subjects')
                      plot_fn = CORR_PLOTS_FN+'_grp-%s_meas-
%s_subjXfeature_plot.pdf'%(G.upper(),Cstr)
```

```
                        plt.savefig(plot_fn, dpi=1200)
                        if os.uname().nodename == 'Aoraki.local' and
DEBUG_VERBOSE:
                              plt.show()
                        plt.cla()
                        plt.close()
                  ##------------- Build Sparse Inverse Matrix ---------------
-------------------------------------------------
                  if C == 'covariance' and Run_GraphicalLassoCV == True:
                        print(' + estimating group=%s SparseInverse
Covariance with GraphicalLassoCV'%(G.upper()))
                        glCV.fit(np.concatenate(grp_ts_list))
                        cov_matrix = glCV.covariance_
                        print(' + group=%s, estimated covariance matrix has
shape {0}.\n'.format(cov_matrix.shape)%(G.upper()),cov_matrix)
                        p=plotting.plot_connectome(glCV.covariance_,
FUNC_ROI_COORDS, edge_threshold='90%',

title="GraphicLassoCV Covariance", display_mode='lyrz',colorbar=True)
                        plot_fn = CORR_PLOTS_FN+'_grp-
%s_GraphicLassoCV_covariance_plot.pdf'%(G.upper())
                        p.savefig(plot_fn, dpi=1200)
                        p=plotting.plot_connectome(-glCV.precision_,
FUNC_ROI_COORDS,

edge_threshold='90%', title="GraphicLassoCV inverse-precision",

display_mode='lyrz',colorbar=True)
                        plot_fn = CORR_PLOTS_FN+'_grp-
%s_GraphicLassoCV_inverse_precision_plot.pdf'%(G.upper())
                        p.savefig(plot_fn, dpi=1200)
                        #plot_matrices(glCV.covariance_, glCV.precision_,
"GraphicalLasso", FUNC_ROI_LABELS_SHORT)


print(' ++ %s: completed DMN connectivity analysis with script: %s'
%(datetime.datetime.now().strftime('%Y%m%d-%H%M'),sys.argv[0]))
```

Appendix C

Consent Forms

nova scotia health authority

Diffusion Weighted Magnetic Resonance Imaging in Patients with Parkinson's disease

Patient consent form – neuroimaging task

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STUDY SPONSORS: Parkinson's Society of Canada, and Dalhousie Department of Psychiatry Research Fund

1. Introduction

You have been invited to take part in a research study. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

Please read this carefully. Take as much time as you like. If you like, take it home to think about for a while. Mark anything you don't understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- Discuss the study with you
- Answer your questions
- Keep confidential any information which could identify you personally
- Be available during the study to deal with problems and answer questions

We do not know if taking part in this study will help you. You may feel better. On the other hand it might not help you at all. It might even make you feel worse. We cannot always predict these things. We will always give you the best possible care no matter what happens.

If you decide not to take part or if you leave the study early, your usual health care will not be affected.

2. Why Is This Study Being Done?
Parkinson's disease (PD) is a neurodegenerative disorder that results in a person having tremor and difficulty moving. By the time PD is diagnosed by a physician, a large number of nerve cells have already been lost. People with PD also have a reduced ability to smell. There is also evidence that people who are at risk for developing PD, but have not yet developed the symptoms also have reduced smell ability. Thus, it is possible that smell ability can be used to diagnose PD before motor symptoms become present. The present study is investigating whether reduced smell ability is present in early stage PD and if it is related to changes in the brain's organization.

'Diffusion-weighted' and 'diffusion-tensor' magnetic resonance imaging (MRI) are special types of brain scans that are sensitive to changes in fluid movements in the brain. They can be used to study whether the brain is behaving normally and can give an indication of abnormalities in the integrity of brain cells in health and disease.

A recent study has shown that people with PD are known to have changes in the integrity of brain cell organization in a very specific region of the brain - that is, the region that is highly involved in the sense of smell. This disorganization could account for the changes in the ability to smell normally but this question must be investigated more fully.

These same smell problems are observed in patients who have been diagnosed with Alzheimer's disease (AD); however, the mentioned brain changes have not been examined in this patient population. In addition, individuals with AD have been shown to exhibit changes to their sleep and activity patterns early in the disease, but the exact nature of these changes, and how early they occur, are unclear. We wish to include a sample of patients with AD in order to determine whether brain organization changes are specific to Parkinson's disease or are similar in both neurological conditions. As well, we aim to characterize the early changes to sleep and activity patterns in AD, which may ultimately aid in earlier and more accurate diagnosis of the disease. We will also be examining patients who have been diagnosed with mild cognitive impairment (MCI). It has been shown that MCI can develop into AD in some individuals.

We will also include a sample of patients with REM sleep behaviour disorder (REMBD) because people with the disorder are known to have an increased risk of developing Parkinson's disease over a period of 5-10 years.

3. Why Am I Being Asked To Join This Study?
You volunteered to participate, and you meet the inclusion criteria.

4. How Long Will I Be In The Study?
You will be asked to meet with investigators once for smell testing and MRI scanning (see below). This visit can be broken down into two visits if it would be more comfortable and convenient for you. Smell testing will require 25 minutes of your time while MRI scanning will take 1.5 hours.

5. How Many People Will Take Part In This Study?
The study is being offered through Nova Scotia Health Authority at the IWK Children's Hospital. Forty-five (45) patients with suspected early-stage dementia of the Alzheimer's type will take part in this study, along with forty-five (45) patients with mild cognitive impairment, with forty-five (45) patients with Parkinson's Disease, forty-five (45) patients with REMBD and sixty (60) neurologically healthy control subjects.

6. How Is The Study Being Done?
There are two parts to the study: smell testing and MRI scanning.

a) Smell testing
This test is a scratch and sniff format of 40 items make up the test. For each item, there is a small patch to scratch to release the odour. Once released, you will be asked to smell the odour and pick one of the four potential answers that are written on the page (multiple choice format). The test determines how well you can identify and name different odours. You will have unlimited time to complete these tasks, but they will likely only take up to 25 minutes to finish.

b) Diffusion Weighted and Diffusion Tensor Magnetic Resonance Imaging (DW and DT-MRI)
You will be asked to lie quietly in the MRI scanner while pictures of your brain are taken. You will need to stay very still while the scan is happening. Magnetic fields will be turned off and on rapidly. When exposed to these changing magnetic fields, the tissues of the body release signals from which a computer makes an image or picture of the internal anatomy of the region being examined. The MRI scan produces no X-rays and is approved by the Health Protection Branch. The entire MRI procedure will be completed in approximately 45-55 minutes. There are no hazardous side effects known to occur as a result of MRI scanning unless you have metal in or near the body part being examined. The MRI scan will take place at the IWK Children's Hospital.

7. What Will Happen If I Take Part In This Study?
First, a clinician will review the study with you, and make sure that you meet the inclusion criteria. If you do, an appointment will be made for both the smell testing and the MRI scanning. If you have been diagnosed with Parkinson's, a qualified occupational therapist will do a short 15-minute assessment to test the progression of your physical symptoms. The findings from this assessment will determine if you can continue participating in the study.

8. Are There Risks To The Study?
There are no known risks and most people experience no discomfort from the smell testing procedures used in this study. All of the odours used are common ones and are not known to be harmful if inhaled.

You may find the interviews and questionnaires you receive during the course of the study upsetting or distressing. You may not like all of the questions that you will be asked. You do not have to answer those questions you find too distressing.

The MRI scans require you to lie in a small space. Some people find this unpleasant. If you do, the examination can be stopped at any time. As well, we will supply you with soft earplugs to reduce the noise from the MRI scanner (the sound it produces is a loud knocking noise). The noise may make it slightly difficult for you to communicate with the MRI technician but you will

be in constant contact via a microphone in the scanner. The technician can pause the machine and allow you to speak to him/her. Also, although extremely rarely, people complain of temporary hearing problems that quickly disappear after the scan is finished.

Some people also experience a phenomenon known as "Peripheral Nerve Stimulation." This is the result of some of the MRI scans that we perform. Peripheral Nerve Stimulation, or PNS as it is known, is a normal and safe side effect of MRI scanning. The most common feelings associated with PNS are tingling sensations in the arms or torso, tapping sensations (like someone is touching your skin), or muscle twitches. While PNS cannot harm you, some people find this unpleasant. If you do experience PNS and feel uncomfortable during the testing, you can stop the scanning at any time. This feeling will subside once you are out of the magnet.

MRI can be dangerous for anyone with metal inside his or her body. Some metal objects may move or heat up due to the scanning procedure used for MRI. We will screen you to make sure that it is safe for you to participate. You must tell us if you have had any surgery, as metal may be left in your body after certain types of surgery.

9. What Happens at the End of the Study?

At the end of the study, your involvement in the study will be over and you will continue to be followed by your regular clinician in the division of Geriatric medicine.

10. What Are My Responsibilities?

As a study participant, you will be expected to:

- Follow the directions of the principal investigator
- Report all medications being taken or that you plan on taking
- Report changes in your health to the principal investigator
- Report any problems that you experience that you think might be related to participating in the study

11. Can I Be Taken Out Of The Study Without My Consent?

Yes. You may be taken out of the study at any time, if:

- There is new information that shows that being in this study is not in your best interests.
- Parkinson's Society of Canada or Dalhousie University Department of Psychiatry Research Fund (the study sponsors), the Nova Scotia Health Authority Research Ethics Board or the Principal Investigator decides to stop the study.
- You do not follow the directions of the Principal Investigator.

You will be told about the reasons why you might need to be taken out of the study.

12. What About New Information?

It is possible (but unlikely) that new information may become available while you are in the study that might affect your health, welfare, or willingness to stay in the study. If this happens, you will be informed in a timely manner and will be asked whether you wish to continue taking part in the study or not.

13. Will It Cost Me Anything?
You will not be paid to be in the study. You will be given a small honorarium to cover any costs that you might incur while participating in the study (e.g., meals and parking).

Research Related Injury
If you become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. Your signature on this form only indicates that you have understood to your satisfaction the information regarding your participation in the study and agree to participate as a subject. In no way does this waive your legal rights nor release the Principal Investigator, the research staff, the study sponsor or involved institutions from their legal and professional responsibilities.

14. What About My Right To Privacy?
Protecting your privacy is an important part of this study. When you sign this consent form you give us permission to:

- Collect information from you
- Collect information from your health record
- Share information with the people conducting the study
- Share information with the people responsible for protecting your safety

The study doctor and members of the research team will see health and study records that identify you by name. Other people may need to look at the health and study records that identify you by name. These might include:

- people working for the sponsor* - You may ask to see the list of persons working with the sponsor (if applicable)
- the Nova Scotia Health Authority Research Ethics Board and Research Quality Associate

Use of records.
The research team will collect and use only the information they need to complete the study. This information will include your:

- date of birth
- sex
- medical conditions
- medications
- the results of tests and procedures you had before and during the study
- information from study interviews and questionnaires

Your name and contact information will be kept secure by the research team at the QEII Site. It will not be shared with others without your permission. Your name will not appear in any report or article published as a result of this study. Information collected for this study will kept as long as required by law. This could be 7 years or more.

If you decide to withdraw from the study, the information collected up to that time will continue to be used by the research team. It may not be removed.
After your part in this study ends, we may continue to review your health records.
We may want to follow your progress and to check that the information we collected is correct.

Information collected and used by the research team will be stored by the Centre for Clinical Research. The Manager of the Centre is the person responsible for keeping it secure. You may also be contacted personally by Research Auditors for quality assurance purposes.

Your access to records
You may ask the study doctor to see the information that has been collected about you.

15. What if I want to quit the study?
If you chose to participate and later change your mind, you can say no and stop the research at any time. If you wish to withdraw your consent please inform the Principal Investigator. All data collected up to the date you withdraw your consent will remain in the study records, to be included in study related analyses.

16. Declaration Of Financial Interest
The sponsor is paying the Principal Investigator and/or the Principal Investigator's institution to conduct this study. The amount of this payment is sufficient to cover the costs of conducting the study. The Principal Investigator has no financial interests in conducting this research study.

17. What About Questions Or Problems?
For further information about the study call **Dr. Kimberley Good** (902-473-4250). Dr. Good is in charge of this study at this institution (she is the "Principal Investigator"). If you can't reach the Principal Investigator, please refer to the attached Research Team Contact Page for a full list of the people you can contact for further information about the study.

The Principal Investigator is **Dr. Kimberley Good**
Telephone: (902) 473-4250

Your Research Coordinator is **Ceire Storey**
Telephone: (902) 473-3147
ceire.storey@nshealth.ca

18. What Are My Rights?
After you have signed this consent form you will be given a copy. If you have any questions about your rights as a research participant, contact the **Patient Representative** at **(902) 473-2133**.

In the next part you will be asked if you agree (consent) to join this study. If the answer is "yes", you will need to sign the form.

19. Consent Form Signature Page

I have reviewed all of the information in this consent form related to the study called:
"Diffusion Weighted Magnetic Resonance Imaging in Patients with Parkinson's disease"

I have been given the opportunity to discuss this study. All of my questions have been answered to my satisfaction.

I agree to allow the people described in this consent form to have access to my health records.

This signature on this consent form means that I agree to take part in this study. **I understand that I am free to withdraw at any time.**

_____ Signature of Participant	_____ Name (Printed)	____ / ____ / ___ Year Month Day*
_____ Witness to Participant's Signature	_____ Name (Printed)	____ / ____ / ___ Year Month Day*
_____ Signature of Investigator	_____ Name (Printed)	____ / ____ / ___ Year Month Day*
_____ Signature of Person Conducting Consent Discussion	_____ Name (Printed)	____ / ____ / ___ Year Month Day*
_____ Signature of Participant's Authorized Legal Representative	_____ Name (Printed)	____ / ____ / ___ Year Month Day*

If the consent discussion has been conducted in a language other than English, please indicate:

_____
Language

_____ Signature of Translator	_____ Name (Printed)	____ / ____ / ___ Year Month Day*

**Note: Please fill in the dates personally*

I will be given a signed copy of this consent form
Thank you for your time and patience!

nova scotia health authority

STUDY TITLE: Is cognitive performance associated with preclinical markers of Parkinson's disease?

Patient consent form

PRINCIPAL INVESTIGATOR Kimberley P. Good, Ph.D.
Department of Psychiatry,
Dalhousie University
4064 AJLB
5909 Veterans Memorial Lane
473-4250

ASSOCIATE INVESTIGATORS: Please see the attached Research Team Contact Page for a full list of the investigators for this study.

STUDY SPONSOR: Canadian Institutes of Health Research

PART A.

Non-Interventional Studies – General Information

1. Introduction

You have been invited to take part in a research study. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

Please read this carefully. Take as much time as you like. If you like, take it home to think about for a while. Mark anything you don't understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- Discuss the study with you
- Answer your questions
- Keep confidential any information which could identify you personally
- Be available during the study to deal with problems and answer questions

We do not know if taking part in this study will help you. You may feel better. On the other hand it might not help you at all. It might even make you feel worse. We

cannot always predict these things. We will always give you the best possible care no matter what happens.

If you decide not to take part or if you leave the study early, your usual health care will not be affected.

PART B.

EXPLAINING THE STUDY

2. Why Is This Study Being Done?

Parkinson's disease (PD) is a degenerative brain disorder that results in a person having a tremor along with difficulty moving and thinking. By the time PD is diagnosed by a physician, a large number of nerve cells have all ready been lost. Between 20 and 40% of patients with PD later develop dementia, which is a deterioration of cognitive functioning (changes in memory, attention, language and/or problem solving) that cannot be accounted for by normal aging. We currently do not know when these changes begin or who might be susceptible. The purpose of this study is to determine whether changes in cognitive functioning occur in the early stages of PD and whether any observed changes can help to predict who is susceptible to developing a dementia. Moreover, in addition to information gathered from the smell testing and diffusion weighted MRI investigation in which you recently participated, we wish to determine whether cognitive testing can improve our ability to identify persons who may be at risk for developing Parkinson's disease.

These same smell problems are observed in patients who have been diagnosed with dementia of the Alzheimer's type; however, the mentioned brain changes have not been examined in this patient population. We wish to include a sample of patients with probable Alzheimer's Disease in order to determine whether the brain organization changes are specific to Parkinson's disease or are similar in both neurological conditions.

The literature indicates that the earlier a person with PD is diagnosed, the better the chance that the newer treatments will be effective. Finding ways to diagnose and treat patients in the early stages of illness are the long-term goals of this line of investigation.

3. Why Am I Being Asked To Join This Study?

You are being asked to participate in this study as you have already participated in an investigation entitled: "Diffusion weighted magnetic resonance imaging in patients with Parkinson's disease". The information obtained in the current study

will be combined with that gathered in the prior study to find markers that may help to facilitate early diagnosis of Parkinson's disease.

4. How Long Will I Be In The Study?

This study involves only one (1) visit and we will ask you to commit two hours of your time for the cognitive testing.

.

5. How Many People Will Take Part In This Study?

The study is being offered through Nova Scotia Health Authority. In total, 268 subjects will participate in this study: We will recruit 45 of each patient group, all who will be between the ages of 45 to 75 years old. The patient group includes those with a diagnosis of dementia (Alzheimer's type), those with Parkinson's Disease, those with REM Behaviour Disorder, and those with Mild Cognitive Impairment. We will also recruit 60 neurologically healthy controls, age 45 to 75 years old. Finally, 28 first-degree relatives of Parkinson's patients aged 40-65 will also be recruited.

6. How Is The Study Being Done?

Participants enrolled in this study will be asked to come to the Abbie J. Lane Building for cognitive testing. This study involves only a short cognitive test battery (described below) that assesses the way you process information.

7. What Will Happen If I Take Part In This Study?

First, a research assistant will review the study with you, and make sure that you meet the inclusion criteria. We will begin by asking you a few questions about your illness, your sleep and bowel patterns. You will then undergo a series of cognitive (thinking) tests. The cognitive assessment involves pencil and paper tests, along with some tasks that take place using the computer. These measures assess your ability to remember lists of words, correctly pronounce words that do not follow common English spelling rules, provide words that conform to a given category, and remember and manipulate information in your mind. Many of these tasks are used frequently to assess brain functioning in patients with PD, while others are experimental. You will be provided with as many breaks that you need in order to easily complete the testing. Moreover, the testing can be done over the course of two days, rather than in one session, if that is easier for you. You are free to choose not to participate in any further testing or study visits at any time.

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8. Are There Risks To The Study?

You may find the interviews, questionnaires and cognitive tests you receive during the course of the study upsetting or distressing. You may not like all of the questions that you will be asked. You do not have to answer those questions you find too distressing. You are free to choose not to participate in any further testing or study visits at any time.

9. What Happens at the End of the Study?

At the end of the study, your involvement in the study will be over and you will continue to be followed by your regular clinician in the division of Geriatric medicine. You will be given a copy of any publications that arise as a result of this study.

10. What Are My Responsibilities?

As a study participant you will be asked to:

- follow the directions of the Principal Investigator, and
- complete the questionnaires associated with this study to the best of your ability.

11. Can I Be Taken Out Of The Study Without My Consent?

Yes. You may be taken out of the study at any time, if:

- there is new information that shows that being in this study is not in your best interest, and/or
- the Nova Scotia Health Authority Research Ethics Board or the Principal Investigator decides to stop the study.

You will be told about the reasons why you might need to be taken out of the study.

12. What About New Information?

It is possible (but unlikely) that new information may become available while you are in the study that might affect your health, welfare, or willingness to stay in the

study. If this happens, you will be informed in a timely manner and will be asked whether or not you wish to continue taking part in the study.

13. Will It Cost Me Anything?

Compensation
You will not be paid to be in the study. You will be given a small honorarium $10/hr to cover any costs that you might incur while participating in the study (e.g., meals and parking).

Research Related Injury
If you become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. Your signature on this form only indicates that you have understood to your satisfaction the information regarding your participation in the study and agree to participate as a subject. In no way does this waive your legal rights nor release the Principal Investigator, the research staff, the study sponsor or involved institutions from their legal and professional responsibilities.

14. What About My Right To Privacy?

Protecting your privacy is an important part of this study. A copy of this consent will be put in your health record.

When you sign this consent form you give us permission to:

- Collect information from you
- Collect information from your health record
- Share information with the people conducting the study
- Share information with the people responsible for protecting your safety

Access to records
The study doctor and members of the research team will see health and study records that identify you by name.

Other people may need to look at the health and study records that identify you by name. These might include:

- people working for the sponsor. You may ask to see the list of persons working with the sponsor
- the NSHA Research Ethics Board and Research Quality Associate

Use of records.
The research team will collect and use only the information they need to

complete the study. This information will only be used for the purposes of this study.

This information will include your:

- age
- sex
- medical conditions
- medications
- results of tests and procedures you had before and during the study
- information from study interviews and questionnaires

Your name and contact information will be kept secure by the research team at Nova Scotia Health Authority. It will not be shared with others without your permission. Your name will not appear in any report or article published as a result of this study. Information collected for this study will kept as long as required by law. This could be 7 years or more.

If you decide to withdraw from the study, the information collected up to that time will continue to be used by the research team. It may not be removed.

After your part in this study ends, we may continue to review your health records. We may want to follow your progress and to check that the information we collected is correct.

Information collected and used by the research team will be stored by the Centre for Clinical Research. The Manager of the Centre is the person responsible for keeping it secure.

You may also be contacted personally by Research Auditors for quality assurance purposes.

Your access to records

You may ask the study doctor to see the information that has been collected about you.

15. What If I Want To Quit The Study?

If you chose to participate and later change your mind, you can say no and stop the research at any time. If you wish to withdraw your consent please inform the Principal Investigator. All data collected up to the date you withdraw your consent will remain in the study records, to be included in study related analyses.

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16. Declaration Of Financial Interest

The sponsor is paying the Principal Investigator and/or the Principal Investigator's institution to conduct this study. The amount of this payment is sufficient to cover the costs of conducting the study. The Principal Investigator has no financial interests in conducting this research study.

17. What About Questions Or Problems?

For further information about the study call **Dr. Kimberley Good**. Dr. Good is in charge of this study at this institution (she is the "Principal Investigator"). Dr. Good's work telephone number is (902) 473-4250. If you can't reach the Principal Investigator, please refer to the attached Research Team Contact Page for a full list of the people you can contact for further information about the study.

The Principal Investigator is: **Dr. Kimberley Good**
Telephone: (902) 473-4250

Your Research Coordinator is **Denise Lewis**
Telephone: (902) 473-3147.
Email: denise.lewis@nshealth.ca

18. What Are My Rights?

After you have signed this consent form you will be given a copy.

If you have any questions about your rights as a research participant, contact the **Patient Representative** at **(902) 473-2133.**

In the next part you will be asked if you agree (consent) to join this study. If the answer is "yes", you will need to sign the form.

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PART C.

19. Consent Form Signature Page

I have reviewed all of the information in this consent form related to the study called:

"Is cognitive performance associated with preclinical markers of Parkinson's disease?"

I have been given the opportunity to discuss this study. All of my questions have been answered to my satisfaction.

I agree to allow the people described in this consent form to have access to my health records.

This signature on this consent form means that I agree to take part in this study. I understand that I am free to withdraw at any time.

_____	_____	_____ / _____ / _____
Signature of Participant	Name (Printed)	Year / Month / Day*
_____	_____	_____ / _____ / _____
Witness to Participant's Signature	Name (Printed)	Year / Month / Day*
_____	_____	_____ / _____ / _____
Signature of Investigator	Name (Printed)	Year / Month / Day*
_____	_____	_____ / _____ / _____
Signature of Person Conducting Consent Discussion	Name (Printed)	Year / Month / Day*
_____	_____	_____ / _____ / _____
Signature of Participant's Authorized Legal Representative	Name (Printed)	Year / Month / Day*

If the consent discussion has been conducted in a language other than English, please indicate:

_____
Language

_____	_____	_____ / _____ / _____
Signature of Translator	Name (Printed)	Year / Month / Day*

Note: Please fill in the dates personally

I Will Be Given A Signed Copy Of This Consent Form
Thank you for your time and patience!

Appendix D

Demographic Questionnaire

Parkinson's Disease Cognitive Study ID#: _____
Demographics and Health Questionnaire Date: _____(D/M/Y)

Collected by: _____

Initials:_____ **Date of Birth:** (Month and Year only) _____ **Age:** _____yrs.

Sex: M F

Education	Last yr completed	Year	Age at completion	Degrees/Spec.Ed./Enrichment, etc.
Elementary				
High School				
Post Sec				

Sense of Smell: Do you have any disruptions to your sense of smell?

How would your rate your sense of smell? ☐great ☐very good ☐good ☐poor ☐absent

If reported to be poor/absent, how long has this been evident? Since: _____year; or

_____age.

Is there anything in particular that you no longer smell?_____

_____

Cognition: This is your ability to plan, reason, and problem-solve.

Y N Have you had cognitive or neuropsychological testing done before?

If yes, when? _____year; or _____age.

If yes, for what reason? _____

Y N Do you notice any changes in your memory, attention, conversation abilities or ability to understand what you hear or read? If yes – since: _____year; or _____age.

If yes, can you describe what you think has changed?

_____

_____

_____

_____

Y N Do <u>your family or friends</u> tell you that they have seen changes in your memory, attention, conversation abilities or ability to understand what you hear or read?

If yes, what do they say has changed?

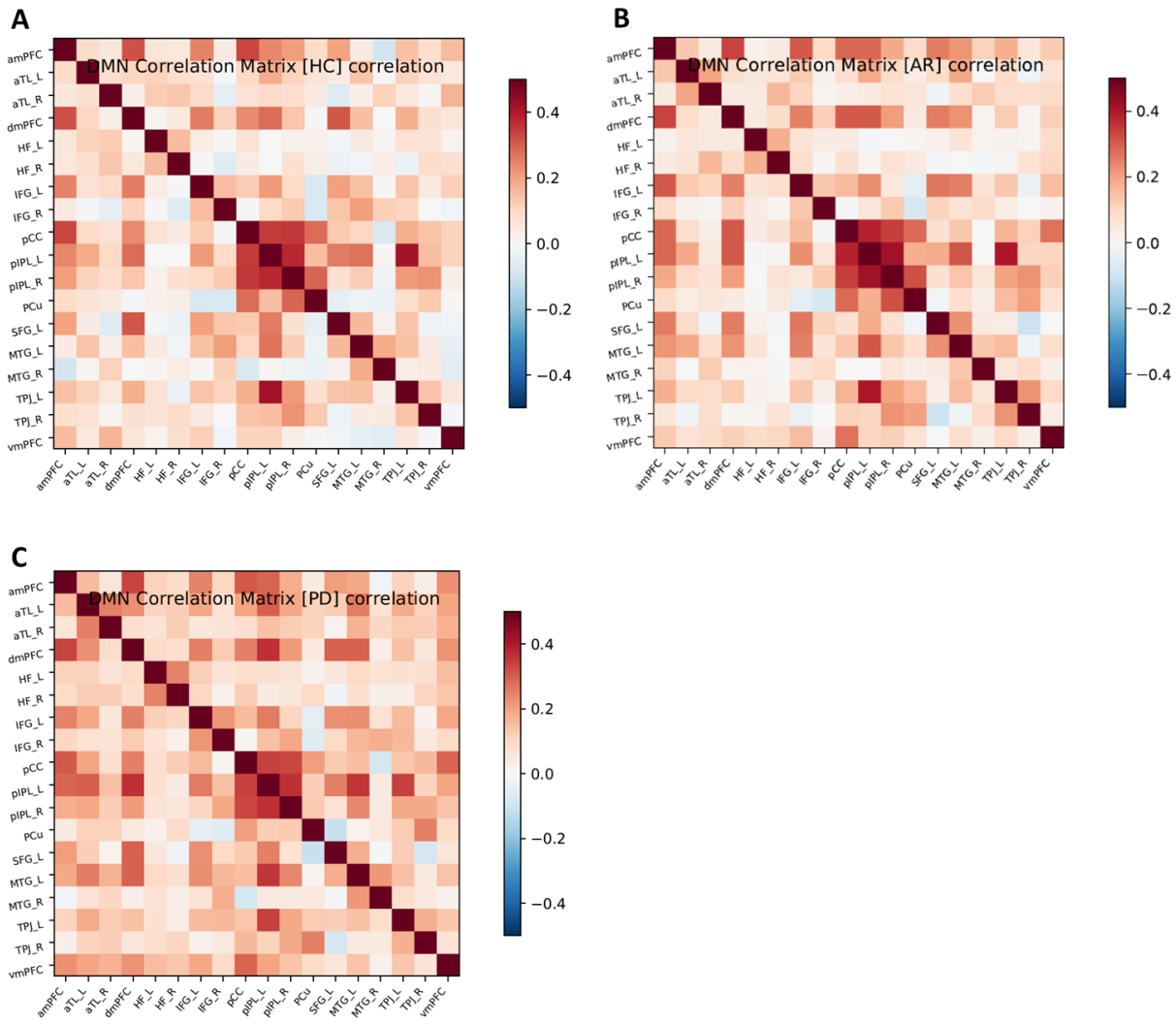
_____

Appendix E

Graphs for Default Mode Network ROI-to-ROI Correlations

Figure E1

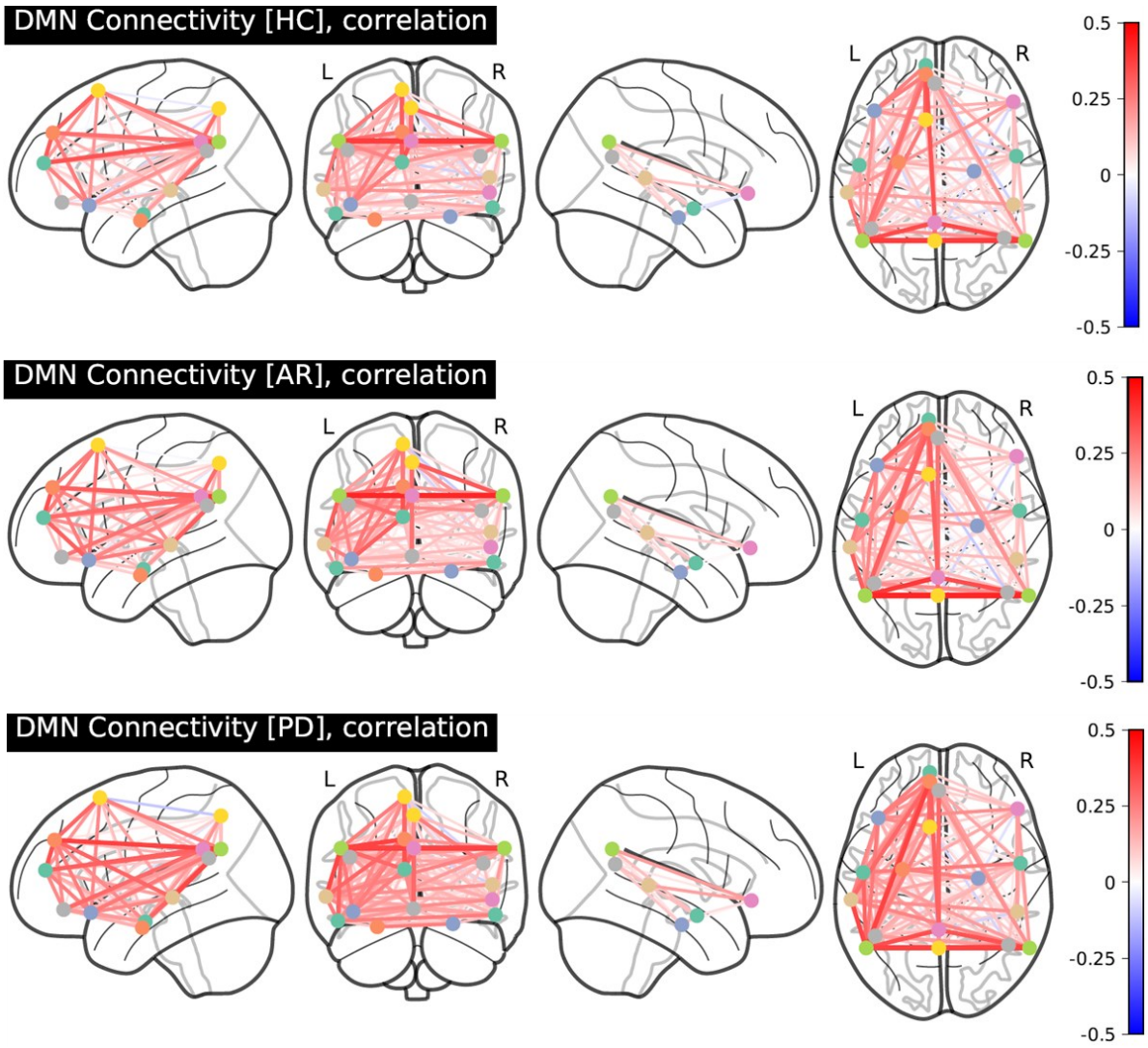
Correlation Matrices between Default Mode Network ROIs for Each Group



Note. (A) Correlation matrix depicting ROI-to-ROI correlations for the healthy control (HC) group. (B) Correlation matrix for the at-risk group (AR). (C) Correlation matrix for the Parkinson's group (PD).

Figure E2

Default Mode Network ROIs Correlation Plots for Each Group



Appendix F

Supplementary Tables

Table F1

Comparison of DMN Functional Connectivity Values between the AR and HC Groups

Edge	Test Statistic	df	*p*	*p* (FDR-corrected)
amPFC - aTL-L	-0.79	51	0.436	0.939
amPFC - dmPFC	-0.41	51	0.685	0.939
amPFC - HF-L	0.85	51	0.398	0.939
amPFC - HF-R	0.09	51	0.927	0.984
amPFC - IFG-L	-1.14	51	0.261	0.939
amPFC - IFG-R	-1.12	51	0.270	0.939
amPFC - pCC	0.87	51	0.387	0.939
amPFC - pIPL-L*	373.00	-	0.682	0.939
amPFC - pIPL-R	0.47	51	0.640	0.939
amPFC - PCu	-0.09	51	0.929	0.984
amPFC - SFG-L	-0.94	51	0.349	0.939
amPFC - MTG-L	**-3.32**	**51**	**0.002**	**0.114**
amPFC - MTG-R	**-4.64**	**51**	**0.000**	**0.003**
amPFC - TPJ-L	-0.51	51	0.614	0.939
amPFC - TPJ-R	0.45	51	0.658	0.939
amPFC - vmPFC**	0.49	38.93	0.631	0.939
aTL-L - aTL-R	-1.93	51	0.060	0.909
aTL-L - dmPFC	0.33	51	0.744	0.939
aTL-L - HF-L	1.29	51	0.204	0.939
aTL-L - HF-R**	0.38	46.04	0.706	0.939
aTL-L - IFG-L	-1.14	51	0.262	0.939
aTL-L - IFG-R	-0.55	51	0.588	0.939
aTL-L - pCC	0.32	51	0.749	0.939
aTL-L - pIPL-L	-0.10	51	0.919	0.984
aTL-L - pIPL-R	0.41	51	0.684	0.939
aTL-L - PCu	0.69	51	0.491	0.939
aTL-L - SFG-L	-0.97	51	0.336	0.939
aTL-L - MTG-L	-0.98	51	0.333	0.939
aTL-L - MTG-R	0.27	51	0.791	0.941
aTL-L - TPJ-L	0.32	51	0.754	0.939
aTL-L - TPJ-R	2.05	51	0.046	0.894
aTL-L - vmPFC	-0.33	51	0.745	0.939
aTL-R - dmPFC	-0.34	51	0.734	0.939

Edge	Test Statistic	df	*p*	*p* (FDR-corrected)
aTL-R - HF-L	1.34	51	0.185	0.939
aTL-R - HF-R	-0.57	51	0.570	0.939
aTL-R - IFG-L	-0.17	51	0.865	0.984
aTL-R - IFG-R**	-1.04	49.32	0.303	0.939
aTL-R - pCC	0.69	51	0.491	0.939
aTL-R - pIPL-L	0.77	51	0.447	0.939
aTL-R - pIPL-R	0.26	51	0.793	0.941
aTL-R - PCu	-0.29	51	0.775	0.941
aTL-R - SFG-L*	391.00	-	0.465	0.939
aTL-R - MTG-L	-0.98	51	0.334	0.939
aTL-R - MTG-R	-0.25	51	0.802	0.941
aTL-R - TPJ-L	-0.53	51	0.598	0.939
aTL-R - TPJ-R	-1.10	51	0.275	0.939
aTL-R - vmPFC	1.11	51	0.272	0.939
dmPFC - HF-L	-0.60	51	0.554	0.939
dmPFC - HF-R	-0.84	51	0.407	0.939
dmPFC - IFG-L	0.13	51	0.894	0.984
dmPFC - IFG-R	-0.09	51	0.928	0.984
dmPFC - pCC	-1.38	51	0.174	0.939
dmPFC - pIPL-L	-0.65	51	0.520	0.939
dmPFC - pIPL-R	-1.66	51	0.104	0.939
dmPFC - SFG-L*	296.00	-	0.336	0.939
dmPFC - MTG-L	-1.29	51	0.204	0.939
dmPFC - TPJ-L	0.81	51	0.422	0.939
dmPFC - TPJ-R	0.98	51	0.330	0.939
dmPFC - vmPFC	-1.02	51	0.313	0.939
HF-L - HF-R*	367.00	-	0.762	0.940
HF-L - IFG-L	-0.50	51	0.621	0.939
HF-L - IFG-R	-0.81	51	0.422	0.939
HF-L - pCC	0.61	51	0.547	0.939
HF-L - pIPL-L	0.13	51	0.897	0.984
HF-L - pIPL-R	0.36	51	0.722	0.939
HF-L - SFG-L	-0.79	51	0.435	0.939
HF-L - MTG-L	-0.48	51	0.633	0.939
HF-L - MTG-R	-0.07	51	0.941	0.984
HF-L - TPJ-L	1.03	51	0.310	0.939
HF-L - TPJ-R	1.17	51	0.250	0.939
HF-L - vmPFC**	-1.21	36.81	0.234	0.939
HF-R - IFG-L	-1.93	51	0.059	0.909
HF-R - pCC	-0.59	51	0.561	0.939

Edge	Test Statistic	df	*p*	*p* (FDR-corrected)
HF-R - pIPL-R	0.10	51	0.921	0.984
HF-R - PCu	-0.39	51	0.701	0.939
HF-R - MTG-L**	-0.33	43.99	0.746	0.939
HF-R - TPJ-R	0.48	51	0.631	0.939
HF-R - vmPFC	-0.39	51	0.697	0.939
IFG-L - IFG-R	0.36	51	0.718	0.939
IFG-L - pCC	-0.59	51	0.555	0.939
IFG-L - pIPL-L	-0.34	51	0.736	0.939
IFG-L - pIPL-R	0.66	51	0.510	0.939
IFG-L - PCu	-0.51	51	0.615	0.939
IFG-L - SFG-L	-1.06	51	0.293	0.939
IFG-L - MTG-L	-1.71	51	0.094	0.939
IFG-L - MTG-R	-1.38	51	0.174	0.939
IFG-L - TPJ-L	0.08	51	0.938	0.984
IFG-L - vmPFC	-0.69	51	0.492	0.939
IFG-R - pIPL-L**	0.71	44.12	0.483	0.939
IFG-R - pIPL-R	0.01	51	0.994	0.997
IFG-R - PCu	0.04	51	0.968	0.997
IFG-R - SFG-L	0.50	51	0.620	0.939
IFG-R - MTG-L	**3.01**	**51**	**0.004**	**0.184**
IFG-R - MTG-R	0.37	51	0.713	0.939
IFG-R - TPJ-L	1.53	51	0.132	0.939
IFG-R - vmPFC	-0.53	51	0.598	0.939
pCC - pIPL-L	-0.67	51	0.505	0.939
pCC - pIPL-R*	324.00	-	0.643	0.939
pCC - PCu	-0.06	51	0.952	0.988
pCC - SFG-L	1.00	51	0.324	0.939
pCC - MTG-L**	-0.61	46.86	0.546	0.939
pCC - MTG-R	-1.49	51	0.143	0.939
pCC - TPJ-L	0.11	51	0.913	0.984
pCC - TPJ-R	0.49	51	0.625	0.939
pCC - vmPFC	**-2.58**	**51**	**0.013**	**0.443**
pIPL-L - pIPL-R*	384.00	-	0.545	0.939
pIPL-L - PCu	-0.42	51	0.680	0.939
pIPL-L - SFG-L	1.25	51	0.217	0.939
pIPL-L - MTG-L	-0.69	51	0.496	0.939
pIPL-L - TPJ-L*	338.00	-	0.831	0.964
pIPL-L - TPJ-R*	324.00	-	0.643	0.939
pIPL-L - vmPFC	0.03	51	0.979	0.997
pIPL-R - PCu	-0.55	51	0.583	0.939

Edge	Test Statistic	df	*p*	*p* (FDR-corrected)
pIPL-R - SFG-L	-0.004	51	0.997	0.997
pIPL-R - MTG-L	-0.25	51	0.804	0.941
pIPL-R - MTG-R	-1.50	51	0.140	0.939
pIPL-R - TPJ-L	0.21	51	0.838	0.964
pIPL-R - TPJ-R	-0.01	51	0.995	0.997
pIPL-R - vmPFC**	-1.43	42.95	0.160	0.939
PCu - SFG-L	-0.61	51	0.548	0.939
PCu - MTG-L	**-2.16**	**51**	**0.036**	**0.812**
PCu - MTG-R	-1.43	51	0.159	0.939
PCu - TPJ-L	-1.06	51	0.294	0.939
PCu - TPJ-R	-1.16	51	0.254	0.939
PCu - vmPFC**	-1.15	39.60	0.256	0.939
SFG-L - MTG-L	-1.28	51	0.206	0.939
SFG-L - TPJ-L	1.72	51	0.092	0.939
SFG-L - TPJ-R	1.23	51	0.223	0.939
SFG-L - vmPFC	-0.26	51	0.796	0.941
MTG-L - MTG-R	0.94	51	0.354	0.939
MTG-L - TPJ-L	-0.11	51	0.910	0.984
MTG-L - vmPFC	**-2.36**	**51**	**0.022**	**0.603**
MTG-R - TPJ-L	-0.57	51	0.570	0.939
MTG-R - TPJ-R	-1.17	51	0.246	0.939
TPJ-L - TPJ-R	-1.26	51	0.212	0.939
TPJ-L - vmPFC	-0.72	51	0.475	0.939
TPJ-R - vmPFC	-0.70	51	0.489	0.939

Note. Test statistic = t-value unless otherwise indicated. Bolded edges had significant functional connectivity differences between the AR group (n = 28) and the HC group (n = 25) before FDR correction ($p < .05$). * Mann-Whitney U test was conducted and corresponding Mann-Whitney U test statistics and p-values are reported. **Welch t-test was conducted and corresponding t-values, degrees of freedom (df) and p-values are reported.

Table F2

Comparison of Functional Connectivity between PD and HC Groups

Edges	$t(46)$	p	Effect size (Hedges' g)
amPFC – MTG-L	2.79	**.008**	0.81
amPFC – MTG-R	1.56	.125	0.45
IFG-R – MTG-L	-0.71	.482	0.20
pCC – vmPFC	2.72	**.009**	0.79
PCu – MTG-L	0.59	.557	0.17
MTG-L – vmPFC	2.93	**.005**	0.84

Note. amPFC = anterior medial prefrontal cortex. MTG-L = left middle temporal gyrus. MTG-R = right middle temporal gyrus. IFG-R = right inferior frontal gyrus. pCC = posterior cingulate gyrus. vmPFC = ventromedial prefrontal cortex. PCu = precuneus.

Appendix G

Instructions for Cognitive Tests

***** Instructions seen in bold font must be said verbatim, as they come from licensed studies ******

Equipment Needed:

-stopwatch – preferably one that shows minutes & seconds to at least 30 minutes

-Spatial Span Display Board

-the Licensed Assessment Sheets for the tasks listed below:

c/ WMS-III

d/ DKEFS – Trails: tests 1 thru 5 incl.

e/ DKEFS Fluency

-Demographics questionnaire

-pens: do not enter data in pencil

Demographics

Fill in all fields in pen, as much as Ppt will answer. RA should write in info as Ppt cites it to the RA. This will ensure that info is written in legibly, and in all possible fields.

WMS-III: [Test #9] Spatial Span -need: Spatial Span Board, Record Form

A/ FORWARD

7Aa/ DISCONTINUE after scores of 0 on both trials of an item, or after you have administered all items of this assessment.

Instructions

7Ab/ Place the Spatial Span Board on the table with the cube numbers facing you, and the board centered at the examinee's midline, so the she can easily reach the cubes.

Say: **Now I want you to do exactly what I do. Touch the blocks I touch, in the right order.**

7Ac/ Use the Record Form for the tapping sequence. Tap out the sequence for Trial 1 at the rate of 1 block per second. Begin with Item 1, Trial 1.

7Ad/ Record the examinee's responses. If the criterion for discontinuing is met, or if all Spatial Span Forward items have been administered, proceed with Spatial Span Backward.

7B/ WMS-III: [Test #9] Spatial Span Backward

7Ba/ DISCONTINUE after scores of 0 on both trials of an item, or after you have administered all items of this assessment.

Instructions

7Bb/Say: **Now I am going to touch some more blocks. This time when I stop, I want you to touch the blocks backward, in reverse of mine. For example: if I touch this one** [Cube 3] **& then this one** [Cube 5]**, what would you do?** If the examinee responds correctly, say: **That's right. Here's the next one. Remember to do them in reverse order.** Then proceed with Item 1.

7Bc/ If the examinee responds incorrectly on the 3-5- example, then you say: **No, I touched this one then this one, so to do it in reverse. Now, let's try another one. If I touched this one** [Cube 9] **then this one** [Cube 1], **what would you do?** Whether the examinee succeeds or fails on the second example, proceed to Item 1, Trial 1.

WMS-III: [Test #11] Digit Span **-** need: Record Form

A/ FORWARD

8Aa/ DISCONTINUE after scores of 0 on both trials of an item, or after you have administered all items of this assessment.

Instructions

8Ab/ Say: **I am going to say some numbers. Listen carefully, and when I am through, I want you to say them right after me. Just say what I say.** Read each dropping your voice inflection slightly on the last digit in the sequence.

B/ BACKWARD

8Ba/ Say: **Now I am going to say some more numbers. But this time when I stop, I want you to say them backward. For example, if I say [7-1-9], what would you say?** If the examinee says [9-1-7], say: **That's right,** and proceed to Trial 1 of Item 1. However, if the examinee responds incorrectly, provide the correct response, and say: **No, you say [9-1-7]. I said [7-1-9], so to say it backward, you would say [9-1-7]. Now, try these numbers. Remember, you are to say them backward: [3-4-8]**

8Bb/ Do not provide any assistance on this example or any of the items. Whether or not the examinee responds correctly [8-4-3], proceed to Trial 1 of Item 1.

WMS-III: [Test #8] Letter-Number Sequencing **-** need: Record Form

9A/ DISCONTINUE after scores of 0 on all 3 trials of an item, or after you have administered all items of this assessment.

Instructions

9B/ Say: **I am going to say a group of numbers and letters. After I say them, I want you to tell me the numbers first, in order, starting with the lowest number. Then tell me the letters in alphabetical order. For example, if I say [B-7], your answer should be [7-B]. The number goes first then the**

letter. If I say [9-C-3], then your answer should be [3-9-C], the numbers in order first, then the letters in alphabetical order.

9C/ **Let's practice.** Administer all the practice trials. Say each combination at a rate of 1 number or letter per second. Allow the examinee ample time to respond. Correct responses are in parentheses.

6-F [6-F]

G-4 [4-G]

3-W-5 [3-5-W]

T-7-L [7-L-T]

1-J-A [1-A-J]

If the examinee makes an error on any practice trials, correct the examinee and repeat the instructions as necessary. Even if the examinee fails all practice trials, continue with the subtest. Proceed to Item 1.

SDMT = Symbol-Digit Memory Task need: SDM Worksheet, stopwatch, pens

10A/ DISCONTINUE AFTER 90 SECONDS OF TASK PERFORMANCE.

10B/ Place the test form is placed before the examinee and then say these instructions:

Please look at these boxes at the top of the page. You can see that each box in the upper row has a little mark in it. Now look at the boxes in the row just underneath the marks. Each of the marks in the top row is different, and under each mark in the bottom row is a different number.

Now look at the next line (examiner points to the line of boxes) **just under the top 2 rows. Notice that the boxes on top have marks, but the boxes underneath are empty. You are to fill each empty box with the**

number that should go there according to the way they are paired in the key at the top of the page.

For example, if you look at the first mark, and then look up at the key, you will see the number 1 goes in the first empty box; so write 1 in the first empty box. Now, what number would you put in the 2nd box? [number 5]. **What number goes into the 3rd box** [number 2]. **That is the idea. You are to fill each of the empty boxes with the numbers that should in them according to the key.**

Now, for practice, fill in the rest of the boxes until you come to the double line; then stop.

10C/ The examiner checks that the examinee understands the task. Any errors made in the first 10 practice responses should be immediately pointed out to the examinee, and corrected. If the examinee *has not understood* the nature of the task, the instructions are repeated with further examples, until the nature of the task is clearly understood .

10D/ The examiner then continues, saying: **Now, when I say "Go!" write the numbers just like you have been doing as fast as you can until I say "Stop" When you come to the end of a line, go quickly to the next line without stopping and so on. If you make a mistake, do not erase, just write the correct answer over your mistake. Do not skip any boxes and work as quickly as you can. Ready? Go !**

10E/ Exactly 90 seconds from starting, the examiner says: **Stop.**

11/ DKEFS – Trails 1 thru 5

-need DKEFS Trails Condition Response Booklets:#1 = Visual Scanning;

#2 = Number Sequencing; #3 = Letter Sequencing; #4 = Number-Letter Switching;

#5 = Motor Speed; stopwatch, pens

11A/ General Instructions

Administer Condition 1 in its entirety, even if the examinee is unable to complete the practice task. For Conditions 2 -5, DISCONTINUE ANY CONDITION FOR WHICH EXAMINEE MAKES 4 ERRORS ON THE PRACTICE TASK.

11B/ Time Allowance per Condition

-Condition 1 = Visual Scanning : 150 seconds = 2min 30sec

-Condition 2 = Number Sequencing: 150 seconds

-Condition 3 = Letter Sequencing : 150 seconds

-Condition 4 = Number-Letter Switching: 240 seconds = 4 min 0 sec

-Condition 5 = Motor Speed : 150 seconds

11C/ Demonstration & Participant Instructions

All Conditions: Place Response Booklet flat on a table, facing the examinee, and hold down top or side edges with your fingers. The examinee MAY LIFT THE PEN FROM THE PAPER AT ANY POINT DURING THE PRACTICE & SCORED TASKS OF ALL 5 CONDITIONS.

11D/ Condition 1 = Visual Scanning. Give the Examinee a pen and point to the practice page and say: **Here are some numbers and letters. I want you to find all of the 3's on this page** [draw a slash through the 3 in the upper-left quadrant, from the examinee's perspective]. **Don't place marks on any of the other numbers or letters, just the 3's. Mark the 3's as quickly as you can without missing any. Go ahead.** Correct & explain any errors. After examinee has completed practice task, say: **Good, now try this one.** Open the response booklet to the 2nd & 3rd pages. Say: **Here are more numbers and letters. Like**

before, I would like you to mark all the 3's on these two pages. Mark the 3's as quickly as you can without missing any. Tell me when you are finished. Ready? Begin. Record the total time in seconds. If the examinee fails to finish the task by 150 seconds, say: **Stop. That's good.** Do not allow examinee to make any marks on the form after the time limit.

11E/ Condition 2 = Number Sequencing. Point to the Practice Page and say: **Here are some numbers and letters. This time I want you to connect *just* the numbers. Begin at number 1** [point to the 1] **and draw a line from 1 to 2** [draw this connection with your finger], **2 to 3** [trace this connection with your finger], **3 to 4** [trace this connection with your finger], **and so on, in order, until you reach the end** [point to the 5]. **Draw the lines as quickly as you can without making any mistakes. Go ahead.** If the examinee makes an error– stop him / her immediately. Write an 'X' over the incorrect connection, explain the error, and point to the correct connection. Ask the examinee to proceed from the last correct number connection.

DISCONTINUE THIS BOOKLET IF THE EXAMINER HAS TO CORRECT THE EXAMINEE 4 TIMES.

Open Condition Booklet #2 to the second & third pages. Place it flat on the table in front of the examinee's midline and say: **On this page are more numbers and letters. Just connect the numbers. Begin at number 1**[point to 1] **and draw a line from the 1 to 2** [trace this connection with your finger], **then 2 to 3** [trace this with your finger], **3 to 4** [trace this with your finger], **and so on, until you reach the end** [point to 16]. **Draw the lines as quickly as you can without making mistakes. Ready? Begin.** Start timing. If the examinee makes an error, stop him immediately. Write an 'X' over the error and *without explaining the error,* ask the examinee to proceed from the last correct number. When 150 seconds has passed say: **Stop. That's good.**

11F/ Condition 3 = Letter Sequencing. Point to the Practice Page and say: **Here are some numbers and letters. This time I want you to connect *just* the letters. Begin at A** [point to the A] **and draw a line from A to B** [draw this connection with your finger], **B to C** [trace this connection with your finger], **C to D** [trace this connection with your finger], **and so on, in order, until you reach the end** [point to the E]. **Draw the lines as quickly as you can without making any mistakes. Go ahead.** If the examinee makes an error– stop him / her immediately. Write an 'X' over the incorrect connection, explain the error, and point to the correct connection. Ask the examinee to proceed from the last correct number connection.

DISCONTINUE THIS BOOKLET IF THE EXAMINER HAS TO CORRECT THE EXAMINEE 4 TIMES DURING EITHER THE PRACTICE SET *OR* THE SCORED SET.

Open Condition Booklet #3 to the second & third pages. Place it flat in front of the examinee's midline, and say: **On this page are more numbers and letters. Just connect the letters. Begin at A** [point to A] **and draw a line from A to B** [trace this connection with your finger], **then B to C** [trace this with your finger], **C to D** [trace this with your finger], **and so on, until you reach the end** [point to P]. **Draw the lines as quickly as you can without making mistakes. Ready? Begin.** Start timing. If the examinee makes an error, stop him immediately. The stopwatch keeps running. Write an 'X' over the error and *without explaining the error,* ask the examinee to proceed from the last correct number. When 150 seconds has passed say: **Stop. That's good.**

11G/ Condition 4 = Number-Letter Switching. Point to the Practice Page and say: **This time I want you to do something different. I want you to switch between connecting the numbers and letters. Begin at number 1** [point to 1), **and draw a line from 1 to A** [trace this connection with your finger], **2 to B** [trace this connection with your finger], **B to 3** [trace this connection with your

finger], **and so on, in order, until you reach the end** [point to D]. **In other words, you will draw a line from a number to a letter to a number, and so on. Do you have any questions? Draw the lines as quickly as possible without making mistakes. Go ahead.** If the examinee makes an error– stop him / her immediately. Write an 'X' over the incorrect connection, explain the error, and point to the correct connection. Ask the examinee to proceed from the last correct connection.

DISCONTINUE THIS CONDITION IF THE EXAMINER HAS TO CORRECT THE EXAMINEE 4 TIMES .THE EITHER THE PRACTICE SET OR THE SCORED SET.

Open Condition Booklet #4 to the second & third pages. Place it flat in front of the examinee's midline, and say: **On this page are more numbers and letters. Do this the same way by *switching* between numbers and letters. Begin at 1** [point to 1] **and draw a line from 1 to A** [trace this connection with your finger], **then A to 2** [trace this with your finger], **2 to B** [trace this with your finger], **and so on, until you reach the end** [point to P]. **In other words, you will draw a line from a number to a letter to a number, and so on. Draw the lines as quickly as possible without making mistakes. Ready? Begin.** Start timing. If the examinee makes an error, stop him immediately. The stopwatch keeps running. Write an 'X' over the error and *without explaining the error*, ask the examinee to proceed from the last correct connection. When 240 seconds has passed say: **Stop. That's good.**

11H/ Condition 5 = Motor Speed. Point to the Practice Page and say: **Here is a dotted line. I want you to start at "Start"** [point to "Start"], **and draw a line over the dotted line as quickly as you can** [trace the first 3 connections with your finger]. **Keep drawing over the dotted line until you reach the end** [point to "End"]. **You don't have to draw the line neatly on the dotted line; just**

draw it as quickly as you can. Make sure your line touches every circle along the path. Do you have any questions? Go ahead.

If the examinee departs from the dotted line and makes an error or haphazard line, stop him / her immediately, explain the error and redirect the examinee to draw over the dotted line.

IF THE EXAMINEE CANNOT COMPLETE THE PRACTICE AFTER 4 CORRECTIONS, DO NOT ADMINISTER THE SCORED MOTOR SPEED CONDITION.

Open Condition Booklet #5 to the second & third pages. Place it flat in front of the examinee's midline, and say: **Good, now let's try this one. Again I would like you to draw over the dotted line as quickly as possible. Start here** [point to "Start"] **and draw a line like this** [trace over the first 3 connections with your finger] **until you reach the end** [point to "End"]. **Remember, it's more important to draw the line quickly than to make it neat. Make sure your line touches every circle along the path. Ready ? Begin.** Start timing. If the examinee departs from the dotted line or makes an error or haphazard line, stop examinee immediately, and *without explaining the error,* redirect the examinee to draw over the dotted line. Allow 150 seconds. Allow the examinee to complete any connection in progress at the time limit, then say: **Stop. That's good.** Include the just-completed connection within the time limit.

12 / DKEFS- Fluency – need DKEFS Response Booklet, stopwatch

12A / Conditions: 1 = Letter Fluency: F / A / S; 2 = Category Fluency: Animals / Boy's Names

12B / General Instructions. The time limit is 60 seconds for every fluency topic to be tested. None of the words can be the names of places, numbers, or verb

variants. Write down every word the examinee states during 60 seconds, in its appropriate 15-second interval box [1 to 15sec; 16 to 30 sec; 31 to 45sec; 46 to 60sec].If the examinee repeats a word, write it down as many times as the person states it. Write down any words the examinee says that are not words belonging to the fluency topic you are timing.

Allowed examples = words can have the same root word, but must be semantically distinct. T = toy, tooth, take, toe, toenail. S = sail, sailboat, slip, slippery.

Disallowed examples: T ≠ tooth, teeth [one is the singular, one is the plural of tooth; so you can only accept *either* tooth *or* teeth. T ≠ Texas, Toronto [these are place names]. T ≠ twelve, thirteen, twenty, twenty-one [these are numbers].

12C/ Conditions: Letter Fluency. Say to the examinee: **I'm going to say a letter of the alphabet. When I say 'begin', I want you to tell me as many words as you can that begin with that letter. You will have 60 seconds before I tell you to stop. None of the words can be names of people, places or numbers. For example, if I gave you the letter *T*, you could say *take, toy, tooth* and so forth, but you should not say *Tom* because that is a person's name; you should not say *Texas* because that is the name of a place; and you should not say *twelve* because that is a number. Also, do not give me the same word with different endings. For example, if you say *take*, you should not say *took* or *taking.* Do you have any questions?**

The first letter is *F.* Ready? Begin. Start timing. On the record form, write the examinee's responses verbatim under the *F* column. Record the responses the examinee gives in the first 15 seconds, in the first box labeled "1-15 seconds," and so forth. After 60 seconds, say: **Stop.**

The next letter is *A.* Ready? Begin. Start timing. Record examinee's responses under the *A* column. After 60 seconds, say: **Stop.**

The next letter is *S.* Ready? Begin. Start timing. Record examinee's responses under the *S* column. After 60 seconds, say: **Stop.**

12D/ Conditions : Category Fluency. Say to the examinee: **Now we are going to do something a little different. This time, I want you to tell me as many animals as you can. It doesn't matter what letter they start with. You will still have 60 seconds before I tell you to stop. Do you have any questions? Ready? Begin.** Start timing. Record examinee's responses under each 15-second interval, as in Letter Fluency. After 60 seconds, say: **Stop.**

Now, tell me as many boys' names as you can . You will have 60 seconds before I tell you to stop. Ready? Begin. Start timing. Record examinee's responses under each 15-second interval, as in Letter Fluency. After 60 seconds, say: **Stop.**