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**FACTORS AFFECTING NEUROPSYCHOLOGICAL
TESTING IN THE ELDERLY AND THE USE OF A NEWLY
DEVELOPED VIRTUAL REALITY TEST**

Implications for the accurate and early diagnosis of
Alzheimer's disease

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Abstract

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Key Words: neuropsychology, Alzheimer's disease, cognition, aging, virtual reality

Neuropsychological testing is one method used in the diagnosis of Alzheimer's disease and other cognitive disorders. However, the testing process may be affected by subtle external factors which if not controlled for may have the ability to affect the scores obtained. The primary aim of this thesis was to investigate the effects of some of these external factors, namely caffeine, non-oily fish consumption and time of day. A secondary aim was to evaluate the use of a novel virtual assessment as a possible tool for the early detection of AD. Healthy elderly participants over the age of sixty with no existing cognitive impairment or neurological condition were recruited to take part. For each external factor investigated participants were required to undertake a cognitive assessment. The results demonstrated that subtle external factors present during a typical testing session have the ability to significantly affect the scores obtained. Scores on one part of the virtual test correlated with existing tests used for the early detection of cognitive impairment and were significantly lower in participants classified as mildly impaired. With further modification this test has the potential to be used as an early detection tool. The results have implications for the interpretation of neuropsychological test scores which may be considered when classifying participants, determining treatment interventions, selecting participants for research and making a diagnosis. These findings have important considerations for psychological and cognitive research that investigates human brain function.

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List of Main Abbreviations

5HT	5- Hydroxytryptamine
Ach	Acetylcholine
AchEIs	Acetylcholinesterase inhibitors
AD	Alzheimer's disease
ADAS	Alzheimer's disease Assessment Scale
ADAS COG	Alzheimer's disease Assessment Scale Cognitive
ADI	Alzheimers Disease International
ADL	Activities of daily living
aMCI	Amnesic Mild Cognitive Impairment
APOE	Apolipoprotein E
APP	A β precursor protein
ART	Alzheimer's Research Trust
A β	Amyloid β protein
BF	Basal Forebrain
CANTAB	Cambridge Neuropsychological Automated Test Battery
CCFS	Caffeine Containing Food Stuffs
CDR	Clinical Dementia Rating Scale
CSF	Cerebral Spinal Fluid
CVLT II	California Verbal Learning Test II
DHA	Docosahexaenoic acid
DKEFS	Delis Kaplan Executive Function System
DLB	Dementia with Lewy Body
DT	Dual Tasks
DTR	Dual Task Ratio
DT T1-T2	Dual Tasks Time 1 - Time 2
EC	Entorhinal cortex

EF	Executive Function
EPA	Eicosapentaenoic acid
FDG	2-[18F] fluoro-2-deoxy-D-glucose
FFQ	Food Frequency Questionnaire
FTD	Frontotemporal Dementia
GDS	Global Deterioration Scale
GLM	General Linear Model
GNT	Graded Naming Test
GP	General Practitioner
GPCOG	General Practitioner Assessment of Cognition
HCy	Homocysteine
HE	Healthy elderly
HRT	Hormone Replacement Therapy
HVLT	Hopkins Verbal Learning Test
HVLT - R	Hopkins Verbal Learning Test - Revised
LCS	Letter Comparison Speed
LTD	Long term depression
LTP	Long term potentiation
MANCOVA	Multivariate Analysis of Covariance
MANOVA	Multivariate Analysis of Variance
MCI	Mild Cognitive Impairment
MEQ	Morningness-Eveningness Questionnaire
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe
MWT	Morris Water Task
n-3 PUFAS	Omega-3 Polyunsaturated fatty acids

naMCI	Non-amnestic mild cognitive impairment
nbM	Nucleus basalis of Meynert
NFTs	Neurofibrillary tangles
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMDA	N-methyl- D -aspartate
NSAIDs	Non-Steroidal Anti Inflammatory Drugs
PAL	Paired Associates Learning task
PCS	Pattern Comparison Speed
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PiB	C-Pittsburgh compound B
PSEN1	Presenilin 1
PSEN2	Presenilin 2
ROS	Reactive Oxygen Species
SCN	Suprachiasmatic Nuclei
SDMT	Symbol Digit Modalities Test
SSRIs	Selective serotonin reuptake inhibitors
TBI	Traumatic brain injury
TD	Topographical Disorientation
TMT	Trail Making Test
TOD	Time of Day
TPT	The Placing Test
VR	Virtual Reality
VREAD	Virtual Reality of Early Detection of Dementia
VVLT-D	Visual Verbal Learning Test with auditory distraction

Chapter 1

Thesis overview

1.1 Introduction and aims

Neuropsychological testing is a research tool used to measure cognitive ability, detect cognitive impairments and diagnose neurological dysfunction (Chapman et al., 2010). Numerous cognitive assessments are available which differ in the domain they assess, the age range they are used for, the level of difficulty and administration time. Research has shown that performance on some assessments can be affected by demographic variables such as the participant's age and education level (Fields et al., 2011; Jacova et al., 2007; Zamrini et al., 2004; Dubois et al., 2007). There are a number of factors, albeit subtle, which still need to be investigated in order to determine their effects on neuropsychological test performance. This is important if an accurate score is to be obtained. These subtle factors, which are prevalent during a typical neuropsychological testing session, may affect performance, skew results and have significant consequences for the interpretation of data.

This thesis explores some of these factors, caffeine and time of day and also investigates the effects of a dietary factor, fish consumption, to determine any influence of this on neuropsychological performance in the healthy elderly. The findings are discussed in the context of Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI). Although these factors were investigated in the healthy elderly, and established AD patients were not recruited, the results obtained may have implications and considerations for the early diagnosis of AD. The thesis also evaluates a newly developed virtual reality based assessment of spatial route learning. The test may have the potential to be used as a tool for the early detection of cognitive impairment in the future.

Each experimental chapter describes the factor investigated and includes an introduction, methods section (including details of materials and procedure), results and discussion. The general discussion chapter then summarises the

main empirical findings, ideas for future work and the implications for the fields of experimental psychology and cognitive neuroscience.

1.2 Overview of thesis structure

The thesis is comprised of nine chapters and the following section gives a brief overview of each:

Chapter two gives an in depth overview of AD and MCI and specifically includes information on the incidence, diagnosis, symptoms, neuropathology, neurochemistry, treatments and prognosis.

Chapter three discusses early diagnosis of AD and why there is a prominent focus by research teams to try to detect and initiate treatment earlier in the disease's trajectory. The chapter evaluates the positives and negatives to this approach, the tools available to researchers and clinicians to implement this and also discusses the available treatment options and lifestyle changes which could be suggested if an earlier diagnosis was to occur. This chapter also evaluates the effects an early diagnosis may have on the individual and their families.

Chapter four discusses neuropsychological testing. Neuropsychological assessments which will form the cognitive batteries delivered to participants in this thesis are discussed including the cognitive domain the test assesses and a description of how the test is administered and scored.

Chapter five is the first experimental chapter which investigates the effect of prior caffeine consumption on neuropsychological testing. Caffeine is famously consumed by the general population (Nawrot et al., 2003; Carrier et al., 2009) and is likely to be prevalent in a person during a usual testing session.

Chapter six, the second experimental chapter, investigates a dietary factor, monthly non-oily fish consumption, in order to investigate any influence of non-oily fish on neuropsychological test performance in the elderly via the use of a four week food diary and a cognitive assessment battery.

Chapter seven, the third experimental chapter, investigates the effect of Time of Day (TOD), another factor often uncontrolled for in a typical testing session. The chapter also investigates whether the administration of caffeine, which is commonly used to prevent feelings of tiredness (Carrier et al., 2009), influences TOD effects on cognitive test scores in the elderly.

Chapter eight evaluates a newly designed virtual reality based assessment of spatial route learning created in collaboration with the University of Bradford School of Computing, Informatics and Media as a possible tool for the early detection of cognitive impairment associated with AD and MCI.

Chapter nine is the discussion chapter which summarises the main findings of the thesis and the implications these have for the field of cognitive neuropsychology and other disciplines. The chapter also provides details of possible further work before concluding the thesis.

Chapter 2

Alzheimer's disease and Mild Cognitive Impairment

2.1 Introduction

The purpose of this chapter is to introduce the reader to Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI). Knowledge of these two conditions is necessary as the thesis progresses as findings are discussed in the context of AD and MCI. The chapter begins with some background information on AD including the symptomology, diagnosis, key neurological and pathological features, risk factors and treatments. The second half of the chapter discusses MCI which is considered to be a prodromal stage of AD (Markwick et al., 2012).

2.2 Background and history

AD was first described in 1906 by Alois Alzheimer (Meinert et al., 2009) and is a chronic, complex neurodegenerative disease (Brousseau et al., 2007). The exact cause of the disease is unknown but it is believed to be due to a combination of genetic and environmental factors (Cole et al., 2009; Cummings and Cole, 2002). AD is currently the most common form of dementia and affects 25% of those over the age of 85 (Carrera et al., 2012). In 2010 the Alzheimer's World Report specified that there were an estimated 36 million people worldwide suffering from the disease. This figure is expected to double within the next twenty years placing a larger responsibility on the global economy and health care resources.

It is sometimes difficult to discriminate between normal aging and pathological abnormalities such as AD (Hedden and Gabrieli, 2004; Petersen, 2011; Albert et al., 2011; Forlenza et al., 2010; Petersen, 2009). This remains a pressing issue as disease diagnosis often occurs at too late a stage when structural damage to the brain has already reached too great an extent for interventions and treatments to be maximally beneficial (Pantel et al., 2004). There is an

urgent need to try to detect the disease as early as possible before initial signs of cognitive symptoms present. This is discussed in detail in chapter three, 'Early Diagnosis'.

AD has been categorised into two main types, early onset AD and late onset (or sporadic) AD (Alzheimer's Association, 2013). Early onset cases usually show symptomatic presentation between the ages of 30-60 years and account for approximately 1-6% of all AD cases (Bekris et al., 2010). Sixty percent of early onset patients have a strong familial history of the disease (Bird, 2008) and certain genes have been implicated in the disease's heritability. Late onset AD accounts for 90% of all AD cases. Although late onset AD can show some evidence of a family history, the only significant gene implicated in this form of the disease so far is the Apolipoprotein gene (APOE) (Roses et al., 1995; Albert et al., 2011). The genetic implications for both sporadic and early onset AD types are discussed in section 2.8.2 'Genetics'.

2.3 Symptoms and prognosis

The primary symptom of AD is memory loss which becomes gradually worse over time (Bird, 2008). The degree of memory impairment is exaggerated relative to normal age related loss which is common in the elderly and can complicate the diagnosis if the patient has always been forgetful. Other symptoms such as confusion, getting lost and personality change vary between individual patients, however, the disease is progressive with an amnesic core (Dubois et al., 2007). An initial episodic memory impairment is present in 86-94% of patients (Dubois et al., 2007). Symptoms gradually worsen over time and a diagnosis at the time of symptomatic onset is sub optimal as underlying pathological changes occur decades beforehand (Forlenza et al., 2010).

Warning signs of early AD, as recommended by the Alzheimer's Association annual report (2013) can include the following:

- Memory loss which begins to disrupt normal everyday life
- Difficulties completing familiar tasks
- Confusion, often with time and place, for example, not knowing what day of the week it is, or forgetting how to return home from the local town

- Decreased spatial awareness or wandering behaviour
- The misplacement of objects and belongings
- Poor judgement, for example, placing trust in strangers
- Difficulty planning or organising simple tasks
- Changes in mood or personality
- Withdrawal from social activities or work

As the disease is progressive patients are usually bracketed into mild, moderate or severe AD (Alzheimer's Association, 2012). The rate of decline is dependent on a multitude of other factors such as family history, presence of co-morbid conditions, particularly stroke and other vascular diseases (Mielke et al., 2011), age at time of diagnosis (Rountree et al., 2013), APOE status (Mielke et al., 2011) and many more (Alzheimer's Association, 2013). Risk factors for AD are discussed in more detail in section 2.8.1 'Risk Factors'. Patients with advanced AD require help with basic activities of daily living (ADL) such as bathing, clothing and eating. In the end stages of the disease the patient often loses mobility, the ability to communicate and fails to recognise their close relatives. Once patients are bed bound for long periods they become susceptible to other infections such as pneumonia. These secondary illnesses are often responsible for cause of death (Ganguli et al., 2005). Disease duration is approximately 7-10 years from initial diagnosis to death (Bekris et al., 2010) and is dependent on factors such as those mentioned above.

2.4 Diagnosis

A true diagnosis of AD cannot be given until a post mortem examination can confirm the presence of specific pathological markers representative of the disease (Mosconi et al., 2008). The National Institute for Health and Clinical Excellence (NICE) has a recommended diagnostic procedure for probable AD in the UK (NICE, 2006a). These guidelines were reviewed in 2011 and state that the following should be undertaken with the General Practitioner (GP) who is usually the first point of call for most patients (Petersen, 2009):

- A comprehensive patient history
- A cognitive and mental examination

- Physical examination and blood analysis
- Review of current medication

The patient's history should include a check of other health complaints noting the presence of vascular disease or associated conditions such as stroke, diabetes or hypertension (Feldman et al., 2008). Cardiovascular diseases increase the likelihood of developing AD (Tong et al., 2012). A psychiatric history or the diagnosis of any other medical conditions which may affect mental state is also needed (Overshott and Burns, 2004). Obtaining an accurate family history can determine whether any immediate relatives possess a history of dementia or AD. Having a first degree relative with AD increases the individual's own risk (Green et al., 2002). Green et al., (2002) showed that African Americans were 1.6 times more likely to develop a dementia if they have a first degree relative with the disease, compared to white Caucasians with a first degree relative. This has implications for considering the patient's race (Green et al., 2002).

The cognitive assessment examines the patient's current cognitive ability and should be carried out using one of the recommended brief cognitive tests such as the Mini Mental State Examination (MMSE), the 7 Minute Screen, the General Practitioner Assessment of Cognition (GPCOG) or the Clinical Dementia Rating Scale (CDR) (NICE, 2011). Scores on these tests can determine whether the patient receives pharmacological intervention (Jacova et al., 2007). The patient's language and educational ability should be considered when scoring these assessments as lower educational level has been identified as a possible risk factor for the development of the disease (Revet et al., 2013; Reitz et al., 2011; Petersen, 2009). The accuracy and reliability of neuropsychological assessments can be affected by many other external factors and this thesis explores some of these in later chapters.

Although memory loss is a normal part of the aging process, subjective memory complaints in the elderly are associated with a higher risk of AD development (Waldorff et al., 2012; Geerlings et al., 1999). The confirmation of a change in cognition by an informant such as a spouse or relative also adds reliability to the diagnosis as their opinion is more objective (Dubois et al., 2007).

Routine blood analysis is useful for the exclusion of abnormalities which are associated with cognitive impairment such as alterations in thyroid hormones (Bird, 2008, Hogervorst et al., 2008) and B vitamins (Andreeva et al., 2011). The patient's current medication should also be checked as well as any symptoms of depression, psychosis, urinary tract infection or syphilis. These may cause the appearance of AD like symptoms, such as confusion or altered cognition (Overshott and Burns, 2004).

Within the past decade there has been a considerable increase in the amount of research investigating possible biomarkers or sensitive techniques to increase the reliability of AD detection and improve the identification of early disease of the disease (see chapter three, 'Early Diagnosis'). Advances in neuro imaging have been promising, however, routine Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) scanning are not used for general diagnosis (NICE, 2006b) but may be recommended to rule out other cerebral pathologies or subtyping (NICE, 2006b). Atrophy of the Medial Temporal Lobe (MTL) as seen on structural MRI scans is common in AD but less frequent in normal aging and, therefore, can be used as a possible identifier in questionable cases (Dubois et al., 2007). Cerebrospinal Fluid (CSF) collection and profile examination is not recommended by NICE for routine diagnosis (NICE, 2011) but abnormalities in CSF amyloid and tau levels are reported in AD cases (Okonkwo et al., 2011). Low levels of amyloid protein 1-42 and high total tau concentrations in the CSF are indicative of an AD profile (Shaw et al., 2009; Petersen et al., 2009; Hansson et al., 2006; Forlenza et al., 2010; Okonkwo et al., 2011). Although the procedure is invasive the results offer reliable indicators of plaque and tau burden (Petersen et al., 2009).

2.5 Pathology

The two main pathological hallmarks of the disease are the presence of extracellular senile plaques composed of amyloid β protein ($A\beta$) and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein (Forlenza et al., 2010). Early studies hypothesised that the presence of these markers alone were sufficient for a diagnosis of AD. However, the development

of improved imaging techniques and other scientific approaches have since shown other abnormalities and changes in neuronal processes.

2.5.1 Senile plaques

The amyloid hypothesis of AD maintains that A β , which is cleaved from the A β precursor protein (APP), plays an initial crucial role in activating the complex cascade of events which leads to the neurodegeneration seen in AD (Pimplikar et al., 2010). A β is a neuro peptide composed of between 39-43 amino acids (Butterfield and Boyd-Kimball, 2004) and contains a β pleated sheet (Revett et al., 2013). The protein is derived by cleavage from APP which is a multifunctional protein involved in cell adhesion and neuritic outgrowth (O'Brien and Wong, 2011). APP is cleaved by two forms of secretase enzymes, the β and γ varieties, to form predominantly A β 40 and A β 42 (Findeis, 2007). Fibrils of the protein aggregate together to form senile plaques, often termed neuritic plaques, which locate in the extracellular space disrupting neuronal communication (Forlenza et al., 2010). Figure one shows a histological slide from an AD patient demonstrating the presence of A β plaques.

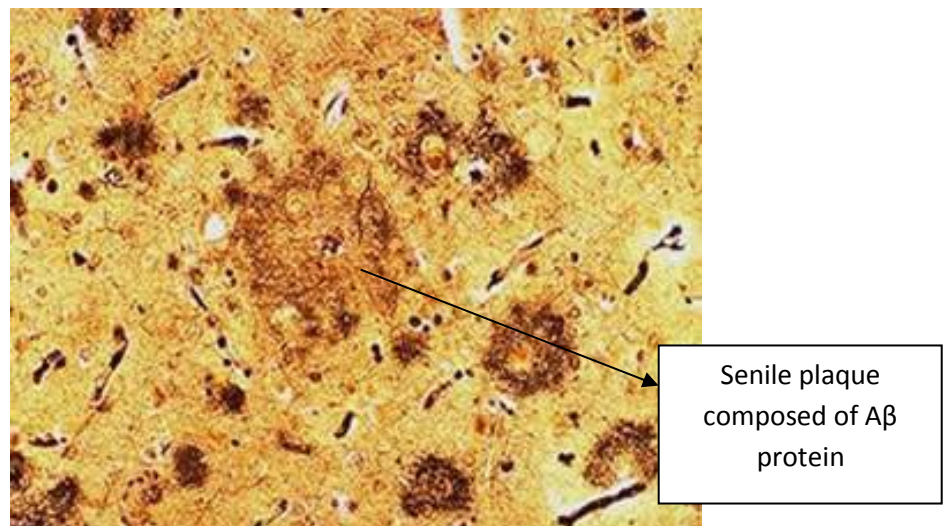


Figure one: histological presence of A β plaques in an AD patient. Figure taken from <http://www.liv.ac.uk/researchintelligence/issue30/alzheimers.html>.

The presence of elevated plasma A β 42 has been linked to an increased risk of developing AD but levels of this longer form of amyloid do naturally increase during aging (Fukumoto et al., 2003). The ratio of the two isoforms is believed to be a more reliable indicator of AD risk (Fukumoto et al., 2003). An increased ratio of the two, (favouring A β 42) predicts an increased risk of AD whereas a lower ratio i.e. a shift towards shorter lengths (A β 40), decreases toxicity (Findeis, 2007). In early onset AD mutations in the gene encoding APP and presenilin 1 and 2 have been discovered (Bird, 2008) and are discussed further in section 2.8.2 'Genetics'.

Initial cell based studies demonstrated that the application of A β to cell media for 24 hours caused massive cell loss, presumably due to the oxidative effects of the protein (O'Brien and Wong, 2011). Multiple studies have now investigated how A β plaques may lead to degeneration and these have noted numerous negative effects on surrounding neurons. This has included synaptic loss (Lacor, 2007), decreases in dendritic spine density (Wilcox et al., 2011), increased oxidative stress (Axelsen et al., 2011), initiation of the inflammatory response (Herrup, 2010), impaired glutamate signalling (Revett et al., 2013), calcium influx (Butterfield and Boyd-Kimball, 2004) and the impediment of long term potentiation (LTP) (Lambert et al., 1998).

As AD patients have elevated extracellular A β levels (Karran et al., 2011), reducing the amyloid burden was predicted to improve the cognitive deficits seen in the disease (Hardy and Higgins, 1992). Since this hypothesis was put forward there has been much research investigating the precise role of the protein in cognitive dysfunction. This has concluded that plaque load does not seem to correlate with cognition (Karran et al., 2011). Further, Gómez-Isla et al., (1996) reported that cognitively normal elderly participants also possess heavy plaque loads, thereby complicating the A β hypothesis.

C-Pittsburgh compound B (PiB) is a radio ligand and tracer which is able to bind to amyloid plaques in vivo (Jack et al., 2008). Studies have consistently reported increased retention of this compound in AD patients and those people with a MCI (Reitz et al., 2011). AD patients have a two fold increase in PiB retention relative to non demented controls of similar ages (Klunk et al., 2004). Areas showing greater retention include the prefrontal cortex (PFC), lateral

temporoparietal cortex, posterior cingulate and the striatum (Edison et al., 2007; Klunk et al., 2004; Forsberg et al., 2007). Those AD patients with greater retention show elevated rates of atrophy (Archer et al., 2006). Although the majority of AD cases possess elevated retention, approximately 30% of the cognitively normal elderly also show retention in cortical regions (Mintun et al., 2006). Consequently, the use of this technique as a possible biomarker for AD does not seem reliable when used alone but could add validity to confirm questionable cases. Petersen et al., (2009) comment that PiB is a useful method for identifying participants who are likely to decline at a rapid rate (Petersen et al., 2009).

Immunisation against A β has also been trialled. In 2010 over 9000 patients were enrolled in such trials (Lemere and Masliah, 2010). Active A β immunisation involves the direct administration of a synthetic version of A β combined with a carrier protein, which when injected, results in the production of antibodies to A β . Passive immunisation uses the direct injection of actual A β antibodies (Fu et al., 2010) and both techniques result in the removal of toxic amyloid from the extracellular space. Trials using animal models of the disease have shown promising results. For example, an early study by Schenk et al., (1999) administered active immunisation with A β 1-42 to PDAPP transgenic mice. These mice express elevated human APP and develop many of the pathological features seen in human AD. Immunisation of young mice (6 weeks old) before pathological abnormalities were present, prevented the formation of A β plaques, and immunisation of older animals (aged 11 months) decreased the rate of progression of typical AD pathology. Active immunisation of Tg₂₅₇₆ mice, which are also genetically modified to develop A β plaques, was less effective in its ability to clear existing plaques in aged mice (Das et al., 2001). Further, 3xTg mice which develop both A β plaques and NFTs showed no reduction of either pathological hallmark after immunisation. The study did report an improvement in animal behavioural measures (Oddo et al., 2006). Similar positive findings have been noted in passive trials (Lemere and Masliah, 2010).

Human clinical trials have also taken place and an initial phase one trial of immunisation using the AN1792 vaccine designed by Elan and Wyeth (Lemere

and Masliah, 2010) showed positive outcomes on one behavioural assessment, the Disability Assessment for Dementia (DAD) rating scale (Bayer et al., 2005). The phase two trial was halted due to the development of meningoencephalitis in 18 of the 300 patients enrolled. Although this was not attributed to the immunisation itself, the activation of cytotoxic T cell immune responses may have lead to an immune reaction in these patients (Fu et al., 2010). The most positive results emerge when animals were immunised prior to the development of AD pathology, implicating immunisation is better as an AD prevention method rather than a potential treatment. Work in this area is ongoing.

2.5.2 Tau

Tau is a neuronal protein encoded on chromosome 17 (Iqbal et al., 2010) and exists in six different isoforms which vary in the number of amino acids they comprise (Wang and Liu, 2008). In the normal brain, tau is responsible for the assembly of neuronal microtubules and the maintenance of their stability (Matus, 1988). In AD tau becomes hyperphosphorylated (Revett et al., 2013; Reitz et al., 2011; Boutajangout et al., 2011) forming paired helical filaments (Iqbal et al., 2010) which impair the proteins ability to bind to and assemble microtubules within the axon and dendrites (Iqbal et al., 2005). Hyperphosphorylation promotes the aggregation of tau into intracellular NFTs (Boutajangout et al., 2011). NFTs consist of twisted ribbons of tau which clump together and form within the neuron (Blackwell et al., 2004) and can be seen in figure two.

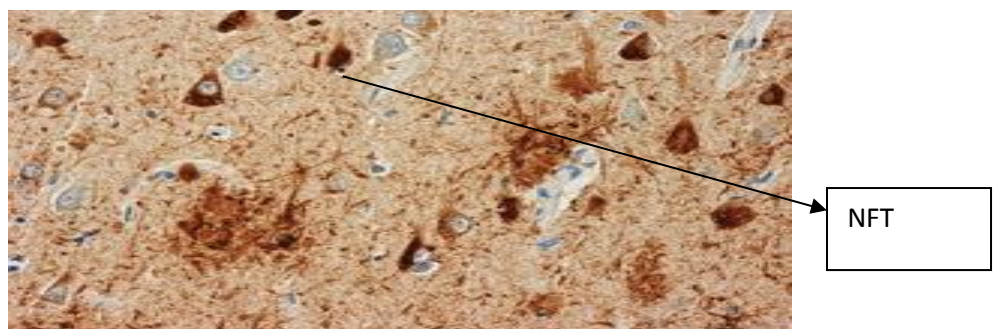


Figure two: histological slide showing the presence of NFTs taken from Callahan et al., (1998)

NFTs are first apparent in the transentorhinal cortex before spreading to the hippocampus proper and association cortices (Braak and Braak, 1991). Studies of AD brains have shown an elevation in the amount of total tau in comparison with normal aged matched controls (Khatoun et al., 1992). The isoform 4 variety of tau is the predominant form detected, although it has been suggested that it may be the ratio of the different isoforms which predicts the level of toxicity induced (Wang and Liu, 2008). As tangles become more widespread, synaptic transmission between neurons becomes impaired. Tangles block the intracellular trafficking of various other intracellular proteins which can lead to the loss of axonal transport and ultimately cell degeneration (Iqbal et al., 2010).

Cognitive decline has been correlated with the number of NFTs (Braak and Braak, 1998; Selkoe and Schenk, 2003) as well as the concentration of total tau in CSF samples, in particular samples from patients who present with mild degrees of cognitive impairment (Arriagada et al., 1992). This suggests that the formation of tau pathology occurs early in the disease process, making the measurement of tau a target for the earlier detection of AD.

Although NFTs can lead to neurodegeneration there has been speculation as to whether they may actually be the product of a protective response. Morsch et al., (1999) presented findings that neurons containing NFTs were able to survive for up to 20 years, implying that the presence of abnormal tau alone does not lead to immediate cell death. In addition, Wang and Liu (2008) believe that NFTs may actually protect neurons and neighbouring cells from oxidative stress. There is decreased oxidative damage in areas with elevated NFTs signifying that they may be a compensatory neuronal response aimed at reducing damage from Reactive Oxygen Species (ROS).

2.6 Anatomy and key neurological processes

2.6.1 Atrophy

Post mortem examinations of AD patients and the use of neuro-imaging have consistently shown generalized atrophy, correlated with neuronal loss, in the temporal, frontal, parietal and hippocampal areas (Whitwell et al., 2007; Hämäläinen et al., 2007; Frisoni et al., 2010). This deterioration is particularly

extensive in the cortex, hippocampus, amygdala, locus coereleus and raphe nuclei (Frisoni et al., 2010; Reid and Evans, 2013) and can be seen in figure three.

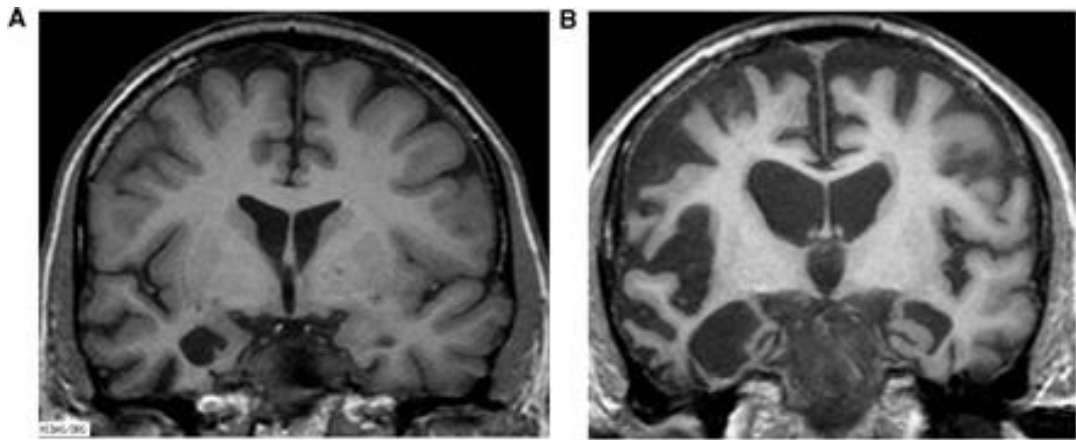


Figure three: temporal lobe atrophy seen in dementia. (A) mild atrophy and (B) severe atrophy. Images have been taken from Chan et al., (2009) in their paper entitled 'The Clinical Profile of right temporal lobe atrophy'.

2.6.2 Neuronal and synaptic alterations

Loss of synaptic structure and function are important in the advancement of AD (Herrup, 2010). Decreased dendritic mass and spine density is present in post mortem studies of AD patients (Uylings and de Brabander, 2002) and these are noted early in the disease process (Yu and Lu, 2012). Synaptic loss is closely correlated with the degree of cognitive impairment the patient presents with (Spires et al., 2005). Loss of synaptic markers is considered a reliable indicator of disease progression and correlates well with clinical symptoms (Selkoe, 2002). There is also evidence of alterations in synaptic processes such as LTP and long term depression (LTD) which are important for learning and memory (Malenka and Bear, 2004). The cause of synapse loss is thought to be due to a combination of factors such as the presence of NFTs, A β , inflammation or oxidative stress (Spires et al., 2005).

2.6.3 Glucose metabolism

The brain uses adenosine tri phosphate (ATP) as its major energy resource (Mosconi et al., 2008). ATP is formed from the mitochondrial oxidation of glucose and a disruption to this process can lead to synaptic dysfunction.

Glucose metabolism, therefore, would appear to be a reliable indicator of neuronal activity or lack thereof (Mosconi et al., 2008). Altered glucose metabolism occurs early in AD and correlates well with clinical manifestations of the disease (De Leon et al., 2001). Disruption to the regulation of these metabolic systems can result in the elevated production of toxic ROS and changes in calcium transport. This leads to over excitability and local tissue damage (Mosconi et al., 2008).

AD is associated with high levels of oxidative stress and early research showed that this too correlates with neuropathological markers of the disease such as tangles and plaques (Lambert et al., 1998). Imaging studies using PET and the glucose tracer, 2-[18F] fluoro-2-deoxy-D-glucose (FDG) have revealed specific hypometabolism in AD in the parieto-temporal regions, posterior cingulate cortices and the frontal lobes (Mosconi et al., 2008). This consistent pattern across research groups has meant that hypometabolism appears to be a reliable indicator of AD (Frisch et al., 2013; Prestia et al., 2013; Minoshima et al., 1997).

2.6.4 Oxidative stress

Oxidative stress refers to the unnecessary oxidation of molecules leading to cellular damage by ROS (Behl and Moosmann, 2002). Neuronal systems are incredibly sensitive to oxidative damage and there are high levels of oxidative stress present in the AD brain (Axelsen et al., 2011). Furthermore, studies have shown low levels of natural antioxidants, such as vitamin A and E in AD patients (Axelsen et al., 2011). The presence of elevated deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), lipids and protein oxidation has been found in both AD patients and individuals with a MCI (Baldeiras et al., 2010). This indicates that oxidative stress occurs early in the disease process (Nunomura et al., 2001). ROS disrupt neuronal processes and induce apoptosis exacerbating the atrophy and neuronal death occurring in AD (Nunomura et al., 2007).

Oxidative stress has been linked to the presence of A β plaques with some discrepancies as to whether A β induces oxidative stress or actually protects against it. Some studies proposed that A β plaques were formed as a protective

response against oxidation (Atwood et al., 2003; Berthon, 2000) as the density of plaques was inversely correlated with markers of oxidative stress (Cuajungco et al., 2000). The majority of research seems to favour the opposite theory and has found that A β plaques actually induce oxidative stress in AD brains (Butterfield and Boyd-Kimball, 2004; Markesbery, 1999). Furthermore, some studies have reported that oxidative stress is present prior to plaque (Pratico et al., 2001) and tangle formation (Resende et al., 2008) and may initiate the production of these markers.

Antioxidants act to inhibit free radical formation, scavenge free ROS and support natural self defence mechanisms (Behl and Moosmann, 2002). Research has investigated whether natural antioxidants such as co-enzyme q10, acetyl carnitine, vitamin E, curcumin and polyphenols are able to minimise the oxidative stress in AD (Feng and Wang, 2012). Most studies investigating the use of the aforementioned antioxidants in animals have shown beneficial effects although results from human trials have been less positive and further research is required (Feng and Wang, 2012).

2.6.5 Inflammation

Neuro-inflammation is present in AD brains and can be confirmed by the presence of elevated levels of activated complement proteins, cytokines, chemokines, free radicals and arachidonic acid (Wilcock, 2012). Inflammatory processes are balanced via negative feedback mechanisms and these can alter during aging and neurodegeneration (Glass et al., 2010). The presence of NFTs and senile plaques has been shown to induce inflammation and activate microglia which exacerbates the problem leading to further localised tissue damage (Pimplikar et al., 2010). The incidence of AD seems to be lower in individuals using Non-Steroidal Anti Inflammatory Drugs (NSAIDs) (Breitner et al., 2009; Herrup, 2010), however, there have been conflicting findings. For example, a meta-analysis by Szekely et al., (2004) showed that individuals taking NSAIDs had a 40% lower incidence of developing AD, whereas Brietner et al., (2009) in their sample of 351 participants, concluded that those participants who were heavy NSAID users were at an elevated risk of dementia. The reasons for these discrepancies are unclear and further work investigating

the length of time patients use NSAIDs, the dose taken, the condition treated and the age of onset medications are initiated is needed. These factors may affect the overall outcomes.

The use of NSAIDs as a possible treatment for established AD patients has yielded inconclusive findings and in general no significant improvements have been noted (Brietner, 2003). This may be due to the fact that inflammation over many years causes too many detrimental effects to the surrounding neuronal tissue which are unable to be reversed by acute NSAID administration. The exact mechanisms of inflammation in AD are beyond the scope of this chapter but it is important to acknowledge that inflammatory processes do seem to contribute to the pathological and anatomical features of AD.

2.7 Neurochemistry

2.7.1 Cholinergic hypothesis

Bartus et al., (1982) put forward the cholinergic hypothesis of memory dysfunction. This stated that the cognitive impairments seen in AD and other dementias were due to disruptions in the activity of the neurotransmitter acetylcholine (Ach). This hypothesis was reinforced by the fact that drugs which block acetylcholinesterase, the enzyme responsible for the breakdown of Ach, improved cognitive performance (Hansen et al., 2008). Likewise, Ach antagonists such as scopolamine interfere with cognitive performance on memory tasks (Auld et al., 2002).

The basal forebrain cholinergic system consists of the nucleus basalis of Meynert (nbM), the horizontal and vertical bands of Broca and the medial septal nucleus (Auld et al, 2002). These structures can be seen in figure four. This system maintains cholinergic input to the limbic and cortical brain structures and densely innervates the hippocampus and its association areas (Brousseau et al., 2007). In AD there is a significant reduction of neurons in the nbM (Whitehouse et al., 1982) accompanied by dense tau deposition (Mesulam et al., 2004). Further, cell loss in the nbM correlates with dementia severity (Perry et al., 1977; Baskin et al., 1999; Grothe et al., 2010; Pappas et al., 2000).

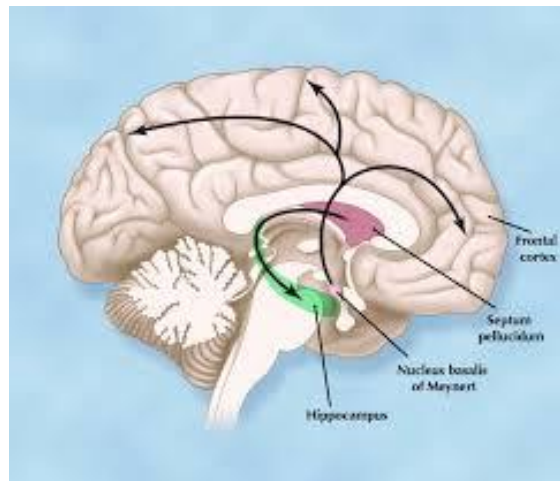


Figure four: projections from the nucleus basalis of Meynert and other cholinergic cell groups in the septum pellucidum to the hippocampus and neocortex. Figure taken from Gauthier (2002).

Early studies such as Bierer et al., (1995) administered cholinergic agonists to both healthy human subjects and rats and found a marked improvement in information processing, learning and memory following drug administration. This was further supported by additional studies such as Levin, (1992) and Nathan and Stough, (2001). Conflicting findings were put forward by Oken et al., (1994). These authors used cholinergic antagonists and found that no attention or memory impairment was apparent in healthy adults and AD patients. Many of these studies recruited healthy participants in order to prove or disprove the cholinergic hypothesis. Increasing cholinergic transmission in individuals who already possess a normal baseline Ach concentration may simply lead to a threshold whereby further stimulation has no additional beneficial effect. Also those authors who did use AD patients when administering cholinergic agonists do not state the stage of the patients' disease and it may be that these patients show no improvement due to advanced pathological changes. There may be a threshold/cut off point in the disease pathway whereby the drugs are only effective up until a certain point.

The drugs used to treat AD, namely the acetylcholinesterase inhibitors (AChEIs), only have modest effects and this has led to the questioning of the cholinergic hypothesis (Dumas and Newhouse, 2011). Enhancing cholinergic transmission may improve the cognitive symptoms of the disease but it will not slow the disease's progression. It has, therefore, been proposed that the cholinergic system may be responsible for the modulation of attention rather

than memory and that other factors and neurotransmitter systems may be involved in the cognitive symptoms of AD (Voytko, 1996; Dumas and Newhouse, 2011; Sarter et al., 2005; Herholz et al., 2008).

2.7.2 Other neurotransmitter systems

There is evidence for a role of serotonin (5-Hydroxytryptamine (5HT)) in the behavioural and cognitive symptoms of AD (Francis et al., 2010). Post mortem studies have reported decreased levels of 5HT and its metabolites (Garcia-Alloza et al., 2005), similarly NFTs and neuronal loss are present in the raphe nucleus, the major serotonergic hub, early in the disease (Francis et al., 2010). Specific loss of the 5HT_{1A} receptor is found in temporal areas and this has been associated with the displays of aggressive symptoms seen in certain patients (Lai et al., 2003). Interestingly, 5HT_{1A} loss in the hippocampus correlated with cognitive decline (Kepe et al., 2006). The serotonergic system appears to be more central to the neuropsychiatric manifestations of the disease. Selective serotonin reuptake inhibitors (SSRIs), which are used for the treatment of depression, have shown some efficacy in treating AD patients with depressive symptoms, noting positive effects on behaviour and cognition (Taragano et al., 1997).

In addition to the 5HT system, the glutaminergic system has also been implicated in AD. There is a decrease in glutaminergic pyramidal neurons in AD (Francis et al., 1993). The hippocampi of AD patients contain lower concentrations of glutamate receptors (Francis, 2003). There is also a decrease in the ability of glial cells to remove excess glutamate from the synaptic cleft leading to over excitability and toxicity (Procter et al., 1988). Inadequate removal of glutamate from the synaptic cleft leads to 'background noise' at glutamate receptors which then affects the functioning of other receptors. This may contribute to certain aspects of cognitive impairment seen in AD (Francis et al., 2010). Memantine, a glutamate receptor antagonist, has proved useful in the management of AD cognitive symptoms (see section 2.9 'Treatment').

2.8 Risk Factors and genetics

2.8.1 Common risk factors

A number of risk factors have been identified for the development of AD and some of the main factors are outlined in table one. These risk factors have received a considerable amount of research interest but there are many more. The consensus so far is that AD is caused by a combination of genetic and environmental factors (Cole et al., 2009, Cummings and Cole, 2002; Bennett et al., 2005; Boutajangout et al., 2011).

Table one: risk factors associated with AD

Risk Factor	Evidence
Age	AD risk increases with age due to a cumulative effect of aging (Rountree et al., 2013).
Gender	Females have a higher risk of developing AD (Hogervorst et al., 2000). This may be due to increased female life expectancy, but may also be a result of decreased oestrogen with age (Markou et al., 2005).
MCI	Those individuals who possess a MCI have an elevated risk of developing AD (Alzheimer's Association, 2013; Petersen, 2009).
Family history	Individuals with a first degree relative who has AD have an increased risk of developing the disease (Green et al., 2002; Mayeux et al., 1991).
APOE gene allele	This gene is implicated in heart disease and also has a high predictability in the development of AD, specifically if the individual possesses the APOE4 allele (Prasanthi et al., 2010). The APOE2 allele seems to be protective against AD (Jorm et al., 2007).
Low levels of physical activity	Individuals with low levels of physical activity have an increased risk of developing AD (Scarmeas et al., 2009).
Cardiovascular disease and high cholesterol	Heart disease, high blood pressure and elevated cholesterol all increase the risk of developing AD (Rosenberg et al., 2008).

Chronic inflammation	Inflammatory markers are present in the AD patient and can induce tissue damage leading to increased neuronal apoptosis and degeneration (Meinert et al., 2009).
Low levels of education	The longer an individual spends being educated the lower the risk of developing AD, this is known as cognitive reserve (Stern, 2012).
Poor diet and obesity	A diet high in saturated fat increases A β load and plaque formation, thereby increasing AD risk (Englehart et al., 2004). The Mediterranean diet which is high in olive oil, fish and low in red meat has shown to be a deterrent against AD (Scarmeas et al., 2009). Increased Body Mass Index (BMI) and being overweight in midlife at approximately age 50 years, increases the risk of developing AD in later life (Kivipelto et al., 2005)
Elevated homocysteine (HCy)	Elevated HCy is a risk factor for the development of AD (Aisen et al., 2008; Smith et al., 2010). HCy is a toxic amino acid and can induce apoptosis and synapse loss. Those individuals with elevated HCy concentrations have a higher risk of developing AD (Lehmann et al., 1999). HCy levels are linked to B vitamins, low B12 levels are associated with elevated HCy. The administration of can decrease levels of HCy (Cunnane et al., 2009) and slow cognitive decline (de Jager et al., 2012).
Head injury	Some studies have reported that a head trauma or traumatic brain injury (TBI) increases the risk of developing AD (Jellinger et al., 2001). The exact reasons behind this are still poorly understood, one study found that TBI initiates the formation of NFTs (Smith et al., 2001).
Low social engagement	Those individuals who have low social engagement, particularly when they reach old age (over 60 years) have an elevated risk of developing AD. The elderly who are isolated and have less frequent contact with relatives or friends are also at an elevated risk (Povova et al., 2012).
Mental activity	The engagement in activities which require a mental effort such as reading, sewing, knitting, completing puzzles and watching television have been associated with a lowered risk of developing dementia and AD (Ruthirakuhan et al., 2012).

2.8.2 Genetics

Early onset AD accounts for 1-6% of all AD cases and has a high genetic heritability (Bird, 2008) with approximate ages of onset between 30-60 years (Bekris et al, 2010). Late onset AD occurs between 60-85 years of age and accounts for over 90% of total AD cases (Bekris et al., 2010). Multiple genes have been implicated in early onset cases and the main three are discussed. For late onset AD a genetic mutation in the APOE gene has been implicated and this too is discussed. The primary genes implicated in early onset AD cases are all related to the functioning and production of A β (Bird, 2008).

2.8.2.1 APP

APP is the amyloid precursor protein from which A β is proteolytically derived (Iqbal et al., 2010). The gene for APP is located on chromosome 21 and so far 32 mutations have been found (Bekris et al., 2010). Mutations within the APP protein account for only 10-15% of early onset cases and do not usually occur in sporadic cases (Bekris et al., 2010).

2.8.2.2 Presenilin 1 (PSEN1)

PSEN1 is a membrane protein located on chromosome 14 (Bekris et al., 2010) and is responsible for the cleavage of APP by γ secretase. Mutations of PSEN1 are the most common cause of early onset AD accounting for 18-50% of autosomal dominant cases (Theuns et al., 2000). At present over 176 PSEN1 mutations have been identified (Bekris et al., 2010). PSEN1 mutations result in a decrease of γ secretase activity leading to an increase in the production of A β ₄₂, the form of amyloid associated with higher toxicity and a higher likelihood of aggregation to form senile plaques. Mutations to this gene lead to the most severe of AD cases and are associated with the earliest ages of onset (Bekris et al., 2010).

2.8.2.3 Presenilin 2 (PSEN2)

PSEN2 also plays an important role in the cleavage of A β and mutations to its structure lead to an elevated production of the A β ₄₂ form (Schneuer et al., 1999). The gene encoding PSEN2 is located on chromosome one and mutations are rare (Bekris et al., 2010).

2.8.2.4 APOE

APOE is implicated in both early and late onset AD. The gene is located on chromosome 19 and exists in four alleles of which possession of the APOE4 genotype carries elevated risk for AD (Bekris et al., 2008). The direct mechanism by which APOE4 is linked to AD is not fully understood, however, APOE plays a role in cholesterol management and metabolism. High cholesterol is a risk factor for AD development (Rosenberg et al., 2008), likewise APOE4 carriers have higher total and low density lipoprotein (LDL) cholesterol levels as well as poorer clearance of cholesterol from neurons (Michikawa et al., 2000). Importantly, Bekris et al., (2010) comment that at least 50% of individuals who carry at least one APOE4 allele do not develop Alzheimer's disease. This suggests that possession of APOE4 alone is insufficient to explain all cases of AD.

2.9 Treatment

2.9.1 Cholinesterase inhibitors

AchEIs are recommended for the management of mild to moderate cases of AD and include donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Reminyl®). All of these drugs work through similar mechanisms of action and inhibit the enzyme acetylcholinesterase which is responsible for the degradation of Ach in the synaptic cleft. This increases the availability of Ach and, therefore, improves memory and cognitive performance. See figure five for a diagram representing this mechanism of action.

There is no evidence to suggest that there are any significant differences between these three drugs in terms of their benefits. Research has shown that the use of AchEIs is able to stabilise and slow cognitive decline in AD patients (Hansen et al., 2008). The recommended dose for each drug varies and side effects are common which can impact on usability and compliance. The most common side effects are gastro intestinal related and include nausea, vomiting, diarrhoea and flatulence (Atri, 2011). Side effects become notably worse as the dose is increased.

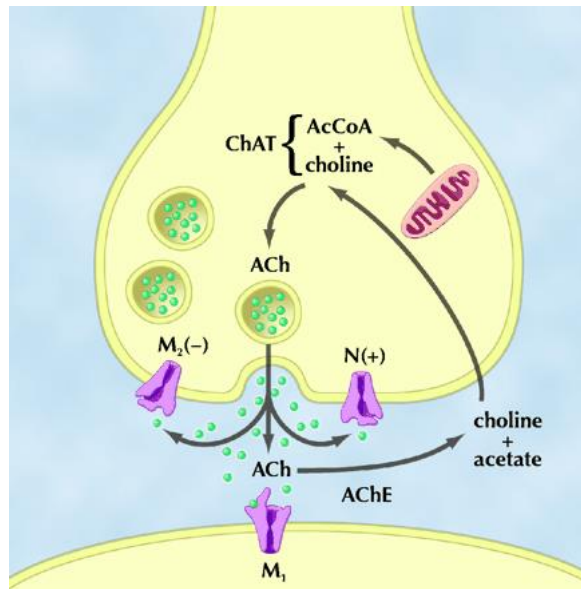


Figure five: cholinergic nerve terminal highlighting the mechanism of action of the AchEIs. Picture taken from Gauthier, (2002)

In the USA a higher maximum dose of 23mg of donepezil has recently been approved. Farlow et al., (2011) noted that in a large trial of 1467 patients those taking the higher dose of 23mg showed significant improvements in cognition after six months compared to those taking 10mg. Gastro intestinal disturbances were more common in those prescribed the higher dose, however, these seemed to stabilize after four weeks. Of the three AchEIs mentioned, galantamine has the most reported side effects, followed by rivastigmine (Lockhart et al., 2009).

Many studies have investigated whether AchEIs can improve or stabilise cognitive performance. Rogers et al., (2000) stated that donepezil was practical and beneficial for up to five years in AD patients. Likewise Evans et al., (2000) and Cameron et al., (2000) gained similar beneficial results. More recent studies such as Molinuevo et al., (2011) demonstrate that patients with mild AD, taking daily donepezil, remained cognitively stable on the MMSE after six months suggesting that donepezil is able to slow the rate of deterioration. In a Spanish cohort, six months of treatment with donepezil was able to improve MMSE score comparative to placebo (Lopez-Pousa et al., 2005). Seltzer et al., (2004) noted significant cognitive improvement following 24 weeks of donepezil on the MMSE and the Alzheimer's Disease Assessment Scale (ADAS).

2.9.2 N-methyl-D-aspartate (NMDA) antagonists

Memantine is a NMDA non competitive receptor antagonist and inhibits glutamate binding (Martorana et al., 2010). Elevated glutamate levels are present in AD and can lead to neuronal toxicity and cell death (Farrimond et al., 2012). In the USA memantine is recommended for the treatment of moderate to severe cases of AD and has less reported side effects than the AchEIs. Negative side effects may include confusion, dizziness and headache (Atri, 2011).

Winblad and Poritis (1999) showed an improvement on the Clinical Global Impression of Change Scale following three months treatment with 10mg of daily Memantine. This study used both AD and vascular dementia patients which show different pathological and cognitive profiles. Reisberg et al., (2003), using a sample of 181 moderate to severe AD patients, reported significant improvements on the ADL scale for those patients prescribed 20mg of memantine in comparison to placebo.

A combination approach has also been trialled to include a treatment profile of a AchEI alongside Memantine. Tariot et al., (2004) recruited 404 participants with a probable diagnosis of AD. All participants had baseline scores of between 5-14 on the MMSE, and had been taking a stable dose of donepezil for at least three months. Those participants who received memantine (20mg per day) in combination with their existing dose of donepezil demonstrated stable cognitive performance, as measured by the MMSE, comparative to baseline, after six months of treatment. The placebo group who continued to take only their prescribed donepezil showed a significant decline in cognitive performance.

Summary

The information provided within the first part of this chapter has shown that AD is a complex disease with numerous associated pathologies, processes, cognitive and behavioural profiles. The progressive nature of AD means that these occur over a long period of time. This chapter will now discuss MCI which is believed to be a transitional stage between healthy aging and AD (Markwick et al., 2012).

2.10 Mild Cognitive Impairment

The term MCI was first used by Reisberg and colleagues in 1988 to describe patients who were cognitively impaired but who did not meet the full AD diagnostic criteria. The term was later adopted by Petersen et al., (1999) who defined a set diagnostic criteria for the condition. MCI is believed to be a prodromal stage of AD or a transitional stage between healthy aging and AD. This sub group of individuals have an increased risk of progression to full AD pathology and symptomology (Petersen et al., 1999; Petersen, 2011; Hughes et al., 2011). The concept of MCI as a precursor to AD came into question when it was discovered that not all those with MCI do indeed develop AD and that the conversion rate, although higher than normal aging (1-2%), (Roberts et al., 2009), was approximately 10-18% per year for individuals aged 70 years or older (Petersen, 2009). In addition, some cases of MCI revert back to a 'normal' cognitive classification (Forlenza et al., 2010; Petersen, 2011). MCI has since been sub typed depending upon the predominant impairment present at baseline. MCI can be subdivided into the amnesic type (aMCI), where there is a predominant impairment in memory, and the non-amnesic type (naMCI), where the predominant impairment falls within another cognitive domain (Petersen, 2011) for example language, spatial skills or naming. The higher conversion rate of the aMCI type to AD has meant that impairments on assessments of memory are the strongest predictors of conversion to AD. However, Mapstone et al., (2003) suggest that in its earliest form, MCI is indeed more likely to present with an impairment in another cognitive domain such as language or spatial ability. This highlights the need for a thorough assessment taxing a variety of cognitive domains at baseline. Recently, Petersen et al., (2013) note that there is a possibility that naMCI may be a preclinical phase of other, non AD dementias.

In the literature, some research has further divided aMCI and naMCI into single domain and multiple domain depending on the number of cognitive domains affected (Hughes et al., 2011). Sub typing the condition enhances the reliability and validity of research so that the clinical and cognitive profiles of each type can be more accurately understood. In the following sections of this chapter MCI will be discussed in terms of its subtypes, diagnosis and symptoms, pathology, anatomy and treatment.

2.11 Symptoms and diagnosis

MCI is defined by clinical, cognitive and functional criteria (Albert et al., 2011). Those with a MCI do not meet the full diagnostic criteria for AD, however, their symptomology is significantly different to the non demented elderly (Forlenza et al., 2010). It can be challenging to distinguish between normal forgetfulness that occurs during aging and a genuine MCI (Petersen, 2011). MCI is usually awarded a CDR score of 0.5, or if the Global Deterioration Scale (GDS) is used, a score of two or three. The core clinical criteria for a diagnosis of MCI include the following points as discussed in Albert et al., (2011).

1. There must be a concern that there has been a change in cognition which can either be obtained from the individual themselves or an informant.
2. There must be a clear impairment in at least one cognitive domain which is greater than would be expected for the individual's age and education level. Wherever possible this should be monitored over time to confirm an objective decline in neuropsychological test performance. In aMCI this change is predominantly apparent in a memory domain, usually episodic memory, however, in naMCI a decline can be seen in another domain such as spatial ability, language or executive function (EF).
3. Despite the above points the individual should still remain independent in the completion of functional activities and daily living tasks. Even though certain errors or changes in tasks may be noted, such as forgetting to pay bills or taking longer to prepare meals, individuals with MCI generally maintain the ability to live in the community relatively unaided.
4. The individual must not be demented and any cognitive changes noted should be mild. There should not be any evidence of severe impairment in social and/or occupational activities.

Objective confirmation of a cognitive deficit is required and this is obtained via the use of a neuropsychological assessment. The MMSE, a test of general cognitive ability, which is a commonly used instrument in the diagnosis of AD, is not reliable for the detection of MCI as it is not sensitive to mild degrees of impairment (Jacova et al., 2007; Petersen, 2011; Mitchell, 2009). It is, therefore, necessary for clinicians to use more sensitive tests. Cases of aMCI

usually demonstrate poor performance on episodic memory tests and delayed recall. These types of assessment can predict to some extent whether a suspected MCI case is likely to progress to a full AD diagnosis (Dubois et al., 2007; Albert et al., 2011). The fact that MCI has different sub types and different cognitive profiles has meant that a thorough assessment of other cognitive domains is required such as EF, attentional control and visuospatial ability. These can be assessed using tests such as the Trail Making Test (TMT), figure copying and digit span respectively (Albert et al., 2011). Neuropsychological assessments are discussed in more detail in chapter four.

2.12 Progression to AD

Confirmations of biomarkers associated with AD are obtainable in some cases depending on the protocol in use by the institution (i.e. the hospital, NHS, clinic). These can be useful indicators of the likelihood that the individual will progress to a full diagnosis of AD (Forlenza et al., 2010). For example, confirmation of A β plaques (Markesbery, 2010), hippocampal and MTL atrophy as seen on MRI (Jack et al., 1999), hypometabolism of tempoparietal regions (Jagust et al., 2009) as measured by PET, a CSF profile which mirrors that seen in AD (Hansson et al., 2006; Shaw et al., 2009) and possession of the APOE4 allele (Artero et al., 2008) all of which are associated with a high probability of progression.

The ability to predict which cases will decline further is complicated by the fact that not all MCI cases go on to develop AD. In addition, progression rates differ for MCI sub types (Albert et al., 2011). There is a higher rate of progression for aMCI than naMCI (Hughes et al., 2011). Markesbery (2010) stresses the importance of considering the source of the samples used in MCI research. For example, those attending memory clinics with a subjective memory complaint are more likely to have a MCI and are also more likely to present with more advanced stages of neuropathology and anatomical changes associated with AD than samples of healthy elderly (HE) participants who are recruited in the community. Of these community dwellers, some may indeed meet the diagnostic criteria for MCI but they are more likely to be in early stages of the

condition as they still continue to live independently (Petersen et al., 2009; Forlenza et al., 2010).

2.13 Neuropathological and anatomical features of MCI

Typical AD pathology namely the presence of A β plaques and NFTs, atrophy, inflammation and hypometabolism are present in MCI supporting the notion that MCI is in fact early AD. Each of these are discussed in the following sections.

2.13.1 A β plaques

Amyloid plaques are present in cases of MCI and measures of total plaque load and plaque distribution fall between the levels expected for healthy aging and AD (Markesbery, 2010). Autopsies from non demented HE participants show similar plaque distributions and levels to MCI participants which has made the presence of A β plaques unreliable as a true pathological marker of MCI (Mufson et al., 2012). Differences between MCI and the HE have however been noted in specific brain areas. For example, in MCI, plaque distribution is significantly greater in the frontal cortex and amygdala (Markesbery et al., 2006). Those MCI cases with low A β 1-42 CSF levels are believed to be at a higher risk of a more rapid progression to AD (Hansson et al., 2006). Recently Petersen et al., (2013) showed that in a group of aMCI participants 43% of the sample did not show any evidence of A β plaques.

2.13.2 Tau

Advanced AD is associated with severe NFT pathology specifically in the nbM, the major cortical cholinergic hub (Mesulam et al., 2004). Mesulam et al., (2004) demonstrated that the formation of NFTs starts during cognitively normal aging and progresses along a continuum, whereby MCI cytopathology is significantly different to normal aging but less severe than in AD. The fact that the cholinergic neurons are affected early in the disease pathway is also important to consider as this supports the cholinergic hypothesis of the disease.

NFTs are significantly greater in number in the amygdalae, entorhinal cortex (EC) and inferior parietal cortex of MCI cases comparative to aged matched controls (Braak and Braak, 1991; Guillozet et al., 2003). The number of NFTs present has been found to correlate well with the level of cognitive impairment the subject demonstrates, making tau load a suitable indicator of MCI status (Forlenza et al., 2010).

In the Religious orders Study which recruited 180 catholic clergy and has a good autopsy rate, evidence of neuropathology associated with AD was reported in 60% of the subjects (Bennett et al., 2005). This study also noted that the presence of vascular disease was also associated with AD pathology. The fact that 40% of subjects did not present with pathological markers typical of AD again questions the prototype of MCI.

2.13.3 Atrophy

Different patterns of brain atrophy have been noted between MCI and HE and aMCI and naMCI. Hippocampal atrophy correlates well with pathology, cognition and behavioural measures of the disease (Shen et al., 2011). MCI cases have been found to exhibit smaller EC and hippocampal volumes relative to aged matched controls (Schuff and Zhu, 2007). A study investigating 153 MCI participants, 218 cognitively healthy individuals and 68 AD patients, revealed significant differences between the three groups on measures of total brain atrophy and hippocampal volumes. Of the MCI cases, those classified as single or multi domain aMCI showed greater hippocampal atrophy relative to naMCI and the HE (He et al., 2009). A similar finding was found by Van de Pol et al., (2009) in a multi centre approach, where MTL atrophy was significantly greater in aMCI relative to naMCI and was also modified by age.

Apostolova et al., (2006) followed a group of MCI participants for three years and carried out regular structural MRI scans. Those participants who developed AD within the follow up period had greater volumetric loss in the CA1 region of the hippocampus. Voxel Based Morphometry has allowed the longitudinal measurements of total brain grey matter and has been able to map the change in grey matter over time in MCI cases. Initial grey matter loss was

predominant in the amygdala, anterior hippocampus and EC before spreading to the entire hippocampus, parietal cortex and finally the frontal cortices (Schuff and Zhu, 2007). Diffusion Tensor Imaging (DTI), a new variant of MRI, has been used to track white matter changes in MCI and has also revealed alterations in hippocampal fibre integrity (Fellgiebel et al., 2004). Furthermore, temporal lobe atrophy correlates well with cognitive performance. Grundman et al., (2002) showed that larger hippocampal volumes were associated with better performance on memory tasks and assessments of general cognitive function.

Structural imaging offers a reliable way to monitor volumetric changes in tissue viability and appears to correlate with disease severity. It should be noted, however, that the EC and hippocampus are affected in other types of dementia such as frontotemporal dementia and vascular disease. Relying on MRI measures alone may not be sufficient to confirm the presence of MCI or the likelihood of progression to AD.

2.13.4 Hypometabolism

Hypometabolic changes are seen in AD and have been confirmed also in cases of MCI. Similar patterns of metabolic abnormality occur in MCI (Petersen et al., 1999) but these are more widespread. FDG-PET scanning has revealed hippocampal hypometabolism in MCI (Mosconi et al, 2009). In naMCI different patterns are observed, whereby, there is a general absence of hypometabolism or mild disturbances in regions around the parieto temporal cortices (Clerici et al., 2009). Amnestic cases show more prominent changes in glucose metabolism. Figure six is taken from Mosconi et al., (2009) and shows regional decreases in cerebral glucose metabolic rates for AD patients, MCI participants who went on to develop AD after two years and stable MCI participants. De Leon et al., (2001) followed 48 HE participants over a period of three years and found baseline reductions in metabolism in the EC could accurately predict future decline.

2.13.5 Inflammation and oxidative stress

As in AD, there is evidence of neuro-inflammation in MCI (Roberts et al., 2009). In a sample of 313 MCI participants and over 1000 cognitively normal elderly participants, MCI was associated with elevated C-reactive protein (CRP) levels, a marker of inflammation (Roberts et al., 2009). Likewise, there is evidence of oxidative stress in MCI cases indicating this too occurs early in the disease process (Nunomura et al., 2007).

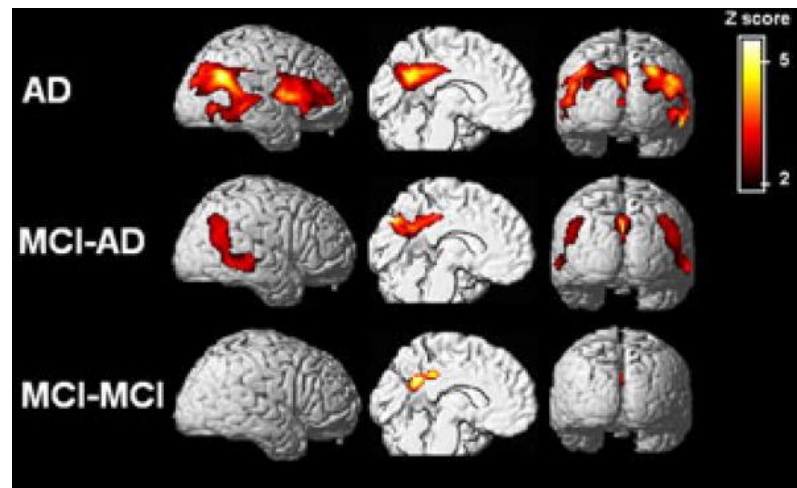


Figure six: regions of hypometabolism (as seen in red) in AD and MCI patients who went onto develop AD, taken from Mosconi et al., (2009)

2.14 Therapeutic interventions and treatment

There are a number of interventions which can be offered in order to prevent further cognitive decline or optimise current cognitive functioning in MCI. A review of intervention approaches by Gates et al., (2011) stated that new innovative programs for the treatment of MCI are vital if clinicians and researchers wish to decrease the number of cases progressing to AD. Currently there is no effective pharmacological treatment for MCI (Aisen et al., 2008). Initially trials with AchEIs were disappointing and in general studies have reported no significant benefit in the delaying of disease progression (Raschetti et al., 2007; Feldman et al., 2007; Winblad et al., 2008). AchEIs such as rivastigmine and donepezil also have significant side effects and are therefore not ideal for long term use (Atri, 2011).

Petersen, (2011) stated that rather than pharmacological treatment, individuals with MCI should be encouraged to increase their involvement in social and

cognitive activities particularly as these are low risk and have shown positive outcomes. Cognitive plasticity refers to alterations in synaptic organisation brought about by cognitive practice such as task learning or mental activity (Sanz Simon et al., 2012). Participation in mentally stimulating tasks is associated with a lower incidence of AD, and may, therefore, offer some degree of protection against cognitive decline (Wilson et al., 2002). Furthermore, cognitive training programs in the HE can decrease the risk of developing cognitive impairment (Valenzuela and Sachdev, 2009). Increased learning potential and cognitive activity in MCI participants has been shown to be associated with a decreased decline in cognitive performance as measured by the Auditory Verbal Learning Test (AVLT) of Learning Potential (Calero and Navarro, 2004). This suggests that cognitively stimulating tasks are useful in MCI through the enhancement of cognitive plasticity mechanisms.

Various intervention programs now exist and include *cognitive stimulation*, which is usually carried out in a group setting to encourage social and cognitive functioning. *Cognitive training*, which implements ways to maximise cognitive performance, and *cognitive rehabilitation* which focuses on ADL where the individual sets their own goals to achieve tasks which they may currently struggle with (Clare et al., 2003).

2.15 Chapter summary

This chapter has outlined the symptoms and diagnostic procedures for both AD and MCI as well as reviewing some of the key pathological, anatomical and neurochemical features of each condition. It is clear that AD is a highly complex neurodegenerative condition with multiple risk factors and underlying processes. This makes detecting and treating the condition difficult. MCI is believed to be a prodromal stage of AD and this is supported by the fact that many MCI cases go on to develop AD and possess some of the key pathological hallmarks of AD. Treating patients at the time of symptomatic onset is sub optimal as changes in the brains systems and underlying pathology have already been altered, sometimes decades beforehand. This is an important issue and therefore earlier diagnosis is needed if these detrimental changes are to be halted, this is discussed in more detail in the following chapter.

Chapter 3

Early diagnosis of Alzheimer's disease

3.1 Introduction

The previous chapter provided an overview of AD and MCI. This chapter will discuss the early diagnosis of AD in more detail including the positives and negatives this approach may have on the patients, their families, the healthcare systems and the economy. The chapter evaluates some possible techniques which could be employed to detect early signs of the disease and what early interventions could then be of use in delaying cognitive decline should a diagnosis occur at an earlier point in the disease pathway.

3.2 The growing burden of AD

Worldwide a number of countries including the UK, USA, and the Netherlands have recognised that AD and other dementias must be prioritised in terms of the treatment, care and support that is made available to patients (Alzheimer's disease International (ADI), 2011). The UK government has recently made AD one of its top priorities due to the significant crisis which is escalating in terms of the social, health and economic impact that the increasing number of diagnosed cases will bring (Department of Health, 2009). There is currently a large treatment gap where it has been estimated that only 20-50% of all AD cases actually receive a formal diagnosis (ADI, 2011). Of the estimated 36 million people suffering from dementia worldwide, there are potentially 28 million who are not diagnosed and are, therefore, not receiving any treatment or support (ADI, 2011).

Without a diagnosis there is a barrier between the patient and their ability to access relevant health care. This makes it difficult to improve the individual's quality of life and prevent any worsening of their symptoms. ADI state in their 2011 world report, which specifically focussed on early diagnosis, that a timely diagnosis is imperative. Early diagnosis may be able to reduce the 'treatment

gap'. Delaying diagnosis wastes valuable time for the patient and may mean that by the time their condition is formally diagnosed they may not gain the maximum benefits from drug interventions.

AD is receiving increased attention and the general population is becoming more aware of the disease, its symptoms and what can be done to help those affected. This has largely occurred due to increased reporting in the media and via organisations such as the Alzheimer's Society, Alzheimer's Research Trust (ART) and ADI who have published fact sheets, magazines and leaflets

Early diagnosis may help to reduce some of the stigma associated with the disease and assist in altering society's views, opinions and attitudes towards AD. People often feel uncomfortable discussing AD (Werner et al., 2013; MacRae, 1999). There are also a number of misconceptions about the disease which creates further barriers for diagnosis and early detection. For example, many believe that losing one's memory is a normal part of the aging process and if they do notice changes in their own memory they are unlikely to seek help for this (ADI, 2011). Many also believe that there is nothing that can be done for AD patients and they do not inform their GP of their symptoms until they are moderate or severe (Department of Health, 2009). Increasing public awareness could encourage carers and those affected to seek help earlier when symptoms are milder. People are more likely to reach out for additional support if it is readily available and accessible to them (ADI, 2011) and hopefully an earlier diagnosis could facilitate and improve this process.

In AD research there has been a prominent focus to try and detect and diagnose the disease as early as possible (Forlenza et al., 2010). Early detection of AD has been recommended as a priority in the most recently published NICE dementia guidelines (Ballard et al., 2011). This was set as a precedence due to the findings from many research groups that the underlying pathological changes associated with the disease could be detected possibly decades before subtle symptoms begin to appear (Forlenza et al., 2010; Dickerson et al., 2011; Petersen, 2009; Sperling et al., 2011). This meant that a diagnosis at the time of symptomatic onset was suboptimal and in the majority of cases too late to alter the disease's progression. The long preclinical phase of the disease, therefore, offers opportunities to intervene earlier (Zamrini et al.,

2004). This approach is seen elsewhere in other medical conditions. For example, in cardiovascular disease high cholesterol can be monitored and treated with statins years before hand, thereby reducing the risk of heart disease or heart attack (Rosenberg et al., 2008).

As mentioned in chapter two, the amnesic form of MCI is likely to be a transitional stage between normal aging and AD (Markwick et al., 2012, Petersen, 2011). These individuals are likely to be in an early stage of the disease, although detection even at this stage may be too late (Petersen, 2009). Finding a definitive cure for AD is as yet impossible and methods to halt the progression may be of benefit both to those affected, the health care systems and the economies worldwide (Petersen, 2009).

3.3 Why is early diagnosis needed?

3.3.1 Increase in expected incidence

The incidence of AD continues to increase on an annual basis (Alzheimer's Association, 2013). Age remains the major risk factor for developing AD (Rountree et al., 2013). The increasing life expectancy of the elderly means that by 2040 there will be over 40 million people affected worldwide (Alzheimer's Association, 2013). This will place an even larger burden on global economies and makes AD care a major concern. Detecting cases as early as possible, whilst individuals still live in the community, could significantly decrease the cost burden for care. These individuals are then more likely to remain independent for longer if their disease pathology can be halted keeping them in the milder stages of the disease for longer (Petersen, 2009).

3.3.2 Decrease the economic burden

The economic costs of AD are huge and in 2010 it was estimated that worldwide \$604 billion were spent on AD care (ADI, 2011). A large proportion of these costs were directly derived from social care and it was further estimated that between 30-50% of those suffering from AD resided in care homes (Knapp and Prince, 2007). With the increasing incidence of the disease, which is likely

to reach 115 million by 2050, care costs will continue to soar (ADI, 2009). Although early diagnosis may initially cost governments additional expense to cover the cost of diagnostic tests and procedures, delaying the need for social care and nursing home placement would benefit the economy in the long run (ADI, 2011). There are currently no studies which have directly compared the benefits in terms of cost for early versus later diagnosis. Likewise, there is currently no direct evidence that earlier initiation of medication can delay institutionalisation and this is mainly because clinical trials have been short in duration (usually between 6-12 months) (Rountree et al., 2013). Despite this it is the expert opinion of many researchers and organisations that early diagnosis would greatly reduce the growing economic burden of AD care (ADI, 2011; Alzheimer's Association, 2013).

3.3.3 Maximise treatment efficacy

Drug treatments for AD which target the cholinergic systems are the most cost effective when symptoms are mild. Early initiation of pharmacological treatments may optimise cognitive symptoms allowing patients to remain in their own homes for longer thus maintaining higher levels of cognitive function (Markwick et al., 2012). The efficacy of the drugs decreases as the disease progresses with limited benefits in severe cases (Gauthier et al., 2013). See chapter two section 2.9 'Treatment' for more detail.

3.3.4 Identify reversible causes of cognitive dysfunction

Some individuals who present with mild cognitive changes may indeed have a MCI or early AD but their symptoms may be caused by another underlying condition such as cardiovascular disease or depression which are treatable and manageable (Overshott and Burns, 2004). Early diagnosis or screening for AD would identify individuals, who if were left untreated, would likely progress to a form of dementia (Ashford et al., 2006). Furthermore, the elderly with generally poor health are at a higher risk of developing AD. Frailty in particular seems to be highly correlated with AD development (Reitz et al., 2011) due to its

association with poor nutrition, lack of exercise and lower levels of social interaction.

3.3.5 Identify MCI and prevent further decline

MCI is a risk factor for the subsequent development of AD (Petersen et al., 2009) but this is complicated by the fact that not all cases will progress to a full AD diagnosis (Chapman et al., 2011). The conversion rate of MCI to AD is approximately 10-18% per year for cases aged 70 years or over (Petersen, 2009). Subjective memory complaints are found in over 50% of those over 65 years of age (Bassett and Folstein, 1993) and are highly predictive of decline to MCI and AD. This highlights the need to screen these individuals and monitor them more closely (NICE, 2006a). If MCI can be detected as soon as possible there may be hope of preventing further cognitive decline or slowing the trajectory to AD. In addition, the MCI population are an ideal cohort for future research and the majority of ethical issues which are encountered when recruiting AD patients are no longer problematic (Ashford, 2006; Gauthier et al., 2013). For example, MCI participants are able to give their informed consent and there are no doubts of competency. Unfortunately, trials of AchEIs in MCI cohorts have not provided beneficial results in their ability to delay disease progression (Gauthier et al., 2013). Although there is no validated treatment for MCI, those affected can be informed about interventions and lifestyle factors which may help maintain ADLs and cognitive ability. For example, cognitive activities, a healthy diet and the rule out of the presence of co morbid medical conditions (Petersen, 2009).

3.4 Considerations for early diagnosis

Doctors are currently not sufficiently reliable in their ability to detect very mild signs of cognitive impairment. Further, GPs reportedly miss over 50% of mild AD cases which then leaves the patient to deteriorate even further to a moderate stage of the disease before an intervention is initiated (Ashford et al., 2006). Another study reported that 91% of mild cases are overlooked in the primary care setting (Valcour et al., 2000). By the time these patients have their

condition formally diagnosed there is then substantial disease related pathology and medications may be of limited benefit. For early, accurate detection to be possible GPs require additional resources and guidance. The GP, who is often the first point of call for suspected AD cases (Petersen, 2009), will ultimately decide whether or not the patient requires a referral onto a specialist memory centre (NICE, 2006a,b). It is imperative that GPs are sufficiently confident and trained in their ability to recognise and document the most subtle of symptoms if the effectiveness of this process is to be maximised. Further, more organised co-ordination and communication between the primary and secondary care centres (i.e. the GP practices and memory clinics) is necessary if early diagnosis is to occur in a timely fashion (ADI, 2011). Valcour et al., (2000) reported that only 20-50% of AD cases were actually documented in primary care practice. Although it is acknowledged that diagnosis is 'easier' and more reliable when symptoms are moderate to severe, it can be challenging to notice mild alterations in cognition (Petersen, 2011). This must be improved and addressed quickly if reliable early diagnoses are to be made (ADI, 2011).

GPs have very little time for each appointment (Cooper et al., 1992; O'Connor et al., 1988). Clinicians often state that they find giving a diagnosis of AD challenging as they do not have sufficient time to sit with the patient and go through all of the available options and treatments. This is particularly difficult when AD is such a sensitive and distressing issue (Werner et al., 2013). In the 1990s it was often the opinion of medical professionals that a diagnosis would bring more harm to the patient and in many cases those affected by AD were not informed (Werner et al., 2013). Turner et al., (2004) carried out a survey of UK GPs which revealed that almost one third felt that they lacked the confidence in their ability to detect and diagnose AD, mainly because they did not have enough specialist specific knowledge regarding early diagnosis. The AD world report 2011 also showed that educational intervention programmes, which were specially designed for general practice, received low levels of participation. For example, only 28% of invited practices in the UK took part even though they were reimbursed for their time, suggesting that this type of education needs to be made mandatory if diagnostic procedures are to be improved (ADI, 2011).

3.5 Implementing early diagnosis

In order to implement early diagnosis there must be tools in place which are sensitive to mild cognitive symptoms (Chapman et al., 2011). Cognitive changes may only be subtle but the underlying brain systems may show a different level of severity. Some have argued that early screening for AD is itself highly expensive. The cost of early identification for one patient in the US has been calculated to be approximately \$4000 and screening may not actually be a cost effective mechanism in the short term (Ashford et al., 2006). However, the UK national audit office recommended in a report published in 2007 that the government should indeed spend to save (National Audit Office, 2007).

There are a number of techniques in use that could detect some of the hallmarks of early AD for example neuro-imaging, blood analysis, immunisation and neuropsychological testing. Researchers disagree which technique is the best and most reliable to use with some suggesting that a combination of biomarkers need to be checked. Unfortunately this increases the cost and the time needed to thoroughly assess each patient. Another important factor to consider is that in the UK waiting times to see specialists can take up to 18 weeks (NHS, 2012) and appointments with a GP are time limited (Cooper et al., 1992). This makes it difficult to obtain a detailed patient history and examination including medical history, family patterns of disease, blood tests and physical examination. The following section discusses some of the possible tools available for use in earlier detection and diagnosis.

3.5.1 Neuro-imaging

The imaging of underlying brain structures implicated in AD can reveal slight volumetric alterations, identifying those individuals who may be at risk but who are currently cognitively normal (Velayudhan et al., 2012; Zamrini et al., 2004). Small alterations in MTL and parietal volumes in cognitively normal elderly participants can identify those who are likely to decline (Morris et al., 2009). Studies which have recruited MCI participants have also demonstrated that those with elevated levels of hippocampal atrophy, specifically in the EC, have an elevated risk of decline to AD. Therefore, the use of imaging as a screening

option would seem viable (Fennema-Notestine et al., 2009; Jack et al., 2004; Varon et al., 2011).

Velayudhan et al., (2012) showed that EC volume was more strongly associated with baseline cognitive performance than total hippocampal volume. AD patients with the most significant tissue loss in this area experienced the most rapid rates of cognitive decline. An earlier study also noted decreased MTL grey matter volumes in a group of 23 cognitively normal individuals who went on to develop a MCI (Smith et al., 2007). Measurements of EC volume may offer a reliable method for early detection.

As discussed briefly in chapter two section 2.5.1 'Senile Plaques', advanced imaging techniques such as PET can utilise radioligands such as PiB which can bind to and detect early deposition of amyloid plaques (Jack et al., 2008). In AD and MCI participants there is increased PiB retention compared to the HE, however, some HE who are classified as cognitively normal also show some levels of retention (Mintun et al., 2006). Using PiB as a biomarker for early detection may not be reliable when used as a stand alone method. It may be useful for the identification of patients who require further, more detailed investigations and neuropsychological testing, to confirm or rule out a suspected diagnosis.

Another potential approach is to focus on the monitoring of whole brain volumes. McDonald et al., (2009) showed that in the non-demented elderly there is minimal whole brain atrophy annually. In MCI cases and AD patients there is, however, considerable atrophy acceleration and this is significantly increased in the medial and lateral temporal and inferior parietal cortex, areas which are implicated in AD. Annual variances in atrophy offer a higher sensitivity to subtle alterations in brain tissue and could be useful for early diagnosis or prediction of future possible AD cases. The authors of this research stress that the interpretation of a single MRI scan does not reliably indicate the rate of subsequent progression nor can it be compared to earlier images (McDonald et al., 2009). Longitudinal follow up annually gives a more accurate picture of underlying processes and possible neurodegeneration (McDonald et al., 2009). Again, the longitudinal monitoring of the elderly over

time is expensive and time consuming and may not be the most economic way of assessing patients for early detection.

In summary, imaging techniques provide an accurate picture of the brain's underlying volume and functioning. Negatives of this approach include the high expense (Zamrini et al., 2004), and the requirement of highly trained staff to carry out the procedure. There is also the risk of exposure to radiation particularly if repeated scans are required. A positive of neuro-imaging is its lack of cultural bias, as seen with some cognitive assessments. Likewise, it is not affected by external variables such as concentration, mood, tiredness, language or education level (Zamrini et al., 2004).

3.5.2 CSF profiling

The CSF is a fluid which is continuously circulated throughout the brain and its cavity ventricles (Bear et al., 2001). Sampling CSF and measuring specific levels of proteins and biomarkers would offer an accurate representation of brain activity and processes (Forlenza et al., 2010). A sample is obtained via lumbar puncture which requires the patient to lie on their side while a sample of CSF is collected via a needle inserted into the dural space of the spine between the lower lumbar vertebrae (Medline Plus, 2011). The procedure takes approximately 20-30 minutes, after which the patient is required to lay flat for a period of preferably four hours (Medline Plus, 2011).

MCI participants with CSF profiles similar to those reliably detected in AD i.e. low levels of A β and high levels of total tau, usually decline at a faster pace than those MCI cases without this typical profile (Petersen, 2009). Hansson et al., (2006) used CSF profiling and accurately divided their sample of MCI participants into those who were at a high probability of decline/non decline based on CSF levels of AD proteins. CSF sampling may, therefore, be a useful tool for early detection but the collection of such samples is invasive with a risk of side effects (Ashford et al., 2006). The report of headaches after a lumbar puncture occurs in 2-4% of patients (Andreassen et al., 2001) and may deter patients from consenting to this procedure. Other side effects may include sore back and less common, more serious adverse effects, can include infection,

haematoma and nerve damage (Medline Plus, 2011). The collection of CSF can only be carried out by trained clinicians and requires further laboratory processing making this option expensive and time consuming. Further, following a lumbar puncture, patients must be kept under observation before being allowed to go home which would require specialist facilities/centres as this type of procedure would not be possible in general practice. NICE do not recommend CSF collection and comparisons for a diagnosis of AD regardless of the stage of disease (NICE, 2006b; 2011).

3.5.3 Blood tests

Obtaining a blood sample is quick, can be carried out by a phlebotomist, has minimal side effects and can be processed quickly and relatively cheaply (O'Bryant et al., 2011). Sampling can be carried out at the GP surgery and does not require admission to a hospital or specialist clinic. Blood analysis would provide a useful way of indentifying possible candidates for research trials and would flag those individuals who may require a more detailed and thorough set of testing via imaging or CSF sampling (O'Bryant et al., 2011).

Amyloid plaques are a hallmark of the disease (Forlenza et al., 2010) and A β can be detected in the serum, but, whether levels in the blood correlate with brain concentrations has been questioned (O'Bryant et al., 2011). One issue in previous research has been the type of blood fraction, (i.e. plasma or serum) that is analysed which can lead to discrepancies in data findings across studies. For example, Mayeux et al., (2003) measured plasma amyloid levels of A β 42 and A β 40 in 530 individuals including AD patients and healthy controls. They reported increased A β 1-42 levels at baseline testing for AD patients and those individuals who went on to develop AD within three years. Luis et al., (2009) on the other hand measured serum A β levels and reported that A β 1-42 was elevated in MCI participants compared to controls but that established AD patients had levels in-between the values for controls and MCI participants.

O'Bryant et al., (2010) used a different approach and created a biomarker risk score using the measurement of serum proteins obtained from blood samples from 197 AD patients and 203 cognitively normal controls. This risk score could

accurately identify 91% of the sample. When demographic variables such as age, education level and clinical laboratory values of other risk factors such as cholesterol were taken into account the accuracy of the algorithm to identify participant's clinical group was increased to 95% (O'Bryant et al., 2011).

Routine blood testing is also useful for highlighting any other reversible deficiencies/abnormalities which could exacerbate cognitive symptoms.

3.5.4 Neuropsychological testing

Mild alterations in cognition are common during aging and it is sometimes difficult to discriminate between normal aging and a pathological abnormality (Hedden and Gabrielli, 2004). Likewise some neuropsychological tests show a decline in performance during normal aging (Sperling et al., 2011). There are many neuropsychological tests available which assess different cognitive domains, although not all tests are validated for the detection of milder degrees of impairment and therefore may not be useful for early diagnosis. Johnson et al., (2009) note that there appears to be an inflection point several years before diagnosis where cognitive deficits first become apparent. Although this occurred approximately three years before diagnosis for visuospatial ability noticeable deficits in verbal and working memory were not detectable until one year before clinical diagnosis. This highlights the need to assess a range of different cognitive domains when making an initial diagnosis (Johnson et al., 2009).

The MMSE is an assessment of general cognition used in the diagnosis of AD but there is currently no one instrument internationally recognised as a tool for the reliable detection and diagnosis of MCI (Guo et al., 2012). In order to detect slight cognitive changes the neuropsychological test used must have high sensitivity and must be able to reliably detect early disease. The MMSE is discussed in more detail in chapter four 'Neuropsychological testing' see page 62 for further details. A more sensitive general test of cognition is the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005). This test, like the MMSE, also assesses multiple cognitive domains but unlike the MMSE, also includes assessments of working memory and EF (Markwick et al., 2012).

Nasreddine et al., (2005) showed that 73% of their sample who possessed an abnormal MoCA score (26 or less out of a possible thirty) had a normal MMSE score (26 or more) creating a conflicting diagnosis based on these results alone. Consequently scoring within the normal range on the MMSE does not necessarily confirm that the individual is AD risk free. This creates important considerations when giving a diagnosis based on a single test score. Both the MMSE and MoCA are discussed in more detail in chapter four 'Neuropsychological testing'.

The fact that general cognitive tests may not thoroughly test specific cognitive domains has meant that many studies have then investigated the HE and those individuals with a MCI to determine whether one cognitive domain appears to be the first affected over another. As alterations in cognitive ability are an early manifestation of the disease, it is feasible that cognitive assessments which are sensitive enough will detect these subtle changes (Chapman et al., 2011). As mentioned earlier Johnson et al., (2009) note that there appears to be an inflection point several years before diagnosis where cognition alters suggesting that regular longitudinal checks from middle age onwards may be needed, however, this is time consuming.

Mapstone et al., (2003) suggest that in its earliest form MCI is indeed more likely to present as an impairment in another cognitive domain such as language or spatial ability. This again highlights the need for a thorough assessment taxing a variety of cognitive domains at baseline. Tests assessing MCI must also be sensitive to change over time in order to accurately monitor the rate of subsequent decline (Stein et al., 2010). This would require longitudinal follow up which is time consuming and adds expense (Carrillo et al., 2009).

Data from imaging studies can complement and narrow down the type of cognitive testing which is required. As previously mentioned research studies sampling participants with MCI (i.e. those who do not yet fully meet the criteria for dementia), have revealed an elevated volumetric decline in the EC of the hippocampus. This area is crucial for memory function (Feczko et al., 2009) and has been implicated in early AD in many research studies. Developments and improvements in imaging techniques have greatly improved the accuracy of

imaging the MTL and as a result these volumetric data can identify anatomical change in the earliest stages of the disease (Pruessner et al., 2002). In addition to a decline in EC volume, the earliest signs of the presence of pathological markers are also found in this area in the form of NFTs. The fact that these participants present with pathological biological markers which are hallmarks of AD suggests they are in a prodromal early stage of the disease. Lesion studies and imaging studies carried out on the EC have found this area to be highly sensitive to tests stimulating visuospatial associative memory. This type of memory typically involves learning the association between a visual stimulus and a distinct spatial location (Égérhazi et al., 2007). Some of the earliest signs of early disease may be detectable by assessments of visuospatial skills (Johnson et al., 2009). An example of an existing test from the Cambridge Neuropsychological Automated Test Battery (CANTAB) which recruits this type of memory is the CANTAB Paired Associates Learning task (CANTAB PAL) (Égérhazi et al., 2007). This test is discussed in detail in chapter four 'Neuropsychological Testing' and could be a useful pre-clinical tool as a AD screening tool in the future.

The most common complaint associated with AD is a deficit in episodic memory (Ahmed et al., 2008) and assessments which tax this domain such as delayed word recall, are useful for the prediction of decline (Dubois et al., 2007; Albert et al., 2011; Jacova et al., 2007). Using this measure alone may not be reliable due to individual variability (Zamrini et al., 2004). In addition, tests of speeded EF such as the Stroop test and the TMT have also proven useful for predicting decline (Chapman et al., 2010). EF relies heavily on the frontal lobes and is crucial for the changing demands and situations experienced in daily living (Schroeter et al., 2012). The frontal lobes are highly sensitive to the aging process and experience a large decline in volume with age (Hedden and Gabrieli et al., 2004). It is, therefore, expected that increasing age would be associated with poorer EF performance. Both the Stroop and TMT require the ability to quickly switch between two stimuli and also have an inhibitory component (Chapman et al., 2010). These tests may also be useful for early diagnosis.

In summary, the border between normal aging and cognitive impairment is not defined. Instead there is a gradual continuous decline in cognition making it

difficult to determine the exact time of disease onset (Carrillo et al., 2009). Consequently, the use of a single cognitive test alone is inadequate for the reliable detection of early cognitive alterations. Negatives of neuropsychological testing include the long administration times for detailed cognitive batteries (Zamrini et al., 2004) and the need for validation and consideration of multiple external factors such as age, education, mood and cognitive reserve (Jacova et al., 2007). There is considerable variability in normal cognitive performance (Zamrini et al., 2004). For example, an individual may have been poor at spatial ability since young adulthood and failure to score well on a test of spatial ability may not signify underlying disease but may simply be due to individual differences (Zamrini et al., 2004). This is one such danger of interpreting a single test score for a diagnosis. Nevertheless, using neuropsychological assessment is less expensive than other procedures such as imaging, is non invasive and safe (Chapman et al., 2011) and has high potential as a tool for use in early screening and diagnosis.

3.6 Recent research for early diagnosis

Korff et al., (2013) recently reported that α synuclein levels were elevated in CSF samples of MCI and AD participants relative to HE controls. The amount of α synuclein present also significantly correlated with participants' MMSE score suggesting that monitoring levels of this protein in CSF samples may also be of clinical relevance for early detection.

Roe and Rentz (2013) investigated glucose metabolism in those with a high cognitive reserve as these individuals are likely to score normally on brief screening instruments creating ceiling effects (Markwick et al., 2012). Unexpectedly the authors found that those with increased cognitive reserve showed patterns of decreased brain glucose metabolism. The results imply that higher levels of education may allow these individuals to cope for longer with underlying detrimental processes associated with AD before symptoms first become apparent.

There is a link between sense of smell and neurodegeneration (Hüttenbrink et al., 2013). Olfactory dysfunction is detectable in early AD (Hüttenbrink et al.,

2013) and in those with a MCI (Wilson et al., 2009). Braak and Braak (1991) note that the EC which plays a role in olfactory processing is one of the earliest affected sites in the disease. Assessments of olfactory function and sensitivity may be useful as an early indicator of potential disease if cognition is normal.

Frost et al., (2013) investigated retinal vascular markers of AD on the premise that the retina is a direct outgrowth of neural tissue and may show signs of AD pathology. In diagnosed AD patients there are visual changes including alterations to the optic disc and retinal arteries. Retinal screening could potentially be useful for early detection. Frost et al., (2013) investigated 148 participants which comprised 125 HE and 25 AD. Clear retinal vasculature abnormalities were seen in the AD participants although further study is needed to determine the earliest point at which these changes are noticeable if this is to be used as an early diagnostic tool.

3.7 What treatments could be offered if diagnosis were to occur earlier?

3.7.1 Pharmacological interventions

As discussed in chapter two AchEIs, including donepezil, rivastigmine and galantamine, and the NMDA non competitive receptor antagonist, memantine, are commonly used as the pharmacological drugs of choice in the treatment of AD and other dementias (Rountree et al., 2013; Atri, 2011). AchEIs increase the concentration of Ach within the synaptic cleft via the inhibition of the acetylcholinesterase enzyme (Rountree et al., 2013) whereas memantine inhibits glutamate binding and this is discussed in chapter 2 'Alzheimer's disease and Mild Cognitive Impairment'. The AchEIs have all shown positive effects in the treatment of mild to moderate AD and memantine has been beneficial in the treatment of moderate to severe cases. All three licensed AchEIs have a similar efficacy for the treatment of mild to moderate cases and there is a great deal of evidence to suggest that these drugs are useful in early cases (Birks, 2006). Research has also shown that these drugs help maintain ADLs and prevent further cognitive decline (Feldman et al., 2001; Holmes et al., 2004). Consequently early diagnosis would be beneficial for the timely prescription of these medications. It is however important for the patient and

their families/carers to understand that these drugs do not bring about a cure and more research is needed to determine the long term effects as current trials have been short in duration over a number of weeks (ADI, 2011). Another issue which has made it difficult to evaluate the use of these drugs in the prevention of further cognitive decline or the stabilisation of cognition is that of compliance. Families often stop medication if they believe they are not seeing an improvement, even though the drugs may be stabilising the patient's symptoms (Geldmacher et al., 2006).

3.7.2 Hormone replacement therapy (HRT)

Women are at an elevated risk of developing AD (Hogervorst et al., 2000). This may be due to their higher life expectancy but may also be a result of changes in oestrogen levels with age (Markou et al., 2005). After the menopause women experience a decrease in their levels of circulating oestrogen and gonadal hormones (Hogervorst et al., 2000; Markou et al., 2005). Research studies have shown that oestrogen has many beneficial mechanisms in the brain including its ability to enhance cholinergic transmission (Gibbs and Aggarwal, 1998; Ryan et al., 2008, Markou et al., 2005), prevent cellular death (Harms et al., 2001), increase dendritic spine density in the hippocampus (Murphy et al., 1998), decrease levels of A β (Ryan et al., 2008) and decrease levels of neurotoxic glutamate (Ryan et al., 2008). Oestrogen also influences certain aspects of cognition including verbal fluency, memory, and spatial skills (Markou et al., 2005; Ryan et al., 2008). Due to these findings it was hypothesised that the administration of HRT to postmenopausal women would benefit cognition and brain health and perhaps decrease the risk of developing AD.

The decline in oestrogen after the menopause can be counteracted through the use of HRT which delivers synthetic oestrogen (Wnuk et al., 2012). A number of different preparations exist which vary in the type of oestrogen used and whether it is combined with a synthetic progesterone (Hogervorst et al., 2000). Early studies carried out in the 1990s appeared to suggest beneficial effects of HRT on dementia risk and cognition. Later studies such as Mulnard et al., (2000) and Espeland et al., (2004) have revealed discrepant findings and have

noted detrimental effects of HRT (Hogervorst and Bandelow, 2010). A meta analysis carried out by Hogervorst and Bandelow (2010) revealed that the strongest effects of HRT were noted on cognitive assessments of verbal memory and in some cases EF and concentration. Duration of HRT was also an important factor, although, the effect of age on treatment outcome was not as strong. Furthermore, the addition of a progesterone was associated with a greater number of negative outcomes (Hogervorst and Bandelow, 2010).

Discrepant findings between studies may be due to the type and dosage of the HRT administered, the duration of the treatment, the cognitive measures and analysis carried out, the participant's age at menopause and treatment onset and also any prior exposure to oestrogenic medications (Ryan et al., 2008). Hogervorst et al., (2000) comment that HRT may only have beneficial effects during a limited window of time or in specific cohorts. For example, surgically menopausal women or those with depressive symptomology. There are also concerns regarding the side effects of HRT such as an elevated risk of certain cancers including breast and ovarian types, pulmonary embolism, coronary heart disease and stroke (Ryan et al., 2008). Currently oestrogens cannot be recommended as an early intervention for preventing cognitive decline in women.

3.7.3 B vitamins

The B vitamins are molecules which are able to bind to and break down Hcy, a toxic sulphur amino acid (Aisen et al., 2008), essential for numerous metabolic actions. Hcy levels significantly increase during aging (Li et al., 2008) and as a consequence of this they have become a therapeutic treatment option to reduce AD risk and progression (Aisen et al., 2008). AD has been linked to an increase in Hcy (Lehmann et al., 1999); further, increased Hcy concentrations in the brain are associated with an increased rate of medial temporal lobe atrophy (den Heijer et al., 2003). If low B vitamins are detected during early diagnosis of AD or MCI, reversing this deficiency may halt further decline and optimise current cognitive function.

den Heijer et al., (2003) investigated Hcy concentrations and brain atrophy in the non demented elderly using a large sample of 1077 participants aged

between 60-90 years. The authors used MRI to visualise global cortical brain atrophy and correlated this with fasting plasma HCy concentrations. Participants with increased HCy had a significantly decreased hippocampal volume bilaterally compared to those with normal HCy values. This was also confirmed in a study by Tseng et al., (2009). Further, increased HCy was also correlated with increased cortical atrophy in general. Multivitamin users constituted 5.9% of the sample and these individuals possessed lower HCy concentrations.

HCy is also associated with vascular dementia, cardiovascular disease and high cholesterol (Smith, 2002; Smith et al., 2010). If lowering HCy via specific vitamin administration could occur, the number of individuals suffering from MCI who go onto to develop AD would be reduced significantly (Smith, 2002).

Whether supplementation with B vitamins is beneficial to cognitive decline, or for use in those diagnosed early, remains unclear as studies have yielded conflicting data (Luchsinger and Mayeux, 2004). Clarke et al., (1998) studied 76 AD patients and 108 HE participants and found that AD patients had lower levels of folate and B12 in blood samples. Aisen et al., (2008) carried out a reliable supplementation study to see if they could shed any light on previous inconsistencies. Their double blind placebo versus treatment experiment took place over a period of 18 months and recruited 409 participants. Participants were recruited from multiple sites in North America from their local health centres. All participants were over the age of 50 and scored between 14 and 26 on the MMSE at baseline. The treatment group were given a daily tablet containing 5mg of folic acid, 1mg of vitamin B12 and 25 mg of B6. The placebo group received a tablet identical in appearance but that contained no active ingredients. All participants received regular checkups at three monthly intervals. In order to determine whether a beneficial effect was observed patients completed the ADAS, a 70 point scale consisting of a series of cognitive tests assessing multiple dimensions including memory, attention, language and orientation. A higher score represents greater impairment. The authors considered a 25% reduction in score to be the equivalent of a beneficial effect.

Of the 409 participants who took part 371 were taking AchEIs and 166 were multivitamin users. Surprisingly, there was an increase in reported cases of depression in the treatment group which the authors believe was not a result of the treatment, rather just the patient's stage in their illness. As expected analysis showed an increased vitamin concentration in the treatment group which correlated with a decrease in Hcy levels in this group also. Overall in the treatment group there was a 27% decrease in Hcy, a 19% decrease in the multivitamin users and a 31% decrease in the non multivitamin users.

Although the authors concluded that there was a significant beneficial effect of taking a high dose vitamin B supplement in reducing Hcy concentration, there was no significant reduction in ADAS score. This may signify that the study needed to have used a more thorough method of determining cognition and should possibly have used a range of tests and assessments rather than one single scale. Participants were also at different stages in disease progression when recruited into the study and this may have impacted on their response to treatment and scores of cognitive function. Recently B vitamin treatment over a period of two years, in comparison to placebo, was able to slow the rate of cognitive decline particularly when baseline Hcy was in the upper quartile range (de Jager et al., 2012) in a group of MCI participants.

Currently there appears to be no significant benefit of B vitamin treatment for early AD or for the prevention of further cognitive decline. However, in their quick referencing guide, NICE do recommended that clinicians should check levels of B vitamins and folate (NICE, 2011).

3.7.4 Vitamin E

Vitamin E is a powerful antioxidant (Uneri et al., 2006) found in a number of dietary foodstuffs including vegetable oils, nuts and seeds (Farina et al., 2012). As previously mentioned in chapter two, AD and MCI are associated with elevated levels of oxidative stress and low levels of antioxidants (Axelsen et al., 2011). High doses of vitamin E (>3000ug/day) are toxic and can cause fatigue and stomach upset. Furthermore, even at low habitual levels there are some health considerations such as altered bleeding rates (Farina et al., 2012).

400ug/day of vitamin E increased prostate cancer risk in men (Klein et al., 2011) and also increased the risk of stroke in another study (Shürks et al., 2010). A recent Cochrane review revealed that across 46 trials, vitamin E administration increased mortality risk significantly (Bjelakovic et al., 2012). Despite this vitamin E has been linked to cognitive function, for example, aged rats show improved cognition following vitamin E administration (Socci et al., 1995). Vitamin E levels are decreased in the serum and CSF of AD and MCI cases (Mangialasche et al., 2012) but this may be due to alterations in dietary intake in these conditions in later stages of the disease.

Farina et al., (2012) carried out a review to determine whether vitamin E would be useful in the treatment of AD and MCI. Early diagnosis may facilitate the use of this micronutrient in AD care. From this review the authors concluded that vitamin E should not be recommended as an early intervention for MCI and AD. Studies such as Sano et al., (1997), Lloret et al., (2009) and Petersen et al., (2005) reported no significant differences between vitamin E and placebo on cognitive outcomes or risk of conversion to AD. There is no evidence at present that vitamin E improves the outcomes of AD patients and it is also associated with a number of serious health side effects (Farina et al., 2012). Although these studies have yielded negative results it may be that the administration of vitamin E in capsule form delivers doses which are too high. It may be better to investigate whether a diet rich in vitamin E containing foodstuffs would be more beneficial to those with MCI or AD.

3.7.5 Ginkgo biloba

Ginkgo Biloba is derived from the Maidenhair tree and is commonly used in Chinese medicine for the treatment of a number of medical conditions (Birks and Grimley Evans, 2009). Ginkgo increases blood supply via its vasodilatory ability, decreases blood viscosity and possesses antioxidant properties (Birks and Grimley Evans, 2009). There have been a number of trials which have assessed the use of Ginkgo as a cognitive enhancer useful in the treatment of dementia. For example Le Bars et al., (1997) and Schneider et al., (2005), These studies have revealed inconsistent results with some earlier studies reporting benefits but with more recent studies showing no positive effects. The

lack of significance does not seem to be modified by dose or trial duration. Despite the lack of positive effects, Ginkgo is safe to use with no reported serious adverse effects and may be suitable as an optional supplement although it cannot be specifically recommended for use in the prevention of further cognitive decline in MCI.

3.7.6 Physical activity

Individuals with high levels of physical activity have a lower risk of developing AD (Podewils et al., 2005; Laurin et al., 2001; Larson et al., 2006). Furthermore, levels of exercise have been shown to correlate with cognitive performance in the elderly (Laurin et al., 2001, Brown et al., 2010). One study has shown that the non demented elderly who are physically active show decreased PiB retention and higher A β 42 levels in the CSF (the opposite profile to AD patients) (Liang et al., 2010). Exercise programs or interventions may, therefore, be useful in decreasing the risk of further decline, maintaining current brain function and improving quality of life for those with an early diagnosis (ADI, 2011). Exercise results in many positive health benefits for AD patients and these include increased resting blood flow to cerebral areas which subsequently increases the delivery of vital nutrients, improved muscular strength, promotion of plasticity mechanisms and increased brain volumes (Ruthirakuhan et al., 2012). All of these mechanisms may improve cognitive performance and maintain ADLs during aging. Exercise programmes are also likely to take part in a group setting thereby facilitating higher levels of social interaction (Ruthirakuhan et al., 2012).

There has been very little research investigating the use of exercise interventions in early AD or MCI but studies which have investigated this have reported positive effects on cardiovascular fitness, behaviour and cognition (Heyn et al., 2004; Teri et al., 2003). It would seem feasible that patients with AD would be able to take part in some form of physical activity even if this was seated for short intervals (ADI, 2011).

3.7.7 Cognitive engagement and stimulation

Engaging in stimulating cognitive activity has been linked to a decreased risk of developing AD (Ruthirakuhan et al., 2012). For example, Wilson et al., (2010) demonstrated that those non demented elderly who were cognitively active had a decreased risk of MCI and AD. Cognitive interventions can benefit AD patients in terms of facilitating improvements in ADLs and cognitive functions (Valenzuela and Sachdev, 2009). There is evidence to suggest that engaging in hobbies such as reading, puzzles, games and watching television for a few hours per week can also decrease the risk of cognitive decline during aging (Hughes et al., 2010). Cognitive engagement can not only decrease the risk of AD but could be useful in the early stages of the disease to prevent further cognitive decline. For example, Treiber et al., (2011), reported that high participation in stimulating activities in early AD slowed the rate of disease progression. Cognitive stimulation and information regarding this type of therapy would be useful in the early diagnosis of AD.

3.8 Additional factors to consider for early diagnosis

Early diagnosis and/or screening for early signs of disease is important for those individuals who are non symptomatic but have a family history of the disease. Possessing a first degree relative with AD increases the individual's own risk of developing the disease by 4-10 % (Cupples et al., 2004). Honea et al., (2010) reported that cognitively normal individuals with a maternal history of AD had decreased grey matter volumes in AD related areas of the brain compared with those individuals who either have a paternal family history or no family history of AD. In those with a maternal history of AD there was significant evidence of progressive atrophy in the parietal and parahippocampal cortices (Honea et al., 2011). The exact reasons why maternal transmission appears to be more significant than paternal cases is still not fully understood but highlights the need to monitor and screen those with a strong family history.

3.9 Pros and cons of early diagnosis

3.9.1 Closer monitoring

An earlier diagnosis would enable the closer monitoring and tracking of the disease's progression (Pesini et al., 2012) but would also allow the clinician to check for reversible causes of cognitive complaints. For example, low levels of B vitamins, infection, depression and/or altered thyroid hormones (Overshott and Burns, 2004). Likewise, earlier detection could help the management of associated conditions which can exacerbate the cognitive problems present in AD such as depression (Overshott and Burns, 2004). Closer monitoring of early AD or MCI cases would allow the clinician to check for, and monitor, other risk factors for subsequent cognitive decline such as poor nutrition, lack of social interaction and/or vascular complaints (such as high cholesterol or high blood pressure) all of which, if left untreated or addressed, could increase the rate of cognitive decline.

The earlier administration of treatments may also halt the disease's progression and optimise cognition whilst underlying brain systems are still relatively intact with normal neurotransmitter concentrations. Early treatment interventions aim to maintain the highest level of cognitive functioning for as long as possible in order to prevent further decline to more severe stages of the disease (Seltzer et al., 2004). The longitudinal monitoring of patients would be beneficial but such monitoring would be expensive and time consuming (Dickerson et al., 2011).

3.9.2 Improved understanding

Receiving an early diagnosis, or knowing that they possess an increased risk of developing AD, allows the patients and their families time to educate themselves about the disease, its symptoms and what they can do to aid their condition. This may include the implementation of certain diet and lifestyle habits, some of which have been discussed in this chapter. Families often report feeling relieved that a diagnosis has been confirmed which can reduce anxieties and can help them to adjust to life with a diagnosis (ADI, 2011). An earlier diagnosis then allows the patient to be fully involved in their own

treatment pathway so that they can make informed decisions about their own care and experience (Ashford et al., 2006).

3.9.3 Reducing harm

Early diagnosis could reduce the numbers of harmful incidents reported in AD populations. For example, in the early stages of AD, patients are known to become lost or forget about electrical appliances for example leaving a frying pan on the stove. This can lead to dangerous risks and the involvement of the emergency services (Ashford et al., 2006).

3.9.4 Time to prepare

An early diagnosis may offer some relief to patients who have been concerned about their memory and would give them time to prepare for the future. For example, making a will if they have not already done so, or giving consent to treatments that at some stage they may not be competent to give or understand (Mattsson et al., 2010). It also allows families to consider safety and security issues with living arrangements, cooking, driving and medication use.

3.9.5 Reducing care costs

Early diagnosis could decrease the cost of AD care for the government but also for the families and individuals affected. Receiving a diagnosis earlier in the disease pathway can allow the patient to continue living independently for longer which would delay the time when additional care such as nursing home placement is required.

3.9.6 Psychological implications

Although positive steps can be taken after an earlier diagnosis, such as closer monitoring and educating, there is a high probability that this may cause increased anxiety or depression in these patients. There may be feelings of

hopelessness and despair (Mattsson et al., 2010) and also the possibility of an increased risk of suicide. It is unclear from previous research whether this is a direct result of feelings of despair at receiving a diagnosis, or a direct consequence of the underlying disease (Draper et al., 2010). Depression and anxiety will be even greater in those individuals who live alone or perhaps do not have the support of close relatives or friends. In addition, depression can accelerate cognitive and physical decline in established AD patients therefore exacerbating the initial cognitive symptoms (Overshott and Burns, 2004).

An early diagnosis, or high likelihood of AD development, may induce symptoms of anxiety and depression in relatives (van der Cammen et al., 2010), placing a higher burden on the family and possibly their ability to care for the patient. There may be legal implications for an early diagnosis, for example, insurance premiums may be more expensive or no longer obtainable. There is the added possibility that early diagnosis may result in the earlier removal of driving licences isolating individuals living in the community even further (Mattsson et al., 2010).

3.9.7 Stigma

AD is perceived negatively by the general public (Gauthier et al., 2013). The elderly reported in a recent survey that AD phobia is the most worrying concern for the aging population and this may indicate that the elderly may not wish to be screened or diagnosed earlier (Draper et al., 2010). Some of the elderly often state that they would rather die earlier than live with a diagnosis of dementia (Mattsson et al., 2010). Information about AD is now more readily available and often the elderly know or are aware of friends and/or relatives who have suffered from the disease. As a result they may be more willing to be screened (Draper et al., 2010). Losing one's memory can have a profound effect on personality, identity and sense of self which can also lead to symptoms of depression (Ashford et al., 2006). Stigma associated issues also create implications for the individual in terms of employment and insurance (Sperling et al., 2011).

3.9.8 Diagnostic uncertainty

No one biomarker test useful for early detection has 100% sensitivity and accuracy and this indicates that false positive diagnoses may occur in some cases. This will cause unnecessary worry or the administration of testing or invasive treatments which are not actually needed. This has the potential to cause unnecessary harm and unwanted side effects and is a prominent issue in the implementation of early detection of AD. Although a diagnosis of MCI could be given not all MCI cases do go onto to develop AD and again this may cause needless anxiety and stigma (Sperling et al., 2011).

3.10 Chapter summary

This chapter has outlined some of the benefits that an early diagnosis could bring. For example, the earlier prescription of AchEIs, information regarding lifestyle and dietary factors such as maintaining B vitamins and antioxidants, using Gingko Biloba, cognitive stimulation and social interaction and using support groups. It has also highlighted some of the potential barriers to early diagnosis including, costs, stigma and lack of diagnostic accuracy. In order to combat these issues GPs must be further educated and trained to become more reliable in their ability to detect mild symptoms because they are often the first point of call for suspected cases of early AD. Stigma surrounding AD must also be tackled by increasing public awareness. Memory clinics need to become more accessible to those with suspected AD in order to correctly identify questionable cases.

One of the main questions posed in this area of research is 'will early diagnosis be beneficial to the patient or just the economy?' Early detection may be able to decrease the time the patient spends in care facilities but it will not prevent subsequent decline until methods are found to halt disease progression. It is clear that no one biomarker seems to be 100% reliable in its ability to diagnose early cases and ideally all elderly individuals should be entitled to imaging and neuropsychological screening. Cost restrictions are currently making this an impossible task at present. The UK government has, however, recently made dementia care in the UK a priority.

Chapter 4

Neuropsychological testing: review and methodology

4.1 Introduction

In the previous chapter neuropsychological testing was discussed as one potential method for the early diagnosis of cognitive impairment. Unfortunately the testing process can be affected by external factors which have the potential to affect the accuracy of the scores obtained. The experiments of this thesis will utilise neuropsychological testing to assess cognitive performance in the HE. This chapter will discuss neuropsychological testing in more detail and will include detailed descriptions of the neuropsychological tests which will be included in the cognitive batteries administered to participants.

Neuropsychological assessment is one method used to diagnose a cognitive impairment (Chapman et al., 2010). The assessment process is useful for clinicians and researchers to distinguish between normal aging and whether a clinical impairment is present (Zamrini et al., 2004). Numerous tests exist which vary in their levels of specificity and the type of cognitive domain that they assess. The scores obtained are used by health care professionals to determine which treatments can be offered, whether pharmacological intervention is initiated and potentially what care programme is delivered in the community (Jacova et al., 2007). Scores from these tests can determine whether individuals are suitable to be included in research trials (Gauthier et al., 2013) which often have very specific inclusion criteria. It is essential for test scores to be accurate to ensure that those recruited are indeed suitable. Patients diagnosed with a cognitive impairment may then be monitored over time and will often be re tested to follow their progression (Overshott and Burns, 2004).

Aging is a continuous process and is progressive in nature. The effects of aging become more noticeable with increasing age due to the cumulative effects which have built up over time (Chan et al., 2009). At present a comprehensive neuropsychological assessment is delivered when the patient

begins to show signs of AD symptoms such as a rapid decline in memory, language, spatial awareness or changes in personality (Brousseau et al., 2007). Testing at this stage is problematic as underlying pathological changes can occur many years, perhaps even decades prior to symptomatic onset (Blackwell et al., 2004; Forlenza et al., 2010). These changes are irreversible and so it is important to catch the disease in its early stages. This will enable treatment to be initiated and maximised with the aim of slowing progression and preventing further cognitive decline (as discussed in chapter three 'Early Diagnosis').

4.2 Brief tests of general cognitive function

Brief tests of general cognitive function are used to determine the presence and severity of any memory impairment (Feldman et al., 2008). They are usually administered by the GP at the GP surgery and take approximately 10-15 minutes to complete. They are quick to administer and are easily accessible to the primary care setting, however, they do have lower sensitivities to milder degrees of cognitive impairment which is somewhat problematic if an early diagnosis is to be made (Feldman et al., 2008). An abnormal score on one of these tests can identify individuals who may require further, more in depth neuropsychological testing, or another type of dementia screening such as neuro-imaging or blood analysis (Jacova et al., 2007). Some brief tests can be affected by the participant's demographic variables such as their age, education level, mood and race (Revett et al., 2013). Examples of brief tests include the MMSE, the MoCA, the Seven Minute Test, the GPCOG and the clock drawing test (CDT), amongst others. For the purpose of this thesis the MMSE and the MoCA will be discussed in further detail. These tests will be used as a part of the cognitive batteries administered to participants in this thesis.

4.2.1 The Mini Mental State Examination (MMSE)

The MMSE was developed in 1975 by Folstein et al., and is a quick assessment of general cognitive ability. The test consists of 19 components which tax a variety of cognitive domains including orientation, short term memory, recall, language, attention, copying and verbal understanding (Folstein et al., 1975). A copy of the MMSE can be seen in figure seven. The MMSE is considered the most widely used cognitive assessment in the primary care setting and is useful for the rule out of suspected dementia cases (Mitchell, 2009). The test takes approximately ten minutes to complete and the patient receives a score out of a possible 30 points. The interpretation of cut offs can vary with some researchers using a cut off of 24 for impairment. According to the NICE dementia guidelines a score of 26 or above signifies that no impairment is present, 21-26 represents mild AD, 10-20 signifies moderate AD, 10-14 equates to moderately severe AD and scores less than ten represent severe AD (NICE, 2011).

The MMSE has been criticised for its inability to reliably detect mild cases of cognitive impairment which is problematic for patients who score on the borderline (Tombaugh and McIntyre, 1992, Tombaugh, 2005). There is only a six point difference between a normal and an abnormal diagnosis and within these six points both MCI and mild AD are also included (Marioni et al., 2011). Another issue with the scoring of the MMSE is the occurrence of ceiling and floor effects (Crum et al., 1993). This creates clusters of patients scoring the test's maximum (30/30), and severe cases clustering at lower scores (<10/30). Demographic variables including age, education, race, culture and language ability have also been shown to affect scores (Ballard et al., 2011; Davey and Jamieson, 2004; Crum et al., 1993; Tombaugh and McIntyre, 1992; Tombaugh, 2005). This is problematic as the test may not be reliably sensitive for those with a large cognitive reserve or a higher level of education (Jacova et al., 2007; Tombaugh, 2005). As mentioned in chapter two, a higher education level (cognitive reserve) has been associated with a decreased risk of developing AD and MCI. As a result it has been suggested that for highly educated individuals, a higher cut off of possibly 27 out of 30 should be used (Markwick et al., 2012) for defining an abnormal score. This may increase the risk of false positive results. In addition, non Caucasian individuals may be at a disadvantage,

particularly if English is not their first language or if culturally, they are not familiar with the phrases or objects that they are asked to remember or recall as a part of the test. The revised NICE dementia guidelines (NICE, 2011) have, therefore, incorporated this and note that MMSE score alone should not be used to give a diagnosis or determine care if the patient is not fluent/familiar with English or is not of Caucasian background (NICE, 2006b).

The NICE dementia guidelines were highly criticised for recommending MMSE cut off scores for the prescription of AchEIs (Davey and Jamieson, 2004). Researchers had argued that the MMSE is not reliably sensitive as a suitable assessment for the determination of treatment interventions (Ballard et al., 2011) or for assessing treatment response over time (Bowie et al., 1999). These guidelines stated that cholinesterase inhibitors should only be prescribed to those patients with a moderate form of dementia which, therefore, implied that a patient with mild cognitive difficulties would not be treated until they continued to decline further. The guidelines also stated that medications should be removed if MMSE score fell below 10 points (NICE, 2006b). This contradicted the widely acknowledged goal that earlier detection of AD was needed. The revised guidance in 2011 still recommends the MMSE as a tool used in the diagnosis of AD but states the following “.... *treatment should be continued only when it is considered to be having a worthwhile effect on cognitive, global, functional or behavioural symptoms...*” (NICE, 2011). A review of neuropsychological testing by Jacova et al., (2007) concluded that the MMSE was not superior and gave no additional advantage in comparison to other brief cognitive screens. Future NICE guidelines may need to place a larger emphasis on the recommendation of other brief cognitive assessments.

Consequently, the MMSE is better as a method for the exclusion of AD rather than a tool which diagnoses the severity of impairment the patient initially presents with (Tombaugh, 2005). Even though researchers and clinicians are aware of the problems associated with the MMSE scoring system it is recommended by NICE to determine whether or not they should consider prescribing medication.

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

Instructions: Ask the questions in the order listed. Score one point for each correct response within each question or activity.

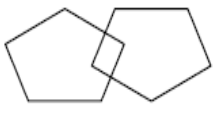
Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day of the week? Month?"
5		"Where are we now: State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible. Number of trials: _____
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, ...) Stop after five answers. Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

Figure seven: a paper copy of the MMSE (Folstein et al., 1975)

4.2.2 The Montreal Cognitive Assessment (MoCA)

The MoCA was specifically created as an assessment for the detection of MCI (Nasreddine et al., 2005). It is a brief cognitive test which assesses short-term memory, visuospatial ability, EF, attention, concentration, working memory, language and orientation to both time and place (Markwick et al., 2012). The MoCA is similar to the MMSE in that it assesses multiple domains of cognitive ability and is scored out of a maximum score of 30; however the cut off for impairment is a score of 26/30 rather than the cut off of 24/30 used in the MMSE (Jacova et al., 2007). A copy of the MoCA can be seen in figure eight.

Studies have reported good internal consistency and retest reliability for the MoCA (Markwick et al., 2012). The MoCA includes more thorough testing of memory, including a delayed recall question and also assesses EF and more complex visuospatial skills. Tests of these two domains have previously been found to be sensitive to early cognitive impairment (Blackwell et al., 2004; Mapstone et al., 2003; Dubois et al., 2007). Unlike the MMSE, the MoCA takes into account if the participant has a lower level of education and awards them an additional point if their level of education falls below 12 years (Nasreddine et al., 2005). This is determined purely by the participant's recollection. A review revealed that the MoCA has excellent sensitivity to detect MCI and AD, 90% and 100% respectively, compared to the MMSE, which has sensitivities of 18% and 78% (Feldman et al., 2008). Nasreddine et al., (2005) reported that the mean score of a sample of MCI participants fell within the normal range on the MMSE but in the abnormal range on the MoCA suggesting that the MoCA is a more reliable method for detecting milder forms of cognitive impairment. Likewise Smith et al., (2007) reported 83% sensitivity for the MoCA to identify MCI participants compared to only 17% by the MMSE.

Recently Markwick et al., (2012) compared the MoCA and MMSE scores from 107 healthy elderly participants with a mean age of 76 years. All of the subjects had an MMSE score of 24 or higher. The MMSE scores for the cohort ranged from 24-30 whereas scores obtained on the MoCA ranged from between 13-30. Of those scoring normally on the MMSE, 40.2% had an abnormal MoCA score, supporting claims that the MMSE may not be sensitive to MCI. These findings were in accordance with previous work such as Nasreddine et al., (2005) and

Pendlebury et al., (2010). Damian et al., (2011) noted in their study that the orientation items of the MoCA correlated the strongest with the degree of cognitive impairment but that the animal naming portion of the assessment did not and was, therefore, possibly too easy. In summary, these findings indicate that the MoCA is a superior cognitive test to the MMSE for the detection of early AD and MCI.

4.2.3 Brief tests: summary

Brief cognitive tests are useful aids for the GP. They provide a quick method of obtaining a picture of a participant's overall global cognitive function. The quick administration time is useful for application in clinics and is not too taxing for the participant. One of the major disadvantages to some, but not all, of these tests is their inability to detect mild degrees of impairment which is crucial if earlier diagnosis is to be implemented. The issue of intra individual variability must also be taken into consideration. For example, is it reliable to state that two participants who both score 28/30 on the MMSE have the same level of cognitive function? What if one patient goes onto to develop AD within the next twelve months but the other patient remains cognitively stable? The fact that this type of problem does indeed occur suggests that the use of one total score alone is not a reliable or accurate enough method to be used for the diagnosis of AD. It may be useful to consider the participant's reaction time and processing speed for each item in order to combat this issue. No single brief cognitive test has been found to be better than the rest and none can be recommended for the differentiation of subtypes, i.e. MCI or AD (Feldman et al., 2008). It may be better to re test the patient regularly over the following six months before a diagnosis is made. However, this is time consuming and delays the administration of treatment if it is indeed required. The remainder of this chapter will now discuss the tests which will be used in the experiments of this thesis.

MONTREAL COGNITIVE ASSESSMENT (MOCA)

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

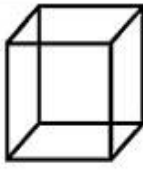
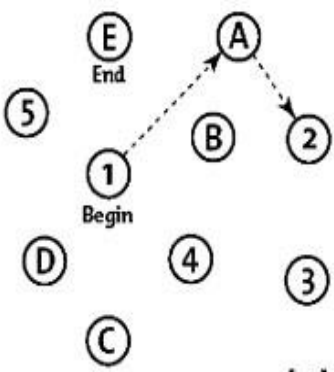
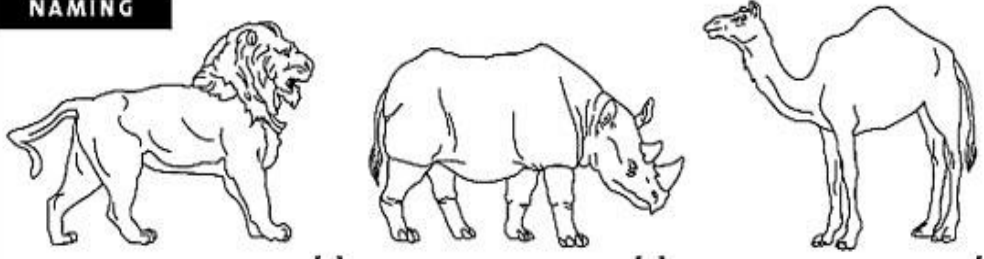
VISUOSPATIAL / EXECUTIVE							POINTS	
	Copy cube <input type="checkbox"/>	Draw CLOCK (Ten past eleven) (3 points)				<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	___/5	
NAMING							<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	___/3
MEMORY	Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.	FACE	VELVET	CHURCH	DAISY	RED	No points	
		1st trial						
		2nd trial						
ATTENTION	Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [] 2 1 8 5 4 Subject has to repeat them in the backward order [] 7 4 2						___/2	
Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors		[] FBACMNAAJKLBAFAKDEAAAJAMOF AAB					___/1	
Serial 7 subtraction starting at 100 [] 93 [] 86 [] 79 [] 72 [] 65		4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt					___/3	
LANGUAGE	Repeat : I only know that John is the one to help today. [] The cat always hid under the couch when dogs were in the room. []					___/2		
Fluency / Name maximum number of words in one minute that begin with the letter F [] _____ (N ≥ 11 words)							___/1	
ABSTRACTION	Similarity between e.g. banana - orange = fruit [] train - bicycle [] watch - ruler					___/2		
DELAYED RECALL	Has to recall words WITH NO CUE	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only	
		[]	[]	[]	[]	[]		
Optional	Category cue							
		Multiple choice cue						
ORIENTATION	[] Date	[] Month	[] Year	[] Day	[] Place	[] City	___/6	
© Z.Nasreddine MD Version November 7, 2004		Normal ≥ 26 / 30			TOTAL		___/30	
www.mocatest.org		Add 1 point if ≤ 12 yr edu						

Figure eight: the Montreal Cognitive Assessment (MoCA). Picture taken from www.mocatest.org.

4.3 CANTAB PAL

The CANTAB battery consists of multiple assessments, one of which is the PAL (Égérhazi et al., 2007). As briefly mentioned in chapter three, the PAL is an assessment of visuo-spatial associative memory and requires the participant to learn an association between a visual stimulus and a spatial location (Égérhazi et al., 2007). This type of task recruits the hippocampus and hence provides an indication of underlying hippocampal integrity (de Rover et al., 2011). The test is delivered via a touch screen computer and takes approximately ten minutes to complete (Wild et al., 2008). The computer based scoring system offers a higher degree of accuracy for the recording of responses compared to traditional pen and paper tests and, therefore, removes some of the potential for subjective researcher error.

The participant is presented with a computer screen depicting six white boxes which open up in a random order. The participant is asked to remember which box a pattern appears in and must record their response by pressing the appropriate box on the touch screen. The task progressively becomes more difficult and the participant is then required to remember the location of two different patterns, then three and then six. The number of errors made at the six pattern stage is of importance as this level has previously been shown to be highly sensitive to MCI and early AD (Blackwell et al., 2004; de Rover et al., 2011). Participants' performance at the six pattern level significantly correlates with subsequent deterioration in global cognitive function over the following eight months (Swainson et al., 2001). Further, the earliest signs of underlying pathology, which are the presence of NFTs, are present in the EC (Braak and Braak, 1991). Imaging studies have found this area of the brain necessary for visuospatial associative learning (Blackwell et al., 2004), therefore, this test may be the most useful for detecting the presence of early neuropathological abnormalities.

Fowler et al., (2002) reported that a six month decline in the PAL performance of participants with mild cognitive difficulties predicted later progression to AD. Likewise, de Rover et al., (2011) report impaired performance in a group of MCI participants on both the four, six and eight pattern stages of the PAL relative to controls. The PAL's high discriminability (Nestor et al., 2004) and usefulness in

identifying early cases of impairment could, therefore, make it a useful pre clinical tool/biomarker (Beddington et al., 2008). The PAL could be used as a dementia screening assessment in the future. The PAL may also be useful for employment in the evaluation of new treatment interventions or strategies (de Rover et al., 2011) to trace longitudinal improvement or deterioration. Its longer administration times mean it is not an ideal option for use by GPs due to their limited appointment times (Cooper et al., 1992). This means it is more likely to be used at specialist clinics by trained neuropsychologists. For the purpose of this thesis the score at the six pattern stage will be recorded.

4.4 The Graded Naming Test (GNT)

The GNT is also a test from the CANTAB battery and is an assessment of semantic memory (McKenna and Warrington, 1980). The test requires the participant to correctly name 30 black and white line drawings presented on a computer screen. A pen and paper version of the GNT is also available. The objects presented in the GNT becoming progressively harder to identify, beginning with easy objects such as a scarecrow, and ending with harder, less easily recognisable objects, such as a retort (Lambon Ralph et al., 2001). The participant receives a score out of 30. It is important to consider that the drawings presented during the GNT may not be cross culturally applicable. This should be considered when administering the test to a non UK sample. Naming and vocabulary skills usually improve with age (Kahlaoui et al., 2012) however some studies have reported a clear semantic deficit in AD and this is mirrored by poor GNT performance by AD patients compared to aged matched controls. For example Lambon Ralph et al., (2001) investigated semantic deficits in 10 AD patients, 10 Dementia with Lewy Body (DLB) patients and 10 cognitively normal controls. AD patients and DLB patients performed similarly on the GNT. The AD sub group, (mean score 20.6), achieved significantly worse scores compared to the cognitively normal controls, (mean score 25.1). Similar results were also reported by Graham et al., (2004) and Thompson et al., (2005).

MCI participants have also shown impaired performance on the GNT suggesting that a semantic deficit occurs early in the disease process (Chan et

al., 1997). Dudas et al., (2005) investigated MCI, AD and cognitively normal participants. Although some of the MCI participants performed normally on the MMSE they were significantly worse than the controls, but better than the AD patients, on the GNT. Likewise, Ahmed et al., (2008) reported poorer performance on the GNT by 32 MCI participants, (mean score 19.7), compared to 37 cognitively normal older adults, (mean score 26.1).

Blackwell et al., (2004) demonstrated in a regression analysis that the number of errors made at the six pattern stage of the CANTAB PAL, the score obtained on the GNT and the subjects age at the time of testing could identify converters to AD with 100% accuracy within a 32 month period using a sample of 40 patients. The results of all of these studies imply that the GNT may, therefore, also be a useful test to be used in the detection of early AD cases.

4.5 The Trail Making Test (TMT)

The TMT (Reitan and Davison, 1974) is a commonly used neuropsychological instrument which assesses multiple cognitive processes (Lezak et al., 2004). The test comprises two parts, the first section, referred to as Trails A or the TMT part A, requires the subject to join a series of numbered circles in increasing numerical order as quickly as possible. This part of the test assesses visual search (O'Rourke et al, 2011) and processing speed. Part B of the test, referred to as Trails B or TMT part B, requires the subject to join a sequence of circles containing numbers and letters, alternating between numbers and letters, in increasing numerical/alphabetical order. This part of the assessment is a measure of EF, particularly visual search and set switching (O'Rourke et al., 2011). The most commonly used scoring system is the time taken to complete each part in seconds (Ashendorf et al., 2008). Other scoring methods which take into account the number of errors, ratio scores (Giovagnoli et al., 1996) and the difference in score between part A and B have also been investigated (Arbuthnott and Frank, 2000). Arbuthnott and Frank (2000) stated that subtraction of the time to complete part A from part B removes the motor component of TMT part A and gives a better index of EF. The time to completion still remains the most common scoring method, as even cognitively

normal participants are known to make errors on this test (Ashendorf et al., 2008).

Some studies have noted main effects of age (Coffey et al., 2001; Tombaugh, 2004) and education (Horton and Roberts, 2001) on this assessment. Hammar and Ardal (2009) found significant effects of depression on TMT score, whereby, depressed individuals took longer to complete both parts of the test. This is an important consideration for AD research as depression is a common co morbid condition in both MCI and AD (Overshott and Burns, 2004). Symptoms of depression can present as a cognitive impairment, highlighting the need to check for depressive symptoms before the interpretation of neuropsychological test scores.

Research has shown that the score on TMT part B may be useful in predicting the likelihood of progression from aMCI to AD. Chen et al., (2000) reported that TMT part B was sensitive to group differences of pre symptomatic AD patients and healthy controls. Further, a combination of delayed word recall scores and TMT part B score were the optimal set of cognitive measures used to predict conversion. Likewise, Ewers et al., (2012) reported that the TMT part B has significant value for the prediction of AD development in MCI cases. In their sample of 81 AD patients and 101 cognitively normal elderly individuals, TMT part B score was one of the best single predictors of subsequent conversion to AD. Similar findings were also found by Samtani et al., (2012).

Brown et al., (2011) found that the processing speed component of TMT part A correlated with greater functional impairment in 394 aMCI participants in comparison with 229 cognitively normal participants and 193 mild AD patients. From their study of 42 MCI participants, Chapman et al., (2011), suggest that measures of speeded EF, such as the TMT part B, may serve as an early detection tool as they are sensitive to conversion from MCI to AD. Impairments in rapid switching between sets may be useful as a potential screening method.

Scores recorded for the purpose of the experiments of this thesis will be the time in seconds taken to complete each part of the test.

4.6 Dual Tasks (DT)

The DT is a pen and paper test of EF. Participants are given an A4 piece of paper depicting a string of connected boxes and are first timed for one minute to put a cross in each box following the direction of the string (Baddeley et al., 1997). Participants then repeat the task but are simultaneously required to remember and repeat strings of numbers based on their digit span score (see section 4.3.7). They are, therefore, required to place a cross in each box at the same time as repeating aloud strings of digits. The scores used in the evaluation of test performance are the difference in the number of boxes crossed between the first and second times (DT T1-T2) and the dual task ratio (DTR) which is calculated as the ratio of boxes crossed to the number of digit strings remembered.

Generally it is expected that performance will decline when two tasks are performed simultaneously due to the larger cognitive demand and the requirement of dividing attention between the two tasks (Nebes et al., 2001). Baddeley et al., (1986) and (1991) stated that in AD patients there is a clear deficit when completing two tasks simultaneously, an effect which is not common in normal aging (Della Sala and Logie, 2001). This is likely to be a result of a deficit in the central executive component of working memory (Baddeley et al., 1991). This would suggest that both AD and MCI participants would be impaired on the DT (Lonie et al., 2009). There are conflicting results surrounding the time of onset of divided attention impairments in AD. Both Baddeley et al., (2001) and Logie et al., (2004) report impaired DT performance in AD patients, even in the early stages of the disease. Furthermore, Baddeley et al., (1991) report that the size of the dual task deficit increases as the disease progresses. Lonie et al., (2009), however, report that in aMCI, there is no impairment of dual task performance in comparison to healthy non demented elderly participants. Likewise, there was no impairment in early AD participants either. Early AD patients were, however, impaired on the TMT part B, an alternative assessment of divided attention which has a speeded component (discussed in the previous section 4.3.3). Further, Greene et al., (1995) reported that dual task impairment occurs only in the later stages of AD when MMSE score falls below 23 out of 30.

The discrepancies between these studies may be due to slight differences in the methodology employed. For example, differences in the time used to complete each stage, Lonie et al., (2009) used a total time of 90 seconds whereas Baddeley et al., (2001) used a longer duration of 120 seconds. It is difficult to make direct comparisons between the data sets. There may have been differences in disease severity between participants groups. The results of these studies, therefore, suggest that the DT is not sufficiently sensitive for the detection of early AD (Lonie et al., 2009) but maybe useful alongside another assessment.

4.7 Stroop Test

The Stroop test was devised in 1935 by Stroop and assesses both EF and processing speed. The Stroop consists of a list of colour words, some of which are presented in an incongruent colour (Trenerry et al., 1989). Participants are given 30 seconds to read down each column of words, first stating the word as the text appears, this stage of the task is referred to throughout this thesis as the 'Stroop Word' and assesses processing speed. Secondly the participant is timed for a further 30 seconds and is required to read aloud the colour of the ink the word is presented in ignoring the semantics of the written word. This is referred to throughout the thesis as the 'Stroop Interference' and this stage involves inhibition of the irrelevant material and tests EF. Subjects are generally slower at the second phase of the test and multiple explanations for this slowing have been suggested such as the creation of a response conflict, failure of response inhibition or failure of selective attention (Lezak, 1995).

Imaging studies have revealed activation of the PFC during completion of the Stroop test with specific activation of the lateral PFC and the anterior cingulate cortex (De Pisapia and Braver, 2006). These areas have previously been implicated during inhibitory tasks of EF (Schroeter et al., 2012).

Previously, a larger interference effect on the Stroop test has been noted in early AD patients relative to the cognitively normal HE. AD patients took longer to complete the second phase of the Stroop and made more errors (Spieler et al., 1996). Hutchinson et al., (2010) replicated these findings in a sample of 64

HE participants and 32 mild AD cases. Duchek et al., (2009) investigated 291 elderly participants and noted more errors in the incongruent trial for those classified as mild AD. These findings suggest that the Stroop test may be useful as an early cognitive assessment for the detection of mild cognitive changes.

Chapman et al., (2010) investigated a group of 43 MCI patients, 14 of which went on to convert to a probable AD diagnosis. Using baseline assessment scores on a battery of tests, the authors investigated which tests were the best predictors of likely conversion. After memory, the second best predictor of conversion was speeded EF performance as measured by the Stroop and the TMT. Convertors were worse at inhibiting irrelevant stimuli and, therefore, had difficulty completing the second stage of the Stroop. The results of this study support the notion that scores on the Stroop may be a useful indicator of the probability of conversion to AD.

In some instances participants have reported that they find this assessment uncomfortable and poor concentration can be detrimental to Stroop performance. Further, visual competence is required and individuals who are colour blind will be unable to complete the task (Lezak, 1995).

4.8 Symbol Digit Modalities Task (SDMT)

The SDMT was devised by Smith in 1968 as an assessment to detect general neuropsychological dysfunction. It is now a popular assessment of choice in multiple medical fields and is minimally affected by education level and language abilities (Lopez et al., 2008). The SDMT involves multiple cognitive strategies such as processing speed, attention, concentration, visual scanning and EF (González et al., 2007).

The test requires the participant to complete a grid using a key of symbols and corresponding numbers (Smith, 1968). After a practice, the participant is given 90 seconds to work through the grid using the key to write the correct number underneath each corresponding symbol. The number of errors and the total number of correctly matched symbol number pairs are noted. A higher score

signifies a better performance. The test can also be completed orally if the participant is unable to complete the written version (Lezak, 1995).

An early study by Pfeffer et al., (1982) [noted in Spreen and Strauss (1998)] found SDMT score to be a better assessment over the MMSE for the discrimination of cognitively normal and abnormal elderly subjects. Richardson and Marottoli (1996) investigated elderly drivers with differing levels of education. They reported that in those elderly with over 12 years of education, aged between 76-80 years, the mean SDMT score was 32.75 and for those aged 81-91, the mean score was 28.84. The results demonstrated that increasing age was associated with poorer performance which is likely to be due to impaired processing speed and EF with age. This is in agreement with Bowler et al., (1992) and Feinstein et al., (1994) who also report that score on the SDMT declines with advancing age. In a Hispanic sample of 106 mild to moderate AD patients, Lopez et al., (2008) revealed a significant increase in SDMT score following 12 weeks treatment with Donepezil highlighting that this test is useful for the monitoring of cognition during drug intervention.

4.9 Digit Span and Digit Score

The digit span assessment is a component of the Wechsler adult intelligence scale revised (WAIS-R) (Wechsler, 1981). Digit span assesses working memory and includes both digit span forwards and digit span backwards. In the forward stage of the task a series of numbers are read aloud to the participant starting with a sequence length of three numbers, increasing to a sequence of nine. Numbers are read aloud by the researcher at a rate of one word per second (Lezak, 1995). The test ends when the subject is unable to recall two sets of a certain length in the same order they were stated by the researcher. For example, if the researcher states 592 the subject repeats 592. In backward span the subject must repeat the sequence of digits in the reverse order, e.g. if the researcher states 592 the subject states 295. The digit span score is the number of digits in this last set. Digit Score is the total number of strings correctly repeated.

Previous research has shown impaired digit span in AD patients relative to non-demented aged matched controls, e.g. Belleville et al., (1996) and Cherry et al., (1996). AD patients are, however, better at digit recall than word recall (Cherry et al., 2002). This is possibly because digit words are shorter, perhaps easier to remember and easier to organise in short term memory. Words in a recall list may be unfamiliar to the subject, longer in length and may place a larger demand on short term space. Cherry et al., (2002) investigated 35 mild to moderate probable AD patients and 38 non demented older adults. The non demented adults were significantly better at digits span forwards and backwards compared to the AD patients. The mean digits forward score for AD patients was 5.1, compared to 7.1 for the control group. AD patients also performed worse at digit span backwards, (mean score 2.6), compared with the control group (mean score 5.7). Better preservation of digit forward ability is likely to be due to the lower demand on long term stores which are deficient in AD.

4.10 Letter Comparison Speed (LCS) and Pattern Comparison Speed (PCS)

The LCS and PCS are assessments of processing speed (Salthouse and Babcock, 1991). In the LCS task participants are asked to state whether two strings of letters are the same or different. The letters are presented in strings of three, six, or nine letters and participants have 20 seconds to complete as many pairs as possible. PCS also assesses processing speed (Salthouse and Babcock, 1991) and participants are required to state whether two geometric shapes (patterns) are the same or different. They are timed for 20 seconds and are required to compare as many pairs as possible.

Both of these assessments involve multiple cognitive processes such as a visual component, short-term memory, the ability to visually compare two stimuli, motor speed and response selection (McCabe and Hartman, 2008). McCabe and Hartman (2008) showed that older adults, aged between 61-81 years, performed worse on both of these tasks in comparison to younger adults aged 18-27 years. Older adults received lower scores which may be due to decrements in processing speed with age but may also be due to impairments

in short term memory. This is in agreement with Salthouse and Babcock, (1991) who also note that increasing age leads to progressive declines in ability.

Lustig et al., (2006) used the LCS and PCS to investigate processing speed in young and older adults. In general, older adults are perceived to be poorer at speeded tasks and this may be due to their inability to ignore distracting stimuli. The pen and paper versions of the PCS and the LCS are deemed to have a high level of distractibility as there are many strings/patterns presented on a single page, therefore, in order to successfully complete the pattern/letter pairs, the subject must ignore the other distracting stimuli above and below. For the LCS, Lustig et al., (2006) reported higher error rates in both groups of subjects as string length increased. For the PCS, however, older adults performed significantly worse than younger adults. It is unclear whether this is due to impaired spatial comparison ability with age or whether older adults are generally slower when assessing and comparing two shapes. AD patients would be expected to be significantly worse at both of these tasks, however, there is little research investigating the use of these tests specifically in MCI or AD sub populations.

4.11 Word recall

Word recall assesses short term memory and there are a number of existing tests available. Due to financial constraints, for the purpose of this thesis a word recall test was created by the researcher using the MRC Psycholinguistics database to control for word length, frequency and imaginability (Coltheart, 1981).

Two of the most widely used word recall assessments are the California Verbal Learning Test (II) (CVLT II) (Delis et al., 2000) and the Hopkins Verbal Learning Test (HVLT) (Brandt, 1991). The CVLT II is an assessment of verbal learning and memory which involves a multi trial approach of list learning (Strauss et al, 2006). The original version was developed by Delis et al., (1987) but has since been improved. The CVLT II can be administered to a large age range of participants between 16-89 years but has been shown to be affected by age, gender and education (Strauss et al., 2006). For the first five trials of the

assessment the participant is required to immediately recall words from list A which consists of 16 words belonging to four separate semantic categories: vegetables, means of transport, animals and furniture. Following this 16 interference words from list B are introduced. There is then a 20 minute delay before participants are required to recall words from list A. A shorter version also exists for those with a severe cognitive impairment. The administration time is approximately 50 minutes and the test is scored by hand although software is available to record responses (Strauss et al., 2006).

The Hopkins Verbal Learning Test – Revised (HVLT-R) is a brief assessment of learning and memory (Brandt and Benedict, 2001). It can be administered to participants aged 16-80+ years and involves six alternate forms of 12 nouns belonging to three semantic concepts. Three learning trials are followed by a delayed recall trial (which is given without forewarning after 20 minutes). There is then a yes/no recognition trial. The delayed recall trial was included in the revised version and was absent from the original (Brandt, 1991). Delayed recall is believed to be more sensitive to MCI and early AD and is also able to predict further cognitive decline (Dubois et al., 2007; Chapman et al., 2011). Age, gender and education effects have been noted on this assessment (Strauss et al., 2006). Brandt and Benedict, (2001) noted impaired performance on the HVLT R in AD patients who made more false positive recognitions. Likewise, Hogervorst et al., (2002) noted that the combined HVLT score was more sensitive as a screen for dementia than the MMSE and was less likely to have a ceiling effect.

AD patients perform poorly on word recall tasks due to deficits in short term memory. As discussed in section 4.2, both the MMSE and the MoCA incorporate a word recall item but the MoCA involves a delayed word recall question (Nasreddine et al., 2005). Delayed recall has been shown to be more sensitive to AD and MCI (Dubois et al., 2007). AD patients struggle to complete word recall assessments. For example, Cherry et al., (2002) investigated 35 probable AD patients and 38 non demented HE participants using a ten item simple word recall task. The probable AD patients recalled significantly less words than the control group with a mean number of words recalled of 1.7, compared to 5.7 words, recalled by the control group. This is in agreement with other studies such as Teng et al., (1989).

For the purpose of this thesis an immediate word recall test was created. This test required the participant to immediately recall as many words as possible from a list of 15 words read aloud by the researcher.

4.12 Chapter summary

This chapter has described and evaluated some common assessments used in the cognitive evaluation of AD and MCI. The onset of cognitive alterations is difficult to determine and the occurrence of an abnormality is not instantaneous but is the result of progressive deterioration (Zamrini et al., 2004). Neuropsychological tests need to be sensitive to early, subtle alterations in cognition if they are to be used as an early diagnostic tool for the detection of AD and MCI. Some cognitive tests, such as the CANTAB PAL, the MoCA and the TMT, have been shown to be sensitive to the detection of MCI and early AD disease stages.

Using a battery of tests which assess multiple cognitive domains is advantageous as brief tests of general cognition may not accurately reflect the participant's actual ability. Mapstone et al., (2003) comment that in its earliest stages MCI is more likely to present as an impairment within another cognitive domain aside from memory. This highlights the need for a thorough assessment taxing a variety of different cognitive domains at baseline.

Neuropsychological testing now includes some computerized assessments such as the CANTAB battery. Traditional pen and paper tests are still widely used and are still valuable. There are strengths and weaknesses associated with using both these types of tests. For example, using a computerized assessment can improve the accuracy of the recording of the participant's responses, especially when recording parameters such as reaction time (Wild et al., 2008). There is also improved noting of errors and less reliance on the opinion of the administrator. Another advantage is the potential cost saving as some computerized assessments do not need a researcher present and are self explanatory. Pen and paper tests are often favoured by the elderly as some older subjects do not have prior computer experience and may feel anxious about completing a computerised test. This may impact on their performance. Tests which require the participant to write a response will require the

participant to be competent at performing a motor response. Computer based assessments could also be useful in the implementation of early screening for AD as they can be quickly applied to large populations (Wild et al., 2008). This would require access to computer facilities and the participant's prior knowledge and use of computers is, therefore, an important factor to consider.

When interpreting test scores there are external factors which need to be considered and this is the main focus of this thesis. An abnormal test score on one occasion does not necessarily signify the presence of underlying disease. The participant's mood, current medication or level of tiredness may affect their performance (Jacova et al., 2007). Some participants may be particularly nervous or anxious which may affect their ability (Zamrini et al., 2004). As briefly mentioned in chapter two, education level can affect score on some assessments. The MoCA accounts for this and awards subjects with an additional point if their education level is less than 12 years (Nasreddine et al., 2005). It is necessary to note the participant's education in years and to take this into consideration when interpreting test scores. Prior exposure to tests is also a factor when longitudinal or repeated testing is used (Zamrini et al., 2004) as practice effects may occur (Blatter and Cajochen, 2007). Age is the major risk factor for the development of a cognitive impairment and, therefore, is a factor which influences performance. Tests of processing speed such as the PCS and the Dual Tasks are affected by age. Age related deficits are common but abnormal scores are exaggerated compared to relative age groups.

Neuropsychological testing is not recommended for a routine work up/diagnosis by NICE. Testing is useful for research trials and for participants whose predominant impairment may not be memory. Neuropsychological testing is useful for categorising MCI and also for the prediction of whether a participant is likely to decline and at what rate (Jacova et al., 2007). The clear advantage of neuropsychological testing is its ability to test a wide range of cognitive domains. The scores obtained can, therefore, be useful in the evaluation and management of treatment and some care based decisions. Table two summarises the tests mentioned in this chapter and the cognitive domains they assess.

Table two: summary of neuropsychological tests by cognitive domain

Cognitive Domain	Test name
General Cognitive Function	MMSE
	MoCA
	GPCOG
	7 Minute Screen
Processing Speed	Stroop Word
	PCS
	LCS
	TMT part A
	SDMT
Executive Function	SDMT
	TMT part B
	Stroop Interference
	Dual Tasks Ratio
	Dual Tasks T1-T2
Semantic Memory	GNT
Short Term Memory	Word Recall
Working Memory	Digit Span and Digit Score
Visuo spatial associative Memory	PAL
Visual Search	TMT
Attention	SDMT

Chapter 5

The effect of prior caffeine on neuropsychological testing in the elderly

5.1 Introduction

The accuracy of neuropsychological testing is vital if a diagnosis is to occur earlier in the disease pathway when symptoms are mild. Neuropsychological testing is a useful diagnostic tool but performance on some tests can be affected by external factors, which if not controlled for or taken into consideration may affect the overall outcome, particularly as scores are often compared to population norms (Lesk et al., 2009). This has the potential to affect test accuracy and the diagnosis given. Such factors can include the patient's mood at the time of testing, age, tiredness and the presence of certain medications or foodstuffs (Jacova et al., 2007).

This chapter explores whether caffeine, one of the world's most commonly consumed stimulants (Nawrot et al., 2003; Butt and Sultan, 2011; Nehlig, 2010), can affect cognitive performance in the elderly. Caffeine is present in a number of commonly consumed foodstuffs such as tea, coffee, soft drinks, chocolate, sweets and also some medications (Nehlig, 2010). Caffeine possesses many different cerebral mechanisms of action and has been shown to minimise and counteract AD associated pathology and processes. Examples include its ability to decrease amyloid plaque load and A β levels (Zeitlin et al., 2011), the ability to inhibit both β and γ secretase (Arendash et al., 2006), decrease the expression of pro inflammatory markers (Cao et al., 2009), and down regulate pro apoptotic signalling factors (Zeitlan et al., 2011) amongst others. As a result of these findings, a number of studies have investigated whether caffeine consumption (in the form of caffeinated foodstuffs) throughout adulthood can affect the overall risk of developing AD in old age (Ritchie et al., 2007; Eskelinen et al., 2009; Lindsay et al., 2002; Van Gelder et al., 2007; Santos et al., 2010).

To date there has been little study into the affect of caffeine on neuropsychological test performance at the actual time that these assessments

are administered. Consequently this chapter explores the effect of 200mg of pure caffeine on some of the neuropsychological tests used in the diagnosis of AD and cognitive impairment.

5.1.1 Caffeine

Caffeine is present in many commonly consumed food stuffs such as tea, coffee, soft drinks, cocoa, sweets and medicines (Nehlig, 2010; Nawrot et al., 2003; Butt and Sultan, 2011). A survey investigating caffeine consumption revealed that 70% of caffeine intake is derived primarily from coffee drinking (Frary et al., 2005). Globally daily habitual caffeine consumption in humans is estimated to fall between 70-350mg/person/day, which is approximately the equivalent of three cups of coffee (Chen et al., 2010). Habitual caffeine consumption does vary with location and culture. In the UK caffeine consumption is estimated to be approximately 4mg/kg of body weight whereas in Denmark consumption is higher at 7mg/kg of body weight (Nawrot et al., 2003). At these concentrations positive effects of caffeine are noted such as an increase in alertness, decreased feelings of subjective tiredness, improved attention and concentration, improved problem solving and faster reaction times (Glade, 2010). In doses exceeding 400mg there is a larger likelihood of side effects which can include anxiety, raised blood pressure, headaches, confusion, tachycardia and gastrointestinal disturbance (Chen et al., 2010; Sweetman, 2009).

5.1.2 Pharmacology and mechanism of action

Chemically caffeine is known as 1, 3, 7 trimethylxanthine and is a non selective adenosine receptor antagonist (Cunha et al., 1995; Constenla et al., 2010). Adenosine acts as a neuromodulator and initiates and controls multiple synaptic functions including the release of other neurotransmitters (Gomes et al., 2011; Rahman, 2009). There are four adenosine receptor subtypes expressed within the brain, including the high affinity A_{1R} and A_{2AR} receptors and the low affinity A_{2B} receptor (Boison, 2008). There is a low abundance of the A_3 receptor and this receptor is not widely expressed (Boison, 2008). A_{1R} receptors have the

highest expression in the cortex, cerebellum and hippocampus (Ribeiro et al., 2002). The A_{2AR} receptors are less widely distributed in these areas but are predominantly found in the olfactory bulb and on striato-pallidal GABAergic neurons (Ribeiro et al., 2002).

Adenosine's predominant action is to decrease excitatory transmission via A_{1R} receptors (Dunwiddie and Haas, 1985) and both the A_{1R} and A_{2AR} control the release of other neurotransmitters including Ach which is depleted in AD (Gomes et al., 2011). Animal studies have shown that adenosine receptors undergo adaptive changes during aging resulting in a decrease in A_{1R} receptor density but an increase in A_{2AR} density in limbic cortex (Cunha et al., 1995, Cunha, 2001). In AD, alterations in the expression of adenosine receptors have also been noted (Cunha and Agostinho, 2010; Gomes et al., 2011). A post-mortem study revealed that there were increased numbers and densities of the A_{1R} and A_{2AR} in the frontal cortex of early AD patients (Albasanz et al., 2008) as well as a redistribution of these receptors particularly in the hippocampus and cortex (Angulo et al., 2003). This pattern was not seen in control samples.

The binding of caffeine to adenosine receptors results in the indirect stimulation of cholinergic transmission in the MTL, specifically the hippocampus, the area prominently affected by AD pathology (Dall'igna et al., 2004). Further supporting the role of caffeine in the modulation of cholinergic transmission, in a group of healthy young adults aged 25-35 years of age, consumption of three cups of coffee was shown to be able to inhibit the effects of scopolamine, an Ach receptor antagonist (Riedel et al., 1995). This suggested that when there is CNS dysfunction (mimicked in this experiment by scopolamine administration) caffeine acts as a cognitive enhancer rather than a stimulant.

The use of adenosine receptor knock out (KO) mice has also provided insight into the functioning of adenosine receptors during caffeine administration. KO A_{2AR} mice, which lack the A_{2AR} receptor, do not show the usual behavioural effects of caffeine administration such as an increase in psychomotor activity, suggesting a role for this particular adenosine receptor and caffeine selectivity (Chen et al, 2010). Furthermore, KO A_{2AR} mice show impaired learning and memory, and mice with an over expression of this receptor perform poorly on

tasks such as the Morris Water Task (MWT) (Gimenez-Llort et al., 2007) which assesses spatial memory and requires the animal to successfully locate a raised escape platform in an opaque water pool (Morris et al., 1982).

5.1.3 Caffeine and risk of AD

Due to caffeine's variety of neuro modulatory and antioxidant properties, there have been a number of longitudinal studies which have investigated whether caffeine consumption over time can impact on the risk of developing AD (Camouse et al., 2005). Some studies have investigated the effects of caffeine containing food stuffs (CCFS) particularly coffee, on the risk of developing AD. For example, Jarvis (1993), utilising a large sample of 9003 participants aged 18 and over revealed that increased coffee consumption was associated with better performance on assessments of reaction time, verbal memory and visuospatial reasoning. The Canadian Study of Health and Aging (CSHA) investigated a large cohort of 6343 individuals over 65 years of age via the use of a risk factor questionnaire. This was designed to gather information on a number of factors which may be linked to the development of cognitive impairment. The questionnaire asked participants about their sociodemographic characteristics, occupational and environmental exposures, lifestyle habits including smoking, alcohol consumption, intake of specific foods (including tea and coffee) and exercise levels. The questionnaire also asked participants about their family and medical history specifically noting any occurrence of head injury or loss of consciousness. After a five year follow up period, the study reported that daily coffee drinking was associated with a decreased risk of developing AD (Lindsay et al., 2002). The Cardiovascular risk factors, Aging and Dementia (CAIDE) study, recruited 1409 participants and reported that consumption of 3-5 cups of coffee per day in midlife was associated with a decreased risk of developing AD by 64% (Eskelinen et al., 2009). The Finland, Italy and the Netherlands Elderly (FINE) study showed that elderly men consuming an average of three cups of coffee per day experienced the lowest decline in cognition in comparison to non-consumers (Eskelinen and Kivipelto, 2010). Interestingly Ritchie et al., (2007) found that drinking three

cups of coffee a day was associated with a smaller decline in verbal cognitive fluency in women only.

Although these studies are useful for investigating risk over time they rely upon the self-report of caffeine consumption which does not take into account the variance in dose or the preparation method used. This may be subject to inaccurate estimation of consumption.

5.1.4 Caffeine and cognitive testing

There has been little investigation into whether immediate prior caffeine consumption at the time of testing can have an effect on neuropsychological test scores during aging. If caffeine does elicit an effect this could have implications for the diagnostic process of AD and the interpretation of neuropsychological data. Individuals who have consumed caffeine prior to being tested may perform differently than on an occasion when they have not. As a result they may score differently which has the potential to affect their diagnosis.

Studies such as Swift and Tiplady (1988) administered 200mg of pure caffeine to a small number of elderly participants and reported improvements in cognition specifically for tasks of attention. However, Lorist et al., (1995) reported no significant differences in cognition between elderly (mean age 64.5 years) and young participants (mean age 21.1 years) after the administration of 250mg of caffeine dissolved in decaffeinated coffee, 90 minutes prior to the neuropsychological assessment. Lesk and Womble (2004) reported strong interactions between the prior consumption of 200mg of caffeine or placebo and phonological priming on the tip-of-the-tongue state in both young participants (Lesk and Womble, 2004) and in a patient with anomic aphasia (Lesk et al., 2007). Given this striking effect of prior caffeine intake on this paradigm Lesk et al., (2009) then investigated prior caffeine intake on neuropsychological test scores in the elderly. This study used a questionnaire to investigate participants' caffeine consumption by simply asking participants about their intake of CCFS (tea, coffee, chocolate and cola), four hours before the testing session. An interaction effect of age and intake of CCFS prior to the

neuropsychological assessment was found on six of the nine assessments used whereby the older elderly (over approximately the age of 80) performed significantly worse if they had consumed CCFS and the younger elderly performed significantly better if they had consumed CCFS. Significant interactions were specifically reported for three assessments of processing speed, PCS (Salthouse and Babcock, 1991), LCS (Salthouse and Babcock, 1991) and the Stroop word (Trenerry et al., 1989), one assessment of semantic memory, the GNT, (McKenna and Warrington, 1980), one assessment of visuospatial associative memory, PAL 6 pattern error score, (Sahakian et al., 1988), one assessment of episodic memory, The Placing Test (TPT), (Anderson et al., 2006) and one assessment of EF, the SDMT total score (Smith, 1968). There were no reported significant interactions for global cognitive function [MMSE, (Folstein et al., 1975)], working memory [digit span and digit score (Wechsler, 1981)], DT T1-T2 or the DTR (Baddeley et al., 1997), which along with the SDMT (which did show significance), is a test of EF (Smith, 1968). There was no main effect of CCFS present as compared to the CCFS absent group on any of the assessments.

The results of Lesk et al's., (2009) study have important implications for the neuropsychological testing process. Another consideration that remains unclear is whether it is the caffeine contained in these foodstuffs which has caused the interaction effect or whether it is because of any of the other substances contained within CCFS. For example, coffee also contains chlorogenic acid, cafestol and kahweol (Eskelinen and Kivipelto, 2010) which are powerful antioxidants (Butt and Sultan, 2011). Furthermore, tea is concentrated in polyphenols which have also been shown to enhance memory (Lindsay et al., 2002) although some studies have reported non-significant effects for tea drinking and overall AD risk (Lindsay et al., 2002; Eskelinen et al., 2009). The preparation method of caffeinated beverages should also be considered as this can influence the final amount of caffeine contained within the drink (Eskelinen and Kivipelto, 2010). For example, brewed coffee contains the highest amounts of caffeine (56-100mg per 100ml) whereas instant coffee and tea contain significantly less (20-73mg) and are dependent on the amount of spoonfuls that are added to each drink (Nawrot et al., 2003). The size of the

drink should also be noted and controlled for in experimental investigations (Stavric et al., 1988).

5.2 The present study: research aims

Given that caffeine is present in all of the CCFS consumed in the Lesk et al., (2009) study, this chapter will specifically investigate the influence of 200mg of pure caffeine on the neuropsychological assessment. In Lesk et al., (2009) the average amount of caffeine intake was calculated to be 70.3mg. The dose of caffeine to be administered in this study is 200mg which is the equivalent of one medium black filter coffee (Starbucks, 2011). This dose is considered to be safe for adults (Nawrot et al., 2003) and similar doses have been used elsewhere in the elderly (Hogervorst et al., 1998; Lorist et al., 1995; Carrier et al., 2009).

If caffeine is able to affect neuropsychological test scores, then by simply asking a participant to abstain from caffeine, or to at least note how much caffeine they have consumed, will allow for more accurate results and clinical interpretation. This chapter extends upon the findings of Lesk et al., (2009) and will investigate whether it is the caffeine contained within CCFS which affects cognitive scores and interacts with age (as seen in Lesk et al., 2009) or one of the other ingredients by specifically investigating the effect of pure caffeine intake prior to the neuropsychological assessment in a group of elderly participants.

5.3 Methods

5.3.1 Participants

Forty participants from the University of Bradford, Division of Psychology, over-60s participant pool were recruited to take part in the study. Ethical approval was obtained from the University of Bradford Research Ethics Committee and all participants gave their informed written consent to take part. The mean age of participants was 73.40 years (\pm 6.55 years) with a mean number of years spent in education of 15.53 years (\pm 3.93 years). The sample consisted of nine males and 31 females.

The inclusion criteria specified that participants must be at least 60 years of age and must not possess a current neurological medical condition or an existing diagnosis of a memory impairment. In order to avoid any adverse reactions to caffeine, participants were required to be regular caffeine consumers with a habitual consumption of between 1-3 cups of coffee per day. Excessive consumers, (defined in this study as a habitual consumption of four or more cups of coffee per day - approximately 400mg of caffeine) were not permitted to take part to reduce the likelihood of withdrawal effects occurring during the abstinence period. In addition, participants must not have been advised to avoid caffeine by their GP or health care professional and must be non-smokers as smoking increases the metabolism of caffeine (Stavric and Gilbert, 1990, Nawrot et al., 2003). Participants were required to be fluent in English and must not have a severe visual impairment.

5.3.2 Design

The study utilised a double blind caffeine vs. placebo experimental design and all the participants completed all of the neuropsychological tests in the same order.

5.3.3 Materials

Neuropsychological assessments

A detailed description of the neuropsychological assessments used in this chapter can be found in chapter four 'Neuropsychological Testing' however a brief description of each test is listed below.

MMSE - the MMSE is a test of general cognitive function assessing a variety of cognitive domains (Folstein et al., 1975). Participants receive a score out of 30.

Immediate word recall - an assessment of short-term memory, requiring the participant to recall as many words as possible from a list of 15 common nouns read aloud by the researcher. This test was created by the researcher using

the MRC Psycholinguistics database to control for word length, frequency and imaginability (Coltheart, 1981).

CANTAB PAL - an assessment of visuo spatial associative memory (Sahakian et al., 1988) which requires the participant to learn an association between a visual stimulus and a spatial location (Égérhazi et al., 2007).

GNT - an assessment of semantic memory (McKenna and Warrington, 1980) which requires the participant to name 30 objects presented in black and white on a computer screen.

SDMT - an assessment of EF which requires the participant to complete a grid using a key of symbols and corresponding numbers (Smith, 1968). After a practice, the participant is given 90 seconds to work through the grid using the key and writing the correct number underneath each corresponding symbol. The number of errors and the total number of correctly matched symbol number pairs are noted.

Stroop Test - this test assesses both EF and processing speed. The Stroop consists of a list of colour words which are all presented in an incongruent colour (Trenerri et al., 1989). Participants are first given 30 seconds to read down each column of words stating the word as the text appears. Secondly they are timed for 30 seconds and are required to read the colour of the ink the word is presented in ignoring the semantics of the written word.

PCS - this test assesses processing speed (Salthouse and Babcock, 1991). Participants are asked to state whether two shapes (patterns) are the same or different. They are timed for 20 seconds and are required to compare as many pairs as possible.

LCS - this is an assessment of processing speed (Salthouse and Babcock, 1991), where participants are asked to state whether two strings of letters are the same or different. The letters are in strings of three, six, or nine letters and participants have 20 seconds to complete as many pairs as possible.

Digit Span and Digit Score - digit span assesses working memory (Wechsler, 1981). A series of numbers are read aloud to the participant starting with a sequence length of three numbers, increasing to a sequence of nine. The test

ends when the subject is unable to recall two sets of a certain length. The digit span is the number of digits in this last set. Digit score is the total number of strings correctly repeated.

DT - this is a test of EF where participants are given a page with a string of boxes and are required to put a cross in each box following the direction of the string (Baddeley et al., 1997). Participants are timed for 60 seconds. Participants then repeat the task but are simultaneously required to remember strings of numbers (based on their digit span score; see previous section). Scores used are the difference in the number of boxes crossed between the first and second times (DT T1-T2) and the DTR which is calculated as the ratio of boxes crossed to the number of digit strings remembered.

5.3.4 Procedure

Participants within the participant pool database were sent an invitation letter and information sheet via the post and were telephoned one week later to enquire if they would be interested in participating. Those who wanted to take part, and met the inclusion criteria, completed a brief telephone checklist to ensure they were regular caffeine consumers, had not been advised by a medical professional to avoid caffeine consumption, were non-smokers and consented that they were aware that they may receive 200mg of caffeine as a part of the study. Those individuals who successfully completed the checklist were given an appointment and told to abstain from caffeinated drinks and foodstuffs for 12 hours prior to the appointment and to abstain from alcohol for 24 hours prior to the appointed time. A confirmation letter outlining each of these points was then sent out to each participant.

On arrival to their appointment the researcher checked with each participant that they had remembered to abstain from caffeine for 12 hours prior and alcohol 24 hours prior. Participants were then administered a cup of peppermint tea which may or may not have contained 200mg of dissolved caffeine. The study was performed double blind and a research assistant prepared and administered the drink. Participants were then required to wait for a period of forty minutes before cognitive testing commenced (this was to

ensure that caffeine levels in the brain had plateaued which occurs at approximately 45 minutes after initial administration (Arnaud,1987; Smith, 2002). Participants were not permitted to consume any food or drink during this time period.

After 40 minutes, cognitive tests of MMSE, word recall, Stroop, PCS, LCS, digit span; DT, CANTAB PAL and GNT were then administered to each participant in the same order. Participants were then thanked for their time and told they may prefer to avoid consumption of caffeinated beverages in the following few hours to avoid any potential side effects of excess caffeine intake.

5.4 Results

The aim of the current study was to investigate whether 200mg of prior caffeine can effect scores on some neuropsychological tests used in the diagnosis of AD and MCI in a group of 40 HE participants over the age of 60 years. Table three shows the demographic characteristics of participants in the caffeine and placebo group. There were no significant differences between the participants in each group in terms of any of the listed demographics.

Table three: Demographics of participants randomly assigned to the caffeine and placebo groups

	Caffeine (n=20)	Placebo (n=20)
Age (mean ±SD)	73.25 (6.52)	73.45 (6.74)
Education (mean ±SD)	15.97 (3.59)	15.00 (4.32)
Males/females	5/15	4/16

There were no main effects of gender or education on any of the tests administered. There were significant main effects of age on the DT T1-T2 interval score and the Stroop Interference score. A regression analysis revealed a negative linear relationship on both of these tests, with scores decreasing as age increased. [DT T1-T2 [$\beta = -0.66$, ($F (1, 38) = 10.34$, $p < 0.01$), $\eta^2 = 0.77$, y-axis intercept = -43.13 with regression correlation coefficient $r = -0.45$, $p < 0.01$];

Stroop Interference [$\beta = -0.69$, ($F(1, 38) = 9.46$, $p < 0.01$), $\eta^2 = 0.66$, y-axis intercept = 78.53 with regression correlation coefficient $r = -0.45$, $p < 0.01$].

5.4.1 Main effects of caffeine vs. placebo

To investigate the effect of caffeine on the tests administered all of the tests were submitted to a multivariate analysis of variance (MANOVA) with all the test scores entered as the dependent variables and caffeine or placebo entered as a fixed factor. There was no main effect of drug group on any of the assessments used.

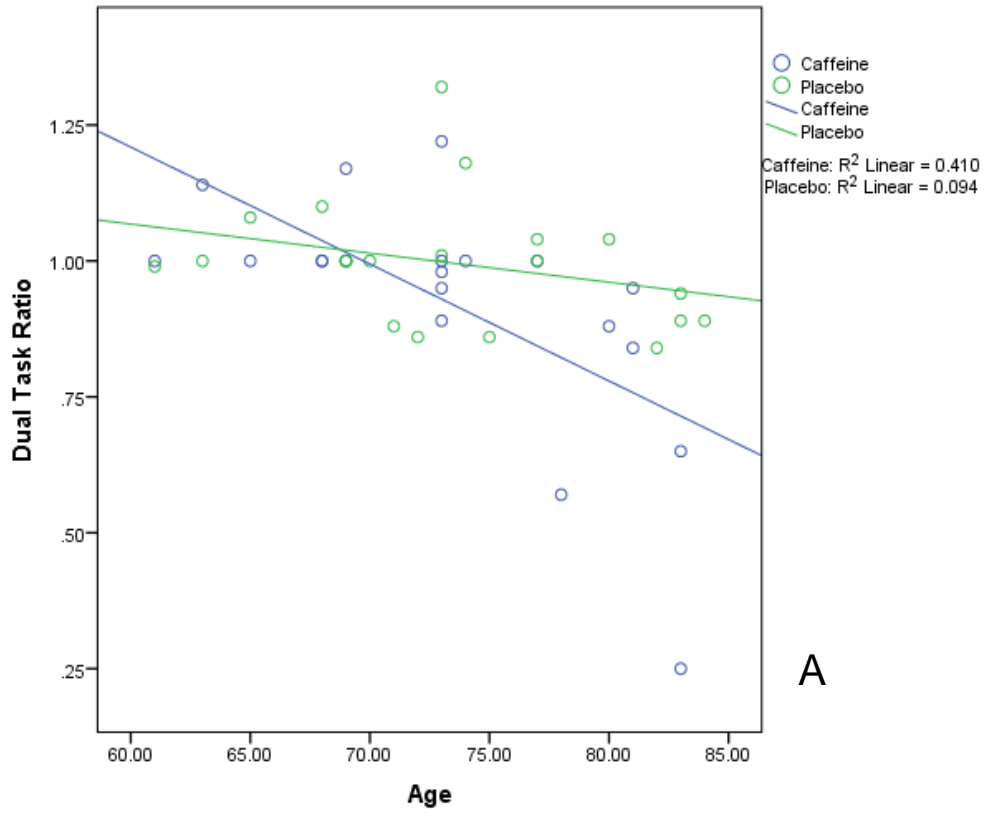
5.4.2 Age interactions

Following on from the results obtained by Lesk et al., (2009) which showed an interaction effect between caffeine, age and scores on the PCS, LCS, Stroop word, GNT, PAL and SDMT total, to formally assess whether prior caffeine consumption had an effect on performance with increasing age, each cognitive test was submitted to a univariate General Linear Model (GLM) with drug group (caffeine or placebo) as a fixed factor and age as a covariate. An interaction term was also included in the model. The results of this can be seen in table four. Figure nine depicts scatter plots of those test scores that showed a significant interaction between drug group and age.

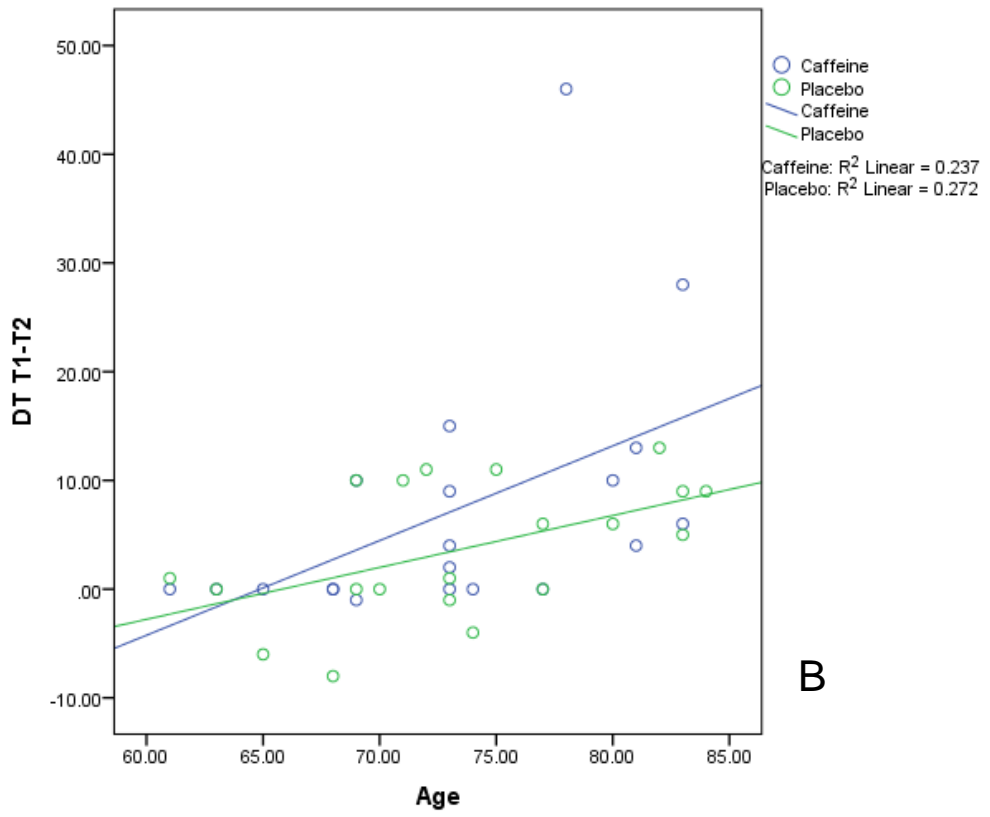
There is a clear interaction effect of drug group x age on the SDMT total score, the DTR and DT T1-T2 interval, the Stroop Interference, the PCS and the LCS. No significant interaction effect is present for the MMSE, digit span and digit score, the PAL, the GNT, word recall or the Stroop word test. There were no significant linear trends for the placebo group on any of the tests administered. Although there is no interaction between drug group and age for the GNT, a regression analysis did reveal a linear decrease in performance with age for those participants who received caffeine (see figure ten).

Table four: Univariate GLM results for the interactions between age and drug group and the outcomes of the linear regression analysis for each drug group separately.

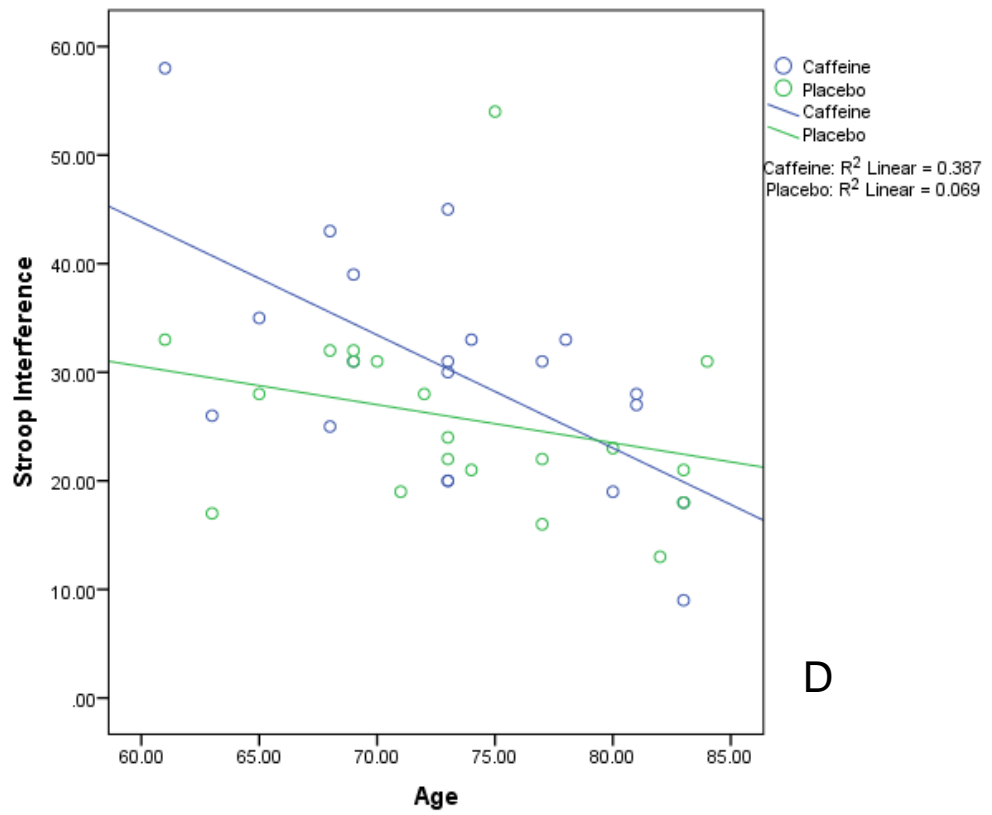
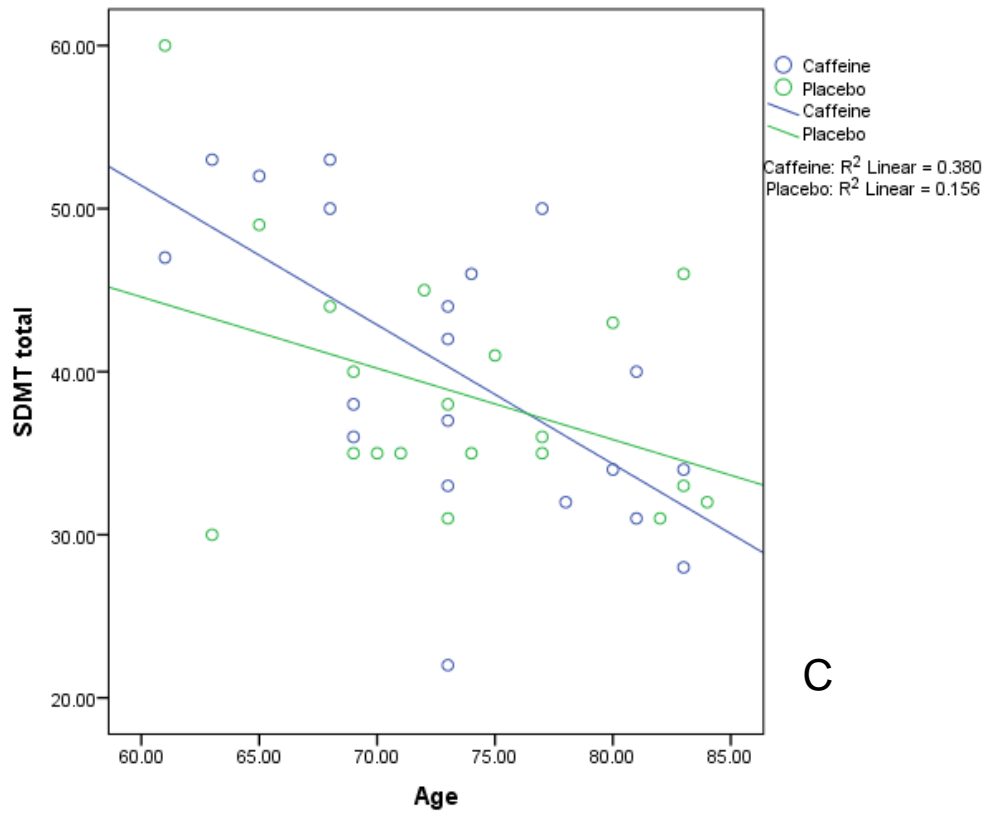
Testing Domain	Assessment	Caffeine	Placebo	P and η^2 value of interaction caff/plac x age	Caffeine regression	Placebo regression
General cognition	MMSE	28.75 \pm 1.21	28.40 \pm 0.94	N.S	N.S	N.S
Visuoassociative memory	CANTAB PAL 6 errors	7.35 \pm 6.40	7.90 \pm 6.67	N.S	N.S	N.S
Semantic memory	Graded Naming Test	26.30 \pm 2.98	25.65 \pm 2.64	N.S	r = 0.44, p = 0.05	N.S
Working memory	Digit Span	6.50 \pm 1.00	6.25 \pm 0.79	N.S	N.S	N.S
	Digit Score	8.95 \pm 1.82	8.60 \pm 1.64	N.S	N.S	N.S
Executive function	Dual Tasks T1-T2	7.30 \pm 11.66	3.65 \pm 6.17	f(2,37) = 6.51, p < 0.01, η^2 = 0.26	r = 0.49, p < 0.05	N.S
	Dual Tasks ratio	0.92 \pm 0.22	0.10 \pm 0.12	f(2,37) = 7.55, p < 0.01, η^2 = 0.29	r = 0.64, p < 0.05	N.S
	SDMT total correct	40.10 \pm 9.03	38.70 \pm 7.45	f(2,37) = 6.67, p < 0.01, η^2 = 0.27	r = 0.68, p < 0.01	N.S
	Stroop Interference	30.05 \pm 10.92	25.80 \pm 9.02	f(2,37) = 5.64, p < 0.01, η^2 = 0.23	r = 0.62, p < 0.01	N.S
Processing speed	PCS	25.25 \pm 3.96	22.45 \pm 4.94	f(2,37) = 3.65, p < 0.05, η^2 = 0.17	r = 0.56, p < 0.05	N.S
	LCS	15.35 \pm 3.72	12.80 \pm 2.95	f(2,37) = 6.91, p < 0.01, η^2 = 0.27	r = 0.58, p < 0.01	N.S
	Stroop word	54.55 \pm 12.61	50.15 \pm 9.99	N.S	N.S	N.S
Short term memory	Word Recall	5.35 \pm 1.76	4.80 \pm 1.44	N.S	N.S	N.S



A



B



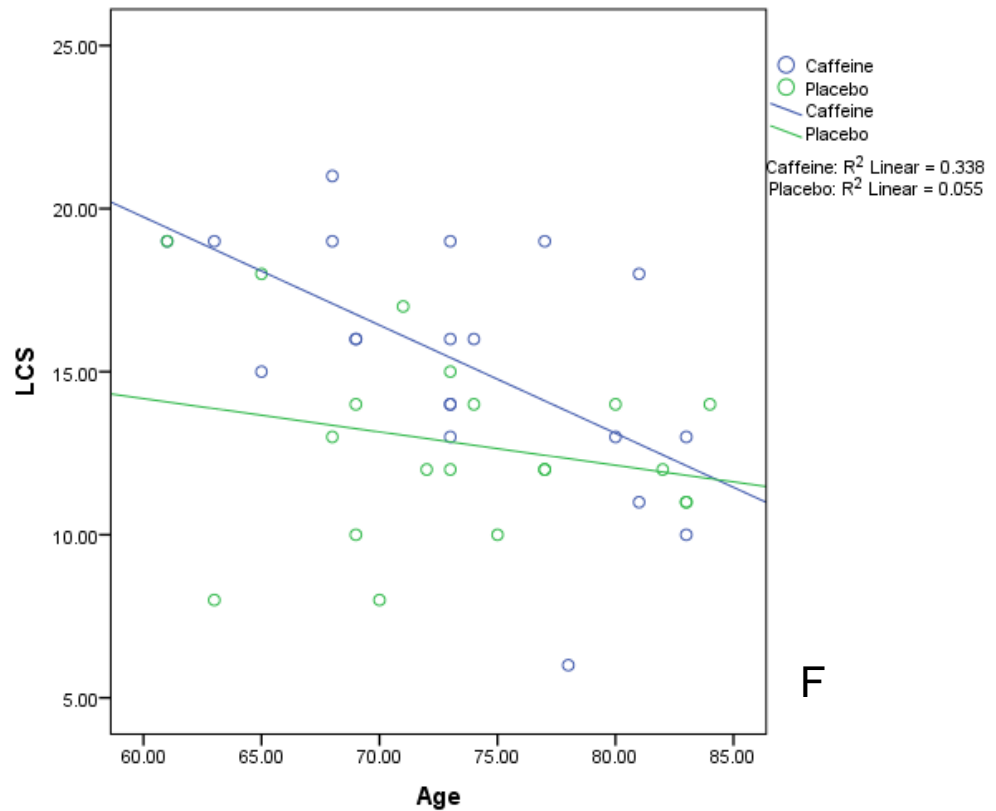
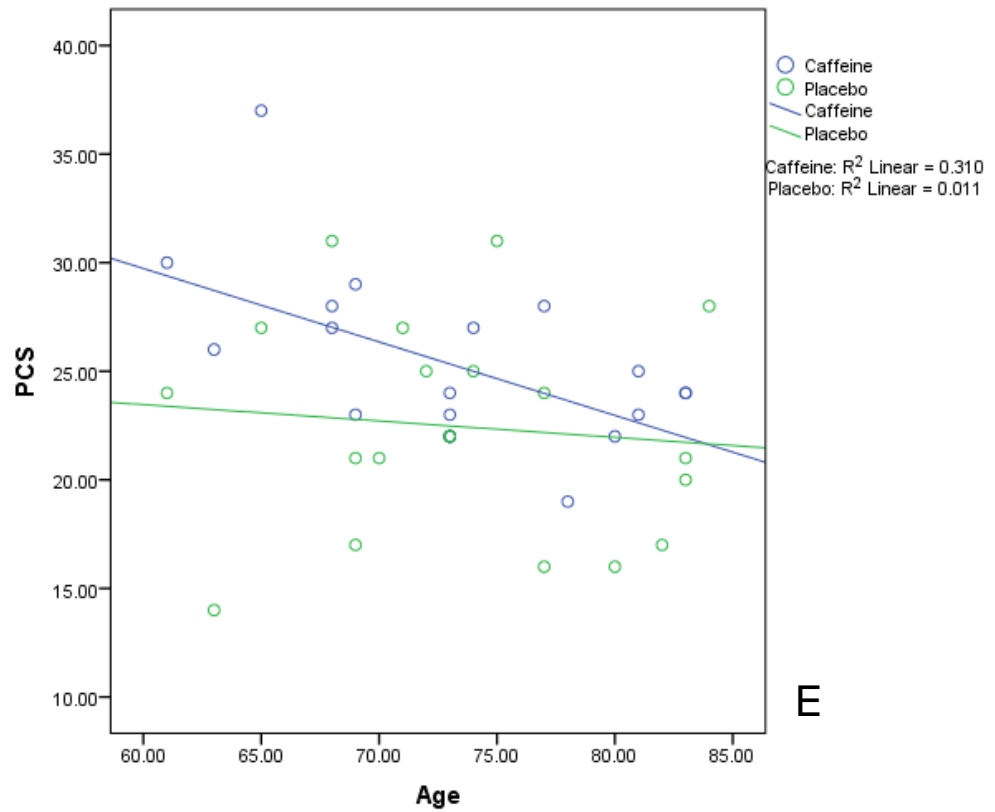


Figure nine: interaction effects between caffeine x age and score for (A) Dual Task ratio, (B) DT T1-T2 interval, (C) SDMT total score, (D) Stroop Interference, (E) PCS and (F) LCS. There is a significant linear decrease in performance with increasing age

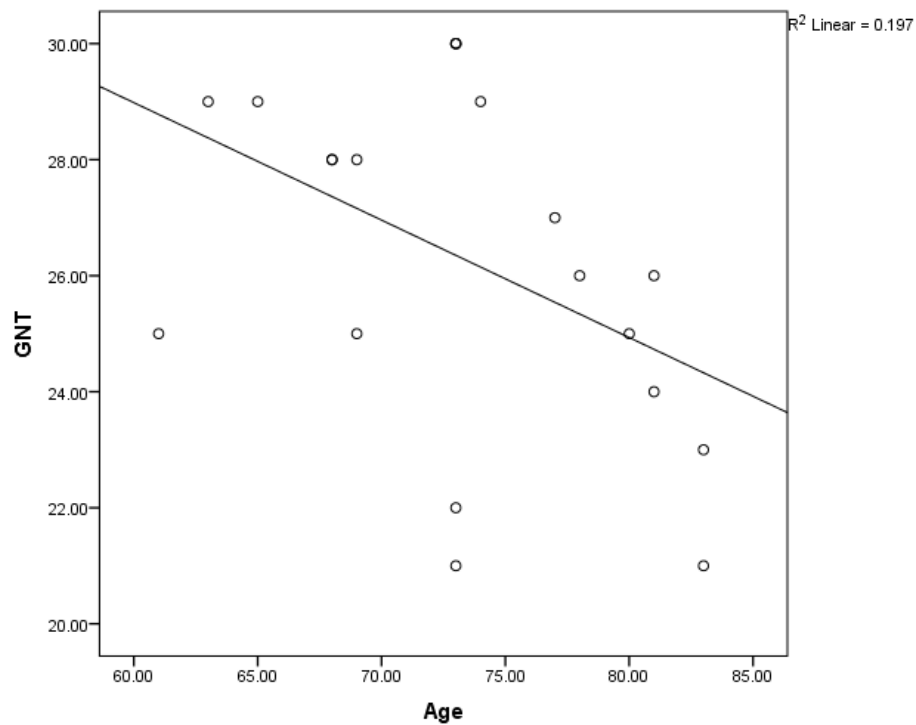


Figure ten - Scores on the GNT for participants in the caffeine condition, demonstrating a linear decrease in GNT performance as age increases, an effect not seen for participants in the placebo condition.

5.5 Discussion

5.5.1 Main findings

The aim of this chapter was to extend upon the results of Lesk et al., (2009) by investigating whether 200mg of pure caffeine could influence score on cognitive tests in the over 60s. The main findings were (1) there was no main effect of caffeine or placebo on any of the tests which were administered (2a) significant interaction effects between drug group, age and tests of **executive function**, (specifically the number of boxes crossed at time point one v time point two of the DT and DTR, the SDMT total score and the score for the Stroop Interference) and **processing speed**, (specifically the scores on the LCS and PCS tasks) were found. The third assessment for processing speed, the Stroop word test, did not show the same interaction effects; (2b) in the tests that did show this interaction, for the caffeine subgroup, participants' scores declined with increasing age, an effect which was not seen in the placebo condition.

Table five summarises the results of the current chapter and the results of Lesk et al., (2009) to allow clear comparisons between the data sets.

Table five: Comparison of significant interactions between caffeine/placebo, age and cognitive scores between Lesk et al., (2009) and the results of the current chapter

Test	Lesk et al., (2009)	Present Study
MMSE	X	X
PAL	✓	X
GNT	✓	X
Digit Span	X	X
Dual Tasks	X	✓
SDMT	✓	✓
Stroop Interference	trend	✓
Stroop word	✓	X
PCS	✓	✓
LCS	✓	✓
Word Recall	X	X

X – no significant interaction, ✓- significant interaction. Tests highlighted in yellow show a discrepancy in result between the two studies.

Consistent with the CCFS subgroup in Lesk et al., (2009), the present study also shows that for the caffeine subgroup there is a significant linear decrease with age and scores on assessments of EF (SDMT, Stroop Interference), processing speed (PCS and LCS) and semantic memory (GNT) revealing that as age increases performance on these assessments decreases. The results also showed a significant linear decrease for the caffeine subgroup with increasing age on one other test of EF, the DTR. Unlike the results of this study, Lesk et al., (2009) also reported significant regressions for the CCFS present subgroup on the PAL six pattern error score, Stroop word, and the TPT. For both the placebo group in this study and the CCFS absent group in Lesk et al., (2009) there were no effects of age on test scores.

The discrepancies between this study and Lesk et al., (2009) particularly in terms of the CANTAB PAL and the GNT are important as these tests have previously been shown to be highly sensitive for their ability to discriminate between AD and other forms of dementia. Blackwell et al., (2004) demonstrated in a regression analysis that the number of errors made at the six pattern stage of the CANTAB PAL, the score obtained on the GNT and the participant's age at the time of testing, could identify converters to AD with 100% accuracy within a 32 month period using a sample of 40 patients. Lesk et al., (2009) reported significant interactions of age and CCFS on the PAL and GNT, whereas in the present study, in which caffeine was more controlled, no significant interactions for the CANTAB PAL or GNT were found. This highlights the importance of noting the effect of prior caffeine intake which may affect scores used in predictive analysis.

The finding that increasing age was associated with a decrease in GNT score for the caffeine subgroup in the present study is important as vocabulary, naming skills and semantic memory usually improve with increasing age (Kahlaoui et al., 2012), nevertheless the present data contradicts this. It is unclear why this is the case but it does highlight that the consumption of small amounts of caffeine prior to testing (200mg is approximately the same amount of caffeine in one strong cup of coffee) can significantly affect the results.

5.5.2 Decline in EF with age

The predominant interactions with caffeine and age in the present study were for tests of EF and processing speed. For those participants who received caffeine, the older elderly perform worse on these tests in comparison with the younger elderly. Importantly there was no change in the placebo group.

EF relies heavily on the frontal lobes and is crucial for the changing demands and situations experienced in daily living (Schroeter et al., 2012). The frontal lobes are highly sensitive to the aging process and experience a large decline in volume with age (Hedden and Gabrieli, 2004; Huag and Eggers, 1991; Resnick et al., 2003). The PFC also experiences alterations in neurotransmitter levels (Hedden and Gabrieli, 2004) and white matter tract integrity during aging. This area is also affected in AD but not in the early stages of the disease (Hedden and Gabrieli, 2004). It is, therefore, expected that increasing age would be associated with poorer EF performance, as can be observed in the present study but only in the caffeine subgroup again supporting the need to investigate the effect of caffeine further.

EF is not a unitary component and consists of different sub domains (Miyake et al., 2000). Some studies have noted impaired EF in the elderly (Carey et al., 2008; Smith and Baltes, 1997) and specific impairments in EF sub domains have also been found including cognitive flexibility (Yang et al., 2006), which is the ability to switch between two stimuli or sets of information, decision making (Denburg et al., 2005) and attention (Verhaeghen and De Meersman, 1998). This chapter did not thoroughly assess all domains of EF, therefore, direct comparisons with these studies are not possible. However, the studies mentioned above do not take into account the prior caffeine consumption of their participants which may have affected the data they have obtained.

Few studies have directly compared EF in the younger elderly with the oldest old. Bakos et al., (2008) investigated differences in EF in these two age groups. Their study comprised a small sample of 10 younger elderly participants aged 60–67 years (mean age 62 years), and 10 ‘oldest old’ participants aged 75-84 years, (mean age 79 years). The study specifically looked at performance on the Stroop test (a test of flexibility/inhibition), digit span (a test of working memory), vocabulary (a test of semantic knowledge) and the Iowa Gambling

Test (IGT) (a decision making task). Significant differences were only found for the measure of decision making and judgement, the IGT, whereby the oldest old performed significantly worse than the younger elderly. The current chapter did not utilise a problem solving based task and direct comparisons are not possible.

5.5.3 EFs in MCI/AD

Tasks which recruit EF have been suggested as useful in the prediction of whether MCI cases are likely to convert to AD (Tabert et al., 2006), as EF impairments are common in the neurodegenerative process (Schroeter et al., 2012). Clark et al., (2012) recruited 71 non-demented elderly participants and followed them longitudinally. Of these 71 participants, 15 showed a significant decline in performance after one year. For this subgroup there was significantly poorer performance at initial baseline testing on two measures of EF, the inhibition/switching component of the Colour–Word Interference Test (CWIT), which measures how quickly the participant is able to switch from reading words aloud to naming the colour of the ink the word is presented in, and the category switching condition of the Verbal Fluency test which assesses the participant’s ability to generate words fluently while simultaneously shifting between semantic concepts (Clark et al., 2012). Other aspects of EF such as spatial planning, as measured by the Tower Test, and spatial fluency, measured by the Design Fluency switching condition of the Delis Kaplan Executive Function System (DKEFS), were spared. These results highlight that EF tests utilising inhibition and task switching in particular may be useful in predicting cognitive decline. Consequently if habitual levels of caffeine can have a significant effect with increasing age, as shown in the present study with 200mg of caffeine, on selected tests of EF, prior caffeine intake must be taken into account when using EF tests for the prediction of decline to AD.

There is little literature specifically investigating the effect of caffeine on executive performance in the elderly. Cropley et al., (2012), using 39 healthy elderly participants revealed that caffeinated coffee (not pure caffeine) improved performance on tasks requiring attentional processes although there was no reported age interaction. Hogervorst et al., (1998) reported that 225mg of

caffeine improved memory performance in the middle aged (age 50-65) but not in the older elderly (66-74) on an assessment of short-term memory and trial one of the Visual Verbal Learning Test with auditory distraction (VVL-T-D). A greater number of words were retained in those middle aged participants who had consumed caffeine. Hogervorst et al., (1998) do comment that their middle aged participants habitually consumed almost twice the amount of caffeine than both the elderly and young participants in their study which may have affected their results. In the present study, in which habitual consumption was similar for all participants, we found no significant interaction between age and short-term memory using a simple immediate word recall task which did not have a distraction component.

Similar to the points of Hogervorst et al., (1998), Schmitt et al., (2003) highlighted the issue of possible interaction effects between caffeine, age and cognitive test scores. The authors suggested that differences in habitual caffeine consumption between the younger elderly and the older elderly may cause reported differences in performance when participants are administered caffeine. Higher caffeine intake in the younger elderly may make them more vulnerable to caffeine withdrawal effects when they are asked to abstain before taking part in experiments. The results of their paper, however, failed to find any interaction effect of caffeine, age and assessments of digit span, word learning, the Stroop and memory scanning (Schmitt et al., 2003). The authors postulate that the lack of interaction effect with age may be due to the low dose of caffeine (100mg) administered. This chapter used a higher dose of 200mg of caffeine and reported significant interaction effects of caffeine, age and assessments of EF and processing speed.

5.5.4 Crossover points

The significant interactions of caffeine/placebo with age and scores on assessments of EF and processing speed show crossover points at different ages. For tests of EF (DT, DT T1-T2, SDMT total and Stroop Interference) the ages at crossover are 69, 64, 77 and 79 years respectively. Differences in the crossover points for these tests may be because EF is not a single unitary

process and EF tasks actually draw upon multiple different strategies all of which may alter with different ages (Schroeter et al., 2012).

For the two assessments of processing speed, PCS and LCS, which showed this significant interaction of caffeine/placebo with age, the crossover point is at an older age of 83 and 84 respectively. The crossover points for the interactions in the Lesk et al., (2009) study were at similar ages to the current study (PCS - 82, LCS - 81, SDMT total - 82). It is unclear as to what the exact underlying processes are which determine the age at which caffeine switches from enhancing to inhibiting performance in the elderly and this would require further, more detailed study.

Yang et al., (2006), using a test re-test paradigm, reported evidence of continued cognitive plasticity in the oldest old even though this was reduced in comparison to the younger old. Argimon and Stein (2005) investigated memory, attention and verbal fluency in a group of Brazilian elderly participants over the age of 80. A higher preservation of cognitive ability was noted in those oldest old who had the highest education level. The present study found no effect of education level on any of the assessments used.

5.5.5 Gender

The present study reported no effect of gender on any of the cognitive assessments administered. In Lesk et al., (2009) a main effect of gender was found on the GNT whereby males performed significantly better than females. No main effect of gender was found in this study for the GNT or any of the neuropsychological assessments used. This may be due to the small numbers of males recruited in this study (n=9). Previously Johnson-Kozlow et al., (2002) reported that for a sample of 890 females a higher lifetime coffee consumption, as measured by questionnaire, was associated with better performance on six out of the 12 assessments that the study employed. There were no benefits of coffee consumption on any of the assessments for the male participants (n = 638). A possible explanation for these effects was that caffeine may be metabolized differently in males and females (Relling et al., 1992). The authors also imply that their results suggest that women are more susceptible to the

cholinergic properties of coffee compared to males (Johnson-Kozlow et al., 2002). Likewise, Ritchie et al., (2007) reported beneficial effects of three or more cups of coffee on verbal memory in a group of women (mean age 73.8 years) compared to women consuming one or fewer cups of coffee per day. There were no reported beneficial effects of coffee drinking in the male participants. The FINE study reported that men who were drinking three or more cups of coffee a day showed the lowest decline in cognition (Eskelinen and Kivipelto, 2010). These mixed findings may be in part due to the different methodologies, assessments and sample sizes used which make it difficult to directly compare the results. Other confounding factors may also have affected the data even though most of these were controlled for, for example the use of HRT in women, use of statins for high cholesterol and use of anti-inflammatory drugs for conditions such as arthritis. All of these have been investigated as possible factors which may affect cognitive performance during aging. All of the studies mentioned have relied upon the self-report of caffeine consumption (predominantly in the form of coffee) over a set period of time. In the present study which was more controlled, 200mg of pure caffeine was administered and no reported gender differences were found.

5.5.6 Possible biological underpinnings

Another speculative explanation for the decline in caffeine's beneficial effects during aging may be due to alterations in adenosine receptor density with increasing age (Cunha et al., 1995). There is a consistent decrease in A_{1R} receptor density during aging and animal studies have also confirmed a predominant increase in the density of A_{2AR} receptors in aged animals (Cunha and Agostinho, 2010). Adenosine modulates the release of several neurotransmitters and adenosine binding in the cortex and hippocampal regions of the brain results in a decrease in the release of Ach (Cunha, 2001; Rahman, 2009). Furthermore, a decrease in the adenosine A_{1R} receptor subtype in the CA1 region of the hippocampus has also been reported in AD patients (Rahman, 2009). A study using post-mortem samples revealed a 40-60% decrease in A_{1R} adenosine receptors (Kalari et al., 1990). The highest reduction in expression was observed in the molecular layer of the dentate gyrus which

forms the major cortical input pathway to the hippocampus. The present study found no interaction between caffeine and tests of memory although research has also confirmed that in early AD both A_{1R} and A_{2AR} receptors are also upregulated in the PFC, the area of the brain associated with executive performance.

The potential role of adenosine in memory became apparent when adenosine agonists were shown to disrupt learning and memory in rodents (Corodimas and Tomita, 2001). The antagonism (blockade) of adenosine receptors by caffeine has the opposite effect and results in an increase in the amount of Ach released from synapses (Dall'Igna et al., 2004). The changes in adenosine receptor expression, namely an up regulation, with increasing age is important for further study as this is a speculative explanation as to why the effects of caffeine alter with increasing age in this sample. An increase in receptor expression may decrease the degree of adenosine blockade that can be initiated by caffeine's antagonism. This could result in a smaller effect both at the receptor site and via the downstream modulation of other neurotransmitter systems. This may ultimately result in a lower performance enhancement from caffeine in the older elderly as demonstrated on some of the assessments used in the present study.

5.6 Conclusion and implications

The results of this preliminary study have shown that the older elderly who received 200mg of caffeine perform significantly worse than the younger elderly on tests of EF and processing speed. Although there are some discrepancies between this study and the data obtained previously in Lesk et al., (2009), both studies have shown that when there is no caffeine in the system (i.e. no prior consumption of CCFS in Lesk et al (2009), and the placebo condition in the present chapter) the results are in agreement i.e. no significant effects of age and no main effects present in the regression analysis. This is interesting and raises the question as to whether participants should be asked to abstain from caffeine and CCFS for at least four hours before being given any neuropsychological assessment to improve the accuracy of the data that is obtained.

Significant interactions with age were only present on tests of EF and processing speed which also has some implications for the diagnostic process of AD and a cognitive impairment. The results have shown that when controlling for caffeine the significant effects on tests that are especially sensitive to cognitive impairment such as the CANTAB PAL that were found in Lesk et al., (2009) are no longer seen. This again highlights the need to control prior caffeine intake when interpreting scores on cognitive tests.

The use of pure caffeine rather than coffee or reported CCFS intake reduced the possibility that the results obtained from this study may be due to other ingredients contained within caffeinated drinks. The double blind nature also removed any subjective encouragement during testing from the researcher who was unaware which experimental condition each participant was assigned to. The specific inclusion criteria and checklist used by the researcher ensured that only those who were suitable to take part did so. Recruiting participants with a similar daily habitual consumption of CCFS (i.e between 1-3 cups of coffee per day) has minimised the likelihood that any interactions with age were due to differences in habitual consumption between participants. This was previously highlighted as a potential issue in studies such as Hogervorst et al., (1998) and Schmitt et al., (2003) whereby the younger participants habitually consumed almost twice as much caffeine than the elderly participants. Furthermore, the exclusion of excessive consumers (defined as >400mg or >4 cups of coffee per day) reduced the possibility that the results obtained may be attributed to a withdrawal affect of caffeine. Using a specific amount of caffeine for each participant (200mg), and asking participants to abstain from caffeine for a set amount of time (12 hours), removed the possibility for variances in dose and also did not rely on the self-report of consumption by the participants which has the potential for errors and bias.

It is important to consider the influence of potential outliers which may have skewed the data obtained and any interpretations made. For example, in figure 9b, one participant obtained a score of 46 on the DT T1-T2 interval. This participant's score is elevated in relation to the rest of the cohort and may, therefore, be significantly influencing the data. A repeat analysis, removing this participant's score, did not affect the significance obtained, i.e there was still a significant interaction between age, caffeine and score on the DT T1-T2 score.

As a result this data point was not removed from the analysis but it is acknowledged that outliers may influence subsequent conclusions.

The scores obtained from neuropsychological test batteries are used to give a diagnosis and discriminate between AD, MCI and other dementias. Scores can also determine whether pharmacological treatment is commenced and what care plan is needed. In addition, neuropsychological testing is used to determine if certain individuals are included in clinical trials and research studies. Test scores must be as accurate and reliable as possible. Although it is acknowledged that age can affect scores on some neuropsychological assessments (Overshott and Burns, 2004; Jacova et al., 2007; Salthouse, 1996) the significant interactions with caffeine and age in this study warrant the control of this as an external variable. Issues with cognitive test batteries have highlighted that scores on these assessments are often compared to population norms and are, therefore, limited in their ability to diagnose (Lesk et al., 2009). Test accuracy and the ability to discriminate between AD and MCI and MCI and healthy cognitive aging are imperative particularly for early identification. Confounding factors should therefore be controlled. Such factors including caffeine should be identified and controlled for when creating normative data to ensure that these sets of data in particular are as accurate as possible, particularly as they will be used for comparison purposes (Lesk et al., 2009).

5.7 Chapter summary of key points

- The presence of caffeine may affect the accuracy of neuropsychological test scores.
- Previously caffeine consumption in the form of CCFS such as tea and coffee longitudinally over decades has been associated with a lower risk of developing AD.
- There has been little investigation into the immediate effects of prior caffeine at the time that tests are administered.
- Previously Lesk et al., (2009) investigated the effect of CCFS consumption four hours prior to the neuropsychological assessment.
- The results showed significant interactions between CCFS and age on some of the tests administered.

- This chapter explored the effects of 200mg of caffeine in the over 60s using a double blind experimental approach.
- Participants who were suitable to take part were asked to abstain from CCFS for 12 hours prior to taking part and alcohol 24 hours prior.
- Participants were randomly assigned to the caffeine or placebo condition and another researcher administered the participant their drink which may or may not have contained 200mg of caffeine. 40 minutes later a cognitive battery was administered.
- The results of the chapter found no main effect of 200mg of caffeine on any of the tests used but there were significant interactions between caffeine, age and tests of EF and processing speed.
- For these interactions the older elderly who had received caffeine performed significantly worse than the younger elderly.
- This has important implications for the accurate interpretation of test scores and should be considered when scoring these tests.
- The exact reasons as to why these particular cognitive domains were affected is unclear. EF is highly associated with frontal lobe functioning and these areas of the brain are highly susceptible to age related changes.
- EF encompasses a number of cognitive processes and further study is needed to determine if particular sub domains are more affected over others.
- The results of this chapter highlight the need to consider the participant's prior caffeine consumption when scoring neuropsychological assessments.
- This is particularly important if the scores obtained are compared to normative data or used for the inclusion/exclusion of individuals for research trials.

Some of the information from this chapter can be found in Walters and Lesk (in prep - a) 'The effect of prior caffeine consumption on neuropsychological testing'.

Chapter Six

The effect of non-oily fish consumption on the MMSE

6.1 Introduction

Diet is a factor which can influence cognitive performance (Cole et al., 2009) and poor diet and malnutrition are risk factors for cognitive impairment in the elderly (Morris, 2009; von Arnim et al., 2010). Chapter five investigated the effect of caffeine on cognitive performance in the elderly. This chapter will investigate another dietary factor, fish consumption, in order to determine any effect of this on neuropsychological test performance in the elderly.

The chapter will extend upon findings by Walters et al., (2011) a study which originated from my MSc research project '*Correlates of dietary patterns and lifestyle on cognitive performance in the over sixty fives*'. This study utilised a dietary and lifestyle questionnaire (see appendix one) and a brief cognitive battery to investigate monthly fish consumption and cognitive performance in a group of elderly participants. The results revealed that participants' consumption of non-oily fish, specifically cod and haddock was trending towards significance as a predictor of their score on the MMSE. Increased consumption of cod and haddock was associated with better performance on the MMSE.

As the MMSE is a diagnostic tool used in the detection of cognitive impairment in the elderly and is recommended for use in the early detection of AD by the NICE dementia guidelines (NICE, 2011), it is important to investigate these preliminary findings in greater detail to determine whether non-oily fish intake is a factor which may influence cognitive performance. Furthermore, there has been little investigation into the effects of non-oily fish intake on cognition in the elderly with most studies investigating total seafood consumption or specifically oily fish consumption (Cunnane et al., 2009). Estimations of food intake provided by food frequency questionnaires (FFQs) may be subject to over and under reporting (Liu et al., 2013). Likewise, portions can differ in size and weight therefore investigating people's estimation of their grams of fish intake per month gives a more reliable indication of actual consumption.

Consequently, this chapter will extend upon the preliminary findings of Walters et al., (2011) by investigating the effect of monthly non-oily fish intake in grams on a more thorough battery of cognitive assessments in a different cohort of elderly participants via the use of a four week food diary. The first part of this chapter will review some of the existing literature which has investigated the effect of fish consumption on cognitive performance in the elderly and will provide further details of Walters et al., (2011).

6.1.1 Fish consumption

Marine fish are divided into oily and non-oily varieties. Table six outlines some of the most commonly consumed types, however, there are many more. Oily fish are referred to as fatty fish due to their higher fleshy fat content (approx 5-20%) relative to non-oily fish (also referred to in the literature as white fish) which have a lower fat content (1-2%) and are known as lean fish (Scientific Advisory Committee on Nutrition, 2004).

Table six: Commonly consumed oily and non-oily fish types

Oily Fish	Non-oily
Sardines	Cod
Salmon	Haddock
Pilchards	Plaice
Mackerel	Sole
Herring	Halibut
Trout	Hake
Fresh Tuna	Tinned Tuna
	Whiting
	Pollack
	Seabass
	Snapper
	River Cobbler
	White fish

(Table adapted from the Scientific Advisory Committee on Nutrition, 'Advice on fish consumption benefits and risks', 2004, page 104

6.1.2 Fish oils

Varieties of oily fish are a rich source of omega-3 polyunsaturated fatty acids (n-3 PUFAS), known as 'fish oils' (Robinson et al., 2010), but these are also found in smaller amounts in non-oily fish. The two most biologically important n-3 PUFAs are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), both of which are crucial for the maintenance of brain structure and function (Devore et al., 2009; Dangour et al., 2010; Robinson et al., 2010; Cunnane et al., 2009). Oily fish is a richer source of n-3 PUFAS comparative to non-oily varieties (Dangour et al., 2010; Danthiir et al., 2011) and smaller amounts of DHA and EPA can also be found in other foods such as meat, eggs, leeks and cereals (Robinson et al., 2010).

DHA is the most abundant n-3 PUFA within the brain and is concentrated in membrane phospholipids (Hashimoto and Hossain, 2011). Approximately 30-40% of fatty acids within the cortical grey matter are composed of DHA (Robinson et al., 2010) which is predominantly derived from the diet and cannot be manufactured within the body (Devore et al., 2009; Robinson et al., 2010).

Levels of DHA in the brain naturally decrease with age (Söderberg et al., 1991) and lower levels of plasma EPA and DHA have been reported in AD (Conquer et al., 2000; Hashimoto and Hossain, 2011). The importance of DHA for brain structure and function lead to the investigation of the association between fish consumption (a rich source of DHA) and the risk of developing AD, as well as the effect of fish intake on cognitive function in general. These areas will now be briefly reviewed.

6.1.3 Fish consumption and cognitive function in the elderly

Van de Rest et al., (2009) used a 126 item FFQ to investigate the effects of fish intake on cognition in a group of 1025 elderly male participants with a mean age of 68 years. They investigated four fish items which included 'dark-meat fish', which consisted of mackerel, salmon and sardines, 'canned tuna', 'other fish' and 'shrimp, lobster and scallops'. The total mean weekly fish consumption was calculated to be 2.4 servings, of which 1.3 servings constituted fatty (oily) fish. At baseline testing increased oily fish consumption was associated with

impaired memory and language. Six hundred and seventy one participants were available at three year follow up and for these participants increased fish consumption was not associated with better cognitive ability. Although the study used a large sample, a thorough cognitive battery and a well validated FFQ, the mean age of participants (68 years) was lower than in other studies. A protective effect of fish may occur only in advancing ages.

Another study by van Gelder et al., (2007) investigated 210 elderly male participants aged between 70-89 years. The study employed experienced dieticians to carry out a dietary checklist with each participant in order to note which fish types were regularly consumed. They assessed general cognition using the MMSE. The main findings indicated that men who did not consume fish experienced four times the decline in MMSE score than those participants who did consume fish after a five year follow up. The study did show that a higher intake of DHA and EPA (not fish portions) was associated with a lower decline in cognition.

Kalmijn et al., (1997) also investigated elderly male participants' fish consumption as a part of the Zutphen Elderly Study. Fish intake was categorised into three groups according to the amount consumed and included 0g per day, 1-20g per day and greater than 20g per day. At baseline testing, for participants consuming greater than 20g of any fish type per day, there was an inverse association with cognitive impairment. After a three year follow up, although fish consumption was inversely associated with cognitive function, this association was not significant.

6.1.4 Fish consumption and risk of dementia

Morris et al., (2003) investigated 815 elderly participants aged between 65 and 94 years, all of which were cognitively normal at baseline recruitment. Those participants who consumed higher amounts of DHA, as assessed by a dietary questionnaire, possessed a lower risk of developing AD compared with those individuals who ate lower amounts. In this study a decreased risk of developing AD by approximately 60% was reported in those participants who consumed at least one fish meal per week compared to those eating fish rarely or never.

Similarly, Kalmijn et al., (1997) found increased dietary fish consumption over a two year period was beneficial at lowering risk of dementia (Kalmijn et al., 1997). A questionnaire based study by Barberger-Gateau et al., (2002) reported a lower risk of dementia in individuals who consumed at least one portion of seafood or fish per week. Roberts et al., (2010) reported that in a cohort of elderly participants (over the age of 70 years), a higher intake of n-3 PUFAs, as assessed by a 128 item FFQ was associated with a reduced likelihood of developing a MCI (Roberts et al., 2010).

Devore et al., (2009) found no effect of high fish intake or n-3 PUFAs on dementia risk over a 10 year follow up in a large cohort of 5395 participants over the age of 55 years. This study used a meal based checklist where participants were asked to indicate foods that they had consumed at least two times per week for the past year. Although no significance was reported the authors did note that cod was the most abundant fish consumed in this cohort. In the Netherlands, where the study was conducted, cod tends to be prepared by frying and this may decrease the beneficial nutritional content of the fish which may help to explain the non significant results that were obtained (Devore et al., 2009).

6.1.5 n-3 PUFA supplementation: AD and cognitive function

Supplementation with DHA and EPA increases serum levels of these two fish oils (van de Rest et al., 2008). Some studies have investigated whether fish oil supplements are able to change AD risk or prevent cognitive decline. The majority of such studies have revealed negative findings and have concluded that increased n-3 PUFA consumption via supplementation methods had no relation with decreased AD risk (van de Rest et al., 2008; Englehart et al., 2004) or cognitive decline (Quinn et al., 2010; Dangour et al., 2010). These results do not concur with the findings of epidemiological studies which have associated high DHA intake with a reduced risk of AD (Cunnane et al., 2013).

Dangour et al., (2010) investigated whether 700mg of n-3 PUFA supplementation for a period of 24 months relative to a placebo (olive oil capsule) could prevent cognitive decline in 867 individuals (mean age = 75

years) . All of the participants were recruited from GP practices in the UK and had MMSE scores at baseline of ≥ 24 out of 30. None of the participants were taking any fish oil supplements at recruitment. Habitual baseline fish consumption was assessed using a dietary questionnaire. After a period of 24 months there was no change in cognitive function between the treatment and placebo groups. There were, however, measurable biological changes in DHA in the treatment group. Although no significance was reported in this study it is possible that those in the placebo arm continued to consume fish which may have naturally increased their intake of fish oils (Dangour et al., 2010). Likewise, the placebo was an olive oil capsule and olive oil has been shown, as a component of the Mediterranean diet, to be beneficial for cognition and healthy aging (Scarmeas et al., 2006). Recently, Martínez-Lapiscina et al., (2013) showed that a diet supplemented with olive oil for a period of six and a half years was associated with significantly better performance on the MMSE and CDT.

Other studies have investigated whether n-3 PUFAS can prevent further decline in diagnosed AD patients and these also revealed no beneficial association (Freund-Levi et al., 2006). Chiu et al., (2008) implemented a double blind treatment vs placebo preliminary study to investigate whether 24 weeks of n-3 PUFA treatment had an effect on cognition in a group of AD and MCI participants. The study found that there was no change between the two groups on the cognitive section of the ADAS-COG. The MCI participants who were administered the treatment showed a significant improvement on this test relative to the placebo group. The short follow up period of 24 weeks may also have influenced the results and a longer follow up may have been needed.

A more recent study by Yurko-Mauro et al., (2010) investigated whether a 24 week supplementation study with 900mg/day of DHA could improve cognitive function as determined by fewer errors on the six pattern stage of the CANTAB PAL after 24 weeks of treatment. The PAL has previously been shown to be sensitive to early cognitive impairment (as discussed in chapter four) (Swainson et al., 2001; Blackwell et al., 2004). The results of the study revealed that there were significantly fewer errors made in the treatment group comparative to the performance of participants in the placebo condition. These beneficial findings

were noted after only a short follow up duration and it is possible that with longer follow up even larger improvements may be seen.

6.1.6 Methods of collecting dietary information

FFQs are frequently used to collect dietary information in a cross sectional manner and usually require participants to estimate their habitual consumption of certain food groups (Christensen et al., 2013; Liu et al., 2013). In Walters et al., (2011) (see section 6.1.1) a lifestyle and dietary questionnaire was created to obtain information regarding participants' intake of a variety of foods including a specific section on fish. FFQs are quick and simple to complete but there are some criticisms of their use as a tool for the collection of dietary information. For example, the issue of self report, which relies on the participants' self estimation of their fish consumption. This creates the potential for errors due to over and under estimation of intake (Liu et al., 2013; Bowman et al., 2012; Arsenault et al., 2009; Cunnane et al., 2009).

Food diaries on the other hand require participants to complete a record of their daily intake of all meals, including food and drinks. By recording meals and snacks as they eat them there is less opportunity for participants to make errors due to estimation (Bowman et al., 2012; Arsenault et al., 2009). Food diaries are considered to be a more reliable method of assessing dietary intake (Legg et al., 2000). Legg et al., (2000) do comment, however, that for any type of dietary data collection method (i.e. FFQs and food diaries) there is the potential for a response bias whereby a participant may provide estimates of intake based on what they believe the researcher is interested in.

There are few studies which have specifically used food diaries as a method of comparing diet with cognitive function in the elderly. To my knowledge the only food diary based study to compare fish intake with cognitive function in the elderly is Kesse-Guyot et al., (2013). This study used a 'food record', which was the equivalent of a 24 hour food diary. The study investigated whether adherence to a Mediterranean type diet in midlife had an effect on cognition after a follow up period of 13 years. The Mediterranean diet includes a large intake of fish (Martínez-Lapiscina et al., 2013). Kesse-Guyot et al., (2013)

recruited 3083 participants with a mean age at baseline of 52 years. Participants were required to complete a 24 hour food record every two months resulting in a total of six records for a one year period across all seasons. From these records the authors calculated a Mediterranean diet score (MDS) for each participant using an algorithm they created. This was based upon the number of certain foods consumed. After a period of 13 years participants were invited to attend a cognitive assessment. At follow up the mean age of participants was 64 years.

Overall there was no effect of a Mediterranean diet on cognitive performance, however, a higher MDS score was associated with better performance on an assessment of semantic fluency and a composite score of cognition. In addition, MDS score correlated with n-3 PUFA intake. A limitation of this study is the absence of baseline assessments of cognitive performance which makes it impossible to investigate whether those with a higher MDS score experienced lower levels of decline on some or all of the tests administered. In addition, 24 hour food diaries completed once every two months may not reflect general daily consumption. Consequently, this method may not offer reliable dietary information although the day of the week that participants were asked to record intake was determined at random by the authors.

6.1.7 Pilot Study: Walters et al., (2011)

As a part of my MSc in Psychology in 2010 I investigated dietary correlates of cognition in the HE with the aim of determining whether certain foodstuffs and/or lifestyle habits were associated with cognitive performance. Using a separate cohort of participants Walters et al., (2011) further investigated preliminary findings from this study of an association between non-oily fish and MMSE score. This showed that increased consumption was associated with improved performance on this test.

Non-oily fish consumption was investigated in 70 participants with a mean age of 72.09 (SD \pm 6.24) years and mean years spent in education of 15.57 (SD \pm 4.86) years. A FFQ was used to assess various aspects of lifestyle and diet and specifically asked participants about their medical history, current medical

conditions, medication use and lifestyle habits [including alcohol consumption, smoking status, levels of exercise and interests and hobbies]. The remainder of the questionnaire asked participants to estimate their daily, weekly or monthly consumption of a variety of different foods and drinks and included a small specific section on fish including non-oily and oily types. A short cognitive battery consisting of the MMSE (Folstein et al., 1975), the Stroop Test (Trenerry et al., 1989), digit span (Wechsler et al., 1981) and immediate word recall was administered. The results revealed that portions of non-oily fish intake was trending towards significance as a predictor of MMSE score ($p = 0.08$). As consumption of non-oily fish increased MMSE score also increased. There was no effect of any variety of oily fish on MMSE score (Walters et al., 2011).

6.2 The present study: research aims

This chapter will further investigate whether non-oily fish consumption is a dietary factor which may influence cognitive performance in the elderly using a more controlled method of collecting dietary information, a four week food diary. This will allow the investigation of whether the same effect as seen in Walters et al., (2011) is present in a different cohort of participants.

6.3 Methods

6.3.1 Participants

All participants from the University of Bradford, Division of Psychology Participant Pool were invited to take part in the study and of these individuals 38 participants were recruited to take part in the study. The inclusion criteria specified that participants must be 60 years of age or older and must not possess an existing diagnosis of any neurological condition or a cognitive impairment. A novel cohort was recruited for the purpose of this study and, therefore, none of the participants had taken part in the previous pilot studies which had investigated trends between non-oily fish and cognitive performance. Data from four participants could not be included in the study due to failure to correctly complete the food diary. The final sample consisted of 11 males and 23 females. The mean age of participants was 72.59 (S.D ± 6.09) years and the

mean years in education was 14.84 (S.D \pm 4.69). Ethical approval was obtained from the University of Bradford Ethics Committee and all participants gave their informed written consent to take part.

6.3.2 Materials

6.3.2.1 Food diary

A four week food diary was created to allow the recording of dietary information for a period of one month. The diary consisted of a 17 page A4 landscape booklet which contained an information sheet, a detailed instruction section, an example of a completed diary sheet and a four week blank diary. The diary was designed to be easy to complete and consisted of a tabular grid format which included a column for the day of the week and four columns for breakfast, lunch, dinner and snacks respectively.

Participants were instructed to record any meal that they consumed which contained a fish item. They were also required to note the type of fish, the weight as stated on the packet or scales, the brand make or the location the fish was purchased from e.g. Morrisons fishmonger. They were also required to note all foods eaten alongside the fish, condiments that were added and how the fish was prepared.

6.3.2.2 Neuropsychological tests

A neuropsychological assessment battery was used to assess cognition and this included the following tests. Further details of each test can be found in Chapter four 'Neuropsychological testing'.

MoCA - an assessment of general cognitive function (Nasreddine et al., 2005).

MMSE - an assessment of general cognitive ability (Folstein et al., 1975).

DT - an assessment of EF (Baddeley et al., 1997).

TMT - an assessment of EF (Reitan and Davison, 1974).

PCS and LCS - assessments of processing speed (Salthouse and Babcock, 1991).

Stroop Test - an assessment of processing speed and EF (Trenerry et al., 1989).

Digit span - an assessment of working memory (Wechsler, 1981).

6.3.3 Procedure

Participants were invited to take part in the study via a postal invitation letter. Those who accepted were invited to attend a brief meeting where they were given the opportunity to ask any questions, sign the consent form and were provided with a copy of the food diary to take home. During this appointment the researcher explained how to complete the diary and what information was required. Participants were asked to complete the diary for a period of four continuous weeks and it was specified that this must be a “typical” four week period and must not be completed if the participant was going on holiday for example. Another appointment was then arranged for the week after the diary had been completed where the cognitive assessment could be carried out. Participants were also instructed not to alter their habitual consumption of fish and provide an honest recall of their fish intake. They were asked to note any supplements that they may take but were not specifically asked to record their medications. A sample of a completed week was provided as a guide and participants were instructed to contact the researcher if they experienced any problems or required assistance in the completion of the food diary. After they had completed the diary for a period of one month they were invited back to the university to complete a cognitive assessment (see section 6.3.2.2 for details).

6.4 Results

6.4.1 Main demographics

The aim of the current chapter was to investigate the effect of monthly grams of non-oily fish intake on cognitive test scores in the HE in particular the MMSE. Table seven outlines the participants’ demographics, table eight shows the

types of fish consumed and the mean monthly consumption values and table nine shows computed values for total, oily and non-oily intake.

Table seven: mean \pm standard deviations for main patient demographics

Demographic	
Age (mean years \pm S.D)	72.59 (6.09)
Education (mean years \pm S.D)	14.84 (\pm 4.69)
Gender (M/F)	11/23

Table eight: mean monthly consumption in grams of each fish type that participants consumed during the study

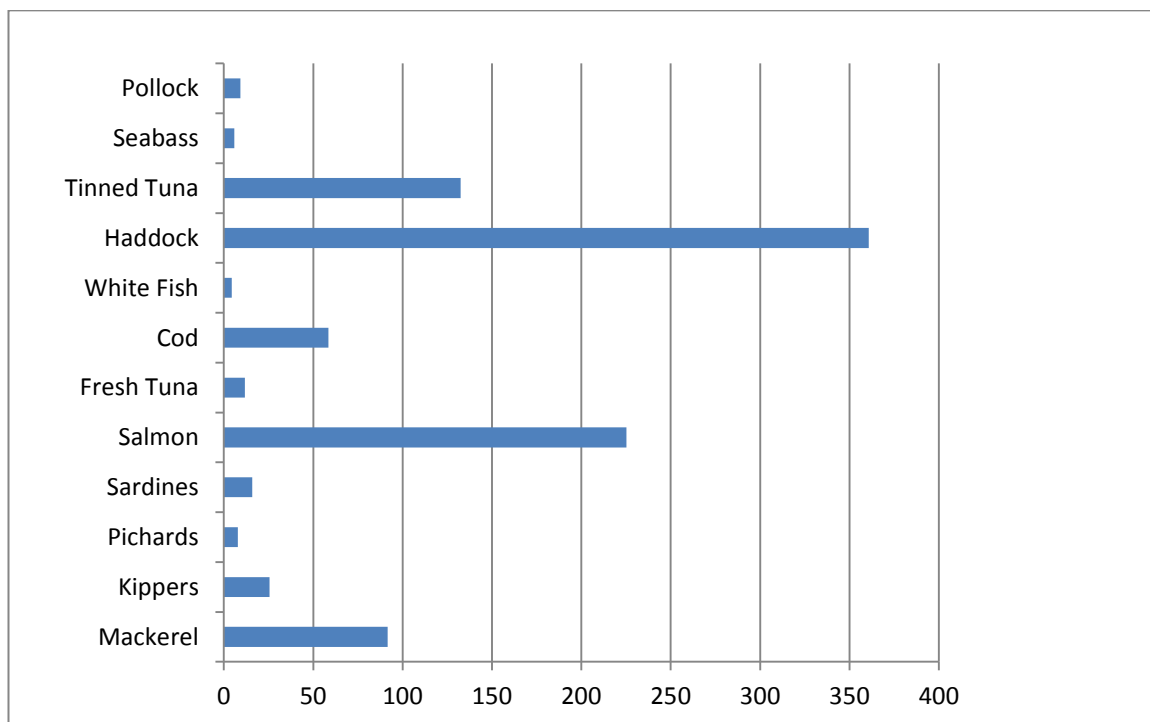


Table nine: mean grams of non-oily, oily and total fish consumed during the study

	Min (g)	Max (g)	Mean (g)	\pmSD (g)
Total non-oily	0	1766.80	571.28	437.60
Total oily	0	1431.00	377.85	378.04
Total fish	289.30	1891.80	949.13	485.36

6.4.2 Neuropsychological tests

6.4.2.1 Main effects of age and education

There were significant main effects of age on the Stroop interference [$F(1,32) = 4.79, p < 0.05$], the PCS [$F(1,32) = 6.35, p < 0.05$], the LCS, [$F(1,32) = 6.64, p < 0.05$], TMT part A [$F(1,32) = 9.54, p < 0.01$] and TMT part B [$F(1,32) = 10.06, p < 0.01$]. Regression analyses revealed that performance decreases with increasing age on all of these tests [Stroop interference - $\beta = -0.34, F(1,32) = 4.79, p < 0.05$, y-axis intercept = 50.94, regression correlation coefficient $r = 0.36, p < 0.05$; PCS - $\beta = -0.29, F(1,32) = 6.35, p < 0.05$, y-axis intercept = 45.67, regression correlation coefficient $r = 0.41, p < 0.05$; LCS. $\beta = -0.17, F(1,32) = 6.64, p < 0.05$, y-axis intercept = 26.85, regression correlation coefficient $r = 0.41, p < 0.05$; TMT part A - $\beta = 1.04, F(1,32) = 9.54, p < 0.01$, y-axis intercept -41.03, regression correlation coefficient $r = 0.48, p < 0.01$; TMT part B - $\beta = 1.66, F(1,32) = 10.06, p < 0.01$, y-axis intercept = -51.67, regression correlation coefficient $r = 0.49, p < 0.01$].

There was a significant main effect of education on the Stroop Word [$F(1, 32) = 4.83, p < 0.05$]. A regression analysis revealed that increased education in years was associated with improved performance on this test [$\beta = 0.92, F(1, 32) = 4.83, p < 0.05$, y-axis intercept = 48.40, regression correlation coefficient $r = 0.36, p < 0.05$].

6.4.2.2 Main effects of gender

Significant main effects of gender were noted on the LCS and MMSE [LCS - $F(1, 32) = 4.31, p < 0.05$], MMSE - $F(1, 32) = 7.97, p < 0.01$]. Independent sample t-tests revealed that the male participants performed significantly worse than female participants on both of these tests, [LCS - $t(32) = -2.08, p < 0.05$, MMSE - $t(32) = -2.44, p < 0.05$]. Independent t-tests revealed that there were no significant differences between males and females in terms of age or education. There were also no significant differences between males and females in their consumption of total, oily or non-oily fish as measured by independent t-tests ($p > 0.05$).

6.4.3 Main effect of total grams of fish consumption

Following the same analytical method as Walters et al., (2011), a MANCOVA with test scores as dependent variables and total grams of fish entered as a covariate, revealed a main effect of total monthly fish intake in grams on the MMSE [$F(1,32) = 4.84, p < 0.05$]. A regression analysis showed that total grams of fish intake could significantly predict MMSE score [$\beta = 0.36, F(1, 32) = 4.84, p < 0.05, y\text{-axis intercept} = 27.62, r = 0.36$]. See figure 11. There was no effect of total grams of fish consumption on any of the other tests administered.

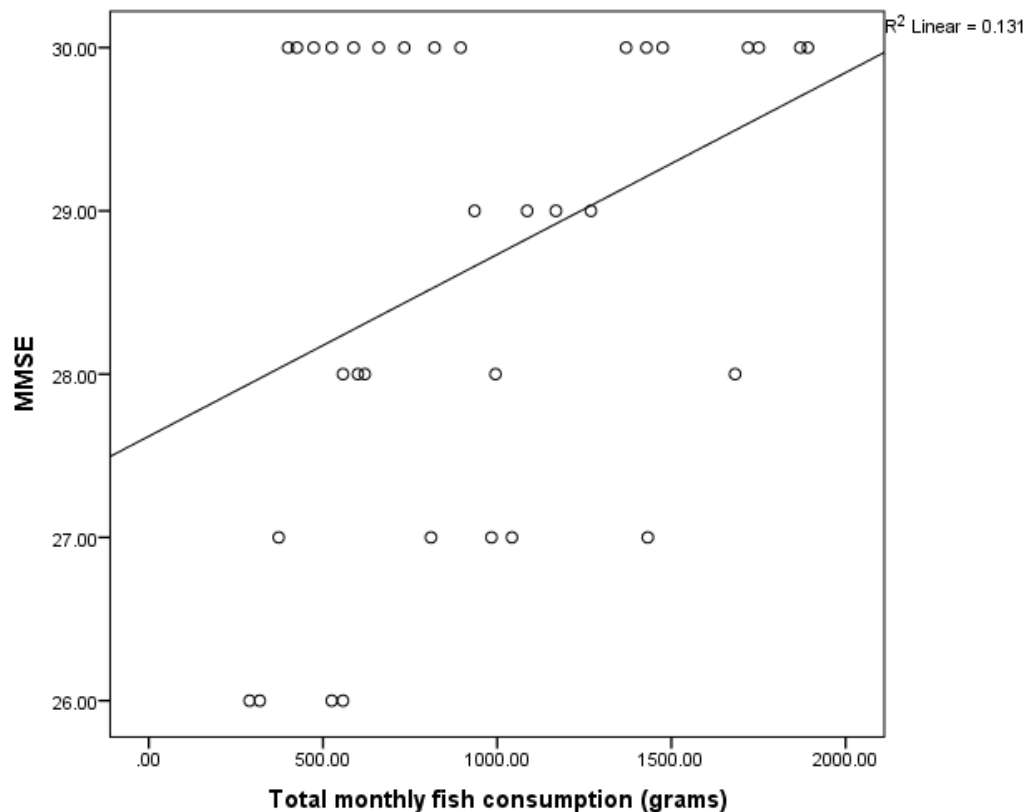


Figure 11: total monthly fish consumption is a significant predictor of MMSE score.

6.4.4 Main effects of oily and non-oily fish consumption

In order to investigate the primary hypothesis and determine whether there were main effects of non-oily fish on any of the assessments used particularly the MMSE, as previously reported in Walters et al., (2011), a MANCOVA was carried out with all test scores entered as dependent variables and total non-oily

fish and total oily fish in grams entered into the model as covariates. This revealed a significant main effect of non-oily fish on the MMSE [$F(1, 31) = 4.09$, $p = 0.05$]. A regression analysis for monthly non-oily fish and MMSE score was not significant, although, a scatter plot showed a positive association between MMSE score and non-oily fish intake in grams. See figure 12.

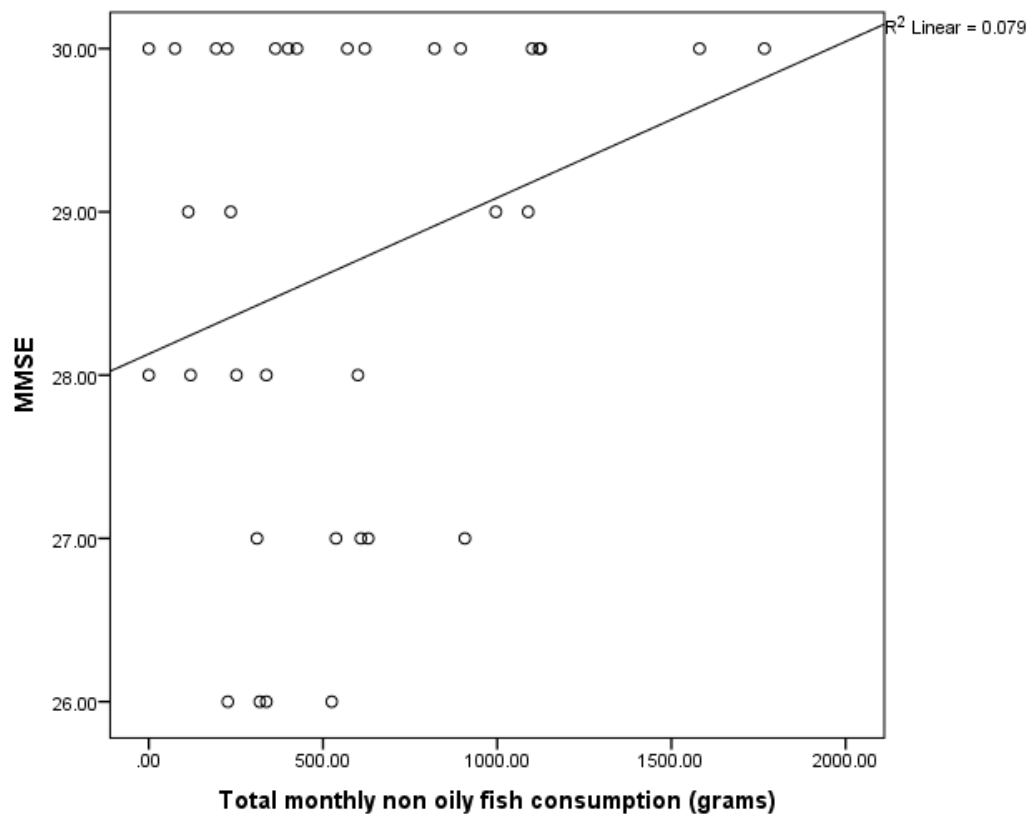


Figure 12: total monthly non-oily fish consumption is associated with an increased MMSE score

6.4.5 Non-oily fish consumption and MMSE score

The main effect of non-oily fish consumption and MMSE score was investigated in more detail to see the influence of any particular non-oily fish type. A univariate analysis with MMSE score entered as the dependent variable and all the separate non-oily fish types in grams [pollock, sea bass, tinned tuna, haddock, white fish and cod] entered as covariates was carried out. This revealed that there was a significant main effect of grams of monthly tinned tuna

consumption and MMSE score [$F(1, 27) = 9.53, p < 0.01$]. There were no effects of any of the other non-oily fish types on MMSE score. A regression analysis revealed that monthly tinned tuna consumption in grams was a significant predictor of MMSE score [$\beta = 0.47, F(1, 32) = 9.15, p < 0.01, y\text{-axis intercept} = 28.19, \text{regression correlation coefficient } r = 0.47$]. Increased consumption is associated with improved score on the MMSE. A scatter plot of these results can be seen in figure 13. There were no main effects of any oily fish type on MMSE score.

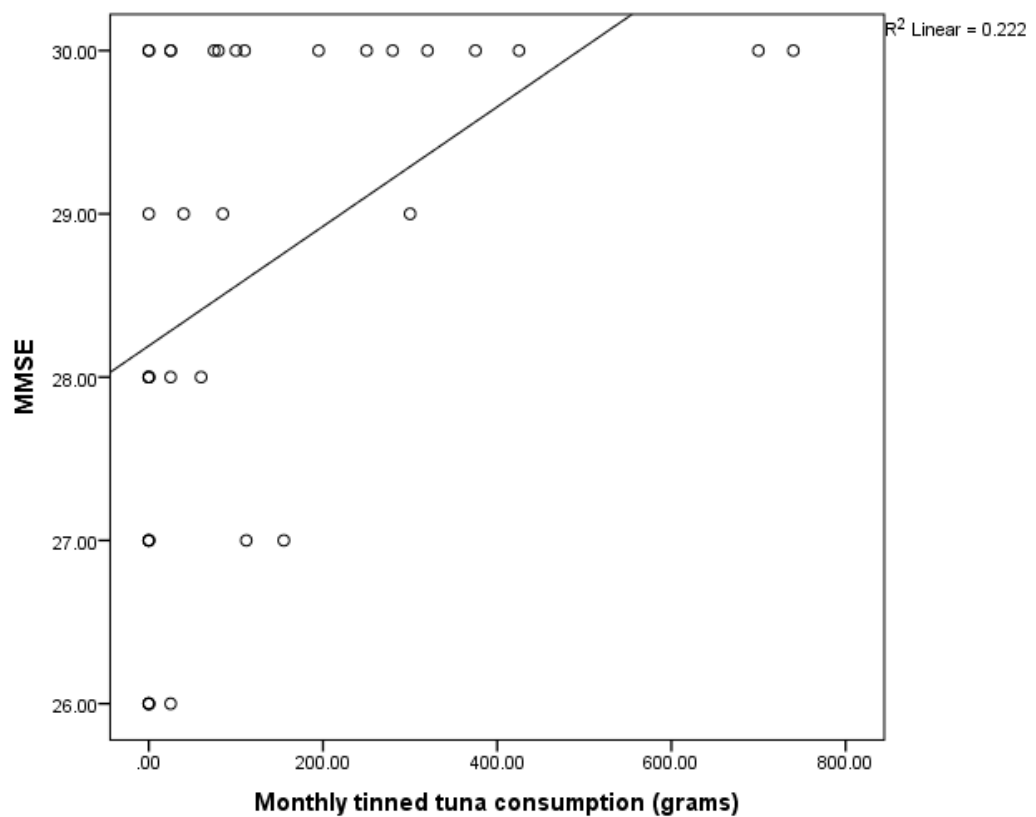


Figure 13: increased monthly tinned tuna consumption is associated with increased MMSE score.

6.4.5.1 Interactions between tinned tuna, age and MMSE score

In order to determine whether the effects of tinned tuna and general cognitive function changed during aging a MANCOVA was carried out and age and grams of tinned tuna consumption were entered into the model as covariates. An interaction term of age x grams of tinned tuna consumption was included. There was a significant interaction between tinned tuna, age and MMSE score [$F(1, 32) = 9.50, p < 0.01$]. In order to graphically represent this interaction tinned tuna consumption was divided into low and high consumption using the median grams of tinned tuna intake. Low tinned tuna consumption in older participants was associated with lower scores on the MMSE. In contrast the older elderly who consumed 'high intake' of tinned tuna perform better on the MMSE. See figure 14 for a scatter plot of this interaction.

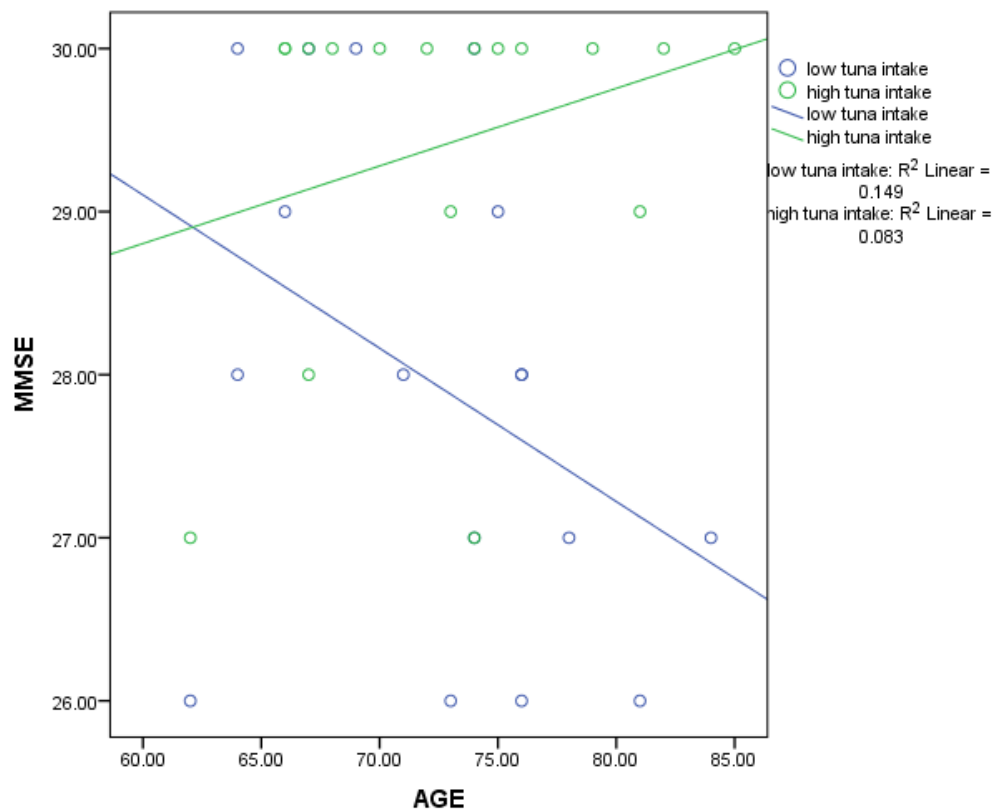


Figure 14: significant interaction between MMSE score, age and tinned tuna consumption. Participants with a low intake of tinned tuna experience a decline in MMSE performance with age whereas those with a high intake show a modest improvement with increasing age.

When the sample was divided based upon low and high tinned tuna consumption in those classified as ‘high intake’ age was not a significant predictor of MMSE performance ($p>0.05$). For participants classed as low tinned tuna consumers a weak trend was observed for age as a predictor of MMSE performance ($p=0.16$).

6.4.5.2 Effect of tinned tuna consumption on MMSE sub domains

It is important to investigate the effect of tinned tuna consumption on the different sub sections of the MMSE to see if the effects are general or whether there is a specific effect given that the test is made up of general cognitive domains. The points of the test were sub categorised according to the domain assessed into orientation (10 points), registration (3 points), attention, calculation and construction (6 points), recall (3 points) and language (8 points) respectively. Table 10 shows the mean, minimum and maximum scores for each sub domain of the MMSE in this cohort of participants.

Table 10: Participants’ mean and SD for scores on the sub domains of the MMSE

MMSE sub domains	Min	Max	Mean	±S.D
Orientation	9	10	9.94	0.24
Registration	3	3	3	0.00
Recall	0	3	2.21	1.09
Attention/Concentration and Calculation	2	6	5.76	0.78
Language	6	8	7.76	0.55

In order to determine whether tinned tuna consumption could predict performance on a specific section of the MMSE a multivariate multiple regression analysis was carried out. This showed that monthly grams of tinned tuna intake was a significant predictor of score on the recall portion only [$\beta = 0.002$, $F(1,32) = 4.00$, $p = 0.05$, y-axis intercept = 1.95, regression correlation coefficient $r = 0.33$] revealing that increased tinned tuna consumption is

associated with a greater number of words recalled on the MMSE recall question. See figure 15.

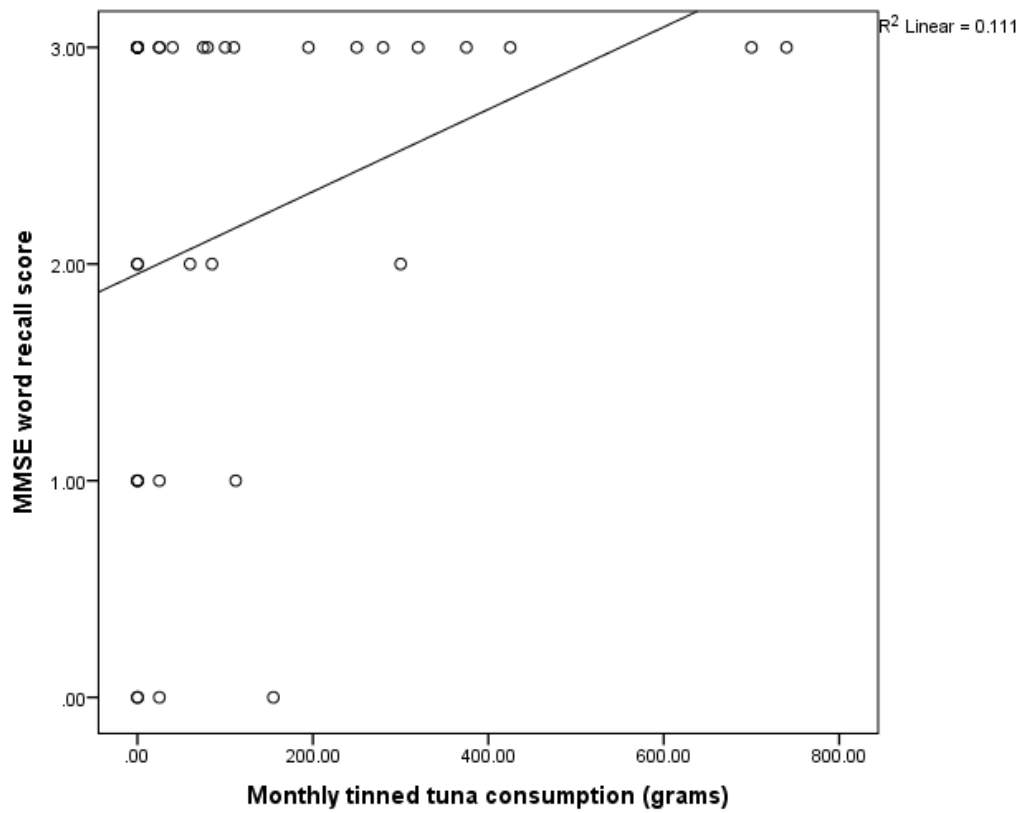


Figure 15: monthly tinned tuna consumption is associated with the word recall portion of the MMSE. Increased tinned tuna intake predicts better performance on this sub domain of the test.

6.5 Discussion

6.5.1 Main findings

In an earlier study Walters et al., (2011) used a FFQ to assess fish intake and a trend towards significance for portions of non-oily fish (cod and haddock) as a predictor of MMSE performance was found. The aim of the current chapter was to extend upon these preliminary findings in a more controlled manor by examining the effects of monthly fish intake (particularly non-oily varieties, in grams, rather than portions) on a neuropsychological test battery in the HE via a more detailed method of data collection, a four week food diary.

The main findings of the study were (1) total monthly fish intake in grams was a significant predictor of MMSE score. (2) a significant main effect of non-oily fish consumption in grams was found for the MMSE (3) of the non-oily fish types consumed, monthly tinned tuna consumption was the only significant predictor of MMSE score, (4) that score obtained on the word recall part of the MMSE was predicted by tinned tuna intake.

6.5.2 Total grams of fish intake predicts MMSE score

Total grams of fish intake (i.e. oily + non-oily varieties) was a significant predictor of MMSE score in this cohort. The more fish consumed the better the participants' performance on the MMSE. There are few studies which have related current fish consumption to cognitive performance and most have focussed on whether fish consumption can predict cognitive decline or dementia and AD risk (e.g. Devore et al., 2009; van Gelder et al., 2007; Morris et al., 2003; Kalmijn et al., 1997; Roberts et al., 2010). Although some of these studies (e.g Van Gelder et al., 2007 and Kalmijn et al., 1997) have used the MMSE as a part of their cognitive assessment, none have specifically investigated which sub section of the test may be associated with fish intake. Nurk et al., (2007) reported in their cohort that fish eaters had significantly better performance on all of the neuropsychological tests administered (including the modified MMSE and TMT).

6.5.3 Tinned tuna as a predictor of MMSE score

Increased monthly tinned tuna intake was associated with better performance on the MMSE. Further investigation of the data revealed that tinned tuna consumption was a significant predictor of participants' score on the word recall portion of the test which assesses short-term memory. Increased intake was associated with a greater number of words recalled on this question.

As an assessment of general cognitive function, the MMSE assesses a number of cognitive processes (Folstein et al., 1975). As discussed in chapter four 'Neuropsychological testing', section 4.2.1, the test consists of 12 items which include orientation, registration, recall, attention, concentration, calculation and language (Nasreddine et al., 2005; Markwick et al., 2012). The test is weighted towards memory and orientation (Alzheimer's Society, 2013) and devotes 16 of its 30 points to these domains. Consequently the MMSE does not tax the full breadth of cognitive processes.

The other tests which were administered as a part of the cognitive battery in this chapter assessed processing speed (specifically the PCS, LCS, TMT part A, Stroop Word), EF (specifically set switching in the TMT part B, inhibition in the Stroop interference and performing two tasks simultaneously in the DT), working memory (specifically the digit span task) and general cognitive function (the MoCA). There was no effect of tinned tuna intake on any of these assessments. The MMSE, which was associated with tinned tuna consumption, does not contain any questions within its 30 points which assess processing speed, working memory or EF. Therefore, the lack of association between tinned tuna and score on the PCS and LCS, digit span, Stroop, DT and TMT concurs. The finding that tinned tuna was a predictor of the number of words correctly recalled on the recall sub-section appears to suggest that tinned tuna is selectively associated with short-term memory performance in this cohort of participants.

Like the MMSE the MoCA also assesses general cognitive performance but there was no association between tinned tuna consumption and MoCA scores in this cohort. The MoCA was specifically designed to detect mild forms of cognitive impairment (Nasreddine et al., 2005) and the domains of cognition that the MoCA taxes differ slightly from the MMSE (Jacova et al., 2007;

Markwick et al., 2012). For example, the MoCA contains a more complex assessment of visuospatial skills whereas the MMSE asks participants to copy two interlinked pentagons, which assesses visual construction (Guerrero-Berroa et al., 2009). The MoCA requires the participant to correctly join a series of circles containing numbers and letters (similar to the TMT part B), copy a 3D cube and draw the face of clock inserting all of the correct numbers and setting the time (a version of the CDT). The MoCA also incorporates questions assessing working memory (digit span) and abstraction (Markwick et al., 2012). It is worth noting that, regarding word recall, unlike the MMSE the MoCA's assessment of this domain requires participants to remember five words, instead of the three words in the MMSE (Nasreddine et al. 2005). The MoCA also allows participants two attempts to repeat these words in the learning phase which differs from the MMSE where they are only asked to repeat each word once. Recall of words in the MoCA occurs after a longer delay of five minutes; therefore, it is argued that the MoCA incorporates a more rigorous assessment of memory (Markwick et al., 2012). Delayed recall has been shown to be a sensitive domain for the prediction of future cognitive decline (Jacova et al., 2007; Chodosh et al., 2002; Albert et al., 2011).

Given the significant differences between the MMSE and MoCA in terms of their structure and testing domains it is difficult to compare the two with regards to tuna's effect on word recall. Further study utilising a thorough assessment of episodic memory using alternative tests will be required to investigate the effect of tinned tuna on short term recall in more detail.

Tinned tuna, unlike fresh tuna, is classified as a 'non-oily' fish (Scientific Advisory Committee, 2004 page 104; British Heart Foundation, 2012). To my knowledge there have been no previous studies which have specifically reported an effect of tuna consumption on MMSE performance or cognitive performance in general. The interactions between tinned tuna, age and MMSE score showed that those participants classified as consuming larger amounts of tuna per month did not experience a decline in MMSE performance with increasing age. The exact reasons for this cannot be determined from the present data and longitudinal follow up of this cohort will investigate whether tinned tuna consumption is associated with any cognitive decline with aging.

6.5.4 MMSE score and Walters et al., (2011)

The results of this chapter were not able to replicate the trend towards significance for cod and haddock portions as a predictor of MMSE score reported in Walters et al., (2011). This is likely to be due to less reliable methods of measuring dietary intake (i.e the FFQ) and the fact that a different cohort of participants were recruited and therefore differences in MMSE scores were obtained.

6.5.5 Methodological considerations

Creating a detailed four week food diary for the dietary recollection of fish intake has removed some of the potential for errors associated with FFQs such as the reliance upon self estimation of intake (Liu et al., 2013; Bowman et al., 2012). Using a battery of cognitive tests allowed the investigation of whether non-oily fish consumption had a domain specific effect on cognitive performance. Assessing dietary fish intake over a period of four weeks may not represent general consumption and intake may vary depending on the time of year. Likewise, current intake may not reflect the individuals' life time intake (Gillette-Guyonnet et al., 2013) and participants may have increased their consumption of fish in midlife because they were aware of the potential health benefits associated with increased fish consumption (Morris et al., 2009). For example, consumption of fish has been recommended for those with heart disease and is associated with a decreased risk of heart attack and coronary disease (NHS, 2013; Morris, 2009; Kalmijn et al., 1997).

Tinned tuna was one of the most commonly consumed fish by this cohort. Participants also consumed larger amounts of salmon and haddock relative to the other varieties but there was no effect of these types of fish on MMSE score. Increased consumption of the other varieties of fish consumed by this cohort may lead to an effect of these fish types on cognitive performance although this cannot be determined by the present data.

The participants in this cohort volunteered to take part in the study. As a result they may not be a true representation of the general population. It is possible that those participants who consumed a greater amount of fish per month were

more likely to possess a healthier diet over all. Wennberg et al., (2012) commented that fish consumption in general seems to be associated with a healthy lifestyle. Cunnane et al., (2009) noted that fish consumers tend to be consumers of other healthy foodstuffs such as fruits and vegetables which are also rich sources of antioxidants. Fish is a major component of the Mediterranean diet (Cunnane et al., 2009; Martínez-Lapiscina et al., 2013), which has in some previous research been associated with a lower risk of developing AD (Scarmeas et al. 2006; von Arnim et al, 2010; Cunnane et al., 2009). Fish is commonly thought of as a healthier dietary option by the general public (Cunnane et al. 2009) and individuals consuming fish, in the case of this chapter, tinned tuna, may be more likely to be aware of other healthier dietary options. This type of diet is likely to protect against other age associated conditions such as diabetes, hypertension and high cholesterol and promote healthy aging overall (Cunnane et al., 2009).

As a non-oily fish, tinned tuna contains lower amounts of fish oils than fresh tuna and other oily fish varieties as the process of 'tinning' reduces the fish oil content to that of other non-oily varieties (NHS, 2011). It is possible that any potential benefits of increased consumption maybe due to other nutrients aside from DHA and EPA within non-oily fish. For example, their high protein content (Cunnane et al., 2009), selenium levels (Cunnane et al., 2009; Mehdi et al., 2013; Gao et al., 2007) vitamin content (Cunnane et al., 2009) or low fat content (Scientific Advisory Committee on Nutrition, 2004).

6.5.6 Implications

The results of this food diary study have shown that a diet which includes a larger amount of non-oily fish, specifically tinned tuna, is associated with improved performance on the MMSE with the significance lying in the MMSE's assessment of short-term recall. The MMSE is recommended as a test of choice for the diagnosis of cognitive impairment by the recently reviewed NICE dementia guidelines (NICE, 2011) and is still used in the early assessment of cognitive impairment (Alzheimer's Association, 2012). The participant's score on the MMSE can be considered when determining diagnosis, treatment and research inclusion. Performance may also be compared with normative data

sets. It is acknowledged that the MMSE has received some criticisms for being insufficiently sensitive to mild forms of cognitive impairment (Mitchell, 2009, Tombaugh and McIntyre, 1994) and the occurrence of ceiling and floor affects are common which creates clusters of scores at the test's maximum and minimum range (Crum et al., 1993). Despite this the MMSE is still widely used by clinicians and researchers to determine overall global cognitive function and detect early cognitive symptoms.

The fact that tinned tuna intake was able to predict MMSE score, specifically the word recall portion of the task, in a sample of older HE adults may have implications for cognitive testing. The findings also add to the current literature investigating dietary factors and cognitive performance in the elderly. The Alzheimer's Association comment that GPs will often ask patients about their dietary intake. Should a strong effect remain with further study this may have implications for the promotion of fish as a modifiable dietary factor which may aid cognitive aging. This is relatively inexpensive and is a method that the individual themselves can implement in the home without the need for consultation with clinicians which is time consuming and expensive. There has been very little literature which has incorporated food diaries as a method of measuring fish intake. The use of the food diary in the present chapter has demonstrated that diaries can be useful tools to collect dietary information from elderly participants when conducting research within the field of cognitive neuropsychology.

6.6 Conclusion and summary

The results of this chapter have shown that a greater monthly intake of tinned tuna is associated with better performance on the MMSE. Furthermore, tinned tuna consumption was a significant predictor of participants' scores on the recall item of the MMSE which assesses short term memory. There was no effect of tinned tuna intake on any of the other assessments administered.

The results have novelly suggested that tinned tuna consumption may be important for both general cognitive performance and short-term memory. This has implications for cognitive research, cognitive aging and the diagnosis of cognitive impairments. The fact that total tinned tuna consumption is associated

with better performance on the MMSE's recall item and none of the other cognitive processes assessed in the battery administered is important. Future study will need to investigate this further via the administration of other cognitive assessments assessing short term memory and word recall.

6.7 Chapter summary of key points

- Fish consumption is a dietary factor which may influence cognitive function and neuropsychological test performance in the elderly.
- Previously, Walters et al., (2011) reported via a FFQ, a trend towards significance for non-oily fish consumption (specifically cod and haddock) as a predictor of MMSE performance in the HE.
- Fish is a rich source of fish oils and non-oily varieties also contain other nutrients which may be associated with brain health.
- A four week food diary was created to enable participants to record all meals containing a fish item as well as specifically recording the grams of each portion. Gram information is likely to provide a more accurate representation of intake in comparison to portions which may differ in size.
- Participants completed the food diary and a cognitive battery in order to extend upon the findings of Walters et al., (2011) and investigate the trend towards non-oily fish intake and MMSE score in a more controlled manor.
- A MANCOVA revealed there was a significant association between non-oily fish consumption and MMSE score.
- Of all the non-oily fish varieties consumed a regression analysis showed that grams of monthly tinned tuna intake was a significant predictor of MMSE score in this cohort.
- Multivariate multiple regression analysis of the MMSE's sub domains showed that tinned tuna consumption was a significant predictor of participants' performance on the word recall portion of the test which assesses short-term memory.
- These preliminary findings are worth future study.

Chapter 7

The effect of time of day on neuropsychological test scores in the elderly

7.1 Introduction

As previously stated in the introductory chapters of this thesis neuropsychological testing forms a part of the diagnostic process for the detection of AD and MCI (Chapman et al., 2010). As the incidence of AD continues to increase it is vital that the diagnostic process is as accurate and reliable as possible if mild alterations in cognition are to be detected at the earliest possible time.

One current issue is that many of the neuropsychological assessments used are influenced by a number of external factors, which if not controlled for or taken into consideration, may have the ability to affect the accuracy of the scores obtained (Overshott and Burns, 2004; Jacova et al., 2007; Forlenza et al., 2010; Ashford et al., 2006). Even subtle external variables may have the ability to affect any data obtained which may have consequences for the interpretation of test scores. So far chapter five of this thesis has explored whether prior consumption of pure caffeine can affect cognitive performance in the elderly (Walters and Lesk, in prep - a; Lesk and Walters, 2012) and chapter six has investigated whether non oily fish, a dietary component can influence cognition. There are many other variables to consider such as the participant's mood (Jacova et al., 2007), their prior consumption of CCFS (i.e. tea, coffee, soft drinks or chocolate) (Lesk et al., 2009), and possibly any medications which they are prescribed (Gray et al., 1999). This chapter will explore the effect of time of day (TOD) on cognitive performance in the elderly. If the time that cognitive tests are administered can affect the score obtained then this has implications for the interpretation of data and ultimately the accuracy of a potential diagnosis. In addition, this chapter will also investigate whether 200mg of caffeine can influence the presence of TOD effects in the elderly, using the data from chapter five.

7.1.1 Peak arousal

Across the 24 hour day there are fluctuations in cognitive performance and this has been linked to the influence of underlying biological circadian rhythms (Froy, 2011). Circadian peak efficiency refers to the TOD that the individual is at their optimal arousal and this alters with age (Hasher et al., 2005) which impacts on the sleep-wake cycle. The elderly often report a decline in their night time sleep quality and quantity (Schmidt et al., 2012), a decreased sleep depth (Bliwise, 2005), increased reports of napping in day light hours (Blatter and Cajochen, 2007) and earlier sleep onset and offset times (Farajnia et al., 2013). These changes result in 75% of older individuals subjectively reporting that they would classify themselves as morning types in comparison to only 7% of younger adults (Yoon et al., 1999). This tendency to prefer mornings is reflected in daily living tasks, for example, Yoon et al., (1999) used a simple questionnaire to evaluate daily living habits in both young and older adults in order to determine whether TOD effects were present. They found that over 80% of the older participants reported that they read newspapers in the morning compared to only 14% of the younger adults. Likewise, over 50% of older adults went shopping during the morning when they were at their optimal arousal. Leirer et al., (1994) also reported that the elderly were more likely to arrange appointments and take their medications in the morning when they believe that they are at their most alert.

7.1.2 Implications for cognitive performance

The preference for morningness seems to begin at approximately age 50 (Schmidt et al., 2007) and has implications for cognitive performance. For example, young adults show improvements in performance during the day with a peak efficiency in the afternoon whereas the opposite pattern is observed in the elderly who experience a marked deterioration in their performance as time spans into the afternoon and evening (Yoon et al., 1999; May et al., 1993; May and Hasher, 1998; Schmidt et al., 2007; Yang et al., 2007). The Morningness-Eveningness Questionnaire (MEQ) (Horne and Ostberg, 1976) is a psychometric index of circadian rhythmicity and scores on this questionnaire typically correlate with the participant's cognitive performance (Winocur and

Hasher, 2004). The questionnaire consists of 19 questions and participants receive a score of between 19 and 86 points. Scores are divided into five potential categorical outcomes which include 'definite evening', 'moderate evening', 'intermediate', 'moderate morning' and 'definite morning'. High scores are associated with morning types and lower scores with evening types. The scores obtained on this questionnaire correlate well with biological measures of circadian rhythmicity including body temperature (temperature increases during mental activity (Weinert, 2010)), sleep cycle and levels of alertness (Hasher et al., 2005; Tankova et al., 1994).

May et al., (1993) administered the MEQ to 210 young adults aged between 18 and 22 years. They reported that only 6% of the sample were classified as 'moderately morning types' and that none of the young participants were defined as 'definitely morning types'. When the authors administered the questionnaire to a sample of older adults, aged between 66 and 78 years, less than 2% of these participants were categorised as 'evening' types. These findings suggest that there appears to be substantial shifts in arousal times during aging.

Hasher et al., (1999) suggest that when the individual is able to choose at what time to take part in an assessment their performance is optimised; hence age related deficits will be more pronounced when older participants are tested at their non-preferred time which for the majority of these individuals is the afternoon (Hasher et al., 1999; Blatter and Cajochen, 2007). Under normal sleep-wake patterns the young outperform the old on most cognitive domains, however, this is not as pronounced when the older adults are assessed at their chosen optimal time i.e. the morning (May and Hasher, 1998). This effect has been noted on assessments of word span (Yoon et al., 1999), sentence recognition (May et al., 1993), cued recall (May et al., 2005), story recall (Winocur and Hasher, 2002) and a false memory paradigm (Intons-Peterson et al., 1999). The elderly tested in the morning do not perform significantly different from young adults also tested in the morning. For participants of any age, cognitive performance will be maximised when testing occurs during the individual's optimal arousal period. This effect has been termed 'the synchrony effect' (Hasher et al., 2002; May et al., 1993; May and Hasher, 1998) and as mentioned earlier, for the majority of young adults peak arousal occurs during

the late afternoon and evening whereas for older adults peak arousal occurs in the morning (Schmidt et al., 2007).

This synchrony effect is also seen on assessments of EF. Whilst younger adults generally outperform older adults at executive tasks, (Ohsugi et al., 2013; Goh et al., 2012) including those with an inhibitory component, (Zacks and Hasher, 1994) these age differences can be reduced when older adults are tested at their preferred TOD, the morning. These findings have implications for neuropsychological testing and May et al., (1993) suggested that variations in cognitive test scores may be exaggerated if participants are tested at their non-optimal time. This is a consideration for the interpretation of scores on cognitive tests and the comparison of scores with normative data.

The forced desynchrony paradigm has also been used to investigate TOD effects on cognition (Blatter and Cajochen, 2007). This involves disrupting the synchrony between the sleep-wake cycle and the circadian rhythms which control various physiological processes within the body over a 24 hour natural light-dark cycle (Blatter and Cajochen, 2007; Schmidt et al., 2007). In humans these two processes interact so that typically individuals are awake during 'light' conditions (i.e. during the day) and asleep during the night when light levels decrease. Forced desynchrony of these processes can, therefore, be implemented by exposing an individual to an artificial sleep-wake cycle which can be achieved by shortening or lengthening the typical 24 hour period (Silva et al., 2010; Blatter and Cajochen, 2007). Although such experiments can be time consuming they are useful for investigating and determining the effects of circadian mis-alignment on cognitive performance during aging, particularly whether alterations to sleep and peak arousal times affect TOD effects. Results from forced desynchrony experiments have shown that younger adults seem to be more affected by sleep loss than older adults and that sleep disturbance in the elderly seems to have less of an effect on cognitive performance (Silva et al., 2010).

Silva et al., (2010) investigated a small sample of 10 younger and 10 older adults who took part in a 20 hour forced desynchrony study. Three weeks prior to the study all subjects were asked to follow sleep-wake schedules consisting of eight hours of sleep (at their habitual times) and 16 hours of time spent

awake. In the third week participants were instructed to abstain from caffeine, nicotine and alcohol and their compliance was checked using toxicological analysis. During the study phase, after three baseline days following the schedule mentioned above, 20 hour forced desynchrony schedules were implemented. Participants were required to spend 13.33 hours awake and 6.67 hours asleep, resulting in 'waking' periods which were four hours earlier than normal. This was completed for a minimum of 18 consecutive days. For the duration of the study younger and older adults resided in private study rooms at the testing location. The results showed that the younger adults reported greater feelings of sleepiness, had longer reaction times and exhibited more moments of lapsed attention during waking hours than the older adults. The authors speculate that the ability of older adults to maintain reaction times and sustained attention comparative to younger adults after desynchrony may be due to alterations in the circadian cycle with age. For example, circadian signals become weaker with increasing age and older adults may, therefore, be better able to cope with periods of sleep deprivation (Silva et al., 2010).

7.1.3 TOD and cognitive domains

Studies investigating TOD effects on cognitive performance in the elderly have revealed that not all cognitive processes are equally affected (Schmidt et al., 2007). Tasks which assess EF seem to be especially vulnerable to TOD effects with significance reported for tasks engaging attentional and inhibitory processes (Hasher et al., 2007; Yang et al., 2007; Winocur and Hasher, 2004). The frontal lobes experience the largest decline in brain volume with age and are also vulnerable to fatigue (Hedden and Gabrielli, 2004; Schmidt et al., 2012; Blatter and Cajochen, 2007). There are, however, substantial individual differences in the rates and patterns of normal brain aging which may explain to some extent the differences in performance seen on some tasks (Bugg et al., 2006; Hasher et al., 2005).

Miyake et al., (2000) suggested that EF incorporates three main processes, working memory, task switching and inhibitory control. Inhibitory control links with attentional control and the ability to ignore distracting stimuli (Yoon et al., 1999). Older adults often complain that they are unable to focus and maintain

attention for long periods of time (Brumback-Peltz et al., 2011). Furthermore, they report feelings of being unable to suppress or ignore distracting stimuli (Hasher and Zacks, 1988). EF tasks tend to be complex and, therefore, are more likely to be sensitive to sleep loss and tiredness (Blatter and Cajochen, 2007).

Deficits of inhibitory control are present in the elderly and in patients with AD (May and Hasher, 1998; Weintraub et al., 2012; Festa et al., 2010). Age related deterioration has been shown to be more severe when older adults were assessed at their non-optimal time on assessments of inhibitory efficiency (Yoon et al., 1999; May and Hasher, 1998). For example, West et al., (2002) investigated 20 young adults and 20 older adults. Ten participants from each age group were tested in the morning (mean age of younger adults – 25.10 years, mean age of older adults – 72.6 years) and the remaining 10 participants from each group were assessed in the evening (mean age of younger adults – 23.70 years, mean age of older adults – 72.9 years). Performance was compared on a working memory task (the Four-box task) which included an inhibitory component. The first portion of the task requires the participant to observe which of the four boxes presented on screen a stimulus (smiley face) will appear in. Once the stimulus appears the participant must press the corresponding box on their response key pad as quickly and accurately as possible. In the second part of the task an inhibitory component is introduced. The participant must still press which box the stimulus appears in but must also simultaneously ignore the box which will contain a distracter shape. The third stage requires the participant to remember which box the stimulus appeared in the previous trial (i.e. 1-back) and the fourth stage requires the 1-back identification of the stimulus but again a distracter is present.

The study also used the MEQ (Horne and Ostberg, 1976) to assess participants' TOD preference and measured their body temperature at varying intervals. Body temperature is decreased at rest and elevated when the subject is mentally active thereby indicating the participants level of arousal (Weinert, 2010). Subjective alertness levels were also noted. Consistent with previous research, the older adults reported a preference for mornings and this was matched by higher subjective ratings of alertness in the morning. As expected older adults performed worse on the inhibitory portion of the task, which

involved ignoring a distracter and focussing on correctly determining the location of the visual target (i.e. the smiley face) as compared to the first stage of the task when no distracter shape was present, when tested in the afternoon (West et al., 2002).

The investigation of TOD effects on other cognitive domains has not been widely investigated, however, TOD does not appear to affect tasks which utilise well established knowledge such as sentence completion or vocabulary (Borella et al., 2011; Yoon et al., 1999). Yoon et al., (1999) noted that vocabulary test scores in both young and older adults did not change considerably across the day. This implies that retrieval of information from semantic long term stores seems to be spared at non-optimal times. Yoon et al., (1999) hypothesise that well learned familiar items of information are not likely to be affected by TOD fluctuations in arousal. The characteristics of the task employed show different vulnerabilities to TOD effects. For example, tasks which are longer in duration, have a high cognitive load and take longer to complete are more likely to be affected by the TOD at which they are carried out (Schmidt et al., 2007; Blatter and Cajochen, 2007). The order in which tests are administered to participants may also influence whether TOD effects are found. For example, participants may perform at their maximum on tests which are administered at the start of a cognitive battery but performance may decline on tests completed at the end of the battery due to increased mental fatigue from sustained attention on previous tests (van der Heijden et al., 2010).

7.1.4 Caffeine and TOD effects

Chapter five investigated whether the consumption of 200mg of pure caffeine consumed 40 minutes before the neuropsychological assessment could affect cognitive test scores in the elderly. Caffeine is a widely consumed stimulant found in many food stuffs such as tea, coffee, chocolate and sweets (Nehlig, 2010). Consumers often report increased subjective energy levels and alertness following caffeine consumption (Lorist and Tops, 2003; Addicott and Laurienti, 2009; Sagaspe et al., 2007). In addition, caffeine is often used purposely to modulate the sleep cycle, for example, by individuals who complete shift work and who are required to stay awake during night time hours (Lorist and Tops,

2003; Samuels and Szabadi, 2008; Sagaspe et al., 2007). Chemically caffeine is a methylxanthine and an antagonist for adenosine receptors in the brain (Cunha, 2001). During prolonged periods of wakefulness levels of cerebral adenosine increase, particularly in the basal forebrain regions (Basheer et al., 2004), until a threshold is reached and sleep is initiated. During sleep adenosine levels begin to fall back to baseline and are at their lowest when the individual awakens (Porkka-Heiskanen and Kalinchuk, 2011). Caffeine's ability to antagonise adenosine receptors prolongs wakefulness and decreases subjective tiredness (Cunha, 2001). At habitual levels, (i.e. at amounts which are commonly consumed during a typical day, but that can vary depending on location and culture (Nawrot et al., 2003)) caffeine administration in humans acts as a neurostimulant (Addicott and Laurienti, 2009) and can antagonise the rise in adenosine levels seen during prolonged periods of wakefulness,. This is particularly apparent in the BF and hypothalamus and counteracts sleep induction, prolonging the time spent awake (Basheer et al., 2004). Tiredness in the afternoon may, therefore, be influenced by the administration of caffeine and may result in a decrease in the TOD effects noted on cognitive performance in the elderly.

Previous research in the elderly has demonstrated that the prior consumption of varying doses of caffeine can impact on neuropsychological test performance (Walters and Lesk in prep - a (see chapter five of this thesis); Lesk and Walters, 2012; Lesk et al., 2009). Lesk et al., (2009) investigated the effects of CCFS on cognitive performance in a group of elderly participants. In those participants who had consumed CCFS up to four hours prior to testing there were significant interaction effects between age and tests of processing speed, semantic memory, visuospatial associative memory and EF. This was not seen for participants that had not consumed CCFS prior to testing. Chapter five of this thesis (Walters and Lesk in prep - a) extended these findings and found significant interaction effects for 200mg of pure caffeine and age on tests of EF and processing speed, whereby the older elderly performed significantly worse than the younger elderly. This effect was not seen in the placebo group. These results highlighted the need to consider prior caffeine consumption when scoring neuropsychological tests or comparing results to normative data. With regards to the present chapter, because CCFS are commonly consumed to

relieve tiredness (Carrier et al., 2009), it is important to investigate whether 200mg of pure caffeine, (equivalent to one strong cup of coffee as stated in chapter five), can interact with the TOD that tests are administered and subsequently affect test scores. Should there be a significant effect, this has implications for the diagnosis of AD and/or MCI. This is particularly important as scores can determine treatment and/or inclusion/exclusion in research and treatment trials.

7.2 The present study: research aims

There has been a large focus on the effects of TOD on tasks of EF and inhibition with little literature investigating TOD effects across all cognitive domains or on assessments which are commonly used at the primary consultation such as the MMSE or the MoCA. The MMSE is currently recommended by NICE as one of the brief cognitive assessments to be used in the diagnosis of dementia (NICE, 2011). Furthermore, the score obtained on this assessment can be used to determine whether or not a patient is prescribed medication (NICE, 2011). An earlier version of the NICE dementia guidelines published in 2006 had recommended that treatment with AchEIs be discontinued if the patient's MMSE score fell below a certain cut off point (<10/30 points). After heavy criticism this was amended in the latest revised version, published in 2011, to state that clinicians should continue to prescribe medication as long as the drugs were still having a benefit on the patient's cognition, behaviour or quality of life (NICE, 2011). Despite these criticisms the MMSE remains the most widely used brief test of general cognitive ability and it is important to investigate the effects of external factors such as TOD on MMSE performance.

This chapter will investigate TOD effects on two data sets obtained from studies carried out during this PhD which administered a battery of assessments taxing a variety of cognitive domains. One of the data sets is that of chapter five 'the effect of caffeine consumption on neuropsychological testing in the elderly' which allows investigation of low doses of pure caffeine and TOD effects on the neuropsychological assessments administered. The second data set is that of

chapter six which investigated the effect of non-oily fish consumption on cognitive performance in the elderly via the use of a food diary.

7.3 Methods

Two data sets were investigated for TOD effects. Participants were recruited from the University of Bradford over-60s participant pool and gave full informed written consent to take part.

Data set one (data from chapter six of this thesis) – the aim of this study was to determine whether monthly non-oily fish consumption, as assessed by the completion of a food diary influenced neuropsychological test performance in the elderly. The mean age of participants was 72.53 (S.D \pm 6.09) years and the mean years spent in education was 14.76 (S.D \pm 4.60). The sample comprised 11 males and 25 females. Inclusion criteria for this study specified that participants must be aged 60 years or over, must be cognitively healthy, i.e. must not possess a current diagnosis of any neurological disorder or memory impairment. The neuropsychological battery administered consisted of the MoCA, digit span forwards, Stroop test, PCS, LCS, DT, TMT and the MMSE.

Data set two (data from chapter five of this thesis) – the aim of this study was to investigate the effect of 200mg of pure caffeine on neuropsychological test performance in the elderly using a double blind treatment vs. placebo design. The mean age of participants was 73.35 (S.D \pm 6.56) years and the mean years in education was 15.53 (S.D \pm 3.93). The sample comprised nine males and 31 females. Inclusion criteria for this study specified that participants must be aged 60 years or over, must not have an existing diagnosis of a memory impairment or neurological disease, must be regular consumers of caffeine (but not have more than 400mg per day). Participants must not have been advised by a health care professional to avoid consuming caffeine. All participants were required to have abstained from CCFS 12 hours prior to attending their appointment and alcohol for 24 hours prior. See chapter five for more details on the methodology. Neuropsychological tests administered included the MMSE, SDMT, Stroop Test, Word Recall, PCS, LCS, PAL, GNT, digit span and the DT.

7.3.1 Materials

Assessments

Detailed descriptions of each cognitive assessment that was administered can be found in chapter four '*Neuropsychological Testing*'. A list of the tests and the domains they assess are given below for reference.

- **MMSE** - an assessment of general cognitive function (Folstein et al., 1975).
- **MoCA** - an assessment of general cognitive function (Nasreddine et al., 2005).
- **Immediate Word Recall** - an assessment of short-term memory created by the researcher.
- **CANTAB PAL** - an assessment of visuo spatial associative memory (Égérhazi et al., 2007).
- **CANTAB GNT** - an assessment of semantic memory (McKenna and Warrington, 1980).
- **SDMT** - a timed assessment of EF (Smith, 1968).
- **Stroop Test** - an assessment of EF and processing speed (Trener et al., 1989).
- **PCS and LCS** - assessments of processing speed (Salthouse and Babcock, 1991).
- **Digit Span and Digit Score** - assessments of working memory (Wechsler, 1981).
- **DT** - a test of EF, (Baddeley et al., 1997).
- **TMT** - a two part test assessing visual attention and EF (Reitan and Davison, 1974). Part A assesses visual attention and Part B assesses EF, specifically flexibility (Ashendorf et al., 2008).

7.3.2 Procedure

Data sets from two studies were analysed for TOD effects. TOD was not controlled for. All participants completed the full battery of neuropsychological tests in the same order. The cognitive batteries differed slightly between data

sets. For data set two it must be noted that participants had abstained from caffeine for 12 hours prior to testing and alcohol for 24 hours prior to testing and were randomly divided into two groups, one group received 200mg of caffeine prior to the neuropsychological assessment and the second group a placebo.

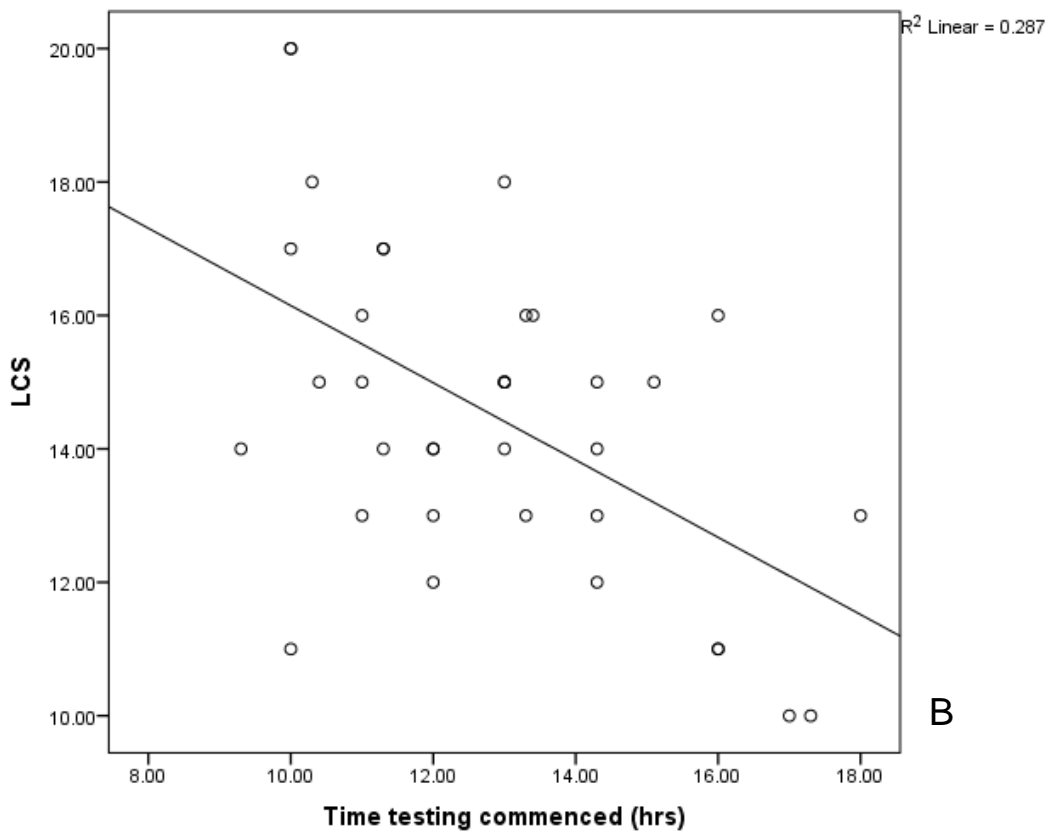
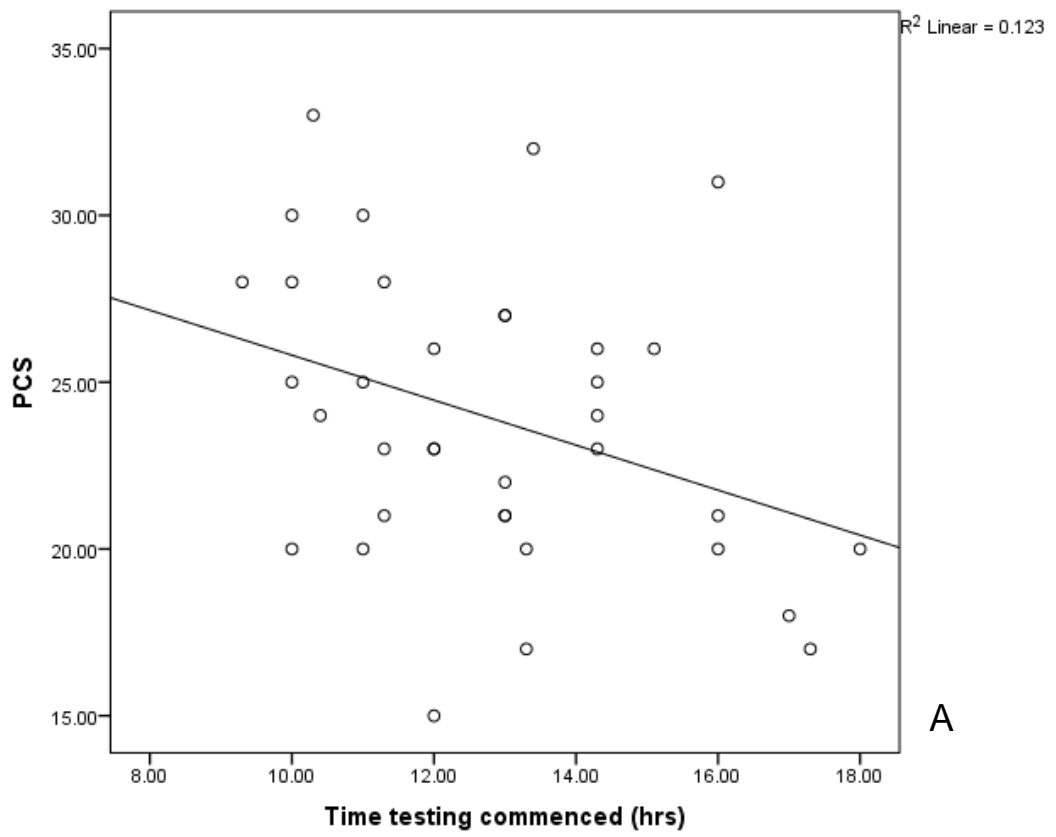
7.4 Results

The aim of the study was to investigate the effect of TOD on two batteries of neuropsychological assessments administered to elderly participants.

7.4.1 Main effects of TOD on cognitive test scores

Data set one:

To formally assess any influence of TOD on test scores a multivariate analysis of covariance (MANCOVA) with test scores as the dependent variables and TOD as a covariate was carried out. Significant main effects of TOD were present for the PCS [$F(1,33) = 4.72, p < 0.05$], LCS [$F(1,33) = 13.51, p < 0.01$] and part A of the TMT [$F(1,33) = 4.29, p < 0.05$]. A regression analysis revealed a linear decrease in performance on all of these assessments as TOD progresses into the afternoon. PCS - [$\beta = -0.35, (F(1,34) = 4.77, p < 0.05)$, y-axis intercept = 32.55 with regression correlation coefficient $r = 0.35, p < 0.05$], LCS, [$\beta = -0.54, (F(1,34) = 13.67, p < 0.01)$, y-axis intercept = 21.94 with regression correlation coefficient $r = 0.54, p < 0.01$] and TMT part A, [$\beta = 0.33, (F(1,34) = 4.23, p < 0.05)$, y-axis intercept = 10.28 with regression correlation coefficient $r = 0.33, p < 0.05$]. Scatter plots of these tests can be seen in figure 16. In addition, a trend towards significance was found for TOD and MoCA score, $p = 0.08$.



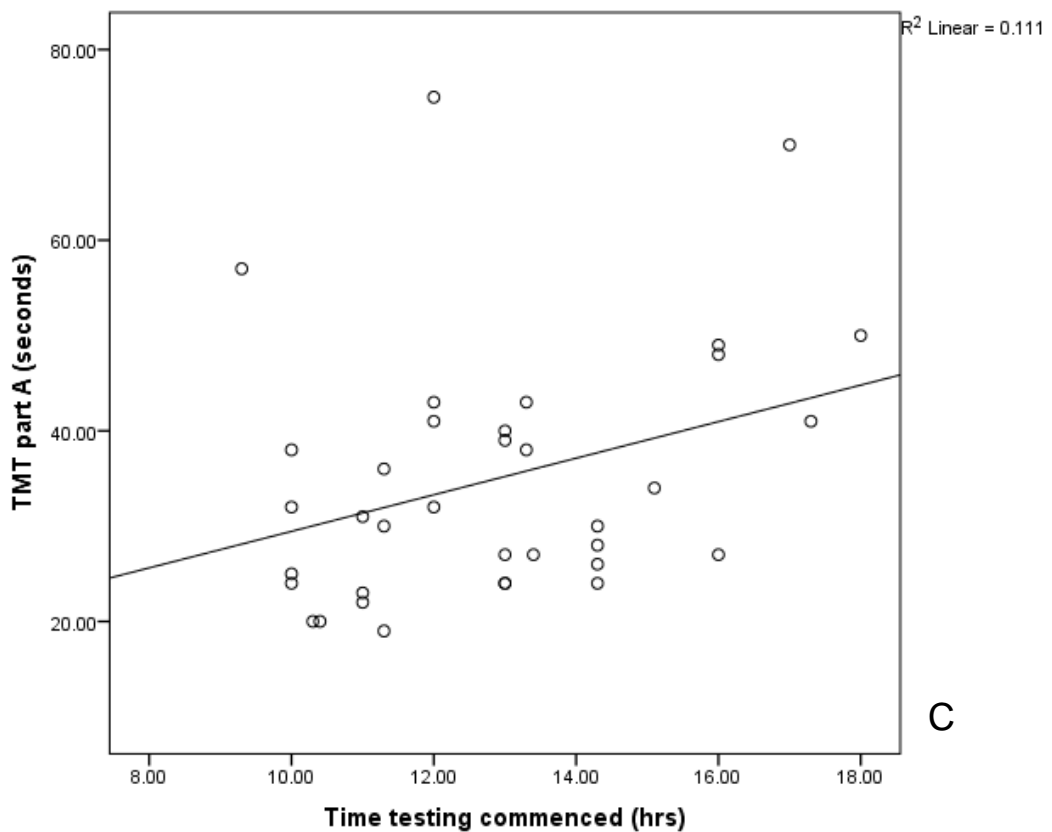


Figure 16: data set one - a linear decrease in performance as time spans into the afternoon on assessments of A - PCS, B - LCS and C - TMT part A. Negative correlations are present for the PCS and LCS signifying a lower number of patterns (PCS) or letter strings (LCS) were correctly compared as TOD progresses. A positive correlation is presented in part A of the TMT signifying that as TOD increases subjects take longer to complete the task.

Data set two:

Following the same analysis method as mentioned in the previous section, data set two revealed significant main effects of TOD on the MMSE [$F(1,38) = 9.01$, $p < 0.01$] and the GNT [$F(1,38) = 7.69$, $p < 0.01$]. Regression analysis revealed that TOD was a significant predictor of MMSE score [$\beta = -0.24$, $F(1,38) = 9.01$, $p < 0.01$, y-axis intercept - 31.55, with regression coefficient $r = 0.44$ $p < 0.01$] and GNT score [$\beta = -0.58$, $F(1,38) = 7.69$, $p < 0.01$] y-axis intercept = 33.18, with regression coefficient $r = 0.41$, $p < 0.01$] revealing a linear decrease in test score as TOD increases for both of these tests. See figure 17 for scatter plots of these results.

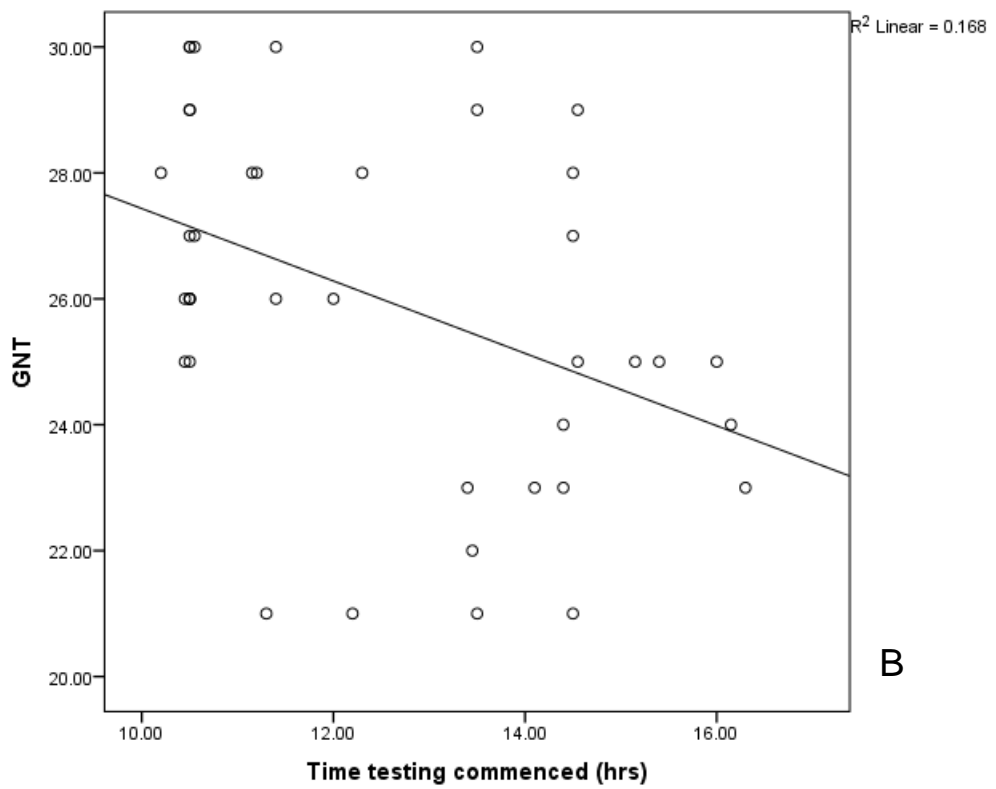
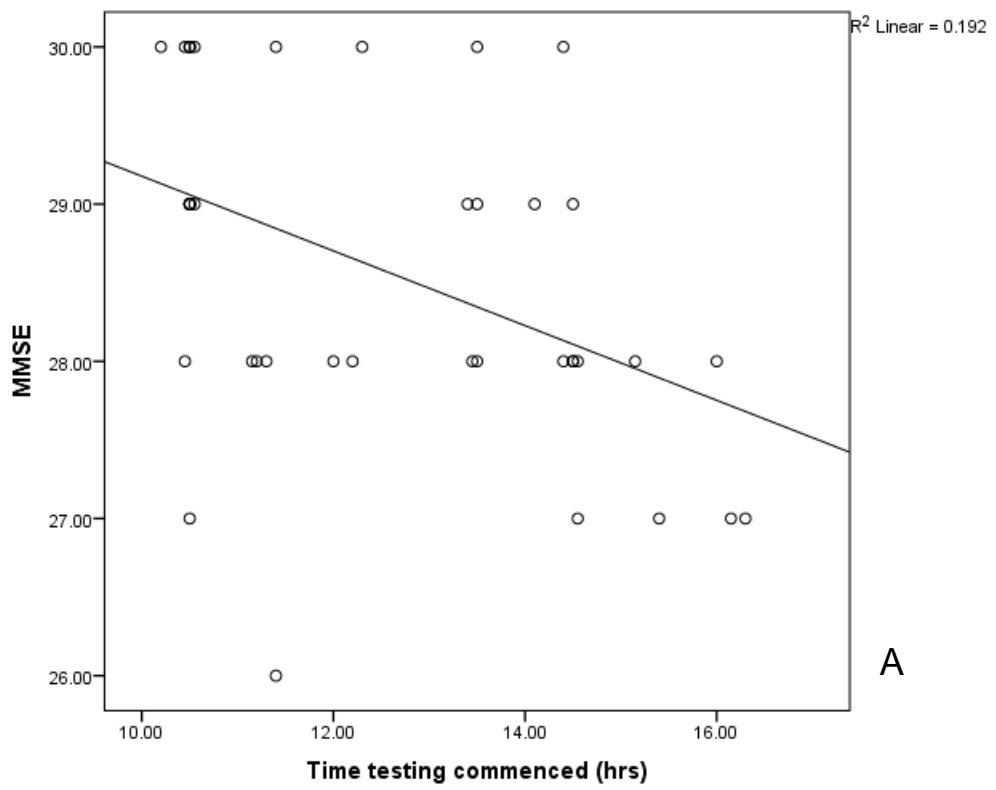
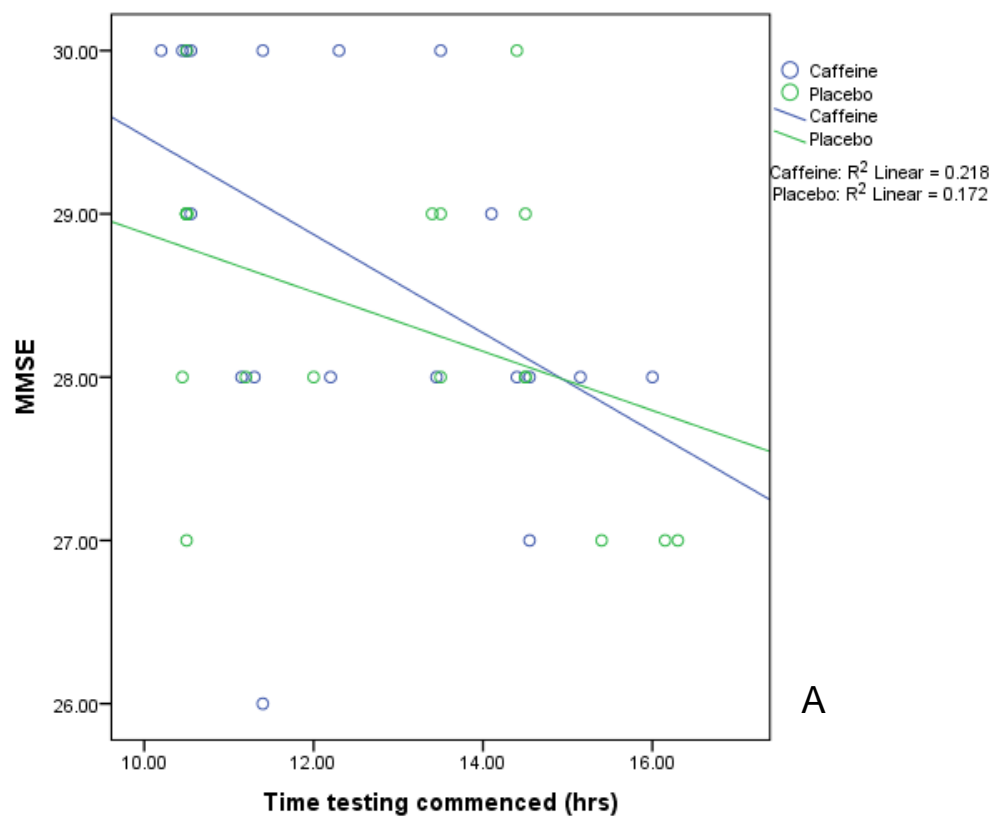


Figure 17: data set two - a linear decrease in performance as time spans into the afternoon for A - the MMSE and B - the GNT. Negative correlations are present signifying that scores on both assessments decrease as TOD progresses.

7.4.2 Data set two: main effects of caffeine vs. placebo and interactions between caffeine and cognitive test scores

There was no significant difference in performance between the caffeine or placebo group on any of the assessments administered (see chapter five for details). To assess prior caffeine consumption and any interaction with TOD all cognitive tests were submitted to a MANCOVA with drug group (caffeine or placebo) entered as a fixed factor and TOD as a covariate. An interaction term was included in the model. Significant interactions between drug group, TOD and both MMSE [F (2, 37) = 4.78, $p < 0.05$] and GNT [F (2, 37) = 3.96, $p < 0.05$] score were found. Scatter plots of these interactions can be seen in figure 18.



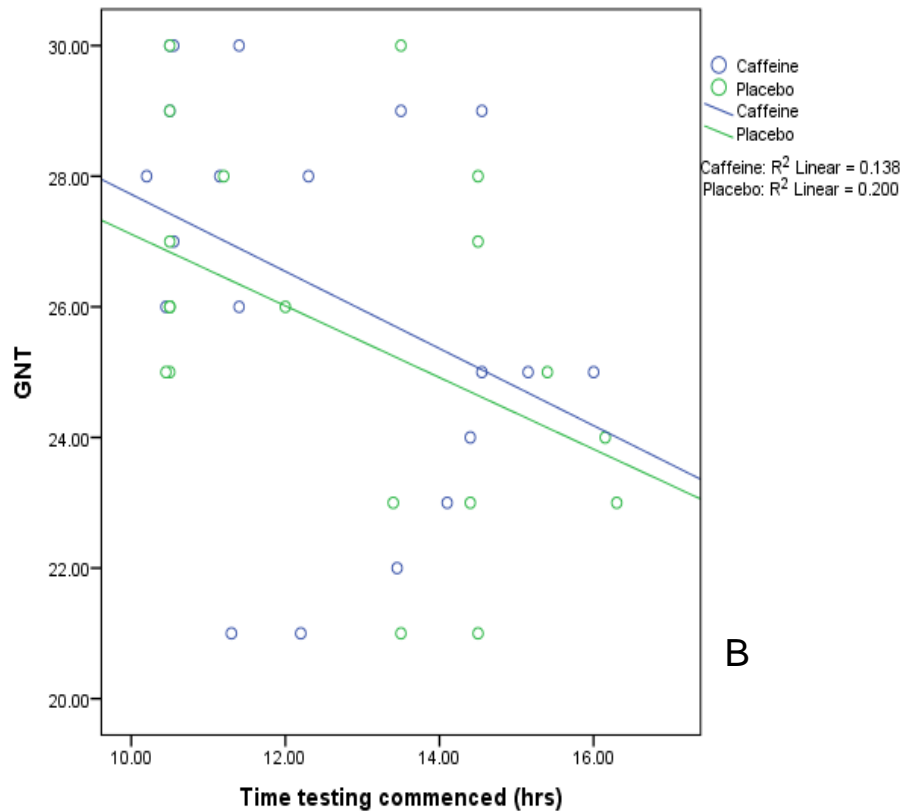


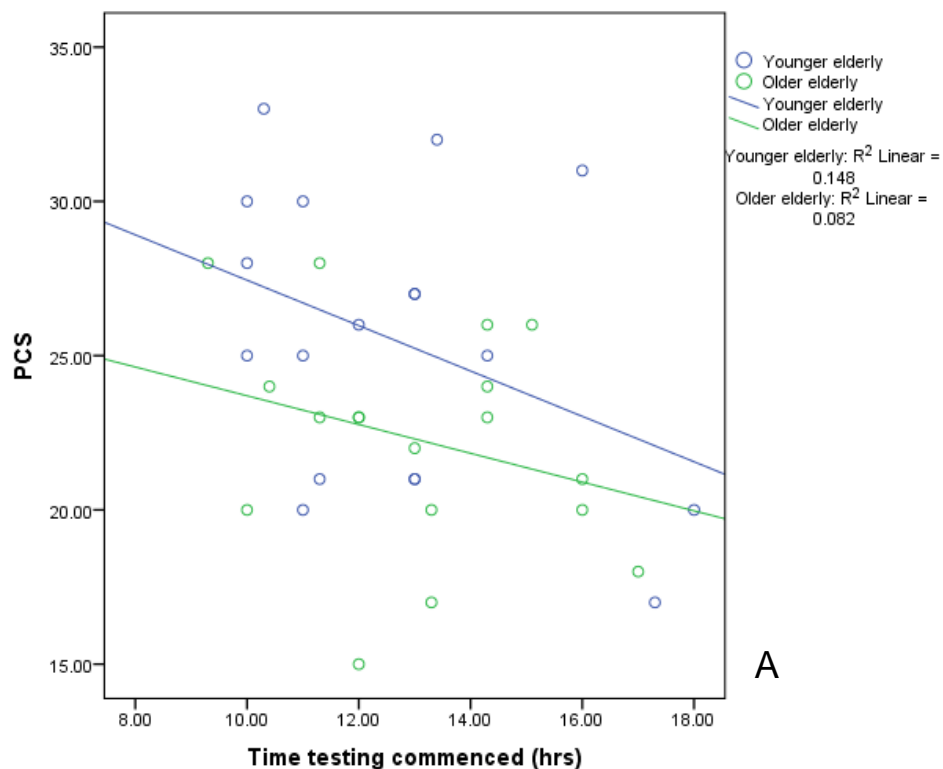
Figure 18: significant interactions between caffeine/placebo, TOD and scores from data set two for A - the MMSE and B - the GNT. There is a significant linear decrease in performance for both the caffeine and placebo group as time spans into the afternoon for both tests. For the MMSE caffeine improves performance in the morning hours however a decline in performance is observed in the afternoon. For the GNT participants who received caffeine perform significantly better than those receiving placebo throughout the morning and afternoon.

7.4.3 Effect of age x TOD x cognitive test scores

Given the age x CCFS interaction effect found in Lesk et al., (2009) and the drug group x age interaction effects found in chapter five of this thesis, this section will now investigate any interaction effects of TOD and age (data set one) and any three-way interactions between caffeine/placebo, age and TOD (data set two).

7.4.3.1 Data set one: age x TOD interactions

A MANCOVA with age and TOD entered as covariates and cognitive test scores as dependent variables was carried out. Significant interactions with age, TOD and scores on the PCS [F (1, 33) = 8.22, $p < 0.01$], LCS [F (1, 33) = 18.79, $p < 0.001$] and TMT part A [F (1, 33) = 9.05, $p < 0.01$] were found. A trend towards significance was obtained for the MoCA ($p = 0.07$), TMT part B ($p = 0.06$) and the Stroop word ($p = 0.07$). For all of these tests the older elderly perform worse than the younger elderly as TOD increases. Interestingly, it is the younger elderly who experience the steepest decline in performance across the time of day variable in comparison with the older elderly. To graphically represent these interactions the median age of the participants (73.5 years) was used to divide the sample into 'younger' and 'older' elderly participants. See figure 19 for scatter plots.



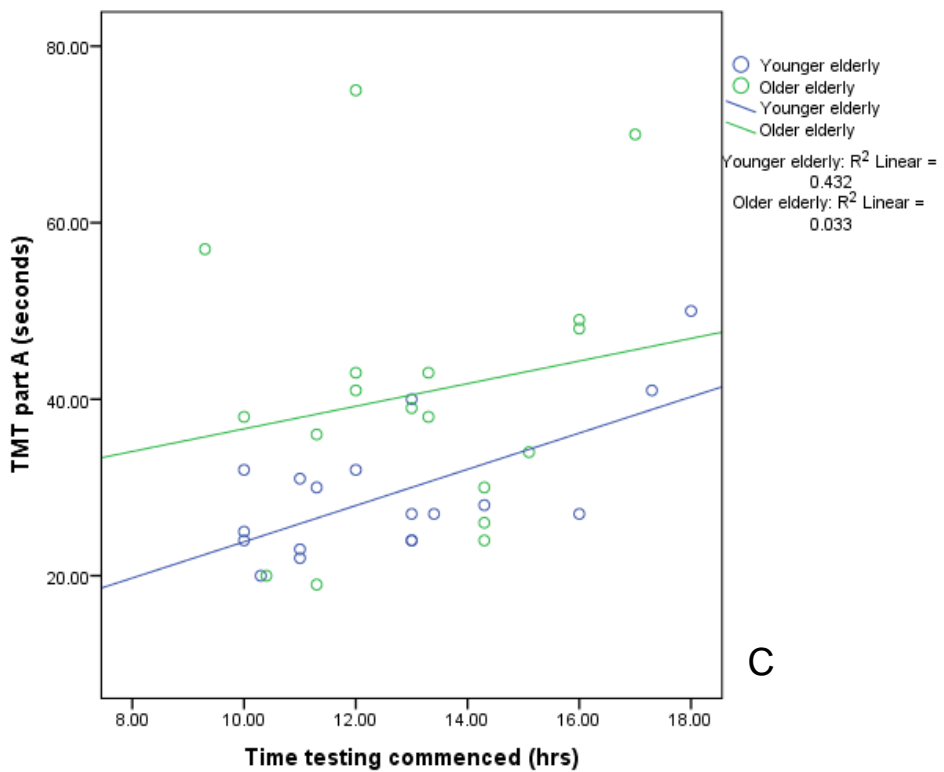
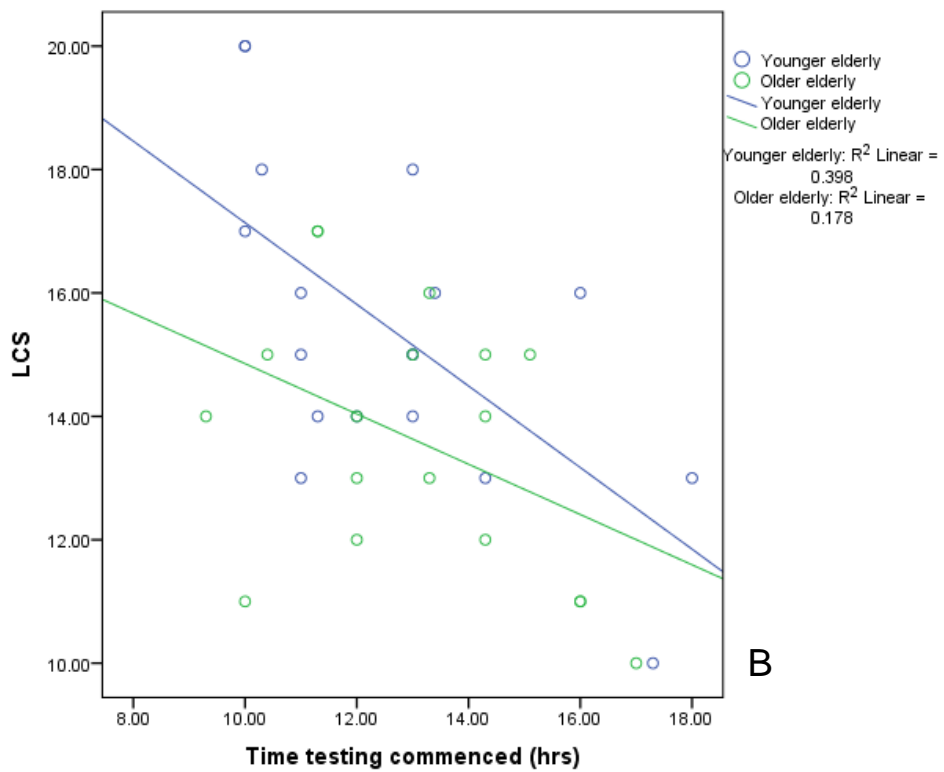
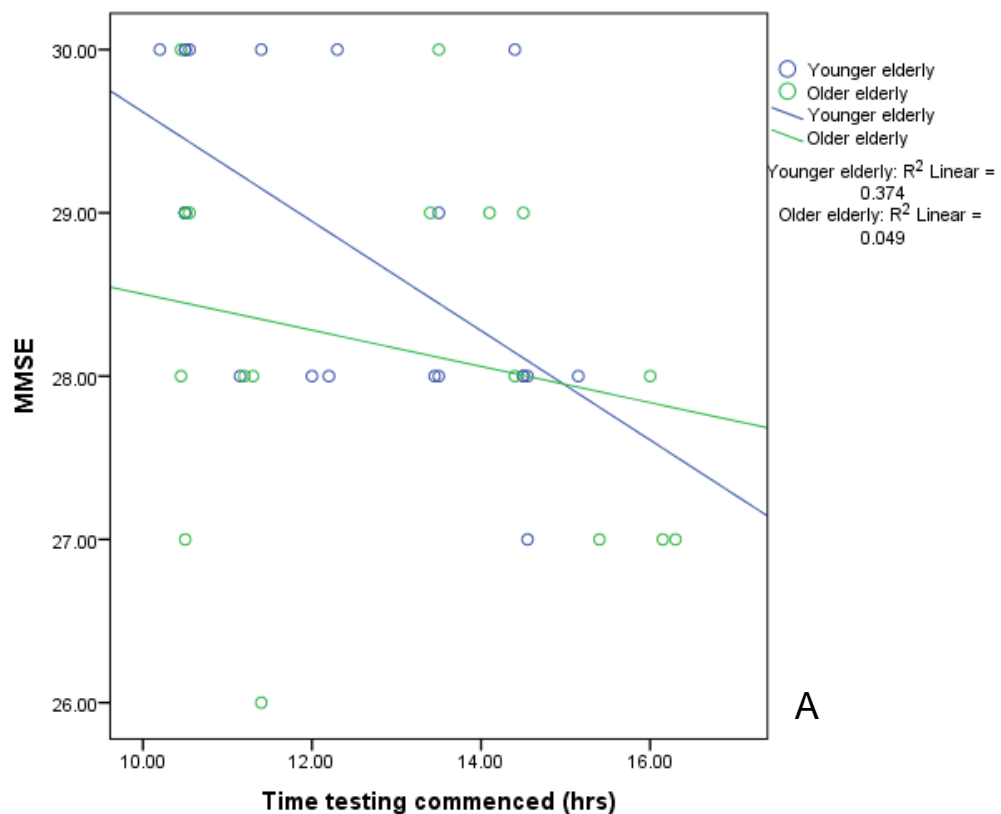
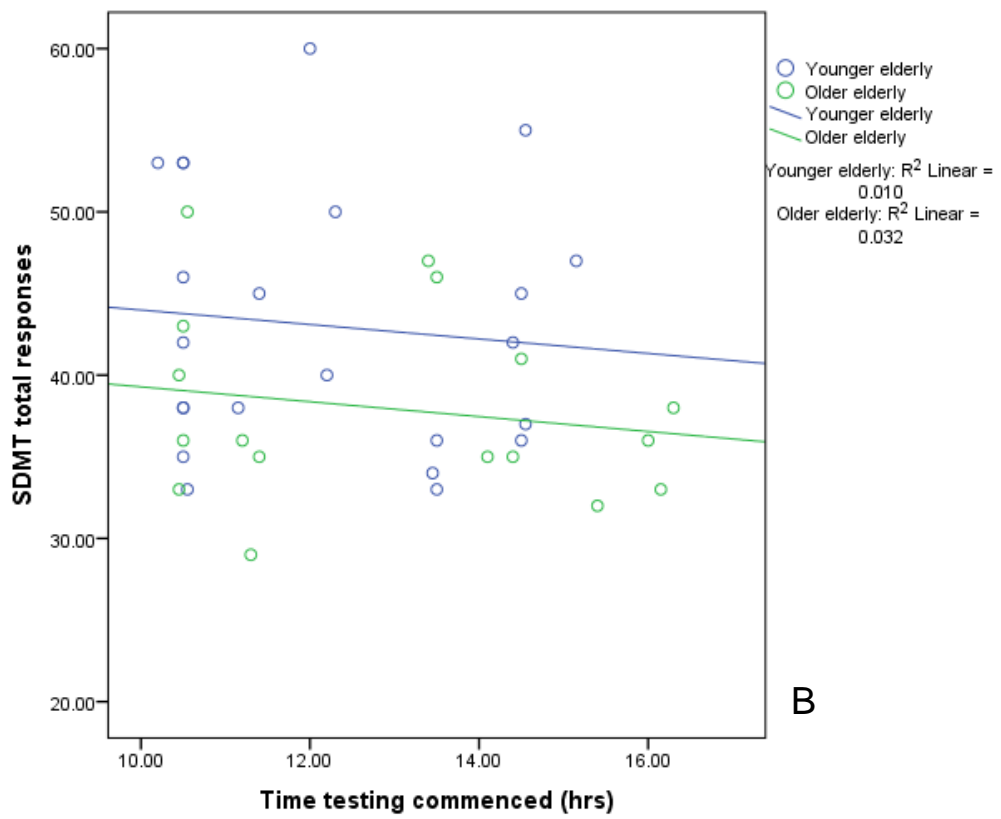


Figure 19 – data set one - significant interactions between TOD, age and A - PCS, B - LCS and C – TMT part A scores. In the PCS and LCS negative correlations are present, the younger elderly perform better than the older elderly as TOD progresses, however, the younger elderly experience a steeper decline in performance as TOD increases. For the TMT part A a positive correlation is seen, the younger and elderly take longer to complete the task as TOD progresses however the younger elderly perform better throughout the day in comparison to the older elderly.

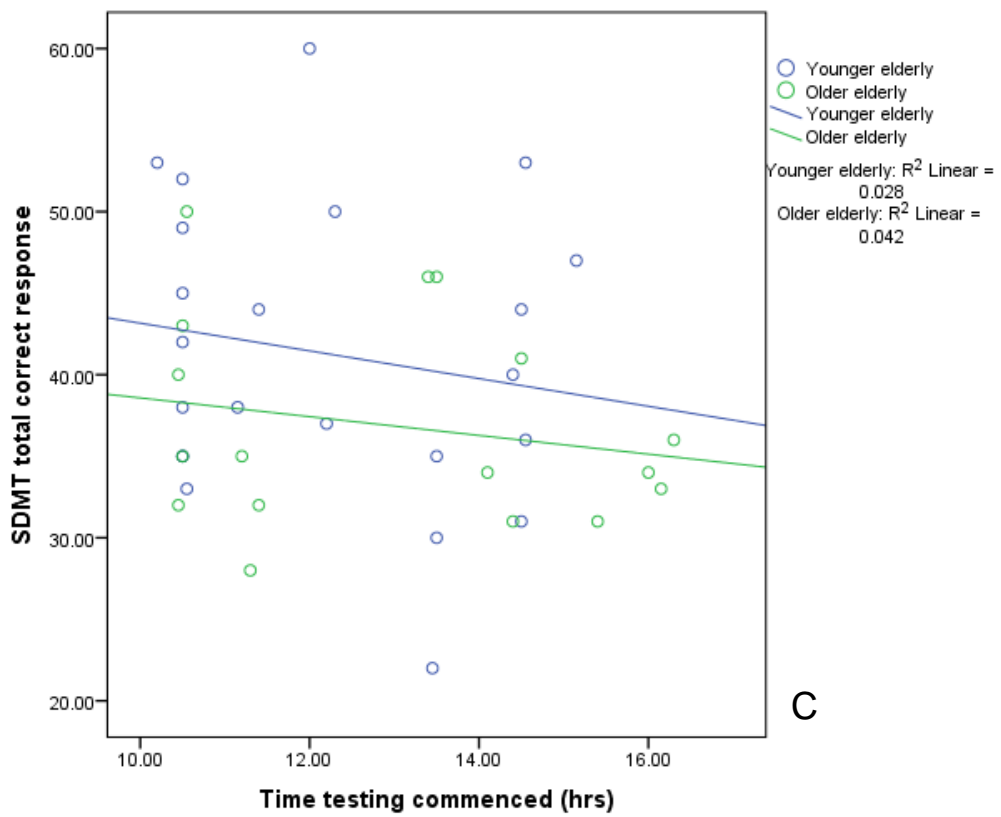
7.4.3.2 Data set two: age x TOD interactions

Using the same method as mentioned previously for data set one, significant interactions with age, TOD and scores on the MMSE [$F(1,38) = 9.97, p < 0.01$], SDMT total responses [$F(1,38) = 7.88, p < 0.01$], SDMT total correct responses [$F(1,38) = 7.96, p < 0.01$] and GNT [$F(1,38) = 10.95, p < 0.01$] were found in data set two. A trend towards significance was obtained for the Stroop word ($p=0.07$), Stroop Interference ($p=0.07$) and the DTR ($p=0.08$). For the MMSE and GNT the younger elderly perform better than the older elderly during the morning, however, a steeper decline in performance is seen for these participants in the afternoon. For the SDMT the younger elderly perform significantly better than the older elderly throughout the day. To graphically represent these interactions the median age of the participants (73 years) was used to divide the sample into 'younger' and 'older' elderly participants. See figure 20 for scatter plots.





B



C

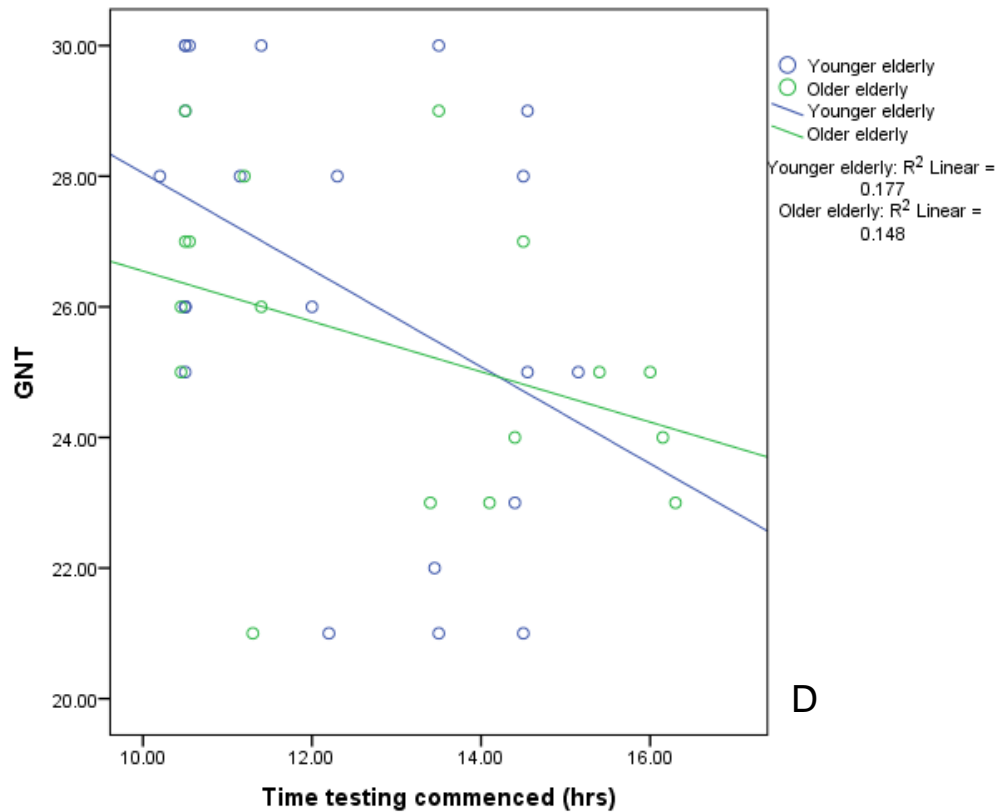
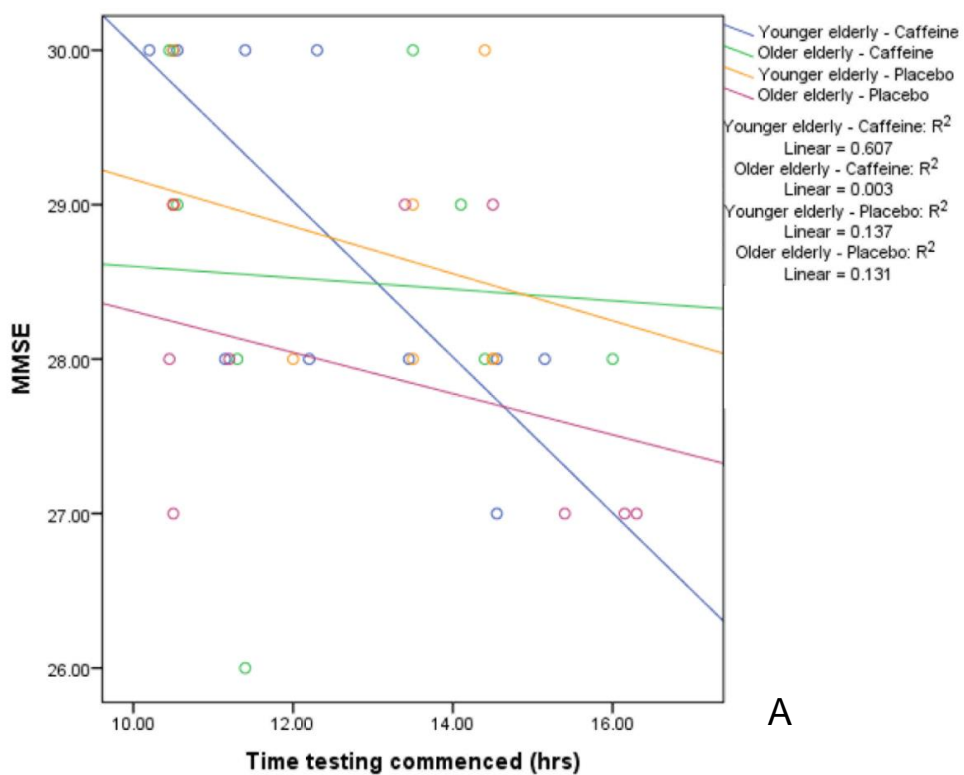


Figure 20 – data set two - significant interactions between TOD, age and A - MMSE, B - SDMT total responses, C - SDMT total correct responses and D - GNT. For all these tests negative correlations are present whereby the younger elderly perform better than the older elderly as TOD progresses however both the younger and older elderly experience a decline in performance as TOD increases. For the MMSE and GNT a steeper decline in performance occurs for the younger elderly in the afternoon.

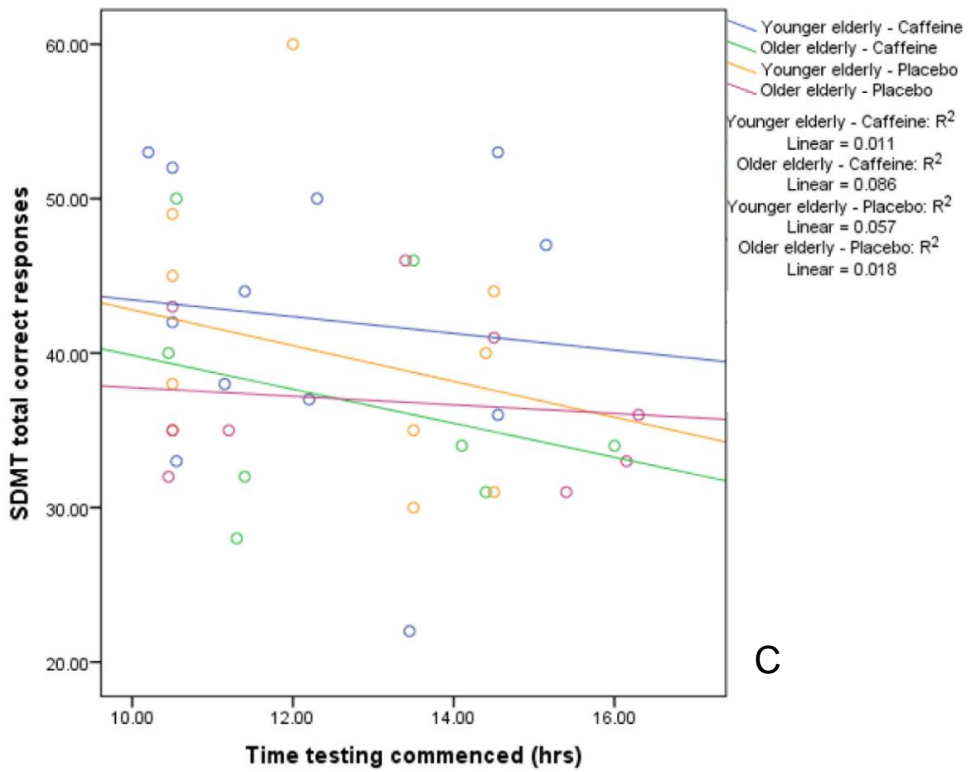
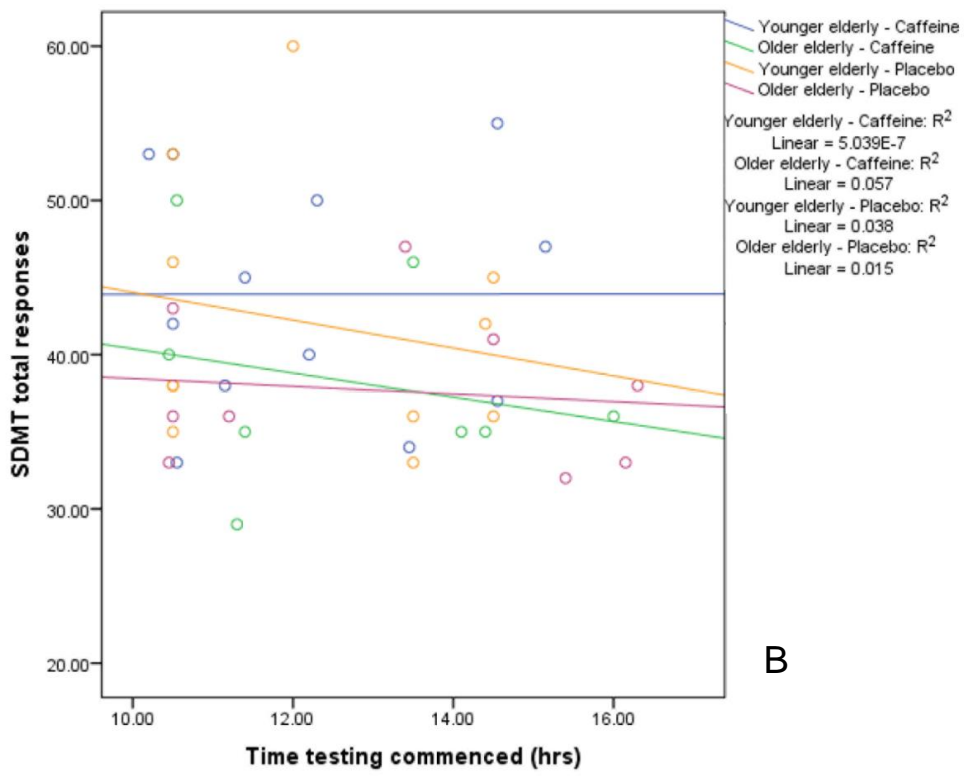
7.4.3.3 Data set two: age x TOD x caffeine/placebo

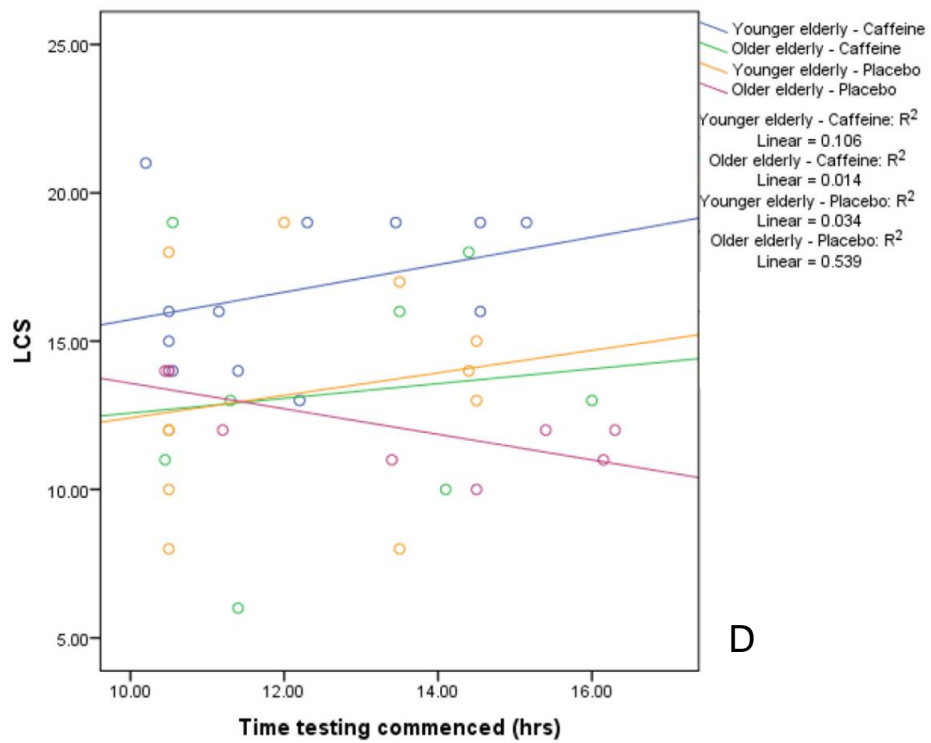
In order to examine any interactions between caffeine/placebo, age and TOD on neuropsychological test scores all of the tests were submitted to a MANCOVA with caffeine/placebo as a fixed factor, age and TOD as covariates and test scores as the dependent variables. Significant three-way interactions between drug group (caffeine/placebo), age and TOD were found on the MMSE [F(2,37)= 5.26, p <0.05], SDMT total responses [F(2,37)= 3.92, p <0.05], SDMT total correct responses (i.e. total responses – errors) [F(2,37)= 3.90, p <0.05], LCS [F(2,37)= 3.42, p <0.05], and GNT [F(2,37)= 5.43, p < 0.01] revealing that the older elderly who had received caffeine, perform worse than

the younger elderly receiving caffeine, however, caffeine in the older elderly maintains performance in the afternoon. Trends towards significance were reported for the PCS ($p=0.06$) and the DTR ($p=0.06$). In order to graphically represent these interactions the median age of the sample (73 years) was used to divide the sample into 'younger' and 'older' elderly participants. Separate scatter plots for each test which showed an interaction were then created and markers of 'younger elderly caffeine', 'older elderly caffeine', 'younger elderly placebo' and 'older elderly placebo' were plotted. See figure 21 for scatter plots of these interactions.

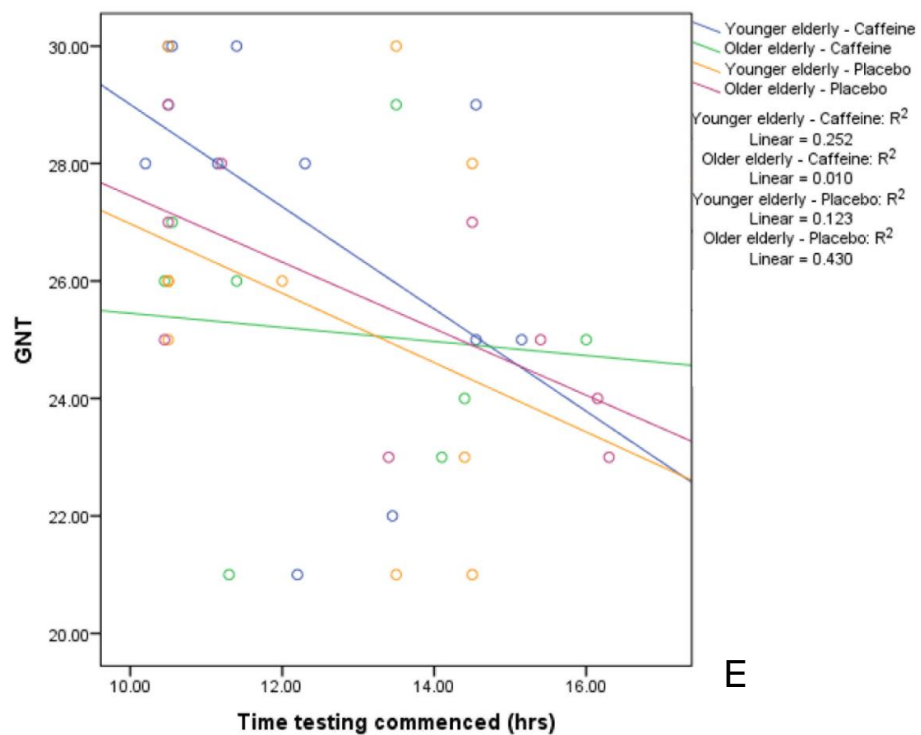


A





D



E

Figure 21 – data set two, significant interactions between caffeine/placebo x TOD x age and score on A – MMSE, B – SDMT total responses, C – SDMT total correct responses, D – LCS and E – GNT. For A – MMSE, in the morning older elderly participants in the caffeine condition perform worse than the younger elderly. In the afternoon the younger elderly participants (caffeine condition) experience a sharp decline in performance whereas the older elderly exhibit only a slight decline. For B - SDMT total responses, C - total correct responses and E - GNT the older elderly participants who received caffeine perform significantly worse than the younger elderly in the caffeine condition throughout the morning and afternoon but do not show as steep a decline in the afternoon. For E LCS, the older elderly (caffeine condition) perform significantly worse than the younger elderly in the caffeine condition throughout the morning and the afternoon.

7.5 Discussion

7.5.1 Main findings

The aim of this chapter was to investigate whether the TOD that elderly participants completed a series of neuropsychological assessments had an effect on the scores obtained. Two data sets were reviewed. Analysis of data set one (data from chapter six of this thesis) showed a significant effect of TOD on two assessments of processing speed, the PCS and LCS, and one assessment of visual attention, the TMT part A. A trend was observed for TOD and performance on the MoCA. Analysis of data set two (data from chapter five of this thesis) showed a significant effect of TOD for one assessment of general cognitive function, the MMSE, and one test of semantic memory, the GNT. All of these assessments, over both data sets, showed a decrease in performance as TOD spanned into the afternoon.

Regarding age, data set one revealed significant interactions between TOD, age and scores on the PCS, LCS and TMT part A, the same tests which showed an effect with TOD alone. The younger elderly experience a larger decline in performance as TOD progresses relative to the older elderly. Although there is a decline in the older elderly's performance as TOD increases this is not as marked relative to the younger elderly. In data set two there were significant interactions between age, TOD and scores on the MMSE, SDMT and GNT. Furthermore, there were significant interactions between TOD, caffeine/placebo, and test scores for the MMSE and GNT in data set two. Significant three way interactions between TOD, age and caffeine/placebo were also found in data set two for the MMSE, SDMT total responses and total correct responses, LCS and GNT. These interactions revealed that the older elderly who had received 200mg of caffeine, performed significantly worse than the younger elderly participants in the caffeine condition but that caffeine did seem to influence the performance of the older elderly in the afternoon. Furthermore, the younger elderly in the caffeine condition experienced a sharp decline in performance on the MMSE and GNT as time spanned into the afternoon, an effect which was not seen for the older elderly

7.5.2 TOD and general cognitive ability

For data set two participants completing the MMSE in the afternoon performed significantly worse than those participants taking the assessment in the morning. The mean MMSE score for participants completing the test in the morning was 28.89 whereas the mean MMSE score for participants taking the test in the afternoon was 28.29. Although this is only a small difference this may be of significant consequence for participants who score on the borderline of an abnormal score. A thorough literature review has shown, that to my knowledge, there are no previous studies which have specifically investigated TOD effects for brief tests of general cognitive ability such as the MMSE and the MoCA. As stated in chapter four the MMSE is comprised of 11 questions and was designed to tax a variety of cognitive domains including orientation, naming, short term memory, sentence generation, instruction and spatial copying (Folstein et al., 1975). The MMSE is currently one of the recommended brief cognitive tests by the NICE dementia guidelines which were updated in 2011 (NICE, 2011). The MMSE is, therefore, widely used in the GP and hospital setting as a tool for dementia diagnosis and the score obtained on this test is considered when determining whether pharmacological intervention with AchEI drugs is to be initiated (NICE, 2011). The test is no longer used to provide cut off scores for the withdrawal of drug treatment.

It is important that MMSE score is accurate and reliable. Previous research has highlighted potential problems with this test as it is not sensitive for the detection of mild degrees of cognitive impairment (Mitchell, 2009; Tombaugh and McIntyre, 1992, Markwick et al., 2012; Jacova et al., 2007) and should not be used alone to diagnose MCI (Tombaugh and McIntyre, 1992). For example, in a group of patients who converted to AD within a 30 month follow up period baseline MMSE scores showed large variations of between 23-28/30 (Blackwell et al., 2004). This creates a conflict between measurable cognitive ability and underlying evidence of disease. Participants who obtained high scores i.e. 28/30 would have been classified as 'no impairment' and this 'label' would not have correlated with underlying pathological signs of disease. As a consequence it has been suggested that the MMSE should be used to rule out suspected dementia and AD (Mitchell, 2009).

In data set one a trend towards significance was noted for TOD and the MoCA whereby there was a linear decrease in performance as time spanned into the afternoon ($p=0.08$). The mean score on the MoCA for participants completing the test in the morning was 28.08 whereas the mean score for participants taking the MoCA in the afternoon was 27.26. The MoCA was not administered to participants in data set two. The MoCA, like the MMSE, is a brief test of general cognitive ability taxing multiple cognitive domains on a 30 point scale (Nasreddine et al., 2005; Jacova et al., 2007). Previously the MoCA has shown good internal consistency, test retest reliability and was able to correctly identify 90% of a sample of 94 MCI participants (Nasreddine et al., 2005). The MoCA is considered to be a superior choice to the MMSE for its ability to detect mild degrees of cognitive impairment (Nasreddine et al., 2005, Jacova et al., 2007; Dong et al., 2012; Lerner, 2012). Further study should employ the MoCA with a larger sample of participants to potentially obtain significance with TOD.

7.5.3 TOD and semantic knowledge

In the present paper, for data set two, a significant effect of TOD was noted on the GNT whereby participants who completed the assessment in the afternoon scored lower than those completing the test in the morning. The mean score for participants completing the GNT in the morning was 27.21 whereas the mean score for those completing the test in the afternoon was 24.86. Previous research has failed to find significant effects of TOD on assessments of semantic knowledge (May and Hasher, 1998), however, the findings of the present chapter contradict this. This may be because all of the participants in this data set had abstained from CCFS for twelve hours prior to testing and some of the participants had then consumed 200mg of prior caffeine which may have influenced TOD affects on GNT performance.

The GNT has previously been shown to be sensitive to the detection of mild degrees of cognitive impairment and early AD (Blackwell et al., 2004, Lonie et al., 2008). As previously mentioned earlier in this thesis, Blackwell et al., (2004) used GNT score in an algorithm created from their data. Using a regression analysis the number of errors made at the six pattern stage of the CANTAB PAL, the score obtained on the GNT and the participant's age at the time of

testing could identify converters to AD with 100% accuracy within a 32 month period using a sample of 40 patients. The significant effect of TOD on GNT score in the present chapter demonstrates that caution should be applied when using such algorithms which have not considered additional external factors such as TOD or prior caffeine consumption. The GNT was not administered to participants in data set one and, therefore, comparisons between the two data sets are not possible.

7.5.4 TOD and processing speed

Data set one revealed significant TOD effects for two assessments of processing speed, the PCS and LCS. The mean PCS and LCS scores for participants tested in the morning were 25.77 and 15.92 respectively and for participants completing the tests in the afternoon the mean scores were PCS - 22.83 and LCS - 13.70. A linear decrease in performance was noted for both assessments as TOD spanned into the afternoon. As far as I am aware there are no other studies which have specifically investigated the effects of TOD on these cognitive tests in the elderly.

Processing speed relies on attention mechanisms and frontal functioning, therefore, it would be expected that this domain would be susceptible to tiredness and TOD effects. Previous research has shown that sleep loss and tiredness have a negative effect on performance for tasks which recruit the PFC (Blatter and Cajochen, 2007; Drummond et al., 2001). When completing these two assessments the participant must actively ignore the patterns (PCS) and letter strings (LCS) above and below the item they are answering in order to complete as many pairs as possible (Lustig et al., 2006). Lustig et al., (2006) investigated this consideration by creating a computerized version of the LCS. Older and younger adults who took part were randomly assigned to two experimental conditions, high distractibility or low distractibility. In the high distraction condition participants viewed two columns of 12 letter strings on a standard PC monitor; in the low distraction condition participants viewed each letter string separately as it appeared alone in the centre of the screen. The authors reported over a 15% reduction in processing speed in the high distraction condition which they attribute to the inability of the older adults to

ignore distracting irrelevant information (i.e. the letters above and below) which slows their processing speed. Those tested in the afternoon may find this inhibition difficult if they are experiencing feelings of tiredness. Inhibition is a component of EF and previous studies have noted TOD effects on inhibitory tasks in the elderly (Schmidt et al., 2007) which is discussed further in the next section.

7.5.5 TOD and EF

Much of the previous literature investigating TOD effects in the elderly has noted significant effects for tasks of EF. The elderly perform significantly worse on these types of assessments in the afternoon and evening relative to when tested in the morning (Zacks and Hasher, 1994; Hasher et al., 2007). For example, May et al., (1993) reported a significant deterioration in the afternoon for older adults completing a simple problem solving task. TOD effects have mostly been found for those EF tasks which have an inhibitory component, i.e. those tests which require the participant to ignore or tune out distracting or irrelevant stimuli (Winocur and Hasher, 2004). Intons-Peterson et al., (1999) and West et al., (2002) have also reported TOD effects on inhibitory tasks in the elderly. The present chapter failed to find TOD effects on assessments of EF in either data set one or two. Specifically, there were no effects of TOD on the DT, Stroop interference or TMT part B in data set one or the SDMT, Stroop Interference and DT in data set two. The discrepancy between the present study and the previous literature regarding TOD effects on EF tasks is interesting. In data set two participants had abstained from caffeine for 12 hours prior to testing and may then have randomly been allocated to the caffeine condition where they then received a further 200mg of caffeine. The absence of TOD effects on EF tasks in data set two may, therefore, be due to the presence of caffeine. Caffeine may influence inhibition and other components of EF during fatigue in the afternoon. The absence of TOD effects in data set one where caffeine was not controlled for may also be due to the consumption of CCFS prior to the participant's arrival at the University.

7.5.6 Interactions between TOD and age on test scores

The interactions between age, TOD and scores on the PCS, LCS, and TMT part A in data set one and the MMSE, SDMT and GNT in data set two, revealed that the older elderly performed significantly worse than the younger elderly as TOD increases. Importantly the younger elderly experience a steeper decline in performance as TOD increases, however, the exact reasons as to why this occurs are not known. With increasing age there is likely to be a larger effect of TOD due to circadian alterations in the hypothalamic supra chiasmatic nuclei (SCN) and other brain areas expressing clock genes (Schmidt et al., 2007; 2012) (see section 7.4.7 of this discussion for more detail). During healthy aging there is a large variability in the rate at which brain tissue degenerates (Bugg and Head, 2011) and this may explain some of the intra individual variation in cognitive performance with age. For example, alterations in brain volume during normal aging are dependent on a number of factors such as educational level (Ho et al., 2011), physical activity (Bugg and Head, 2011), smoking status (Durazzo et al., 2012), HCy levels (Jochemsen et al., 2013), high cholesterol (Enzinger et al., 2005), alcohol consumption (Enzinger et al., 2005) the presence of co-morbid medical conditions including depression (Geerlings et al., 2012) or diabetes (Enzinger et al., 2005), cognitive activity (Ruthirakuhen et al., 2012) and genetics (e.g APOE allele, Honea et al., 2009). All of these factors could, therefore, subsequently affect the rate at which brain volume declines with increasing age and potentially create slight variations in cognitive performance in the HE at different times of day.

7.5.7 Caffeine and TOD interactions

The second aim of this chapter was to investigate whether caffeine, a commonly consumed stimulant present in a variety of foods (Nehlig, 2010), interacted with the TOD that assessments were delivered to affect overall test scores. Caffeine consumption (e.g. in the form of CCFS) is often used to relieve feelings of tiredness and increase levels of alertness (Sharwood et al., 2013; Salín-Pascual et al., 2006; Wright et al., 2013). Caffeine could, therefore, have the potential to influence TOD effects on cognitive performance and perhaps affect performance in the afternoon. In data set two (data from chapter five of

this thesis), significant interactions were found between TOD, caffeine, and participants' MMSE and GNT scores.

Those participants who had consumed 200mg of pure caffeine 40 minutes prior to the completion of the MMSE in the morning performed better than those who received placebo. In the afternoon a linear decrease in performance was observed for both subgroups. However, those participants who received caffeine performed worse as time spanned later into the afternoon (at approximately time 14.00 hours). The fact that the presence of 200mg of caffeine (which is the equivalent to one medium filter coffee (Starbucks, 2011)) was able to affect performance and interact with the time that tests were completed further indicates the importance of controlling for, or at least noting, the presence of external variables such as CCFS and TOD. For the GNT, an assessment of semantic memory (McKenna and Warrington,1980), both the caffeine and placebo group show a linear decrease in performance with time. However, the caffeine sub-group perform significantly better suggesting that caffeine may decrease TOD effects on certain tests depending on the testing domain.

Chemically caffeine is a methylxanthine (Cunha, 2001) and these compounds are known to alter the circadian rhythm resulting in a phase shift or delaying effect which then impacts on the sleep-wake cycle (Miller et al., 1995). Mitchell and Redman (1992) noted that caffeine was able to improve performance on a visual serial search task but only for participants completing the assessment in the morning. Although the data sets analysed in the current chapter did not use a visual search assessment as a part of the cognitive battery delivered, participants in the caffeine condition did perform better on the MMSE and GNT in the morning. Another consideration is that there may be circadian variations in the rate of absorption of pharmacological substances, such as caffeine, and that this may alter at different times of the day (Miller et al., 1995; De Giorgi et al., 2013). Should this be the case, participants who received caffeine in the afternoon may show a differential effect from those who received caffeine in the morning but it remains unclear how this would impact on the results obtained in this chapter.

Three way interactions between caffeine, age and TOD were investigated and plotted in figure 21. Due to the small sample size of this cohort and the subsequent division of participants into categories to graphically represent the interactions, firm conclusions of the effect of caffeine and age across the time of day variable can not be accurately determined. Interestingly, the younger elderly experience a performance enhancement from caffeine in the morning hours on the MMSE and GNT. However, a steep decline in their performance is noted in the afternoon, an effect which is not seen for the older elderly in the caffeine condition. A speculative reason as to the interactions between age and caffeine may be due to alterations in adenosine receptor expression with age (Cunha, 2001), whereby there may be changes in the degree of adenosine antagonism that caffeine can induce. This is speculative and more research is needed to clarify this. Given that the consumption of caffeine, even at low doses can influence feelings of tiredness and levels of alertness (Wright et al., 2013, Nehlig, 2010) it would be expected that caffeine consumption in the afternoon may influence the individuals susceptibility to TOD effects. Further study will aim to extend upon these preliminary findings. The recruitment of additional participants will allow a more thorough investigation of these three way interactions and will increase the number of participants present in each category.

7.5.8 Underlying cerebral mechanisms: circadian rhythms and TOD

As mentioned briefly in the introduction of this chapter, TOD effects on cognitive performance can be linked to underlying biological circadian rhythms (Froy, 2011). Circadian rhythms are 24 hour cyclical processes (Hasher et al., 2005) controlled by complex cellular mechanisms originating in the SCN (Froy, 2011; van der Heijden et al., 2010), commonly referred to as the central 'pacemaker' (Gritton et al., 2013). A number of other brain areas have also been found to express 'clock' genes which are protein transcripts that are activated by downstream messengers of the master clock (the SCN) and are linked to functions associated with timing e.g. body temperature, hormone secretion, sleep cycle amongst others (Froy et al., 2011). Clock gene expression has been found in the PFC, amygdala, ventral tegmental area, hippocampus and striatum (Wright et al., 2012) suggesting that functions associated with timing and

alertness involve a coordinated response from multiple brain areas. Whilst circadian rhythms have been found to influence numerous bodily processes such as temperature (Schmidt et al., 2007), eating behaviours (Froy, 2007), cardiovascular activity (Yang, 2010), sleep (Schmidt et al., 2007) and hormonal secretions (e.g. Kennaway et al., 2012), they are also present in human cognitive functioning (Schmidt et al., 2007; Froy, 2011; van der Heijden et al., 2010; Wright et al., 2012). This creates variations in cognitive performance depending on the TOD that cognitive assessments are administered (Hasher et al., 2007). Disruptions to the circadian clock are common during aging (Froy, 2011; Tranah et al., 2011) and circadian disturbances and SCN alterations are seen in AD patients (Volicer et al., 2001) leading to disturbances in the sleep-wake cycle. This is often a common reason for the decision to move the patient to nursing home facilities (Satlin et al., 1995, Tranah et al., 2011).

Increasing age is associated with neuronal changes in the SCN (Hofman and Swaab, 2006; Farajnia et al., 2013) as well as alterations in the electrophysiology and neurochemical activity of neurons within this area (Froy, 2011). This results in a decreased number of neuronal projections to the frontal cortex and hippocampal areas (Samuels and Szabadi, 2008) and alters synaptic density, affecting overall cognitive performance (Usher et al., 1999). Disruptions between the SCN and other areas of the brain involved in circadian rhythms may, therefore, contribute to alterations in cognitive function and may explain the larger TOD effects noted on the performance of older adults and the cognitive difficulties of AD patients (Cermakian et al., 2011).

Melatonin is a hormone produced by the pineal gland during darkness and influences the time of sleep onset (Wu and Swaab, 2007). Alterations in melatonin secretion occur during aging and recently a decrease in CSF melatonin levels have been found in AD patients (Pandi-Perumal et al., 2012) and in individuals with MCI (Cardinali et al., 2012). This suggests that early circadian disruption may be an important marker for evidence of cognitive impairment (Cardinali et al., 2012). Furthermore, melatonin decreases have been recorded before cognitive symptoms manifest but it is still unclear whether this decline in production is a result of, or a cause of, the neurodegeneration seen in AD (Zhou et al., 2003).

Cardinali et al., (2012) administered 3-24mg of fast release melatonin to 61 MCI participants in combination with their other medication (this varied between participants but consisted of AchEIs and antidepressant drugs). Melatonin was administered prior to bed time for a period of 15-60 months. A further 35 MCI participants acted as controls and did not receive melatonin treatment. After the follow up period, those participants in the treatment group exhibited better performance on all tests within a neuropsychological battery which included the MMSE and TMT, suggesting that melatonin may be useful as an add on treatment for cognitive impairment. Concentrations of melatonin may influence sleep quality and quantity in the elderly which may then subsequently influence peak arousal times and the individuals susceptibility to TOD affects on cognitive tasks. In the HE weakened circadian activity is also linked to a higher risk of mortality (Paudel et al., 2010). Tranah et al., (2011) showed that in a large sample of 1282 women, alterations to the circadian clock, specifically a weakened circadian rhythm, were consistently related to the subsequent development of MCI and dementia after 2-4 years of follow up. Even though this is beyond the scope of this thesis it highlights potential neurobiological underpinnings that in the future may lead to potential explanations of the results central to this chapter.

Although melatonin is a factor which contributes to the elderly having earlier habitual sleep onset times and the tendency to be easily disturbed during their sleep (Froy, 2011) there are other factors which may influence sleep patterns and arousal levels in older adults. These may include levels of exercise, diet, stress, presence of co-morbid disorders such as depression, amongst others which may be important considerations when calculating and determining peak arousal times and the individual's likelihood of demonstrating a change in performance with TOD.

7.5.9 Methodological considerations

This chapter has only investigated the effect of TOD on cognitive test scores. Participants were not asked about their TOD preference, level of subjective tiredness, sleep patterns or the number of hours of sleep they obtained the night before testing. The study did not, therefore, take into consideration

participants' peak arousal time and this is important for future study. Although significant TOD effects have been noted it is important to consider other variables which impact on an individual's need for sleep or their levels of tiredness. For example, amounts of daily exercise (Bugg et al., 2006), dietary patterns (Peuhkuri et al., 2012), medication use (Pagel, 2013), caffeine consumption (Porkka-Heiskanen and Kalinchuk, 2011) and stress levels (Meerlo et al., 2008). All of these factors have the ability to alter or mask the underlying circadian variations in cognitive performance and should ideally be controlled for to determine true TOD effects. Physical activity has been shown to maintain performance on tasks of EF in the elderly (Bugg et al., 2006). Improved cardiovascular fitness is able to increase the amount of blood flow to the frontal and prefrontal cortices, delaying age related deficits seen on executive tasks. Previously, physical activity has been shown to delay or prevent cognitive decline (Albert et al., 1995; Yaffe et al., 2001; Laurin et al., 2001). TOD effects may be modulated by mental fatigue, and Bugg et al., (2006) hypothesised that mental fatigue in the afternoon may be modulated by cardiovascular fitness. In their study the sedentary elderly experienced a significant decline in working memory in the afternoon hours relative to the more active elderly who performed similarly throughout the day (Bugg et al., 2006).

Analysis of the two data sets in this chapter did not show consistent TOD effects for certain tests. For example, TOD effects on the MMSE were only noted in data set two and not data set one. Likewise, significant TOD effects were noted for the PCS and LCS in data set one and not data in set two. This is most likely because in data set two participants had been asked to abstain from CCFS 12 hours prior to the assessment, this was not the case for participants in data set one. This is also why the data sets were analysed separately, i.e. that caffeine is known to have a striking influence on tiredness (Basheer et al., 2004) and also highlights the importance of taking self report measurements of tiredness/alertness into account.

7.6 Conclusion and summary

From the present data within this chapter and the previous literature in this area it is apparent that there are TOD effects on some neuropsychological test

scores depending on the nature of the task and the type of cognitive domain it assesses. This is an important consideration for the accuracy of cognitive test scores which, as mentioned in previous chapters have the potential to be affected by external factors. Despite this scores are often used to decide diagnosis, treatment, care, research inclusion and comparisons with normative data. Furthermore, the significant interactions between TOD, caffeine and MMSE and GNT score in the present study also highlight the need to take participants prior caffeine consumption **and** its interaction with TOD into consideration.

The data sets reviewed in this chapter did not ask participants about their sleep patterns or subjective feelings of tiredness, therefore, peak arousal times were not calculated. Significant TOD effects for assessments of general cognitive function, i.e. the MMSE and the MoCA (trending) have implications for the diagnosis of dementia in the UK as the MMSE is one of the most widely used instruments recommended by the NICE dementia guidelines (NICE, 2011).

The TOD effects obtained in this chapter have practical and theoretical considerations for clinicians and neuropsychologists who may use cognitive assessments as a part of their diagnostic outcomes or research along with the wider field of experimental psychology and cognitive neuroscience. This is important if scores are compared to population norms which may not have considered TOD. Likewise, for those scores which are to be used to determine whether an individual is to be included in a research trial or treatment intervention. Wherever possible clinicians and neuropsychologists should aim to deliver cognitive assessments at the individual's peak arousal time to avoid or minimise any effects of TOD. For the majority of elderly individuals this typically occurs during the morning.

7.7 Chapter summary of key points

- The time that cognitive tests are delivered may influence the participant's performance.

- Circadian rhythms control multiple bodily processes across the 24 hour day and give rise to peak efficiency periods. This is the time when the individual is maximally aroused and this alters with age.
- For older adults peak arousal occurs in the morning, as a result older adults tested in the afternoon may perform differently as to when assessed in the morning.
- Caffeine may also influence the presence of TOD affects as it is commonly used to relieve feelings of tiredness.
- This chapter explored two sets of data obtained from this thesis to determine the presence of TOD effects. The chapter also aimed to investigate any interactions between caffeine and TOD from the data obtained in chapter five of this thesis.
- Main effects of TOD day were found in data set one (data from chapter seven) on the PCS, LCS and TMT part A.
- Main effects of TOD were found for the MMSE and GNT in data set two (data from chapter five).
- Across both data sets performance on these tests decreased as TOD progressed.
- In data set two there were significant interactions between caffeine, TOD and performance on the GNT and MMSE. Significant three way interactions were also noted in data set two for age x TOD x caffeine x score on the MMSE, SDMT, LCS and GNT.
- The findings have shown that TOD is a factor which influences performance on some cognitive tests depending on the cognitive domain assessed.
- Significant interactions between caffeine and TOD highlight the need to control prior caffeine intake when interpreting neuropsychological data.
- The results of this chapter have implications for psychological research which may use scores to determine outcomes or further research inclusion.

Some of the information within this chapter can be found in Walters and Lesk (in prep - b) 'The effect of TOD on cognitive performance in the over 60s'.

Chapter 8

Evaluating a new virtual reality based test of spatial route learning for the early detection of cognitive impairment

8.1 Introduction

The previous experimental chapters of this thesis have explored factors which may affect neuropsychological testing in the elderly and in particular whether these factors can influence the scores obtained. This is important as cognitive test scores may be used to detect early cognitive impairment and may, therefore, have implications for the early detection of AD. In order for an early diagnosis to be possible the assessments and tools used by clinicians must be sensitive to the earliest of cognitive changes (Mapstone et al., 2003). The cognitive domain the task targets is an important factor to consider when detecting such alterations in cognitive function as certain areas of the brain experience larger volumetric declines and increased AD related pathology in the earliest stages of the disease's trajectory.

Tests which stimulate these areas are more likely to identify early impairments if the underlying area is already compromised. Any assessments that are used must also be able to accurately discriminate between participant sub-groups i.e. whether the participant has AD, MCI, another form of dementia or whether they are cognitively 'normal'. The symptoms the patient presents with often involve a number of cognitive, behavioural and social complaints. This, accompanied by the fact that many other forms of dementia such as vascular dementia, frontotemporal dementia and semantic dementia, present with similar initial symptoms makes an accurate diagnosis challenging in the early stages (Karantzoulis and Galvin, 2011). This chapter investigates and evaluates a newly created assessment of spatial route learning as a possible novel tool for the accurate, early detection of AD.

8.1.1 Spatial memory

Research studies sampling participants with MCI (i.e. those who do not fully meet the criteria for AD) have revealed an elevated volumetric decline in the EC of the hippocampus (Blackwell et al., 2004). This area is crucial for memory function (Feczko et al., 2009) and navigation (Laczó et al., 2009) and has been implicated in early AD in a number of research studies including Shen et al., (2011) and Gómez-Isla et al., (1996). Developments and improvements in imaging techniques have improved the accuracy of imaging MTL structures and as a result these volumetric data can identify anatomical change in the earliest stages of AD (Pruessner et al., 2002). In addition to a decline in EC volume the earliest signs of the presence of pathological markers are also found in this area in the form of NFTs (Braak and Braak, 1991; de Calignon et al., 2012). As mentioned in chapter two the fact that these individuals present with pathological biological markers which are hallmarks of AD suggests they are, in fact, in a prodromal early stage of the disease.

Lesion studies and imaging studies carried out in combination have found the EC to be highly sensitive to tests stimulating visuospatial associative memory (Smith and Milner, 1981; Owen et al., 1996). This type of memory typically involves learning the association between a visual stimulus and a distinct spatial location and is recruited in navigational tasks such as remembering routes (Tippett et al., 2009). An example of an existing test which recruits this type of memory is the CANTAB PAL (Blackwell et al., 2004) which requires the participant to learn an association between a visual stimulus and a spatial location (Égérhazi et al., 2007) (see chapter four 'Neuropsychological Testing'). The number of errors made at the six pattern stage of the PAL has been shown to be highly sensitive to MCI and early AD (Blackwell et al., 2004). Performance at this level significantly correlates with the subsequent deterioration in global cognitive function over the following eight months (Swainson et al., 2001). This suggests that spatial associative tasks may be sensitive to the earliest degeneration occurring in AD (Laczó et al., 2009).

Some of the earliest studies of spatial memory were investigated using rodents. The Morris Water task (MWT) (Morris et al., 1982) is one of the most influential and will be discussed as it is a useful model applicable to AD. The MWT was

developed by Morris and colleagues in 1982 as a task which relates hippocampal functioning, aging and spatial learning (Morris et al., 1982, Lithfous et al., 2013). The task requires the animal to successfully locate a raised escape platform in an opaque water pool. Once the animal has learnt the location of the platform they are then much faster at reaching the platform on their subsequent applications to the tank. Aged rats are impaired at this task (Foster, 2012; D'Hooge and De Deyn, 2001) and animals with a hippocampal lesion show an even greater impairment (Foster, 1999). Aged rats took longer to locate the escape platform, travelled longer distances before finding the platform and required a greater number of trials to successfully complete the task (Lithfous et al., 2013).

Genetic AD mouse models have also demonstrated deficits in spatial memory and navigational ability on these types of task (Foster, 2012). The TgCRND8 mice, which possess amyloid induced synaptic dysfunction resulting in the expression of an APP variant linked to AD and plaque deposition (Chishti et al., 2001), perform poorly in comparison to control mice on the Barnes maze (an alternative to the MWT) (Lithfous et al., 2013). The Barnes maze, like the MWT, assesses spatial learning and memory (Barnes et al., 1980). The animal is required to locate a lower darkened chamber beneath a bright open platform via one of 18 holes (Sunyer et al., 2007). These findings have suggested that the presence of AD pathology impairs the animals' ability to learn new spatial scenarios.

8.1.2 Brain structures involved in spatial memory and navigation

The hippocampus is crucial for visuo associative spatial memory and successful navigation through the environment (Burgess et al., 2002; Lithfous et al., 2013). The cognitive map theory by O'Keefe and Nadel (1978) proposed that in rodents the hippocampus is composed of specific 'place cells' which collate to form a cognitive map. Landmarks or environmental cues caused the activation of certain 'place cells' within the cognitive map and a similar system is believed to be present in humans (Knierim et al., 1995; Laczó et al., 2009).

The processing of spatial stimuli is further sub-divided into allocentric and egocentric processing (Burgess et al., 2002). Egocentric processing occurs when spatial information is collated with the individual at the centre and this type of processing involves the parietal cortex (O'Keefe and Nadel, 1978). Allocentric processing on the other hand occurs via an environment driven framework supported by the hippocampus (Burgess et al., 2002). An important consideration of spatial processing, which appears to affect the specific brain structures recruited, is the difference between 2-dimensional (2D) and 3-dimensional (3D) images or environments (Burgess et al., 2002). This has implications for carrying out spatial tasks. For example, a participant with a spatial impairment, who may be disorientated when taking a novel route, may still perform normally on an assessment of table top object locations (Burgess et al., 2002). 2D spatial assessments activate the right parahippocampal gyrus and lesions to this area impair performance (Bohbot et al., 1998), whereas 3D environments activate a separate region of the hippocampus (Burgess et al., 2002).

In order to specifically investigate which brain areas and pathways are involved during spatial route learning tasks Maguire et al., (1998) created a 3D virtual environment of a town and asked the participant to manoeuvre along several routes whilst undergoing PET imaging. During successful navigation of the area there was significant right parahippocampal and hippocampal activation. The participant's accuracy correlated with right hippocampal and right inferioparietal cortex activation. The speed at which the participant navigated through the virtual town correlated with the degree of caudate activation and PFC activation occurred when participants took a 'detour' which the authors suggest helped with the planning and organising of a new unfamiliar route (Maguire et al., 1998).

When the same experiment was performed by brain damaged patients, those with right temporal lobectomy were significantly impaired whereas those with a left temporal lobectomy performed intermediately between controls and right lobectomy patients (Spiers et al., 2001). The results of these experiments support the role of the right hippocampus and parahippocampus in successful navigation and route learning.

8.1.3 Aging and spatial navigation

Alterations to the brain structures involved in route learning occur during aging and this may impair successful navigation (Raz et al., 2004). Data from previous research has shown that the elderly report self-perceived deficits in their navigational abilities (Cushman et al., 2008) which may lead to this cohort avoiding new routes or places which are unfamiliar to them. A review of studies which have recruited HE participants by Klencklen et al., (2012) revealed that older adults are significantly worse in comparison to younger adults at navigational tasks. Jansen et al., (2010) and Rodgers et al., (2010). Jansen et al., (2010) investigated spatial knowledge acquisition in young (20-30 years), middle aged (40-50 years) and older (60-70 years) adults via the use of a virtual reality (VR) environment. Participants were required to learn a specified route, recall the presence of certain landmarks and were then asked to draw the route using a pen and paper. The results revealed that the elderly participants took longer to learn the route, were worse at recalling landmarks and made more errors when attempting to draw the specified route.

Rodgers et al., (2010) evaluated a new spatial test based on the Barnes maze which was initially designed to test spatial ability in mice (Barnes et al., 1980) (see section 8.1.1 for more detail). Their sample included 45 community dwelling older adults aged between 55-85 years and 54 younger adults aged 18-35 years. They reported that the older adults were slower at completing the task and used a different strategy to the younger adults, whereby they preferred to rely upon on egocentric route learning which relies upon route cues.

Navigational deficits become particularly striking when the elderly are tested in new unfamiliar environments (Devlin, 2001). Clinically relevant deficits in spatial memory and route learning have been implicated in the early stages of dementia (Klein et al., 1999) leading to reports of wandering in these patients (McShane et al., 1998).

8.1.4 Navigation in AD and MCI

Navigational deficits are more pronounced in AD patients (Lithfous et al., 2013) but similar deficits are also present in individuals with a MCI (Cushman et al.,

2008). Since navigational and spatial deficits are present in MCI, which is believed to be a prodromal state of AD (Petersen et al., 1999), testing this particular memory domain could improve the earlier diagnosis of AD. Furthermore, those MCI participants with navigational difficulties seem to be at an elevated risk of conversion to an AD diagnosis (Laczó et al., 2011) compared with MCI participants with intact spatial memory. This has important implications for the treatment and subsequent monitoring of these individuals.

Topographical disorientation (TD) refers to problems orientating oneself to the surrounding environment (Barrash et al., 2000) and produces difficulties navigating to specific locations and recalling routes (Barrash et al., 2000). TD is a common problem in AD resulting in many patients becoming lost or unsure of their surroundings (Cherrier et al, 2001; Lithfous et al., 2013; Serino and Riva, 2013). TD may be due to a failure of the brain areas involved in the encoding of spatial layouts or may reflect a more general deficit in spatial processing (Cherrier et al., 2001). Barrash et al., (2000) comment that TD results when the integrity of the inferior medial occipital, occipitotemporal cortices or the right medial temporal areas are compromised either by lesions or the aging process. Research studies using AD patients have linked TD with other measures of neuropsychological assessment suggesting that numerous brain areas contribute to the manifestation of TD symptoms. For example, Henderson et al., (1989) investigated a group of 28 probable AD patients whose carers had stated that they became lost on a regular basis. Using a regression analysis this study showed that memory performance but not attention, language or disease severity was a significant predictor of spatial disorientation.

Passini et al., (1995) investigated navigational ability in a large hospital setting with 14 mild to moderate AD patients and 28 HE controls. All of the AD patients failed to navigate to a set location without making errors, the participants were recorded along their route and AD patients showed impaired problem solving abilities.

TD could also be linked to problems with optic flow. Optic flow provides a moving individual with information on the stimuli surrounding them. It also aids their navigation and orientation through their environment (Duffy, 2009). Tetewsky and Duffy (1999) and Duffy (2009) report that there is significant

impairment in measurements of optic flow in AD patients. This implies that there may be deficits in visuospatial processing contributing to the TD experienced in these patients.

TD is one of the earliest symptoms of AD (Lithfous et al., 2013; Cherrier et al., 2001; Morris, 1993; Serino and Riva, 2013). Approximately 25% of AD patients present with initial TD and this increases to 50% within the first three years of the disease being diagnosed (Pengas et al., 2010). These figures are likely to be under represented as some patients often attempt to hide their TD by using new strategies and avoiding new routes or places (Serino and Riva, 2013). As a result, carers often only become aware of spatial difficulties when the patient is away from their home setting such as on holiday, when they fail to remember or learn new places and routes (Pengas et al., 2010).

Whilst some of the HE may experience TD in new surroundings; AD patients are often disorientated in familiar environments such as their home (Cherrier et al., 2001; Cushman et al., 2008; Serino and Riva, 2013). This restricts daily living tasks and results in patients becoming lost when out and about, wandering, and taking the wrong direction on journeys (Head and Isom, 2010). This has the potential for serious consequences and causes anxiety for the carers and families of such individuals.

In Cherrier et al's., (2001) paper, mild to moderate AD patients (with a mean Dementia Rating Scale score of 107) and a group of elderly control participants learnt an outdoor route whilst accompanied by a researcher. The purpose of this study was to determine whether AD patients experienced problems with spatial memory and orientation rather than simply having an inability to recognise landmarks along the chosen route. AD patients were significantly worse at the route learning test. As the authors predicted, the results confirmed that there were clear deficits in spatial orientation and processing. Strong associations between their route learning test scores and scores on an established route learning task (the Money Road Map test) were noted. These findings were consistent with previous work which has linked TD to the impaired optic flow seen in AD (Tetwesky and Duffy, 1999). All of the AD patients in this sample were impaired at recognising the route, however, they performed almost as well as the control participants in their ability to recall the presence of

landmarks (Cherrier et al., 2001). These results support the suggestion that spatial orientation is impaired in the disease.

Duffy (2009) suggests that visual information processing is impaired in AD and a number of studies have shown that both AD and MCI populations have problems in visual motion processing, which are thought to underlie their navigational difficulties (Mapstone et al., 2003; Tetewsky and Duffy, 1999; O'Brien et al., 2001; Dubinsky et al., 2000; Uc et al., 2004). Testing navigational deficits using 'real-world' methods can be difficult and requires long administration times (Duffy, 2009). Sheehan et al., (2006) investigated navigational deficits using a real world situation. These authors recruited mild AD patients and healthy controls and accompanied them on a short 30 minute walk close to where they reside. The participants were then asked to complete the route again, the researcher accompanied them but did not correct any wrong turns or errors. Clear deficits were seen for the AD patients and this group experienced significant way finding difficulties leading them to become lost. The results of the study demonstrated the reliance on external cues as many of the patients stated that they knew to look out for certain signs or landmarks to help them return home. This implies that rather than reliance on visuospatial memory, memory for familiar objects seems to play a significant role in route planning and spatial orientation. The authors recognise the limitations to this approach such as the use of subjective recordings by a researcher and the fact that environmental cues may change on a daily basis, Such cues may include the weather, external traffic noise and/or the presence of others and may affect the ability of the participant to remember their route (Sheehan et al., 2006). This technique relies upon the participant being mobile and the experience itself could become stressful and tiresome particularly if the patient becomes disorientated or lost.

8.1.5 Virtual reality environments

In recent years the use of computer technology has increased and virtual environments are now used to replicate real-world tasks (Tippett et al., 2009). VR has been recognized as a technology in a variety of domains including phobias (Garcia-Palacios et al., 2002), fear of flying (Banos et al., 2002),

acrophobia (Coelho et al., 2009), fear of public speaking (Slater et al., 2006) and motor rehabilitation (Holden, 2005).

Previously, Werner et al., (2009) examined the feasibility and the validity of a virtual action planning supermarket (VAP-S) for the diagnosis of individuals with a MCI. The study investigated deficits in EF rather than route learning, and recruited 30 MCI and 30 control participants. Participants were instructed to purchase seven items from a list and told to pay for these items at the virtual counter. When using the virtual supermarket those with a MCI made more pauses and took longer trajectories through the supermarket. They also performed a greater number of incorrect actions than the controls and the authors explain this is likely to be due to impaired EF skills such as planning ability and the employment of a task strategy. Score on the VAP-S alone was unable to accurately discriminate the MCI participants from the controls, although using one of the VAP-S scores (trajectory duration) in combination with the participant's MMSE score identified 93% of the controls and 80% of the MCI participants. The authors suggest that the small sample size may have contributed to the inability of the VAP-S to accurately discriminate all of the participants in each clinical group.

Pengas et al., (2010) also used a virtual environment with the aim of accurately discriminating between AD patients and controls. This was assessed using three tasks, one of which was the Virtual Route Learning Test (VRLT), a virtual town consisting of four routes. The study recruited 69 participants which included 32 with a MCI, 22 with mild to moderate AD, 15 with semantic dementia and 35 controls. The authors also further split the MCI participants into different subtypes consisting of 14 aMCI, 15 multi-domain MCI and three naMCI. The results revealed that the VRLT achieved 95% sensitivity and 94% specificity in discriminating AD patients from control participants. Tippet et al., (2009) examined performance on the 'Sunnybrook city VE' in a group of 18 elderly controls (mean age 69 years) and eight MCI individuals (mean age 72 years). The VR task consisted of a fictional city block which the participants were required to navigate through and the authors also administered a neuropsychological test battery. Scores on the virtual route task were unable to discriminate between the MCI and control participants. The authors suggested

that the task may not have been challenging enough and those with a MCI may have found the task too easy.

Cushman et al., (2008) investigated the efficacy of using a VR environment when assessing navigational performance in participants with early AD and MCI as well as young adults and HE controls. The authors used a real world navigational task where the participant was taken on a wheel chair route around a hospital lobby by an experimenter. They were instructed to attend to the environment and completed a series of questions at the end of the tour. The participants also completed a virtual version of the same lobby route, and were assessed again. The results showed correlations between real-world navigational deficits and those from the VR environment across all groups, highlighting the potential efficacy of using VR as a test for dementia and AD. None of the measures were able to predict which clinical group the individual belonged to.

The results of these studies confirm that AD patients perform significantly worse than MCI participants and aged matched controls on these types of task (Tippett et al., 2009; Cushman et al., 2008; Pengas et al., 2010; Werner et al., 2009). Some studies have also demonstrated that MCI participants take longer to complete VR tasks, make more errors and revisit locations many times when learning a new route in comparison to the HE (Werner et al., 2009). Currently none of the existing research which has used VR has been able to discriminate with 100% accuracy between participant sub-groups; for example, whether the participant completing the task is HE, MCI, or AD. As mentioned previously it is vitally important for a test to be 100% accurate at identifying the participant's clinical group if it is to be considered for an early diagnostic tool.

Some studies, for example Werner et al., (2009) and Pengas et al., (2010), confirmed deficits in MCI individuals but were unable to correctly identify which clinical group they belonged to based on their score on the VR test only. The test required participants' scores on an additional test to correctly identify them. This creates a more time consuming, complex scenario for clinicians to interpret. Furthermore, Pengas et al., (2010) revealed that 70% of their MCI participants were impaired at the VR assessment. If the remaining 30% of this cohort were able to successfully pass the test and perform with no significant

difference from the HE their assessment is not sensitive to the early deficits seen in AD.

8.1.6 Advantages and disadvantages of using a virtual environment

There have been some criticisms that VR does not sufficiently mimic real world situations (Burgess et al., 2002), however, it does offer some advantages over standard pen and paper assessments. For example, VR gives the experimenter control over the number and the type of stimuli the environment contains as well as control over the complexity which allows them to observe the participants response in more detail. Using a computerized system allows for the accurate recording of data and the number of errors made without the reliance of subjective assessments and note taking from a researcher.

Pen and paper tests of spatial navigation have been criticised as they lack ecological validity which limits their reliability for comparisons to real world navigational difficulties (Werner et al., 2009). Virtual environments provide the user with a high level of immersion and subjects often report that they felt as though they were actually within the 'game' or 'scene' which can cover a much larger spatial area than standard tests (Duffy, 2009; Weniger et al., 2011). Virtual tasks are also more useful than real world navigational tasks which, although may be better than standard pen and paper tasks, can be impractical as they often rely on public areas such as a hospital lobby, or the floor of a building. These places are likely to change on a daily basis in terms of the number and presence of others, level of noise, smell and sound (Pengas et al., 2010).

Another advantage of virtual assessments is their potential to be used across multiple centres allowing for the direct comparison of results at different clinical settings. Virtual tests may also be useful tools for the differentiation between dementia subtypes, for example, patients with semantic dementia are often misdiagnosed as having AD (Dubois et al., 2007). Topographical memory is usually preserved in semantic dementia compared to AD, therefore, these types of tests may be useful at confirming/ruling out other types of dementia (Pengas et al., 2010). The mis-diagnosis of semantic dementia can lead to the inclusion

of these individuals in AD research trials and studies which has the potential to affect the results obtained.

When recruiting elderly participants for VR tasks there is often a concern regarding their level of previous computer experience. A study by Moffat et al., (2001) showed that prior computer experience only impacted on the fluency of manoeuvres through a virtual world. These same researchers gave their HE community dwelling sample (mean age 57.8 years) extensive training and practise sessions to ensure that all participants were comfortable and competent at the task before they commenced.

A limitation of using a virtual environment for this type of assessment is that it does not allow the participant to recruit cues from the external environment which they may use in real world tasks. For example, audio stimuli or sensory information via perceived touch, as there is no actual movement through space (Moffat et al., 2001). This also restricts their visual field and the richness of sensory input available. Despite this, researchers are still obtaining significant and important results when using VR in a variety of different fields such as dementia research (Man et al., 2011), stroke rehabilitation (Laver et al., 2011 and Cikajlo et al., 2012), depression (Meyerbröker et al., 2011) and rehabilitation following head trauma (Levac et al., 2011).

8.2 The present study: research aims

This chapter will preliminary evaluate the use of a newly developed VR test, Virtual reality of early detection of dementia (VREAD), which was created in collaboration with the University of Bradford, School of Computing, Informatics and Media in a small sample of HE participants. The study will examine whether score on this novel task of spatial route learning correlates with existing neuropsychological tests used in the diagnosis of early cognitive impairments seen in AD. A second aim is to investigate whether the test can discriminate accurately between the HE and people with a suspected MCI.

8.3 Methods

8.3.1 Participants

Thirty one participants (10 males and 21 females) were recruited to take part in the piloting of VREAD. Inclusion criteria specified that participants must be aged 60 years or over and should not possess an existing neurological disorder or clinically diagnosed memory impairment. Prior computer experience was not essential. None of the participants had an uncorrectable visual impairment. All participants were recruited from the University of Bradford, Division of Psychology over-60s participant pool, and the study was approved by the University of Bradford Ethics Committee. All participants gave their informed written consent before taking part. Of the 31 subjects recruited, nine were classified as having a MCI. In this study MCI was defined as a score of 27 or less on the MMSE and/or a probability of 80% or more on the algorithm created by Blackwell et al., (2004) and/or a score of greater than 11 errors on the CANTAB PAL 6 pattern stage.

8.3.2 Materials

8.3.2.1 Virtual reality of early detection of dementia (VREAD)

The virtual task, VREAD consisted of three modules: VR Practice, VR Park and VR Games. VR Park and VR Games both contain five different levels. Each level is progressively harder. VREAD was administered on a standard PC and participants were required to use the cursor keys on a standard key board to manoeuvre through each level. Participants were seated throughout the experiment. There was no time limit for each level.

8.3.2.2 Neuropsychological tests

In addition to VREAD a short battery of neuropsychological tests was administered to each participant to obtain a profile of their cognitive function. A detailed description of each of these assessments can be found in chapter four 'Neuropsychological testing' and a brief explanation of each is given.

GNT - a test of semantic memory (McKenna and Warrington, 1980).

CANTAB PAL - an assessment of visuospatial associative memory (Égerházi et al., 2007).

MMSE - an assessment of general cognitive ability (Folstein et al., 1975)

Immediate word recall - an assessment of short-term memory and

8.3.2.3 Questionnaires

Participants were requested to complete a VREAD feedback questionnaire following their participation. The questionnaire was specifically designed by colleagues in the Informatics Department and aimed to gain information about the participants' acceptance and satisfaction of the virtual environment. The questionnaire assessed the participants (i) ease of learning the controls, (ii) ease of movement, (iii) ability to understand the instructions and (iv) freedom of movement. See appendix two for a copy of this questionnaire.

8.3.3 Procedure

Participants were invited to take part in the study via a postal invitation letter and those who were willing to participate were invited to attend a testing session at the University. A unique user ID was given to each participant to anonymise the data collected. This number was entered into the VREAD system at the start of the testing session. Each participant was trained to use the arrow keys on a standard keyboard during the VREAD practice module (these are the keys needed to carry out the test). When participants were comfortable at using these keys the experiment commenced. Participants were assigned to see one of the two modules: VR Park or VR Games. In each level of the VREAD participants repeated the exercise of reaching a target destination three times by following a thick red line attached to the path. The VREAD consisted of five different levels of difficulty ranging from easy to complex. Level one was considered to be the easiest with each level becoming progressively harder requiring more complex navigation. The levels of difficulty were defined based on the number of junctions, paths and target destinations in each route which users had to achieve in the testing phase. Extra destinations were added as a 'dummy' to make the path more complex and difficult. VR

Park had the target destinations of a typical park layout and included a playground, picnic area, art gallery, park square and a mini garden. For the second module, VR Games, target destinations were typical outdoor games such as a giant chessboard, giant board games, lawn bowls, mini golf and a picnic area. Both modules had the same number of levels and landmarks and the VR Parks path was mirrored to the VR Game path. Pilot testing showed that the VR scores were significantly worse on VR Park and so it was decided to continue with just this version.

Each participant was given three attempts at locating the destination using a red line present on the path as a guide. There was no time limit for this, after the third attempt with the line present, the line was removed and the participant was tested on their ability to recollect the given path. All data were collected and recorded during this phase. Lastly the data was exported to the information system to be analysed. See figure 22 for screenshots of the VR environment. After completing the VREAD each participant was then administered the accompanying neuropsychological test battery in the same order.



Figure 22: screen shots taken from one of the VREAD levels. On the left the presence of a red line used to guide the user to a set location can be seen. On the right there is no red line present and the user must remember the route from memory.

8.3.4 Analysis of VREAD data

Movement measures in VREAD were based on underlying path squares, which consisted of underlying grids unknown to users along each route. These path squares were systematically numbered and were used to calculate the participants' overall scores at each level. During the testing phase, data were collected automatically in real time comprising the following five attributes: (i) correct path, (ii) incorrect path, (iii) correct sequences, (iv) incorrect sequences and (v) time. An overall percentage score was calculated based on path sequences and path squares for each participant. The higher the overall percentage score the more accurate the participant was at locating the target destination and following the correct route of underlying path squares.

8.4 Results

The aim of the present study was to evaluate a newly developed VR environment, VREAD, as a novel test of spatial navigation for the potential use in early detection of AD. Each participant completed a battery of neuropsychological assessments as well as all five levels of VREAD. The descriptive statistics for the participants can be seen in table 11 and the descriptives for each neuropsychological test and VREAD levels are shown in tables 12 and 13 respectively.

Table 11: Demographics for those classified as HE and MCI

Characterisitics	HE (n = 22)	MCI (n = 9)
Age, years (mean \pm S.D)	69.77 (4.60)	72.67 (6.56)
Gender (M:F)	5:17	5:4
Education, years (mean \pm S.D)	15.50 (6.04)	20.07 (6.26)
Possessed previous computer experience	6	2

Independent t tests revealed that there were no significant differences between the HE or MCI participants in terms of age or years in education ($p>0.05$). A chi square test showed that there was no significant difference in the gender distribution between the two diagnostic categories ($p>0.05$).

Table 12: mean and S.D for each neuropsychological assessment

Test	MIN	MAX	Mean	±S.D
MMSE	26	30	28.84	1.21
Word Recall	3	10	5.55	1.84
PAL 6 Errors	0	49	10.13	11.14
GNT	19	30	25.9	3.14

Table 13: mean and SDs for each level of the VREAD

VREAD Level	MIN (%)	MAX (%)	Mean (%)	±S.D.
1	60	100	97.90	8.34
2	6.41	100	93.00	23.42
3	41.67	100	95.06	12.98
4	19.35	100	94.60	16.92
5	56	100	89.80	13.56

The results of all the VREAD levels and all the neuropsychological tests (MMSE, CANTAB PAL 6 error score, GNT and word recall) were submitted to a correlational analysis using SPSS. Of the five levels of VREAD, level four (VR

4) significantly correlated with participants' scores on both the CANTAB PAL six pattern stage and the GNT, $r = -0.45$ $p < 0.02$ and $r = 0.43$, $p < 0.02$ respectively. There was a trend towards significance for the word recall test ($p=0.069$). No significant correlation was seen for VR4 and the participant's MMSE score. No other level of VREAD significantly correlated with any of the neuropsychological test scores. Scatter plots for these significant associations are shown in figures 23 and 24.

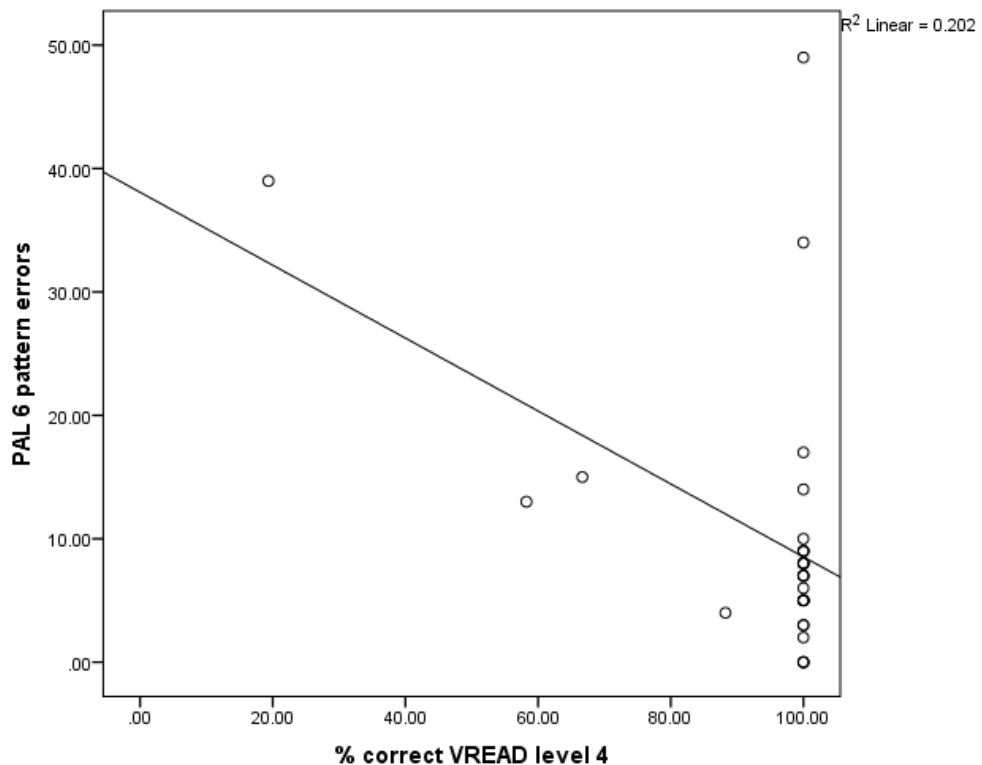


Figure 23: scatter plot of the participants' number of errors made on the six pattern stage of the CANTAB PAL and the overall percentage score on level four of VREAD. As the number of errors on the PAL increased performance on VR4 decreases demonstrating a negative correlation.

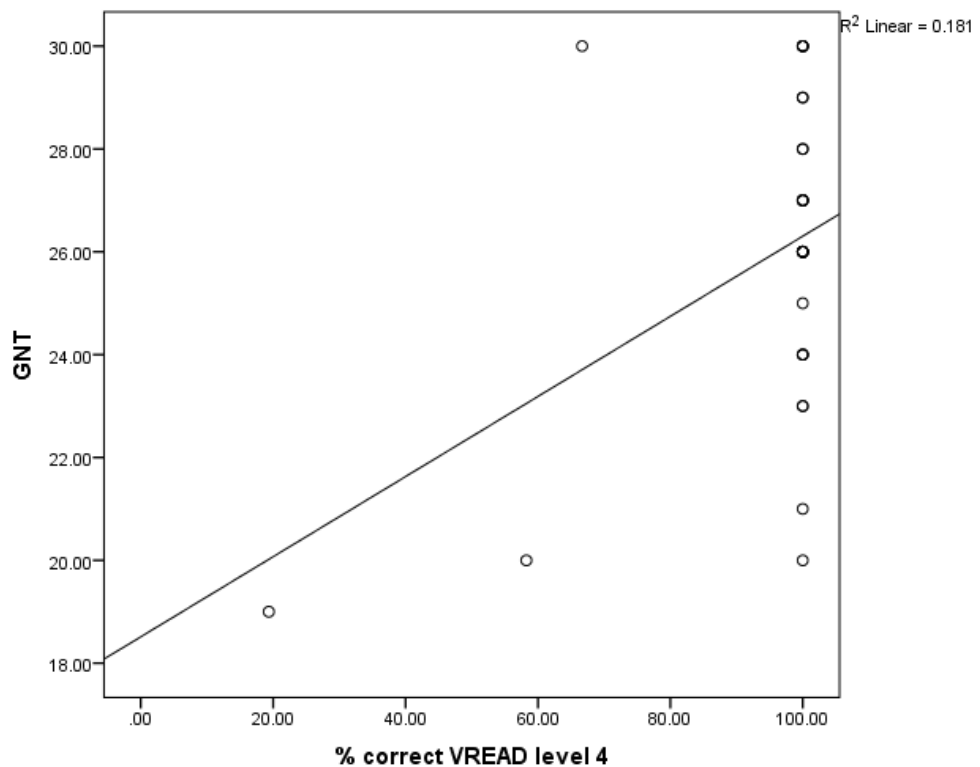


Figure 24: scatter plot of GNT score out of a maximum of 30 against VR4 score. As VR4 score increases so does performance on the GNT.

Given the importance of VR 4, a general linear model (GLM) univariate analysis of variance, was used to investigate the effects of age, gender and education on the scores of VR 4. There were no significant main effects noted. A multivariate analysis was used to investigate age, gender and education on the neuropsychological tests and again no significance was reported.

8.4.1 Discrimination between groups

In total there were 22 HE participants and nine MCI. Figure 25 shows that the mean score for VR 4 was lower in the MCI group compared with the HE group. An independent-samples t-test showed that this difference was significant $t(29) = 3.17, p < 0.01$, suggesting that VR 4 was able to discriminate between the two groups.

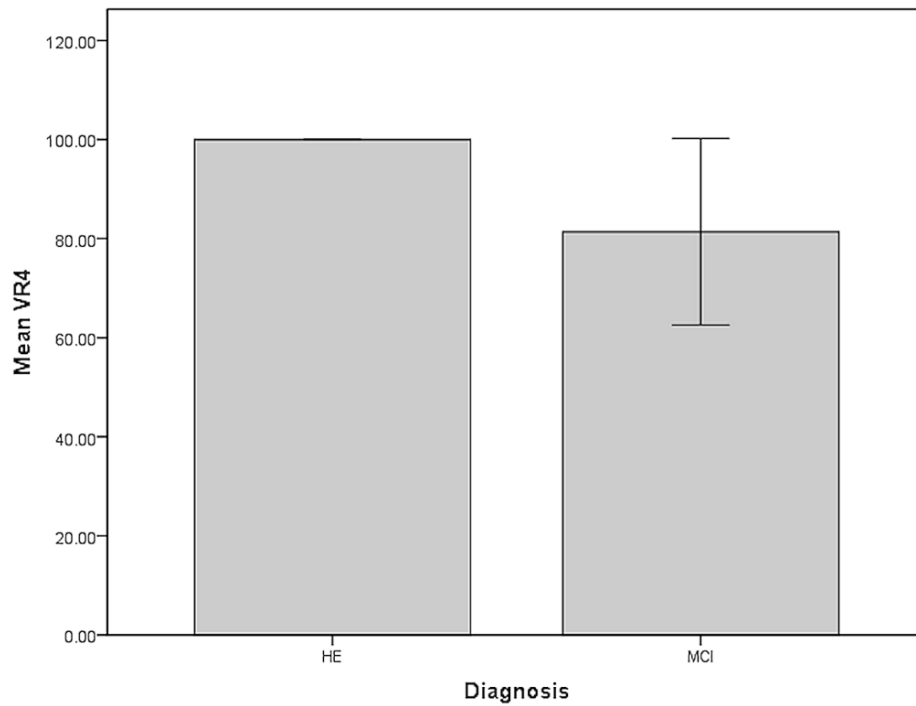


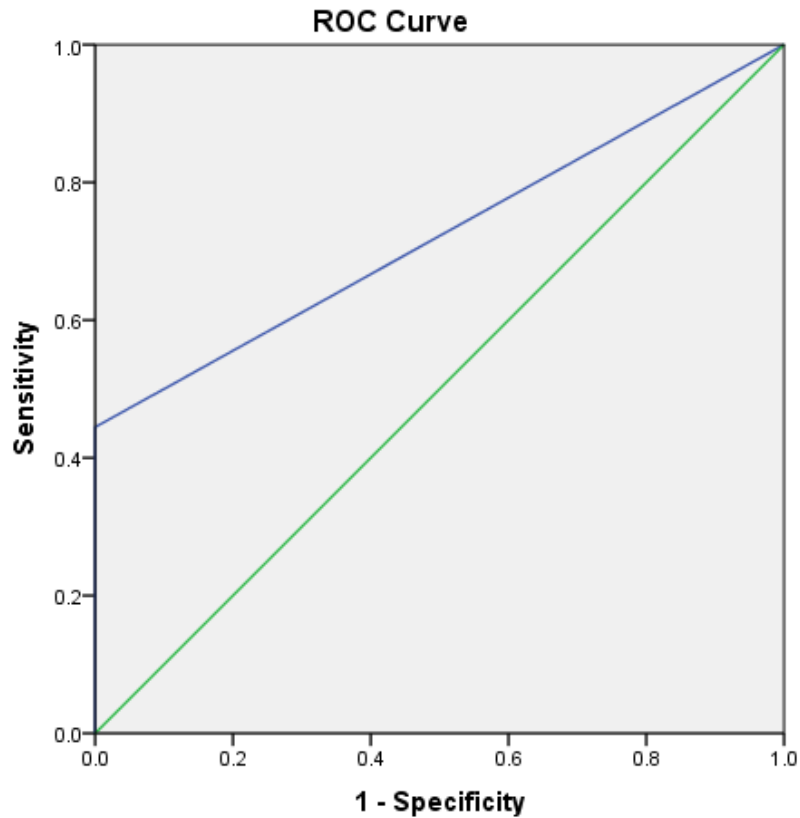
Figure 25: histogram of mean VR4 scores for the two diagnostic groups of participants, HE – healthy elderly and MCI – Mild Cognitive Impairment.

8.4.2 Previous computer experience

Eight participants reported that they possessed previous VR experience (of these individuals, two were classified as MCI). An independent samples t-test of VR4 score carried out between those that had previous VR experience and those that had not was non-significant $t(29) = 0.29, p > 0.05$.

8.4.3 Sensitivity and Specificity of VR4 to MCI

In order to examine the ability of VR4 to discriminate between a diagnosis of HE vs MCI a Receiver Operating Characteristics (ROC) curve was created in SPSS. The area under the curve was trending towards significance, $p = 0.06$, see figure 26.



Diagonal segments are produced by ties.

Figure 26: ROC curve for the sensitivity and specificity of VR4

A selected cut off score which gave the highest sensitivity and specificity was chosen which was the equivalent to a value of 94% on VR4. Logistic regression analysis using the optimal cut off value as the dependent variable (re coded as below '0' and equal or above '1' the cut off score of 94%) was then carried out. In this stepwise backward regression analysis potential confounding variables such as age, gender, education and previous computer experience were included along with diagnosis. This determined whether the cut off needed to be modified as a function of one or more of these variables. The model revealed that none of these variables were predictive of VR4 performance.

8.5 Discussion

8.5.1 Main findings

The aim of the present study was to evaluate whether a new VR environment, VREAD, could be used as a novel test of spatial memory useful for investigating cognition in the elderly. A secondary aim explored how VREAD scores correlate with established neuropsychological tests of cognitive function for this age group, particularly the CANTAB PAL and GNT which are sensitive to early cognitive dysfunction. The overarching aim is to use this test as a method for quick, early, accurate diagnosis of a cognitive impairment. VR is becoming a useful method for assessing participants on a wide range of tasks particularly in the health care domain (Tippett et al., 2009; Broeren et al., 2002; Boejen and Grau, 2011; Greenleaf and Tovar, 1994; Khalifa et al., 2006). This chapter evaluated the use of a novel VR based assessment, VREAD, as a potential tool for the accurate, early detection of AD. Each participant carried out five levels of the VREAD and the results obtained demonstrate that the percentage correct scores on VR 4 significantly correlated with the CANTAB PAL 6 pattern error score and the GNT score. There was also a trend towards significance for the word recall task ($p=0.069$). Score on VR 4 did not correlate with participants' MMSE score which is important as the MMSE has previously been noted not to be sensitive to mild degrees of cognitive impairment (Pasquier, 1999; Cullum et al., 2000; de Jager et al., 2008) which is problematic as it is widely used in the GP setting. In addition, the mean score on VR 4 was significantly lower in the MCI group suggesting that in its preliminary format VR 4 is able to successfully discriminate between participants (according to whether they were classified as HE or MCI).

Blackwell et al., (2004) used a combination of CANTAB PAL 6 pattern error score, GNT score and the subjects' age at the time of testing and incorporated these into a regression analysis in order to create an algorithm to predict the likelihood of a decline to a diagnosis of AD. Using these variables the algorithm was able to accurately predict with 100% which participants converted to AD and those that stayed as questionable dementia over a 32 month period. In the present study, VR 4 score significantly correlated with these two well

established tests but showed no effect of age suggesting that it could be used as a more straightforward method of testing cognition and cognitive decline.

Like the results of this chapter other studies using MCI participants have also demonstrated impairments in route learning (Klein et al., 1999), although, the criteria for MCI may differ across studies. MCI participants have, on imaging scans, presented with an elevated volumetric decline in the EC of the hippocampus in the MTL, an area responsible for visuo spatial associative memory (Dubois et al., 2007). Imaging techniques and lesion studies have shown that this deterioration occurs early in the disease pathway and therefore tests which are sensitive to visuo spatial associative memory and route learning should show deficiencies in performance if this area is affected.

Tombaugh and McIntyre (1992) commented that poor performance on neuropsychological tests may simply be due to increasing age within the sample. The results of this study demonstrate that age was not significantly associated with score on VR 4. Likewise, participants' scores at this level revealed no association with MMSE score. Baseline score on the MMSE can vary immensely. Blackwell et al., (2004) found that in a group of AD convertors baseline score varied from between 23 and 28 out of a possible 30. Individuals scoring 28 would have been classified as normal and this is problematic as their score does not correlate with underlying changes in brain pathology. The NICE dementia guidelines currently recommend the MMSE as a tool for the detection of suspected cognitive impairment and the score obtained can be used to determine whether a patient receives pharmacological treatment (Denning, 2009). Previously NICE stated that drugs should only be given to those individuals with a moderate form of dementia which meant that a patient with a mild impairment would not be treated until they continued to decline further. After a review in 2011, NICE now recommend treatment is administered to patients with a mild form of the disease if the clinician believes it will prove beneficial. Furthermore, patients who did receive drug treatment would have their medication removed when they declined past a score of 10/30 even if they found the treatment beneficial. The accuracy and reliability of scores obtained on the MMSE can be affected by external factors such as the participant's mood, education level and age (Jacova et al., 2007).

Although some previous studies have reported gender effects on navigational ability, with males performing better than females, (Galea and Kimura, 1992; Ward et al., 1986; Astur et al., 1998; Maguire et al., 1999) there were no main effects of gender in this study. This may be due to the small numbers of males recruited ($n = 10$) and this is a factor which requires further investigation. Sandstrom et al., (1998) suggest that gender differences in performance on route learning tasks are likely to be due to the different strategies employed by males and females. For example, males seem to be better at encoding geometrical information whereas females place a heavier reliance on landmark cues (Sandstrom et al., 1998; Galea and Kimura, 1992). Future study using VREAD could incorporate a recognition memory task to specifically investigate whether participants recall the presence of landmarks along the route. Widmann et al., (2012) incorporated a similar approach and found that AD participants were impaired at landmark recall compared with the HE controls.

An important quality of neuropsychological tests should be their ability to correctly identify a participant's clinical group. The score obtained on VR 4 was significantly worse in those participants with a MCI in comparison with the HE suggesting that this novel assessment can discriminate reliably between the two groups. Although the area under the ROC curve in figure 26 showed a trend towards significance, stepwise backward logistic regression analysis revealed that neither age, gender, education, prior computer experience nor diagnosis could significantly predict performance on VR4. This result is likely to be influenced in part by the small sample size of the cohort which resulted in a small number of individuals classified as MCI. This is likely to have limited the statistical power. Future studies using the VREAD test, particularly VR4, need to recruit larger samples to investigate the specificity and sensitivity of the test in further detail and should control for age across both diagnostic categories. It is clear from the results of this study that a number of participants scored 100% which could potentially be obscuring participants that have a more subtle impairment. The results do still successfully show a difference in performance between the two experimental groups but future study making the VR test harder and more challenging would be needed to avoid this.

It remains unclear why VR 4 was the level of the VREAD which correlated with the scores on the neuropsychological tests. VR 5 was more complex although

no significance was obtained for this level. Each level of VREAD became progressively harder based on the number of turns and the number of objects such as trees, benches, street lights, fences and buildings. The overarching aim is to have a simple test with one level which can be administered quickly in clinical settings and this will require further study.

In some previous investigations researchers have raised concerns about the possible side effects of using a virtual task. This included participants reporting feelings of disorientation or nausea during the task (Moffat et al., 2001). In their sample of 133 individuals Moffat et al., (2001) reported that 13 participants withdrew due to feelings of nausea and dizziness during their VR test. This prior knowledge could affect willingness to participate and may make subjects anxious should they be made aware of this which needs to be an important consideration. However, this particular issue was not problematic in this study and there were no reports of illness, headaches or nausea as assessed by the usability questionnaire (Wan Shamsuddin et al., 2011a; Wan Shamsuddin et al., 2011b).

Previous criticisms of virtual environments have included the issue of extrapolation to the real world and whether or not a false 'world' can be thought of as a reliable way to learn and encode newly formed routes or cognitive maps (Werner et al., 2009). Some participants reported that they found the VREAD test too easy and stated that they did not feel they required three practice attempts at each trial. None of the participants commented that they were unable to use the apparatus and each individual was allowed a practice session prior to commencing the actual assessment so as to familiarise themselves with the keyboard and virtual environment. Although it is often assumed that the elderly possess low levels of computer experience (Head and Isom, 2010) in this sample, those who possessed prior use of games consoles or computer gaming devices, may have moved more fluently through the test but this did not contribute to their ability to memorise and learn each route ($p>0.05$). This may change should a more difficult version be used and again is an important consideration which will need to be checked.

8.5.2 Ability of VREAD to predict cognitive decline

The ability of a neuropsychological test to predict cognitive decline is important to identify individuals who may need closer monitoring and intervention. MMSE scores (but not CANTAB PAL scores due to financial restrictions) were obtained one year after baseline testing. None of the participants classified as HE in this chapter had declined to an abnormal MMSE score and as a result it has not been possible to assess whether level 4 of the test could predict cognitive decline although this is something which deserves further study and longitudinal follow up will take place.

8.6 Conclusion and summary

The self report of navigational deficits is common in the elderly and the ability to encode and remember new routes is impaired in those individuals who have a cognitive impairment. The fact that deficits in route learning are common in the early stages of AD and in participants with a MCI makes tests of navigation interesting tools for earlier detection. The results of this chapter which has preliminary tested a newly developed VR based assessment, VREAD, have shown that this task could have the potential to be used as a possible method for the detection of an early cognitive impairment after some further modifications are addressed. Significant correlations were found between level four of this test, the CANTAB PAL 6 pattern error score and the GNT. There was no association with the MMSE which has previously been criticised for its inability to detect mild changes in cognition (Tombaugh and McIntyre, 1992). VR 4 was also significantly lower in those participants classified as MCI compared to the HE sub group. The ability to accurately discriminate between participant sub groups is vital for the accurate diagnosis of AD and MCI.

Future study following on from these preliminary findings is needed and should focus on investigations into whether the VR test can predict future cognitive decline. This will require detailed longitudinal follow up of these participants. Furthermore, the sample size of 31 participants with nine of these having MCI is too small, along with the high ceiling effect of the current version, to verify the

utility of the test in a clinical setting at present but given these promising preliminary results then further study will aim to achieve this.

8.7 Chapter summary of key points

- The domain a task assesses is a factor which can affect the overall accuracy of detecting an early impairment.
- Visuospatial associative memory and route learning activate the EC, one of the earliest brain regions to be affected by AD pathology.
- Impairments in route learning are common in the elderly but are more pronounced in AD and MCI.
- VR assessments are useful for the testing of route learning.
- A novel virtual environment VREAD was evaluated in a sample of HE participants who were >60 years of age with no pre diagnosed cognitive impairment or neurological condition.
- Scores on level 4 of VREAD significantly correlated with CANTAB PAL and GNT scores.
- There were no associations between VR 4 and MMSE score or age, education or gender.
- VR4 was able to discriminate between HE and MCI participants.
- VR4 may be useful for early detection of cognitive impairments.
- Future experiments need to investigate how the task can be made more complex, for example, by decreasing the number of practice levels and determining why VR4 and not the other levels seems to be the most sensitive to navigational difficulties.

Some of the information within this chapter can be found in Wan Shamsuddin et al., (in prep) 'Using a virtual environment to assess cognition in the elderly'

Chapter Nine

General discussion and summary

9.1 Introduction

This thesis has presented experiments which have aimed to investigate some subtle external factors which may affect performance on neuropsychological test batteries during typical testing conditions in the healthy elderly. In addition a newly developed virtual reality based test was evaluated as a possible novel tool for the early detection of AD. All of the experimental chapters followed an experimental quantitative methodology which utilised cognitive batteries consisting of a variety of neuropsychological tests, chosen to specifically tax different cognitive domains and which are commonly used assessments in research settings. All of the experiments within the thesis recruited HE participants; however, the findings may have implications for the use of neuropsychological testing in AD and MCI research. The thesis has illustrated that external factors such as prior caffeine consumption (chapter five), tinned tuna consumption (chapter six), and time of day (TOD) (chapter seven) can influence the scores obtained on some neuropsychological tests. The thesis has also presented preliminary findings that VREAD, a novel virtual assessment of spatial route learning, could be useful as a tool to detect early cognitive impairment in the future (chapter eight). This final chapter collates the main findings from the experimental chapters and discusses the implications these may have. The chapter will also provide a summary of the thesis as a whole and will address some methodological considerations and possible ideas for future research.

9.2 Motivation

In recent years there has been a prominent focus to try to diagnose and treat AD as early as possible. Signs of the disease including pathological markers and changes in underlying cerebral systems can be detected years, possibly even decades before cognitive symptoms first become noticeable (Forlenza et

al., 2010; Dickerson et al., 2011; Petersen, 2009; Sperling et al., 2011). Neuropsychological testing is one method by which early detection can be implemented and it is commonly used in the diagnosis of a cognitive impairment associated with AD or MCI (Chapman et al., 2010). It is crucial that the scores obtained on neuropsychological tests are accurate. It is important, therefore, that external factors which may influence performance are controlled for or at least considered when interpreting participants' performance.

A number of demographic factors have been investigated previously, such as education, age and ethnicity (Fields et al., 2011), and are now usually taken into consideration when evaluating neuropsychological performance. There are still many other variables which may affect the participant's performance. It is useful to determine the effects of these especially when test scores are used in research studies and to determine diagnosis, treatment and care. Furthermore, normative data which is commonly referred to for comparison purposes may not have controlled for such factors (Lesk et al., 2009).

9.3 Main findings

Caffeine, TOD and diet (non-oily fish consumption) are all common lifestyle factors which have the potential to influence and skew data obtained on psychological assessments. These factors are routinely prevalent in the daily life of most individuals attending a cognitive assessment and were, therefore, investigated as factors which may affect neuropsychological testing in the HE.

Chapter five showed that 200mg of prior caffeine interacted with age and scores on assessments of EF and processing speed. This revealed that the older elderly who had received caffeine showed a steeper decline in performance with increasing age, an effect which was not present on any of the assessments for participants in the placebo condition (Walters and Lesk in prep - a; Lesk and Walters, 2012). These findings were not attributed to an alertness effect of caffeine as there was no main effect of caffeine compared to placebo on any of the assessments and caffeine did not improve performance in the older elderly. Chapter six investigated a dietary factor, monthly non-oily fish consumption. Although this was not an immediate prior factor which can be controlled for in

experimental situations it is important to determine the effects of dietary factors on cognitive performance in the elderly. Grams of monthly tinned tuna consumption was a significant predictor of MMSE score, specifically the word recall item of the task which assesses short-term memory. Chapter seven demonstrated that the TOD that some neuropsychological tests are delivered can affect the participants performance. TOD also interacted with the participants' age and prior caffeine consumption on certain assessments (Walters and Lesk, in prep - b).

Chapter eight's evaluation of the newly created virtual reality based assessment, VREAD, created in collaboration with the University of Bradford, School of Computing, Informatics and Media, showed that scores on level four of the test were associated with the participant's performance on the PAL and GNT. Both of these tests been shown to be sensitive to early cognitive impairments (Blackwell et al., 2004; de Rover et al., 2011). Score on level four was also significantly lower in those participants classified as MCI compared to the HE suggesting that in its preliminary form VREAD could distinguish between clinical groups.

Previous research which has investigated dietary factors such as caffeine and fish consumption in the elderly has taken a longitudinal approach and has investigated participants' habitual consumption of CCFS and fish intake on their risk of developing AD or likelihood of cognitive decline (for example Ritchie et al., 2007; Eskelinen et al., 2009; Lindsay et al., 2002; van Gelder et al., 2007; Santos et al., 2010 and Morris et al., 2003; Devore et al., 2009; Kalmijn et al., 1997; van Gelder et al., 2007). Studies investigating the effects of caffeine have relied upon the self report of CCFS consumption and these substances (such as tea, coffee, chocolate and soft drinks) may contain other active compounds which may also be able to influence cognitive performance. For example, polyphenols are found in tea (Lindsay et al., 2002) and chlorogenic acid, cafestol and kaweaol are active ingredients present within coffee (Eskelinen and Kivipelto, 2010). The administration of pure caffeine 40 minutes prior to the neuropsychological assessment in chapter five has reduced the possibility that any findings were due to other ingredients contained within CCFS and has allowed the immediate effects of caffeine on cognition in the elderly to be studied. Due to financial restrictions it was not possible to measure

participants' salivary caffeine concentrations prior to testing and Hogervorst et al., (1998) excluded three participants from analysis because their prior caffeine salivary levels were slightly greater than 2.0mg/l suggesting that these individuals had not adhered to the abstinence protocol. This should be considered in future research.

In all of the experimental chapters participants' TOD preference and peak arousal time was not pre determined. Chapter seven reviewed data sets from chapters five and six. The hypothesis of these chapters was not to investigate peak arousal or time of day effects on performance and, therefore, testing time was not controlled for. The participants were given some choice as to when to attend the neuropsychological assessment and so it is reasonable to assume that at least some of these individuals took part at their preferred TOD even though this may not have been their optimal arousal period. Likewise, for all of the experimental chapters of the thesis TOD preference and peak arousal may have been important factors to consider.

The creation of a food diary to obtain details of participants' monthly fish consumption in chapter six was constructed to reduce the possibility of errors in self-estimation which often arise when using FFQs (Bowman et al., 2012). Food diaries as far as I am aware have not been used previously in research specifically investigating fish consumption and their use in cognitive research in the elderly is also limited. Food diaries are considered to be a more reliable method for obtaining dietary information in comparison to FFQs (Legg et al., 2000) and, therefore, it is likely that a more accurate account of monthly fish consumption was obtained. The use of gram information rather than portion numbers further strengthened the participants' accounts of their actual intake.

The preliminary findings of chapter eight revealed that some participants found the VREAD test too easy (Wan Shamsuddin et al., 2011b) and this is concurrent with the high ceiling effect obtained in the early levels of the test. This suggests that VREAD needs to be adjusted and made more challenging and complex. The majority of the HE participants were able to perform at ceiling and this currently limits the application of VREAD as an early detection tool.

9.4 Future work

This thesis has established that some external factors can influence neuropsychological scores. As discussed throughout this thesis it is important to consider subtle external factors when scoring psychological assessments and making comparisons with normative data. The results obtained have provided a solid basis for future work.

The findings of chapter five, i.e. the significant interaction effects between caffeine/placebo, age and assessments of EF and processing speed, could be extended upon to confirm these findings using more rigorous tests of each domain. Caffeine is often deliberately consumed to improve attention and maintain alertness (Sharwood et al., 2013; Wright et al., 2013) both of which are components of EF. EF encompasses a number of sub functions including, attention, inhibition, problem solving, task switching, dual tasks, reasoning and the ability to plan and devise strategies (Goh et al., 2012) which were not investigated in chapter five. Previous research has demonstrated that some of these processes are affected by age more than others (Goh et al., 2012). For example, Hasher et al., (2007) demonstrated that older participants show a reduced ability to inhibit or ignore distracting stimuli. This thesis found significant affects of caffeine and age on the DT and the SDMT. The DT specifically assesses participants' ability to process two tasks simultaneously (Baddeley et al., 1997) and the SDMT assesses attention, visual search and concentration (González et al., 2007). It is important to establish whether similar interactions with caffeine and age are found on other tests assessing different EF domains to determine whether caffeine's affects are indeed selective. Examples of tests which could be investigated may include the TMT [specifically the difference between TMT part A and TMT part B which would assess task switching ability (Reitan and Davison, 1974)], the Tower of London test which assesses planning and decision making (Shallice, 1982) and the Wisconsin card sorting test which assesses goal switching and decision making (Berg, 1948).

Likewise, further study is needed to extend upon the effect of caffeine on processing speed. The LCS and PCS are classified as tests of processing speed (Salthouse and Babcock, 1991) but the pen and paper versions of both

tests have high levels of distractibility. Whilst confirming whether two patterns or strings of letters are the same or different the participant must also ignore the patterns and strings above and below and it could be argued that a certain degree of inhibition (a sub domain of EF) is also required when completing these tasks (Lustig et al., 2006). In chapter five one other test of processing speed, the Stroop Word, did not show the same interaction suggesting that the effects of caffeine are selectively dependent on executive processes only.

The cross over point for each test which showed a significant interaction between caffeine, age and test score occurred at a different age depending on the cognitive test used. For example, the PCS and LCS had older cross over ages of 83 and 84 years respectively, however, for the assessments of EF, the DTR, DT T1-T2, SDMT and Stroop Interference had ages at cross over of 69, 64, 77 and 79 years respectively. Furthermore, there is little literature which has specifically investigated the effect of prior caffeine on the cognitive performance of those with a MCI and this too could be important.

A preliminary analysis (data of chapter five) was carried out and classified participants as high or low performers based upon their total z-scores for overall performance. In the tests that showed the significant interactions with caffeine and age in chapter five an interesting differential pattern of results was observed, whereby those classified as low performers showed poorer performance on caffeine whereas those classified as high performers scored better on caffeine. This was partly expected as the majority of younger participants were classified as high performers. This does deserve further work in a larger sample and will be the topic of future study.

The finding that tinned tuna consumption was a predictor of MMSE performance in chapter six, specifically the recall item of the test, also requires further study to determine the effect of tinned tuna intake on other assessments of short-term memory and recall. None of the other assessments administered in chapter six tested episodic, short-term memory or word recall and it is possible that the benefits of increased tuna intake are selective to memory processes. This needs to be further investigated and confirmed using more rigorous testing of these domains with appropriate neuropsychological tests and a larger sample.

Although food diaries provide more reliable data than FFQs which rely on self estimation (Bowman et al., 2012; Arsenault et al., 2009), the diary in chapter six was only completed for a period of four weeks and previous researchers have commented that fish consumption may vary depending on the time of year and season (Cunnane et al., 2009). Consequently, the study could be continued over a longer period of time to confirm if the current findings are still present. A thorough investigation into memory and tuna intake should now be implemented based upon these initial findings. It is possible that similar findings may be present in a cohort of participants who eat larger amounts of other types of fish, of which only smaller amounts were consumed in the cohort recruited in chapter six. Recruiting participants with a MCI would also determine any effects of tinned tuna consumption in individuals with impaired cognitive performance.

Although some previous studies have investigated whether total fish consumption is associated with the subsequent level of cognitive decline over follow up, it would also be useful to further investigate whether non-oily fish consumption (and/or tinned tuna consumption specifically) is able to predict cognitive decline in those with lower monthly intakes. This will be the focus of ongoing study which will utilise the longitudinal follow up of this cohort.

There are many other external factors which may affect performance on neuropsychological tests in the elderly prior to their administration aside from those studied in this thesis. Prior caffeine, smoking and alcohol were controlled for in chapter five by asking participants to abstain for a set amount of time before attending the assessment and only non smokers were recruited. Other factors to consider include the participants prior intake of any medications (Gray et al., 1999), water (which may influence hydration level) (Sécher and Ritz, 2012), mood and emotional state (Jacova et al., 2007), prior food intake (Maisto et al., 2011), particularly glucose (Gorby et al., 2010), stress and cortisol levels (Shansky and Lipps, 2013); homocysteine and B12 (Smith et al., 2010; de Jager et al., 2012) and their overall general health. Future study investigating the effects these may have on cognitive performance at the time that tests are administered is needed.

The findings of chapter eight were preliminary and further research is needed for the test to be used as a tool for the detection of cognitive impairment. The

results suggested that VREAD does have the potential to be used as a tool for the detection of a cognitive impairment after some changes in the administration and design of the task are made (Wan Shamsuddin et al., 2011a). The high ceiling effect which was found suggests that VREAD is currently too easy and, therefore, not sufficiently challenging enough for the HE. This has meant that the majority of participants were able to score close to the tests maximum score implying that the tasks difficulty and complexity requires some alterations and adjustment. Ideally VREAD should consist of one level which would reduce the administration time and make the test more suitable for clinical use where time constraints are present for each appointment. Although the use of a single cognitive test score should not be used to determine overall outcome, if the assessment assesses a cognitive domain which is known to be affected by early underlying brain alterations concurrent with AD (i.e. the EC which is recruited during visuo associative memory testing), and also an area which sees the earliest deposition of AD related pathology (i.e. the presence of tau tangles (Blackwell et al., 2004)) this would improve the validity and reliability of such results and the administration of long test batteries would not be necessary. Test re test reliability is also needed for VREAD. Continued use of VREAD addressing these points is warranted. However, there are few tests using VR in early diagnosis and this is an important preliminary investigation.

Some participants noted in the VREAD feedback questionnaire that they found three practice attempts at each level unnecessary (Wan Shamsuddin et al., 2011b) and the task could be repeated with only one practice at each level to determine whether this would affect performance. Task difficulty could also be evaluated by making each route longer with more complex paths to remember. Similarly additional parameters could be assessed, for example, the recall of landmarks or the participants' capability to draw the followed route on paper. The time taken to complete each level was not factored into the overall score on VREAD and this could be important. For example, two participants who scored the same may have taken significantly different lengths of time to complete each level and this should be considered in future continuations of this research. The ability of VREAD to predict later cognitive decline has also not yet been determined and this would be important should the task be modified and applied to a larger cohort.

9.5 Neuropsychological implications

Small habitual amounts of caffeine (200mg), which is the equivalent to a medium filter coffee (Starbucks, 2011) and is a commonly consumed amount, had the ability to affect cognitive performance on a number of neuropsychological assessments in the over 60s in chapter five (Walters and Lesk in prep - a; Lesk and Walters, 2012). Caffeine impaired performance in the older elderly compared to the younger elderly. The older elderly participants who received caffeine may have scored lower than on an occasion when they had not been administered caffeine. For example, those participants over the age of 80 years who scored 27 and 32 respectively on the SDMT in chapter five may have scored closer to 40 if they had received placebo. Even a small difference in the points awarded may affect the interpretation of the results which is particularly important on assessments such as the MMSE where the borderline between normal cognition, mild impairment and AD only spans over a six point difference (Tombaugh, 2005).

These findings have implications for the interpretation of test scores and the accuracy of neuropsychological data and are applicable to any experiment which utilises cognitive tests. For the placebo condition there was no main effect or age interaction and this finding was similar to the results of Lesk et al., (2009) who also reported no significant effect of placebo (i.e. CCFS absent) on any of the assessments using a similar cognitive battery to that of chapter five. Together these results signify that researchers should ask their participants to abstain from caffeine for a set period of time or at least take note of any prior consumption before they take part in a neuropsychological assessment to ensure that the results obtained are the most controlled. This is not just applicable to aging research but to any cohort undergoing a cognitive assessment. Hogervorst et al., (1998) comment that usually cross sectional studies do not control for the participant's caffeine consumption. This is problematic particularly for aging research where age effects may be exacerbated by caffeine administration. The results of chapter five are also important in terms of the use of and creation of normative data which often serves as a comparison and may not have considered participants' prior caffeine consumption (Lesk et al., 2009). The results of chapter five are consistent with Lesk and Womble (2004) and Lesk et al., (2007) who also found

striking effects of 200mg of prior caffeine in young participants on two studies using phonological priming of the tip-of-the tongue-state, one in young participants and one investigating an anomic aphasic patient.

The finding that monthly tinned tuna consumption (classified as a non-oily fish (NHS, 2013)) can predict general cognitive performance as measured by the MMSE, and can specifically predict the number of words recalled on the recall sub-domain in chapter six has important implications for the consideration of the effects of dietary factors on cognitive performance. Tinned tuna is classified as a non-oily fish because the 'canning/tinning' process removes the majority of its fish oil content (NHS, 2013). Previous research has failed to report an effect of white fish or non-oily fish intake on cognitive performance in the elderly or associated this type of fish with a reduced risk of developing a cognitive impairment. The NHS and British Heart Foundation recommend that adults consume two portions of fish per week, one of which must be oily, although they clearly state that tinned tuna does not classify as a portion of oily fish (NHS, 2011; British Heart Foundation, 2012). The findings of this thesis may have implications for how tinned tuna is indeed classified regarding advice given to the general public. Whether or not non-oily fish intake, specifically tinned tuna, can predict future cognitive decline is yet to be determined.

Like previous work on circadian rhythms and TOD effects, chapter seven confirmed that TOD can affect performance on some cognitive domains in the elderly. The significant TOD effects noted on the MMSE are important for AD related research as this test is currently recommended as a diagnostic tool for the detection of AD and dementia in the UK (Ballard et al., 2011). TOD is, therefore, an important factor which affects the neuropsychological testing process and should be considered in studies related to cognitive aging if elderly participants are assessed later in the afternoon at their 'off peak' arousal time. TOD effects were also noted on the GNT, an assessment of semantic memory (McKenna and Warrington, 1980), which has been recognised as a useful tool for detecting mild cognitive alterations (Blackwell et al., 2004). Blackwell et al., (2004) entered GNT score as one component of an algorithm created from their data. If participants were assessed at their 'off peak' arousal period their score may not reflect their actual semantic ability and as a result the accuracy and reliability of the algorithm may have been affected.

If researchers and clinicians are aware that TOD effects in cognition do exist (Schmidt et al., 2007; Wright et al., 2012; Froy, 2011) and that cognition is optimal at peak arousal times (Froy, 2011; Hasher et al., 2005), wherever possible efforts should be made to ensure that participants are assessed at their preferred TOD. For the majority of the elderly this is in the morning (Schmidt et al., 2007). This is important to avoid exaggerated age differences particularly if test scores are to be used to determine research inclusion/exclusion and or diagnosis. Although it may not be possible for researchers or clinicians to administer assessments in the morning for every participant, they could at least note the time the examination takes place and consider this.

TOD effects on cognitive performance should be considered in any study within the fields of experimental psychology, cognitive neuroscience or cognitive psychology which tests human brain function and performance. The findings of this thesis are not only relevant to research which recruits elderly participants but are applicable to participants of any age range. The same issues should be considered when cognitively assessing children and younger adults. Teenagers and young adults should be assessed at their peak arousal time which for the majority of these individuals is in the afternoon or evening and not the early morning (Schmidt et al., 2007; Hasher et al., 2005). Likewise, young children have peak arousal times in the early morning (Schmidt et al., 2007; Hasher et al., 2005). Hasher et al., (2005) commented that currently the majority of examinations for younger adults and the teaching of critical subjects such as mathematics take place in the early morning at approximately 9.00am due to the common belief that performance is maximal after waking. This misconception could disadvantage the performance of children and teenagers and highlights that TOD affects have important considerations for not only cognitive psychology but also for other fields of research and the education systems.

The results of chapter eight have shown that VR based assessments are useful tools in the elderly with positive feedback being received in the feedback questionnaire (Wan Shamsuddin et al., 2011a; Wan Shamsuddin et al., 2011b). Deficits in route learning do occur in early AD (Lithfous et al., 2013; Cherrier et al., 2001; Morris, 1993; Serino and Riva, 2013) and, therefore, assessments of

this type of ability may be useful in the earlier detection of cognitive impairment. The preliminary findings of chapter eight have shown that score on level four of VREAD is significantly correlated with performance on other tests which have previously been shown to be sensitive to early cognitive symptoms. This implies that VREAD may be suitable as a clinical tool, however, it is acknowledged that improvements and alterations to this preliminary version would be required before this can be considered further. This is important particularly as some participants classified as MCI were able to score maximally on this level. Improvements to the tests sensitivity and specificity are therefore needed and this will be the focus of future study.

9.6 Wider implications of the thesis

There is a prominent focus to try to detect and treat AD as early as possible, as signs of the disease including pathological markers and changes in underlying cerebral systems can be detected years, possibly even decades before the first cognitive symptoms become noticeable (Forlenza et al., 2010; Dickerson et al., 2011; Petersen, 2009; Sperling et al., 2011). Neuropsychological testing is one method by which early detection may be implemented and it is commonly used in the diagnosis of a cognitive impairment (Chapman et al., 2010) and the mapping of the impairments trajectory over time. Neuropsychological testing is used in the diagnosis and research of other medical conditions such as Parkinson's disease (O'Claassen and Wylie, 2012), stroke (Suzuki et al., 2013), other dementias (Fields et al., 2011) e.g Lewy Body Dementia, semantic dementia and FTD (Lambon Ralph et al., 2001), schizophrenia (Schaub et al., 2013), autism (Losh et al., 2009), Huntington's disease (Paulsen, 2011), TBI (Dean and Sterr, 2013) and child development amongst others.

This thesis has investigated subtle external factors which are prevalent during a typical testing condition and which may have the ability to influence neuropsychological test scores with significant consequences. Although the thesis has focussed on the implications for AD and MCI related research and has studied elderly cohorts using high level neuropsychological testing used in research settings, the findings are important for any type of study which uses cognitive testing as a part of its methodology.

Clinicians and researchers may use neuropsychological test scores to aid their interpretation of the participant's general cognitive ability and performance may be considered when making a diagnosis or determining whether more in-depth cognitive testing is required (Jacova et al., 2007). The outcome of neuropsychological testing may influence the decision to initiate treatments (e.g. MMSE score and AchEIs, Ballard et al., 2011), recommend social interventions and/or lifestyle changes or select participants for research trials (Zamrini et al., 2004). It is crucial that the scores obtained on these tests are as accurate as possible and represent the participant's actual level of cognitive function particularly for the early detection of AD. The findings of an effect of caffeine and TOD have implications for the collection and interpretation of population norms, which are often referred to during the clinical interpretation of a participant's performance (Lesk et al., 2009; Fields et al., 2011) but may not have considered or taken into account external variables such as caffeine and tiredness. Subsequently this may limit their use as a comparison. Current policy, including the NICE dementia guidelines, has stated that clinicians should take into account external variables which may affect the participant's performance (NICE, 2011). It is feasible that researchers and clinicians could ask participants to abstain from CCFS before being assessed or could simply note whether their participant has consumed caffeine. Researchers can also easily check that participants are well rested and have not had a reduced amount of sleep the night before their assessment.

If performance can be affected by subtle external factors present during a typical testing session, as demonstrated in this thesis, this has implications for all of the processes mentioned above. External factors should be considered and investigated further so that these can be controlled for when assessing participants on neuropsychological tests.

The positive feedback received regarding VREAD has suggested that virtual assessments, particularly spatial route based tests, are suitable for application in elderly cohorts (Wan Shamsuddin et al., 2011a; Wan Shamsuddin et al., 2011b). Whereas some previous research has highlighted concerns about the prior computer experience of the elderly and the possibility that there may be the potential for anxiety related to using computer based tools, this was not problematic in this thesis and even the elderly who did not possess any prior

experience of computers successfully completed the task. Although it is clear that in its current form VREAD is not suitably challenging, the findings have implicated that with further work the test does have the potential to be used as a cognitive assessment in the future.

9.7 Thesis conclusion

This thesis has highlighted that subtle external factors that are prevalent during typical testing conditions can skew neuropsychological data with significant consequences. This signifies the need for researchers to consider such factors when using cognitive batteries as a part of their research, and to wherever possible control for these. Evaluation of a novel virtual neuropsychological test has shown that this may be a useful tool for the detection of cognitive impairment after further alterations to the tests design are made.

Although the thesis has implications for the neuropsychological assessment of AD and cognitive impairments in the elderly, the findings are also applicable to the field of experimental psychology and cognitive research as a whole. The consideration of these subtle every day external factors are important for any experimental paradigm in human brain research. The thesis has investigated the effects of external factors on tests commonly used in elderly populations for the detection of cognitive impairment but these factors may also affect performance on other cognitive batteries used in the diagnosis of other medical conditions and or other age groups or cognitive processes.

The accuracy and reliability of neuropsychological test scores is vital if researchers are to continue to interpret scores, and compare these with normative data sets, determine treatment, evaluate performance over time, select participants for research inclusion, predict cognitive decline or design new interventions. Future research investigating the factors mentioned in this thesis and the study of other external variables which may affect cognition will add valuable knowledge to the field of neuropsychology and cognitive neuroscience and further improve the accuracy of neuropsychological testing.

References

- Addicott, M.A. and Laurienti, P.J. (2009) 'A comparison of the effects of caffeine following abstinence and normal caffeine use'. *Psychopharmacology (Berl)*, 207:3, pp.423-431
- Ahmed, S., Arnold, R., Thompson, S.A., Graham, K.S. and Hodges, J.R. (2008) 'Naming of objects, faces and buildings in Mild Cognitive Impairment'. *Cortex*, 44:6, pp.746-752
- Aisen, P.S., Schneider, L.S., Sano, M., Diaz-Arrastia, R., van Dyck, C.H., Weiner, M.F., Bottiglieri, T., Jin, S., Stokes, K.T., Thomas, R.G. and Thal, L.J. Alzheimer's Disease Cooperative Study (2008) 'High dose vitamin B supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial'. *Journal of the American Medical Association*, 300:15, pp.1744-1783
- Aisen, P.S., Petersen, R.C., Donohue, M., Gamst, A., Raman, R., Thomas, R.G., Walter, S., Trojanowski, J.Q., Shaw, L., Beckett, L.A., Jack, C.R., Jagust, W., Toga, A., Saykin, A.J., Morris, J.C., Weiner, M.W. and Alzheimer's Disease Neuroimaging Initiative (2010) 'Clinical core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans'. *Alzheimer's & Dementia*, 6:3, pp. 239-246
- Albasanz, J.L., Perez, S., Barrachina, M., Ferrer, I. and Martín, M. (2008) 'Up-regulation of adenosine receptors in the frontal cortex in Alzheimer's disease'. *Brain pathology*, 18:2, pp.211-219
- Albert, M.S., Jones, K., Savage, C.R., Berkman, L., Seeman, T., Blazer, D. and Rowe, J.W. (1995) 'Predictors of cognitive change in older persons: MacArthur Studies of successful aging'. *Psychology and Aging*, 10:4, pp.578-589
- Albert, M.S., DeKosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo,

M.C., Thies, B. and Phelps, C.H. (2011) 'The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease'. *Alzheimer's & Dementia*, 7:3, pp.270-279

Alzheimer's Association. (2012) 'Alzheimer's disease facts and figures'. *Alzheimer's & Dementia*, 8:2, pp.131-168

Alzheimer's Association. (2013) '2013 Alzheimer's disease facts and figures'. *Alzheimer's & Dementia*, 9:2, pp.208-245

Alzheimer's Disease International. (2009) '*World Alzheimer Report 2009*'. Prince, M.J. and Jackson, J., eds. London: Alzheimer's Disease International.

Alzheimer's Disease International. (2011). *World Alzheimer Report*. Prince M.J., Bryce, R. and Ferri, C., eds. London: Alzheimer's Disease International.

Alzheimer's Society. (2013) *The Mini Mental State Examination (MMSE)* [online] Available at: <http://www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=121> [Accessed 1st June 2013]

Anderson, E.J., de Jager, C.A. and Iversen, S.D. (2006). 'The placing test: Preliminary investigations of a quick and simple memory test designed to be sensitive to pre-dementia Alzheimer's disease but not to normal ageing'. *Journal of Clinical and Experimental Neuropsychology*, 28:6, pp.843-858

Andreasen, N., Minthon, L., Davidsson, P., Vanmechelen, E., Vanderstichele, H., Winblad, B. and Blennow, K. (2001) 'Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice'. *Archives of Neurology*, 58:3, pp.373-379

Andreeva, V.A., Kesse-Guyot, E., Barberger-Gateau, P., Fezeu, L., Hercberg, S. and Galan, P. (2011) 'Cognitive function after supplementation with B vitamins and long-chain omega-3 fatty acids; ancillary findings from the

SU.FOL.OM3 randomized trial'. *American Journal of Clinical Nutrition*, 94:1, pp.278-286

Angulo, E., Casadó, V., Mallol, J., Canela, E.I., Viñals, F., Ferrer, I., Lluís, C. and Franco, R. (2003) 'A1 adenosine receptors accumulate in neurodegenerative structures in Alzheimer disease and mediate both amyloid precursor protein processing and tau phosphorylation and translocation'. *Brain Pathology*, 13:4, pp.440-451

Apostolova, L.G., Lu, P.H., Rogers, S., Dutton, R.A., Hayashi, K.M., Toga, A.W., Cummings, J.L. and Thompson, P.M. (2006) '3D mapping of Mini-Mental State Examination performance in clinical and pre-clinical Alzheimer's disease'. *Alzheimer disease and Associated Disorders*, 20:4, pp.224-231

Arbuthnott, K. and Frank, J. (2000) 'Trail making test, part B as a measure of executive control: Validation using a set-switching paradigm'. *Journal of Clinical and Experimental Neuropsychology*, 22:4, pp.518-528

Archer, H., Edison, P., Brooks, D.J., Barnes, J., Frost, C., Yeatman, T., et al. (2006) 'Amyloid load and cerebral atrophy in Alzheimer's disease: an 11C-PIB positron emission tomography study'. *Annals of Neurology*, 60:1, pp.145-147

Arendash, G.W., Schleif, W., Rezai-Zadeh, K., Jackson, E.K., Zacharia, L.C., Cracchiolo, J.R., Shippy, D. and Tan, J. (2006) 'Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production'. *Neuroscience*, 142:4, pp.941-952

Argimon, I.I. and Stein, L.M. (2005). 'Cognitive abilities in older seniors: a longitudinal study'. *Cadernos de Saúde Pública*, 21:1, pp.64-72

Arnaud, M.J. (1987) 'The pharmacology of caffeine'. *Progress in drug research*, 31, pp.273-313

Arriagada, P.V., Growdon, J.H., Hedley-Whyte, E.T. and Hyman, B.T. (1992) 'Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease'. *Neurology*, 42:3, pp.631-639

Arsenault, L.N., Matthan, N., Scott, T.M., Dallal, G., Lichenstein, A.H., Folstein, M.F., Rosenberg, I. and Tucker, K.L. (2009) 'Validity of Estimated Dietary Eicosapentaenoic Acid and Docosahexaenoic Acid Intakes Determined by Interviewer-Administered Food Frequency Questionnaire Among Older Adults With Mild Moderate Cognitive Impairment or Dementia'. *American Journal of Epidemiology*, 170:1, pp.95-103

Artero, S., Ancelin, M-L., Portet, F., Dupuy, A., Berr, C., Dartigues, J-F., Tzourio, C., Rouaud, O., Poncet, M., Pasquier, F., Auriacombe, S., Touchon, J. and Ritchie, K. (2008) 'Risk profiles for mild cognitive impairment and progression to dementia are gender specific'. *Journal of Neurology Neurosurgery and Psychiatry*, 79, pp.979-984

Ashendorf, L., Jefferson, A.L., O'Connor, M.K., Chaisson, C., Green, R.C. and Stern, R.A. (2008) 'Trail Making Test errors in normal aging, mild cognitive impairment, and dementia'. *Archives of Clinical Neuropsychology*, 23:2, pp.129-137

Ashford, J.W., Borson, S., O'Hara, R., Dash, P., Frank, L., Robert, P., Shankle, W.R., Tierney, M.C., Brodaty, H., Schmitt, F.A., Kraemer, H.C. and Buschke, H. (2006) 'Should older adults be screened for dementia?' *Alzheimers and Dementia*, 2:2, pp.76-85

Astur, R.S., Ortiz, M.L. and Sutherland, R.J. (1998) 'A characterization of performance by men and women in a virtual Morris water task: a large and reliable sex difference'. *Behavioural Brain Research*, 93, pp.185-190

Atri, A. (2011) 'Effective pharmacological management of Alzheimer's disease'. *The American Journal of Managed Care*, 17:suppl 1, pp.S346-355

Atwood, C.S., Obrenovich, M.E., Liu, T., Chan, H., Perry, G., Smith, M.A. and Martins, R.N. (2003) 'Amyloid-beta: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-beta'. *Brain Research Reviews*, 43:1, pp.1-16

Auld, D.S., Kornecook, T.J., Bastianetto, S. and Quirion, R. (2002) 'Alzheimer's disease and the basal forebrain cholinergic system: relations to β -amyloid peptides, cognition, and treatment strategies'. *Progress in Neurobiology*, 68:3, pp.209-245

Axelsen, P.H., Komatsu, H. and Murray, I.V.J. (2011) 'Oxidative Stress and Cell Membranes in the Pathogenesis of Alzheimer's Disease'. *Physiology*, 26:1, pp.54-69

Baddeley, A., Logie, R., Bressie, S., Della Sala, S. and Spinnler, H. (1986) 'Dementia and Working Memory'. *The Quarterly Journal of Experimental Psychology*, 38A, pp.603-618

Baddeley, A.D., Bressi, S., Della Sala, S., Logie, R. and Spinnler, H. (1991) 'The decline of working memory in Alzheimer's disease: A longitudinal study'. *Brain*, 114:pt 6, pp.2521-2542

Baddeley, A., Della Sala, S., Papagno, C. and Spinnler, H. (1997) 'Dual-task performance in dysexecutive and nondysexecutive patients with a frontal lesion'. *Neuropsychology*, 11:2, pp.187-194

Baddeley, A.D., Baddeley, H.A., Bucks, R.S. and Wilcock, G.K. (2001) 'Attentional control in Alzheimer's disease'. *Brain*, 124:8, pp.1492-1508

Bakos, D.S., Pinheiro de Paula Couto, M.C., Melo, W.V., de M.P.Parente, M.A., Koller, S.H. and Bizarro, L. (2008) 'Executive functions in the young elderly and oldest old: preliminary comparison emphasizing decision making'. *Psychology and Neuroscience*, 1:2, pp.183-189

Baldeiras, I., Santana, I., Proença, M.T., Garrucho, M.H., Pascoal, R., Rodrigues, A., Duro, D. and Oliveira, C.R. (2010) 'Oxidative Damage and

Progression to Alzheimer's Disease in Patients with Mild Cognitive Impairment'. *Journal of Alzheimer's Disease*, 21:4, pp.1165-1177

Ballard, C., Corbett, A. and Sharp, S. (2011) 'Aligning the evidence with practice: NICE guidelines for drug treatment of Alzheimer's disease'. *Expert Review of Neurotherapeutics*, 11:3, pp.327-329

Banos, R.M., Botella, C., Perpiñá, C., Alcañiz, M., Lozano, J.A., Osma, J. and Gallardo, M. (2002) 'Virtual Reality Treatment of Flying Phobia'. *IEEE Transactions on Information Technology in Biomedicine*, 6:3, pp.206-212

Barberger-Gateau, P., Letenneur, L., Deschamps, V., Pérès, K., Dartiques, J-F. and Renaud, S. (2002) 'Fish, meat and risk of dementia: cohort study'. *British Medical Journal*, 325:7350, pp.932-933

Barnes, C.A., Nadel, L. and Honig, W.K. (1980) 'Spatial memory deficit in senescent rats'. *Canadian Journal of Psychology*, 34:1, pp.29-39

Barrash, J., Damasio, H., Adolphs, R. and Tranel, D. (2000) 'The neuroanatomical correlates of route learning impairment'. *Neuropsychologia*, 38, pp.820-836

Bartus, R.T., Dean III, R.L., Beer, B. and Lippa, A.S. (1982). 'The cholinergic hypothesis of geriatric memory dysfunction'. *Science*, 217:4558, pp.408-414

Basheer, R., Strecker, R.E., Thakkar, M.M. and McCarley, R.W. (2004) 'Adenosine and sleep-wake regulation'. *Progress in Neurobiology*, 73:6, pp.379-396

Baskin, D.S., Browning, J.L., Pirozzolo, F.J., Korporaal, S., Baskin, J.A., Appel, S.A. (1999) 'Brain choline acetyltransferase and mental function in Alzheimer disease'. *Archives of neurology*, 56, pp.1121-1123

Bassett, S.S. and Folstein, M.F. (1993). 'Memory complaint, memory performance, and psychiatric diagnosis: a community study'. *Journal of Geriatric Psychiatry and Neurology*, 6:2, pp.105-111

Bayer, A.J., Bullock, R., Jones, R.W., Wilkinson, D., Paterson, K.R., Jenkins, L., Millais, S.B. and Donoghue, S. (2005) 'Evaluation of the safety and immunogenicity of synthetic Abeta42 (AN1792) in patients with AD'. *Neurology*, 64:1, pp.94-101

Bear, M.F., Connors, B.W. and Paradiso, M.A. (2001) '*Neuroscience, Exploring the Brain*'. USA:Lippincott Williams and Wilkins

Beddington, J., Cooper, C.L., Field, J., Goswami, U., Huppert, F.A., Jenkins, R., Jones, H.S., Kirkwood, T.B., Sahakian, B.J., and Thomas, S.M. (2008) 'The mental wealth of nations'. *Nature*, 455(7216), pp.1057-1060

Behl, C. and Moosmann, B. (2002) 'Antioxidant neuroprotection in Alzheimer's disease a preventative and therapeutic approach. *Free radical biology and medicine*, 33:2, pp.182-191

Bekris, L.M., Millard, S.P., Galloway, N.M., Vuletic, S., Albers, J.J., Li, G., Galasko, D.R., DeCarli, C., Farlow, M.R., Clark, C.M., Quinn, J.F., Kaye, J.A., Schellenberg, G.D., Tsuang, D., Peskind, E.R. and Yu, C.E. (2008) 'Multiple SNPs within and surrounding the apolipoprotein E Gene influence cerebrospinal fluid apolipoprotein E protein levels'. *Journal of Alzheimer's disease*, 13:3, pp. 255-266

Bekris, L.M., Yu, C.E., Bird, T.D. and Tsuang, D.W. (2010) 'Genetics of Alzheimer disease'. *Journal of Geriatric Psychiatry and Neurology*, 23:4, pp.213-227

Belleville, S., Peretz, I. and Malenfant, D. (1996) 'Examination of the working memory components in normal aging and in dementia of the Alzheimer type'. *Neuropsychologia*, 34:3, pp.195-207

Berg, E.A. (1948) 'A simple objective technique for measuring flexibility in thinking'. *Journal of General Psychology*, 39, pp.15-22

Bennett, D.A., Schneider, J.A., Wilson, R.S., Bienias, J.L., Berry-Kravis, E. and Arnold, S.E. (2005) 'Amyloid mediates the association of apolipoprotein E e4 allele to cognitive function in older people'. *Journal of Neurology, Neurosurgery and Psychiatry*, 76:9, pp.1194-1199

Bennett, D.A., Scheider, J.A., Bienias, J.L., Evans, D.A. and Wilson, R.S. (2005) 'Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions'. *Neurology*, 64:5, pp.834-841

Berthon, G. (2000) 'Does human beta A4 exert a protective function against oxidative stress in Alzheimer's disease?' *Medical Hypotheses*, 54:2, pp.672-677

Bierer, L.M., Haroutunian, V., Gabriel, S., Knott, P.J., Carlin, L.S., Purohit, D.P., Perl, D.P., Schmeidler, J., Kanof, P. and Davis, K.L. (1995) 'Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits'. *Journal of Neurochemistry*, 64:2, pp.749-760

Bird, T.D. (2008) 'Genetics Aspects of Alzheimer Disease'. *Genetic Medicine*, 10:4, pp.231-239

Birks, J. (2006)'Cholinesterase inhibitors for Alzheimer's disease'. *Cochrane Database of Systematic Reviews*,(1):CD005593

Birks, J. and Grimley Evans, J. (2009) 'Ginkgo biloba for cognitive impairment and dementia'. *Cochrane Database of systematic reviews*, (1): CD003120

Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.G. and Gluud, C. (2012) 'Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases'. *Cochrane Database of Systematic Reviews*, 14:3, CD007176

Blackwell, A.D., Sahakian, B.J., Vesey, R., Semple, J.M., Robbins, T.W. and Hodges, J.R. (2004) 'Detecting dementia: novel markers of preclinical

Alzheimers Disease'. *Dementia and Geriatric Cognitive Disorders*, 17(1-2), pp. 42-48

Blatter, K. and Cajochen, C. (2007) 'Circadian rhythms in cognitive performance: methodological constraints, protocols, theoretical underpinnings'. *Physiology and Behaviour*, 90:(2-3), pp.196-208

Bliwise, D. (2005) Normal aging. *In: Kryger, M, H., Roth, T. and Dement, W.C., eds. Principles and Practice of Sleep Medicine*. 4th ed. Philadelphia: Saunders. pp.24-38

Boejen, A. and Grau, C. (2011) 'Virtual reality in radiation therapy training'. *Surgical Oncology*, 20:3, pp. 185-188

Bohbot, V.D., Kalina, M., Stepankova, K., Spackova, N., Petrides, M. and Nadel, L. (1998) 'Spatial memory deficits in patients with lesions to the right hippocampus and to the right parahippocampal cortex'. *Neuropsychologia*, 36:11, pp.1217-1238

Boison, D. (2008) 'The adenosine kinase hypothesis of epileptogenesis'. *Progress in Neurobiology*, 84:3, pp.249-262

Borella, E., Ludwig, C., Dirk, J. and de Ribaupierre, A. (2011) 'The influence of time of day on interference, working memory, processing speed, and vocabulary: Age differences in adulthood'. *Experimental Aging Research*, 37, pp.76-107

Boutajangout, A., Sigurdsson, E.M. and Krishnamurthy, P.K. (2011) 'Tau as a Therapeutic Target for Alzheimer's Disease'. *Current Alzheimer Research*, 8:6, pp.666-677

Bowie, P., Branton, T., and Holmes, J. (1999) 'Should the mini mental state examination be used to monitor dementia treatments?' *Lancet*, 354:9189, pp. 1527-1528.

Bowler, R., Sudia, S., Mergler, D., Harrison, R. and Cone, J. (1992) 'Comparison of digit Symbol and symbol digit modalities tests for assessing neurotoxic exposure'. *The Clinical Neuropsychologist*, 6:1, pp.103-104

Bowman, G.L., Silbert, L.C., Howieson, D., Dodge, H.H., Traber, M.G., Frei, B., Kaye, J.A., Shannon, J. and Quinn, J.F. (2012) 'Nutrient biomarker patterns, cognitive function and MRI measures of brain aging'. *Neurology*, 78:4, pp.241-24

Braak, H. and Braak, E. (1991) 'Neuropathological staging of Alzheimer related changes'. *Acta Neuropathology*, 82, pp.239-259

Braak, H. and Braak, E. (1998) 'Evolution of neuronal changes in the course of Alzheimer's disease'. *Journal of Neural Transmission Supplementum*, 53, pp.127-140

Brandt, J. (1991) 'The Hopkins Verbal Learning Test: Development of a new memory test with six equivalent forms'. *Clinical Neuropsychologist*, 5, pp.125-142

Brandt, J. and Benedict, R.H.B. (2001). '*Hopkins Verbal Learning Test-Revised*. Lutz, FL: Psychological Assessment Resources.

Breitner, J.C.S. (2003) 'NSAIDs and Alzheimer's disease: how far to generalise from trials'. *The Lancet Neurology*, 2:9, pp.527

Breitner, J.C.S., Haneuse, S.J.P.A., Walker, R., Dublin, S., Crane, P.K., Gray, S.L. and Larson, E.B. (2009) 'Risk of dementia and AD with prior exposure to NSAIDs in an elderly community-based cohort'. *Neurology* 72:22, pp.1899-1905.

British Heart Foundation (2012) *Go fish* [online] Available at <<http://www.bhf.org.uk/heart-matters-online/april-may-2012/nutrition/oily-fish.aspx>> [Accessed 9th September 2013]

Broeren, J., Björkdahl, A., Pascher, R. and Rydmark, M. (2002) 'Virtual reality and haptics as an assessment device in the post acute phase after stroke'. *Cyberpsychology and Behaviour*, 5:3, pp.207-211

Brousseau, G., Rourke, B.P. and Burke, B. (2007) 'Acetylcholinesterase Inhibitors, Neuropsychiatric Symptoms, and Alzheimers Disease Subtypes: An Alternate Hypothesis to Global Cognitive Enhancement'. *Experimental and Clinical Psychopharmacology*, 15:6 pp.546-554

Brown, A.D., McMorris, C.A., Longman, R.S., Leigh, R., Hill, M.D. and Poulin, M.J. (2010) 'Effects of cardiorespiratory fitness and cerebral blood flow on cognitive outcomes in older women'. *Neurobiology of Aging*, 31:12, pp.2047-2057

Brown, P.J., Devanand, D.P., Liu, X., Caccappolo, E. and Alzheimer's Disease Neuroimaging Initiative. (2011) 'Functional impairment in elderly patients with mild cognitive impairment and mild Alzheimer disease' .*Archives of general psychiatry*, 68:6, pp.617-626

Brumback-Peltz, C., Balasubramanian, A.B., Corrada, M.M. and Kawas, C.H. (2011) 'Diagnosing dementia in the oldest-old'. *Maturitas*, 70:2, pp.164-168

Bugg, J.M., DeLosh, E.L. and Clegg, B.A. (2006) 'Physical activity moderates time of day differences in older adults working memory performance'. *Experimental Aging Research*, 32:4, pp.431-446

Bugg, J.M. and Head, D. (2011) 'Exercise moderates age-related atrophy of the medial temporal lobe'. *Neurobiology of Aging*, 32:3, pp.506-514

Burgess, N., Maguire, E.A. and O'Keefe, J. (2002) 'The human hippocampus and spatial and episodic memory'. *Neuron*, 35:4, pp. 625-641

Butt, M.S. and Sultan, M.F. (2011) 'Coffee and its consumption: Benefits and Risks'. *Critical Reviews in Food Science and Nutrition*, 51:4, pp.363-373

Butterfield, D.A. and Boyd-Kimball, D. (2004) 'Amyloid β -peptide(1-42) Contributes to the Oxidative Stress and Neurodegeneration found in Alzheimer Disease Brain'. *Brain Pathology*, 14, pp.426-432

Calero, M.D. and Navarro, E. (2004) 'Relationship between plasticity, mild cognitive impairment and cognitive decline'. *Archives of Clinical Neuropsychology*, 19:5, pp.653-660

Callahan, L.M., Chow, N., Cheetham, J.E., Cox, C. and Coleman, P.D. (1998) 'Analysis of Message Expression in Single Neurons of Alzheimer's Disease Brain'. *Neurobiology of Aging*, 19:1,s1, pp.99-105

Cameron, I., Curran, S., Newton, P., Petty, D. and Wattis, J. (2000) 'Use of donepezil for the treatment of mild-moderate Alzheimer's disease: an audit of the assessment and treatment of patients in routine clinical practice'. *International Journal of Geriatric Psychiatry*, 15:10, pp.887-891

Camouse, M.M., Hanneman, K.K., Conrad, E.P and Baron, E.D. (2005) 'Protective effects of tea polyphenols and caffeine'. *Expert review of Anticancer Therapy*, 5:6, pp.1061-1068

Cao, C., Cirrito, J.R., Lin, X., Wang, L., Verges, D.K., Dickson, A., Mamcarz, M., Zhang, C., Mori, T., Arendash, G.W., Holtzman, D.M and Potter, H. (2009) 'Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice'. *Journal of Alzheimer's Disease*, 17:3, pp.681-697

Cardinali, D.P., Vigo, D.E., Olivar, N., Vidal, M.F., Furio, A.M. and Brusco, L.I. (2012) 'Therapeutic application of melatonin in mild cognitive impairment'. *American Journal of Neurodegenerative Disease*, 1:3, pp.280-291

Carey, C.L., Kramer, J.H., Josephson, S.A., Mungas, D., Reed, B.R., Schuff, N., Weiner, M.W. and Chui, H.C. (2008) 'Subcortical lacunes are associated with executive dysfunction in cognitively normal elderly'. *Stroke*, 39:2, pp.397-402

Carrera, I., Etcheverría., Fernández-Novoa., Lombardii, V., Cacabelos, R. and Vigo, C. (2012) 'Vaccine Development to Treat Alzheimer's Disease

Neuropathology in APP/PS1 Transgenic mice'. *International Journal of Alzheimers Disease*, 2012:376138

Carrier, J., Paquet, J., Fernandez-Bolanos, M., Girouard, L., Roy, J., Selmaoui, B. and Filipini, D. (2009) 'Effects of caffeine on daytime recovery sleep: A double challenge to the sleep-wake cycle in aging'. *Sleep Medicine*, 10, pp. 1016-1024

Carrillo, M.C., Blackwell, A., Hampel, H., Lindborg, J., Sperling, R., Schenk, D., Sevigny, J.J., Ferris, S., Bennett, D.A., Craft, S., Hsu, T. and Klunk, W. (2009) 'Early risk assessment for Alzheimer's disease'. *Alzheimer's and Dementia*, 5, pp.182-196

Cermakian, N., Lamont, E.W., Boudreau, P. and Boivin, D.B. (2011) 'Circadian clock gene expression in brain regions of Alzheimer's disease patients and control subjects'. *Journal of biological rhythms*, 26:2, pp.160-170

Chan, A.S., Butters, N. and Salmon, D.P. (1997) 'The deterioration of semantic networks in patients with Alzheimer's disease: a crosssectional study. *Neuropsychologia*, 35:3, pp.241-248.

Chan, D., Anderson, V., Pijnenburg, Y., Whitwell, J., Barnes, J., Scahill, R., Stevens, J.M., Barkhof, F., Scheltens, P., Rossor, M.N. and Fox, N.C. (2009) 'The clinical profile of right temporal lobe atrophy'. *Brain*, 132:part5, pp.1287-1298

Chapman, R.M., Mapstone, M., Porsteinsson, A.P., Gardner, M.N., McCrary, J.W., DeGrush, E., Reilly, L.A., Sandoval, T.C. and Guillily, M.D. (2010) 'Diagnosis of Alzheimer's disease using neuropsychological testing improved by multivariate analyses'. *Journal of Clinical and Experimental Neuropsychology*, 32:8, pp.793-808

Chapman, R.M., Mapstone, M., McCrary, J.W., Gardner, M.N., Porsteinsson, A., Sandoval, T.C., Guillily, M.D., DeGrush, E. and Reilly, L.A. (2011) 'Predicting conversion from Mild Cognitive Impairment to Alzheimer's disease

using neuropsychological tests and multivariate methods'. *Journal of Clinical and Experimental Neuropsychology*, 33:2, pp.187-199

Chen, J.F., Yu, L., Shen, H.Y., He, J.C., Wang, X. and Zheng, R. (2010) 'What knock-out animals tell us about the effects of caffeine'. *Journal of Alzheimer's Disease*, 20:suppl 1, S17-S24

Chen, P., Ratcliff, G., Belle, S.H., Cauley, J.A., DeKosky, S.T. and Ganguli, M. (2000) 'Cognitive tests that best discriminate between presymptomatic AD and those who remain nondemented'. *Neurology*, 55:12, pp.1847-1853

Cherrier, M.M., Mendez, M. and Perryman, K. (2001) 'Route learning Performance in Alzheimer Disease Patients'. *Neuropsychiatry, Neuropsychology and Behavioural Neurology*, 14:3, pp.159-168

Cherry, B.J., Buckwalter, J.G., and Henderson, V.W. (1996) 'Memory span procedures in Alzheimer's disease'. *Neuropsychology*, 10, pp.286-93

Cherry, B.J., Buckwalter, J.G. and Henderson, V.W. (2002) 'Better preservation of memory span relative to supraspan immediate recall in Alzheimer's disease'. *Neuropsychologia*, 40:7, pp.846-852

Chishti, M.A., Yang, D.S., Janus, C., Phinney, A.L., Horne, P., Pearson, J., Strome, R., Zuker, N., Loukides, J., French, J., Turner, S., Lozza, G., Kunicki, S., Morissette, C., Paquette, J., Gervais, F., Bergeron, C., Fraser, P.E., Carlson, G.A., George-Hyslop, P.S. and Westaway, D. (2001) 'Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695'. *The Journal of Biological Chemistry*, 276:24, pp.21562-21570

Chiu, C.C., Su, K.P., Cheng, T.C., Liu, H.C., Chang, C.J., Dewey, M.E., Stewart, R. and Huang, S.Y. (2008) 'The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: A preliminary randomized double-blind placebo-controlled study'. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 32, pp.1538-1544

Chodosh, J., Reuben, D.B., Albert, M.S and Seeman, T.E. (2002) 'Predicting cognitive impairment in high-functioning community-dwelling older persons: MacArthur Studies of Successful Aging'. *Journal of the American Geriatric Society*, 50:6, pp.1051-1060

Christensen, S.E., Möller, E., Bonn, S.E., Ploner, A., Wright, A., Sjölander, A., Bälter, O., Lissner, L. and Bälter, K. (2013) 'Two New Meal-and Web-Based Interactive Food Frequency Questionnaires: Validation of Energy and Macronutrient intake'. *Journal of Medical Internet Research*, 15:6 e109, doi 10.2196/jmir2458

Cikajlo, I., Rudolf, M., Goljar, N., Burger, H. and Matjačić, Z. (2012) 'Telerehabilitation using virtual reality task can improve balance in patients with stroke'. *Disability and Rehabilitation*, 34:1, pp.13-18

Clare, L., Woods, R.T., Moniz Cook, E.D., Orrell, M. and Spector, A. (2003) 'Cognitive rehabilitation and cognitive training for early-stage Alzheimer's disease and vascular dementia'. *Cochrane Database of Systematic Reviews*, 4, CD003260

Clark, L.R., Schiehser, D.M., Weissberger, G.H., Salmon, D.P., Delis, D.C. and Bondi, M.W. (2012) 'Specific measures of executive function predict cognitive decline in older adults'. *Journal of the International Neuropsychological Society*, 18:1, pp.118-127

Clarke, R., Smith, A.D., Jobst, K.A., Refsum, H., Sutton, L., Ueland, P.M. (1998) 'Folate, vitamin B12 and serum total homocysteine levels in confirmed Alzheimer's Disease'. *Archives in Neurology*, 55:11, pp.1449-1455

Clerici, F., Del Sole, A., Chtit, A., Maggiore, L., Lecchi, M., Pomati, S., Mosconi, L., Lucignani, G. and Mariani, C. (2009) 'Differences in hippocampal metabolism between amnesic and non-amnesic MCI subjects: automated FDG-PET image analysis'. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 53:6, pp.646-657

Coelho, C.M., Waters, A.M., Hine, T.J. and Wallis, G. (2009) 'The use of virtual reality in acrophobia research and treatment'. *Journal of Anxiety Disorders*, 23:5, pp.563-574

Coffey, C.E., Ratcliff, G., Saxton, J.A., Bryan, R.N., Fried, L.P. and Lucke, J.F. (2001) 'Cognitive correlates of human brain aging: a quantitative magnetic resonance imaging investigation'. *Journal of Neuropsychiatry and Clinical Neurosciences*, 13:4, pp.471-485

Cole, G.M., Ma, Q.L. and Frautschy, S.A. (2009) 'Omega-3 fatty acids and dementia'. *Lancet*, 81:2-3), pp.213-21

Coltheart, M. (1981). 'The MRC Psycholinguistic database'. *The Quarterly Journal of Experimental Psychology Section A: Human Experimental Psychology*, 33:4, pp.497-505

Conquer, J.A., Tierney, M.C., Zecevic, J., Bettger, W.J. and Fisher, R.H. (2000). 'Fatty Acid Analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment'. *Lipids*, 35, pp.1305-1312

Constenla, A.R., Cunha, R.A. and de Mendonça, A. (2010) 'Caffeine, adenosine receptors, and synaptic plasticity'. *Journal of Alzheimer's disease*, 20:suppl 1, pp.S25-34

Cooper, B., Bickel, H. and Schäufele, M. (1992) 'The ability of general practitioners to detect dementia and cognitive impairment in their elderly patients: A study in Mannheim'. *International Journal of Geriatric Psychiatry*, 7:8, pp.591-598

Corodimas, K.P. and Tomita, H. (2001) 'Adenosine A1 receptor activation selectively impairs the acquisition of contextual fear conditioning in rats'. *Neuroscience*, 115, pp.1283-1290

Cropley, V., Croft, R., Silber, B., Neale, C., Scholey, A., Stough, C. and Schmitt, J. (2012). 'Does coffee enriched with chlorogenic acids improve mood and

cognition after acute administration in healthy elderly? A pilot study'. *Psychopharmacology*, 219:3, pp.737-749

Crum, R.M., Anthony, J.C., Bassett, S.S. and Folstein, M.F. (1993) 'Population-based norms for the Mini-Mental State Examination by age and educational level'. *Journal of the American Medical Association*, 269:18, pp.2386-2391

Cuajungco, M.P., Goldstein, L.E., Nunomura, A., Smith, M.A., Lim, J.T., Atwood, C.S., Huang, X., Farrag, Y.W., Perry, G. and Bush, A.I. (2000) 'Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of abeta by zinc'. *The Journal of Biological Chemistry*, 275:26, pp.19439-19442

Cullum, S., Huppert, F.A., McGee, M., Dening, T., Ahmed, A., Paykel, E.S. and Brayne, C. (2000) 'Decline across different domains of cognitive function in normal ageing: results of a longitudinal population-based study using CAMCOG'. *International Journal of Geriatric Psychiatry*, 15:9, pp.853-862

Cummings, J.L. and Cole, G. (2002) 'Alzheimer Disease'. *Journal of the American Medical Association*, 287:18, pp.2335-2338

Cunha, R.A., Constantino, M.D., Sebastião, A.M. and Ribeiro, J.A. (1995) 'Modification of A1 and A2a adenosine receptor binding in aged striatum, hippocampus and cortex of the rat'. *Neuroreport*, 6:158, pp.3-8

Cunha, R.A. (2001) 'Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors'. *Neurochemistry International*, 38:2, pp.107-125

Cunha, R.A. and Agostinho, P.M. (2010) 'Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline'. *Journal of Alzheimer's disease*, 20: suppl 1, S95-116

Cunnane, S.C., Plourde, M., Pifferi, F., Bégin, M., Féart, C. and Barberger-Gateau, P. (2009) 'Fish, docosahexaenoic acid and Alzheimer's disease'. *Progress in Lipid Research*, 48:5, pp.239-256

Cunnane, S.C., Chouinard-Watkins, R., Castellano, C.A. and Barberger-Gateau, P. (2013) 'Docosahexaenoic acid homeostasis, brain imaging and Alzheimer's disease: Can we reconcile the evidence?' *Prostaglandins, leukotrienes and essential fatty acids*, 88:1, pp.61-70

Cupples, L.A., Farrer, L.A., Sadovnick, A.D., Relkin, N., Whitehouse, P. and Green, R.C. (2004) 'Estimating risk curves for first-degree relatives of patients with Alzheimer's disease: the REVEAL study'. *Genetics in Medicine*, 6:4, pp.192-196

Cushman, L.A., Stein, K. and Duffy, C.J. (2008) 'Detecting navigational deficits in cognitive aging and Alzheimer disease using virtual reality'. *Neurology*, 71:12, pp.888-895

Dall'igna, O.P., Hoffmann, A., da Silva, A.L., Souza, D.O. and Lara, D.R. (2004) 'Beta-amyloid treatment sensitizes mice to amphetamine-induced locomotion but reduces response to caffeine'. *Neuro-degenerative Diseases*. 1:1, pp.38-43

Damian, A.M., Jacobson, S.A., Hentz, J.G., Belden, C.M., Shill, H.A., Sabbagh, M.N., Caviness, J.N. and Adler, C.H. (2011) 'The Montreal Cognitive Assessment and the mini-mental state examination as screening instruments for cognitive impairment: item analyses and threshold scores'. *Dementia and Geriatric Cognitive Disorders*, 31:2, pp.126-131

Dangour, A.D., Allen, E., Elbourne, D., Fasey, N., Fletcher, A.E., Hardy, P., Holder, G.E., Knight, R., Letley, L., Richards, M. and Uauy, R. (2010) 'Effect of 2-y n-3 long chain polyunsaturated fatty acid supplementation on cognitive function in older people: a randomized, double blind, controlled trial'. *The American Journal of Clinical Nutrition*. 91:6, pp.1725-1732

Danthiir, V., Burns, N.R., Nettelbeck, T., Wilson, C. and Wittert, G. (2011) 'The older people, omega-3, and cognitive health (EPOCH) trial design and methodology: A randomised, double-blind, controlled trial investigating the effect of long-chain omega-3 fatty acids on cognitive ageing and wellbeing in cognitively healthy older adults'. *Nutrition Journal*, 10:117

Das, P., Murphy, M.P., Younkin, L.H., Younkin, S.G. and Golde, T.E. (2001) 'Reduced effectiveness of A β 1–42 immunization in APP transgenic mice with significant amyloid deposition'. *Neurobiology of Aging*, 22:5, pp.721-727

Davey, R.J. and Jamieson, S. (2004) 'The validity of using the mini mental state examination in NICE dementia guidelines'. *Journal of Neurology, Neurosurgery and Psychiatry*, 75, pp.343-344

Dean, P.J.A. and Sterr, A. (2013) 'Long-term effects of mild traumatic brain injury on cognitive performance'. *Frontiers in Human Neuroscience*, 7:30, epub, doi 10.3389/fnhum.2013.00030

de Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D.H., Kopeikina, K.J., Pitstick, R., Sahara, N., Ashe, K.H., Carlson, G.A., Spires-Jones, T. and Hyman, B.T. (2012) 'Propagation of tau pathology in a model of early Alzheimer's disease'. *Neuron*, 73:4, pp. 685-697

De Giorgi, A., Mallozzi Menegatti, A., Fabbian, F., Portaluppi, F. and Manfredini, R. (2013) 'Circadian rhythms and medical diseases: Does it matter when drugs are taken?' *European Journal of Internal Medicine*, epub ahead of print: doi 10.1016/j.ejim.2013.0.019

de Jager, C.A., Lesk, V.E., Zhu, X., Marsico, M. and Chandler, J. (2008) 'Episodic memory test constructs affect discrimination between healthy elderly and cases with mild cognitive impairment and Alzheimer's disease'. *Alzheimer's and Dementia: Journal of the Alzheimer's Association*, 4:4:pp.T554-T555.

de Jager, C.A., Oulhaj, A., Jacoby, R., Refsum, H. and Smith, A.D. (2011) 'Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment

in mild cognitive impairment: a randomized controlled trial'. *International Journal of Geriatric Psychiatry*, 27:6, pp. 592-600

de Leon, M.J., Convit, A., Wolf, O.T., Tarshish, C.Y., DeSanti, S., Rusinek, H., Tsui, W., Kandil, E., Scherer, A.J., Roche, A., Imossi, A., Thorn, E., Bobinski, M., Caraos, C., Lesbre, P., Schlyer, D., Poirier, J., Reisberg, B. and Fowler, J. (2001) 'Prediction of cognitive decline in normal elderly subjects with 2-[¹⁸F]fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG/PET)'. *Proceedings of the National Academy of Sciences of the United States of America*, 98:19, pp.10966-10971

den Heijer, T., Vermeer, S.E., Clarke, R., Oudkerk, M., Koudstaal, P.J., Hofman, A. and Breteler, M.M. (2003) 'Homocysteine and brain atrophy on MRI of non-demented elderly'. *Brain*, 126: pt1, pp.170-175

De Pisapia, N. and Braver, T.S. (2006) 'A model of dual control mechanisms through anterior cingulate and prefrontal cortex interactions'. *Neurocomputing*, 69, pp.1322-1326

de Rover, M., Pironti, V.A., McCabe, J.A., Acosta-Cabronero, J., Sergio Arana, F., Morein-Zamir, S., Hodges, J.R., Robbins, T.W., Fletcher, P.C., Nestor, P.J. and Sahakian, B.J. (2011) 'Hippocampal dysfunction in patients with mild cognitive impairment: A functional neuroimaging study of a visuospatial paired associates learning task'. *Neuropsychologia*, 49:7, pp.2060-2070

Delis, D. C., Kramer, J. H., Kaplan, E. and Ober, B. A. (1987) '*California Verbal Learning Test*'. San Antonio, Tex.: The Psychological Corporation.

Delis, D.C., Kramer, J.H., Kaplan, E., and Ober, B.A. (2000) '*California Verbal Learning Test*' - Second Edition, Adult Version. San Antonio: The Psychological Corporation

Della Sala, S. and Logie, R.H. (2001) 'Theoretical and practical implications of dual-task performance in Alzheimer's disease'. *Brain*, 124:8, pp.1479-1481

Denburg, N.L., Tranel, D. and Bechara, A. (2005). 'The ability to decide advantageously declines prematurely in some normal older persons'. *Neuropsychologia*, 43, pp.1099-1106

Dening, T. (2009) 'Prescribing policy with dementia drugs: The UK NICE experience'. *Maturitas*, 64:2, pp.59-60

Department of Health. (2009) *Living well with dementia. A National Dementia Strategy*. London: Department of Health

Devlin, A.S. (2001) *Mind and Maze: Spatial Cognition and Environmental behaviour*. Westport: Greenwood press.

Devore, E.E., Grodstein, F., van Rooij, F.J.A., Hofman, A., Rosner, B., Stampfer, M.J., Witteman, J.C.A. and Breteler, M.M.B. (2009) 'Dietary intake of fish and omega -3 fatty acids in relation to long term dementia risk'. *American Journal of Clinical Nutrition*, 90:1, pp.170-176

D'Hooge, R. and De Deyn, P.P. (2001) 'Applications of the Morris water maze in the study of learning and memory'. *Brain Research Brain Research Reviews*, 36:1, pp.60-90

Dickerson, B.C., Stoub, T.R., Shah, R.C., Sperling, R.A., Killiany, R.J., Albert, M.S., Hyman, B.T., Blacker, D. and de Toldeo-Morrell, L. (2011). 'Alzheimer-signature MRI biomarker predicts AD dementia in cognitively normal adults'. *Neurology*, 76:16, pp.1395-1402

Dong, Y., Lee, W.Y., Basri, N.A., Collinson, S.L., Merchant, R.A., Venketasubramanian, N. and Chen, C.L. (2012) 'The Montreal Cognitive Assessment is superior to the Mini-Mental State Examination in detecting patients at higher risk of dementia'. *International Psychogeriatrics*, 24:11, pp.1749-1755

Draper, B., Peisah, C., Snowden, J. and Brodaty, H. (2010) 'Early dementia diagnosis and the risk of suicide and euthanasia'. *Alzheimer's & Dementia*, 6:1, pp.75-82

Drummond, S.P.A., Gillin, J.C. and Brown, G.C. (2001) 'Increased cerebral response during a divided attention task following sleep deprivation'. *Journal of sleep research*, 10:2, pp.85-92

Dubinsky, R.M., Stein, A.C. and Lyons, K. (2000) 'Practice parameter: risk of driving and Alzheimer's disease (an evidence-based review): report of the quality standards subcommittee of the American Academy of Neurology'. *Neurology*, 54:12, pp.2205-2211

Dubois, B., Feldman, H.H., Jacova, C., DeKosky, S., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P.J. and Scheltens, P. (2007) 'Research criteria for the diagnosis of Alzheimer's disease: revising NINCDS-ADRDA criteria'. *The Lancet Neurology*, 6:8, pp.6734-746

Duchek, J.M., Balota, D.A., Tse, C.S., Holtzman, D.M., Fagan, A.M. and Goate, A.M. (2009) 'The utility of intraindividual variability in selective attention tasks as an early marker for Alzheimer's disease'. *Neuropsychology*, 23:6, pp.746-758

Dudas, R.B., Clague, F., Thompson, S.A., Graham, K.S. and Hodges, J.R. (2005) 'Episodic and semantic memory in mild cognitive impairment'. *Neuropsychologia*, 43, pp.1266-1276

Duffy, C.J. (2009) 'Visual motion processing in aging and Alzheimer's disease: neuronal mechanisms and behaviour from monkeys to man'. *Annals of the New York Academy of Sciences*, 1170, pp.736-44

Dumas, J.A. and Newhouse, P.A. (2011) 'The cholinergic Hypothesis of Cognitive Aging Revisited Again: Cholinergic Functional Compensation'. *Pharmacology, Biochemistry and Behaviour*, 99:2, pp.254-261

Dunwiddie, T.V. and Haas, H.L. (1985) 'Adenosine increases synaptic facilitation in the in vitro rat hippocampus: evidence for a presynaptic site of action'. *The Journal of Physiology*, 369, pp.365-377

Durazzo, T.C., Insel, P.S., Weiner, M.W. and the Alzheimer Disease Neuroimaging Initiative (2012) 'Greater regional brain atrophy rate in healthy elderly subjects with a history of cigarette smoking'. *Alzheimer's & Dementia*, 8:6, pp.513-519

Edison, P., Archer, H.A., Hinz, R., Hammers, A., Pavese, N., Tai, Y.F., Hotton, G., Cutler, D., Fox, N., Kennedy, A., Rossor, M. and Brooks, D.J. (2007) 'Amyloid, hypometabolism, and cognition in Alzheimer disease: an [11C]PIB and [18F]FDG PET study'. *Neurology*, 68:7, pp.501-508

Égerházi, A., Berecz, R., Bartók, E. and Degrell, I. (2007) 'Automated Neuropsychological Test Battery (CANTAB) in mild cognitive impairment and in Alzheimer's disease'. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31:3, pp.746-751

Engelhart, M.J., Geerlings, M.I., Meijer, J., Kiliaan, A., Ruitenberg, A., van Swieten, J.C., Stijnen, T., Hofman, A., Witteman, J.C. and Breteler, M.M. (2004) 'Inflammatory proteins in plasma and the risk of dementia: the Rotterdam study'. *Archives of Neurology*, 61, pp.668-672

Enzinger, C., Fazekas, F., Matthews, P.M., Ropele, S., Schmidt, H., Smith, S. and Schmidt, R. (2005) 'Risk factors for progression of brain atrophy in aging: six year follow-up of normal subjects'. *Neurology*, 64:10, pp.1704-1711

Eskelinen, M.H., Ngandu, T., Tuomilehto, J., Soininen, H. and Kivipelto, M. (2009) 'Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study'. *Journal of Alzheimer's Disease*, 16:1, pp.85-91

Eskelinen, M.H. and Kivipelto, M. (2010) 'Caffeine as a protective factor in dementia and Alzheimer's disease'. *Journal of Alzheimer's Disease*, 20, (suppl 1), pp.167-174

Espeland, M.A., Rapp, S.R., Shumaker, S.A., Brunner, R., Manson, J.E., Sherwin, B.B., Hsia, J., Margolis, K.L., Hogan, P.E., Wallac, R., Dailey, M., Freeman, R. and Hays, J. Women's Health Initiative Memory Study. (2004) 'Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study'. *Journal of the American Medical Association*, 291:24, pp.2959–68

Evans, M., Ellis, A., Watson, D. and Chowdhury, T. (2000) 'Sustained cognitive improvement following treatment of Alzheimer's disease with donepezil'. *International Journal of Geriatric Psychiatry*, 15:1, pp.50-53

Ewers, M., Walsh, C., Trojanowski, J.Q., Shaw, L.M., Petersen, R.C., Jack, C.R. Jr, Feldman, H.H., Bokde, A.L., Alexander, G.E., Scheltens, P., Vellas, B., Dubois, B., Weiner, M., Hampel, H.; North American Alzheimer's Disease Neuroimaging Initiative (ADNI) (2012) 'Prediction of conversion from mild cognitive impairment to Alzheimer's disease dementia based upon biomarkers and neuropsychological test performance'. *Neurobiology of Aging*, 33:7, pp.1203-1214

Farajnia, S., Deboer, T., Rohling, J.H.T., Meijer, J.H. and Michel, S. (2013) 'Aging of the Suprachiasmatic Clock'. *The Neuroscientist*, epub ahead of print, doi: 10.1177/1073858413498936

Farina, N., Isaac, M.G., Clark, A.R., Rusted, J. and Tabet, N. (2012) 'Vitamin E for Alzheimer's dementia and mild cognitive impairment'. *Cochrane database of systematic reviews*, 11, CD002854

Farlow, M., Veloso, F., Moline, M., Yardley, J., Brand-Schieber, E., Bibbiani, F., Zou, H., Hsu, T. and Satlin, A. (2011) 'Safety and tolerability of donepezil 23 mg in moderate to severe Alzheimer's disease'. *BMC Neurology*, 11:57

Farrimond, L.E., Roberts, E. and McShane, R. (2012) 'Memantine and cholinesterase inhibitor combination therapy for Alzheimer's disease: a systematic review'. *BMJ Open*, 2, e000917

Feczko, E., Augustinack, J.C., Fischl, B. and Dickerson, B.C. (2009) 'An MRI-based for measuring volume, thickness and surface area of entrorrhinal, perirhinal, and posterior parahippocampal cortex'. *Neurobiology of Aging*, 30:3, pp.420-431

Feinstein, A., Brown, R. and Ron, M. (1994) 'Effects of practice on serial tests of attention in healthy subjects'. *Journal of Clinical and Experimental Neuropsychology*, 16:3, pp.436-447

Feldman, H., Gauthier, S., Hecker, J., Vellas, B., Subbiah, P., Whalen, E.; Donepezil MSAD Study Investigators Group. (2001) 'A 24-week, randomized, double-blind study of donepezil in moderate to severe Alzheimer's disease'. *Neurology*, 57:4, pp.613-620

Feldman, H.H., Ferris, S., Winblad, B., Sfikas, N., Mancione, L., He, Y., Tekin, S., Burns, A., Cummings, J., del Ser, T., Inzitari, D., Orgogoza, J.M., Sauer, H., Scheltens, P., Scarpini, E., Herrmann, N., Farlow, M., Potkin, S., Charles, H.C., Fox, N.C. and Lane, R. (2007) 'Effect of Rivastigmine on delay to diagnosis of Alzheimer's disease from mild cognitive impairment: the InDDEX study. *The Lancet Neurology*, 6:6, pp.501-512

Feldman, H.H., Jacova, C., Robillard, A., Garcia, A., Chow, T., Borrie, M., Schipper, H.M., Blair, M., Kertesz, A. and Chertkow, H. (2008) 'Diagnosis and Treatment of Dementia: 2 Diagnosis'. *Canadian Medical Association Journal*, 178:7, pp.825-836

Fellgiebel, A., Wille, P., Müller, M.J. Winterer, G., Scheurich, A., Vucurevic, G., Schmidt, L.G. and Stoeter, P. (2004) 'Ultrastructural hippocampal and white matter alterations in mild cognitive impairment: a diffusion tensor imaging study'. *Dementia and Geriatric Cognitive Disorders*, 18:1, pp.101-108

Feng, Y. and Wang, X. (2012) 'Antioxidant Therapies for Alzheimer's Disease'. *Oxidative Medicine and Cellular Longevity*, 472932, pp.1-17

Fennema-Notestine, C., McEvoy, L.K., Hagler, D.J. Jr, Jacobson, M.W., Dale, A.M. and the Alzheimer's Disease Neuroimaging Initiative (2009) 'Structural neuroimaging in the detection and prognosis of pre-clinical and early AD'. *Behavioural Neurology*, 21:1, pp.3-12

Festa, E.K., Heindel, W.C. and Ott, B.R. (2010) 'Dual-task conditions modulate the efficiency of selective attention mechanisms in Alzheimer's disease'. *Neuropsychologica*, 48:11, pp.3252-3261

Fields, J.A., Ferman, T.J., Boeve, B.F. and Smith, G.E. (2011) 'Neuropsychological assessment of patients with dementing illness'. *Nature Reviews Neurology*, 7:12, pp.677-687

Findeis, M.A. (2007) 'The role of amyloid β peptide 42 in Alzheimer's disease'. *Pharmacology and Therapeutics*, 116:2, pp.266-286

Folstein, M.F., Folstein, S.E. and McHugh, P.R. (1975) 'Mini Mental State - A practical method for grading the cognitive state of patients for the clinician'. *Journal of Psychiatric Research*, 12:3, pp.1449-1455

Forlenza, O.V., Diniz, B.S. and Gattaz, W.F. (2010). 'Diagnosis and biomarkers of predementia in Alzheimer's disease'. *BMC medicine*, 8:89, pp.1741-7015

Forsberg, A., Engler, H., Almkvist, O., Blomquist, G., Hagman, G., Wall, A., Ringheim, A., Långström, B. and Nordberg, A. (2008) 'PET imaging of amyloid deposition in patients with mild cognitive impairment'. *Neurobiology of Aging*, 29:10, pp. 1456-1465

Foster, T.C. (1999) 'Involvement of hippocampal synaptic plasticity in age-related memory decline'. *Brain Research reviews*, 30, pp.236-249

Foster, T.C. (2012) 'Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca^{2+} channels in senescent synaptic plasticity'. *Progress in Neurobiology*, 96:3, pp.283-303

Fowler, K.S., Saling, M.M., Conway, E.L., Semple, J.M. and Louis, W.J. (2002) 'Paired associate performance in the early detection of DAT'. *Journal of the International Neuropsychological Society*, 8:1, pp.58-71

Francis, P.T. (2003). 'Glutamatergic systems in Alzheimer's disease'. *International Journal of Geriatric Psychiatry*, 18:S1, S15-S21

Francis, P.T., Sims, N.R., Procter, A.W. and Bowen, D.M. (1993) 'Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: investigative and therapeutic perspectives'. *Journal of Neurochemistry*, 60:5, pp.1589-1604

Francis, P.T., Ramírez, M.J. and Lai, M.K. (2010) 'Neurochemical basis for symptomatic treatment of Alzheimer's disease'. *Neuropharmacology*, 59:4-5, pp.221-229

Frary, C.D., Johnson, R.K. and Wang, M.Q. (2005) 'Food sources and intakes of caffeine in the diets of persons in the United States'. *Journal of the American Dietetics Association*, 105:1, pp.110-113

Freund-Levi, Y., Eriksdotter-Jonhagen, M., Cederholm, T., Basun, H., Faxen-
Irving, G., Garlind, A., Vedin, I., Vessby, B., Wahlund, L.O. and Palmblad, J. (2006) 'Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer Disease: OmegAD study: a randomized double blind trial'. *Archives of Neurology*, 63, pp.1402-1408

Frisch, S., Dukart, J., Vogt, B., Horstmann, A., Becker, G., Villringer, A., Barthel, H., Sabri, O., Müller, K. and Schroeter, M.L. (2013) 'Dissociating memory networks in early Alzheimer's disease and frontotemporal lobar degeneration - a combined study of hypometabolism and atrophy'. *PloS One*, 8:2, e55251

Frisoni, G.B., Fox, N.C., Jack, C.R.Jr, Scheltens, P. and Thompson, P.M. (2010) 'The clinical use of structural MRI in Alzheimer disease'. *Nature Reviews Neurology*, 6:2, pp. 67-77

Frost, S., Kanagasalingam, Y., Sohrabi, H., Vignarajan, J., Bourgeat, P., Salvado, O., Villemagne, V., Rowe, C.C., Macaulay, S.L., Szoeker, C., Ellis, K.A., Ames, D., Masters, C.L., Rainey-Smith, S., Martins, R.N. and AIBL Research Group (2013) 'Retinal vascular biomarkers for early detection and monitoring of Alzheimers disease'. *Translational Psychiatry*, 3, e233

Froy, O. (2007) 'The relationship between nutrition and circadian rhythms in mammals'. *Frontiers in Neuroendocrinology*, 28(2-3), pp.61-71

Froy, O. (2011) 'Circadian Rhythms, Aging, and Life Span in Mammals'. *Physiology*, 26, pp.225-235

Fu, H.J., Liu, B., Frost, J.L. and Lemere, C.A. (2010) 'Amyloid-beta immunotherapy for Alzheimer's disease'. *CNS and Neurological Disorders Drug Targets*, 9:2, pp.197-206

Fukumoto, H., Tennis, M., Locascio, J.J., Hyman, B.T., Growden, J.H. and Irizarry, M.C. (2003) 'Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels'. *Archives of Neurology*, 60:7, pp.958-964

Galea, L.A.M. and Kimura, D. (1992) 'Sex differences in route-learning'. *Perspectives and Individual Differences*, 14, pp.53-65

Ganguli, M., Dodge, H.H., Shen, C., Pandav, R.S., DeKosky, S.T. (2005) 'Alzheimer Disease and Mortality'. *JAMA Neurology*, 62:5, pp.779-784

Gao, S., Jin, Y., Hall, K.S., Liang, C., Unverzagt, F.W., Ji, R., Murrell, J.R., Cao, J., Shen, J., Ma, F., Matesan, J., Ying, B., Cheng, Y., Bian, J., Li, P. and Hendrie, H.C. (2007) 'Selenium level and cognitive function in rural elderly Chinese'. *American Journal of Epidemiology*, 165:8, pp.955-965

Garcia-Alloza, M., Gil-Bea, F.J., Diez-Ariza, M., Chen, C.P., Francis, P.T., Lasheras, B. and Ramirez, M.J., (2005) 'Cholinergic-serotonergic imbalance contributes to cognitive and behavioural symptoms in Alzheimer's disease'. *Neuropsychologia*, 43:3, pp.442-449

Garcia-Palacios, A., Hoffman, H., Carlin, A., Furness, T.A. and Botella, C. (2002) 'Virtual reality in the treatment of spider phobia: a controlled study'. *Behaviour Research and Therapy*, 40:9, pp.83-93

Gates, N.J., Sachdev, P.S., Singh, M.A.F. and Valenzuela, M. (2011) 'Cognitive and memory training in adults at risk of dementia: a systematic review. *BMC Geriatrics*, 11:55

Gauthier, S. (2002) 'Advances in the pharmacotherapy of Alzheimer's disease'. *Canadian Medical Association Journal*, 166:5, pp. 616-623

Gauthier, S., Leuzy, A., Racine, E. and Rosa-Neto, P. (2013) 'Diagnosis and management of Alzheimer's disease: Past present and future ethical issues'. *Progress in Neurobiology*, epub ahead of print doi 10.1016/j.pneurobio.2013.01.003

Geerlings, M.I., Jonker, C., Bouter, L.M., Ader, H.J. and Schamnd B. (1999) 'Association between memory complaints and incident Alzheimers Disease in elderly people with normal baseline cognition'. *American Journal of Psychiatry*, 156:4, pp.531-537

Geerlings, M.I., Brickman, A.M., Schupf, N., Devanand, D.P., Luchsinger, J.A., Mayeux, R. and Small, S.A. (2012) 'Depressive symptoms, antidepressant use, and brain volumes on MRI in a population-based cohort of old persons without dementia'. *Journal of Alzheimer's disease*, 30:1, pp.76-82

Geldmacher, D.S., Frolich, L., Doody, R.S., Erkinjuntti, T., Vellas, B., Jones, R.W., Banerjee, S., Lin, P. and Sano, M. (2006) 'Realistic expectations for treatment success in Alzheimer's disease'. *Journal of Nutrition and Healthy Aging*, 10:5, pp.417-429

Gibbs, R.B. and Aggarwal, P. (1998) 'Estrogen and basal forebrain cholinergic neurons: implications for brain aging and Alzheimer's disease-related cognitive decline'. *Hormones and Behaviour*, 34:2, pp.98-111

Gillette-Guyonnet, S., Secher, M. and Vellas, B. (2013) 'Nutrition and neurodegeneration: epidemiological evidence and challenges for future research'. *British Journal of Clinical Pharmacology*, 75:3, pp.738-755

Gimenez-Llort, L., Schiffmann, S.N., Shmidt, T., Canela, L., Camón, L., Wassholm, M., Canals, M., Terasmaa, A., Fernández-Teruel, A., Tobeña, A., Popova, E., Ferré, S., Agnati, L., Ciruela, F., Martínez, E., Scheel-Kruger, J., Lluís, C., Franco, R., Fuxe, K. and Bader, M. (2007) 'Working memory deficits in transgenic rats overexpressing human adenosine A2A receptors in the brain'. *Neurobiology of Learning and Memory*, 87:1, pp.42-56

Giovagnoli, A.R., Del Pesce, M., Mascheroni, S., Simoncelli, M., Laiacina, M. and Capitani, E. (1996) 'Trail making test: normative values from 287 normal adult controls'. *Italian Journal of Neurological Sciences*, 17:4, pp.305-309

Glade, M.J. (2010) 'Caffeine-Not just a stimulant'. *Nutrition*, 26:10, pp.932-938

Glass, C.K., Saijo, K., Winner, B., Marchetto, M.C. and Gage, F.H. (2010) 'Mechanisms underlying inflammation in neurodegeneration'. *Cell*, 140:6, pp.918-934

Goh, J.O., An, Y. and Resnick, S.M. (2012) 'Differential trajectories of age-related changes in components of executive and memory processes'. *Psychology of Aging*, 27:3, pp.707-719

Gomes, C.V., Kaster, M.P., Tomé, A.R., Agostinho, P.M. and Cunha, R.A. (2011) 'Adenosine receptors and brain diseases: neuroprotection and neurodegeneration'. *Biochimica et Biophysica Acta*, 1808:5, pp.1380-1399

Gómez-Isla, T., Price, J.L., McKeel, D.W. Jr., Morris, J.C., Growdon, J.H. and Hyman, B.T. (1996) 'Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease'. *Journal of Neuroscience*, 16:14, pp.4491-4500

González, H.M., Whitfield, K.E., West, B.T., Williams, D.R., Lichtenberg, P. and Jackson, J.S. (2007) 'Modified-Symbol Digit Modalities Test for African Americans, Caribbean Black Americans and Non-Latino Whites: Nationally representative normative data from the National Survey of American Life'. *Archives of Clinical Neuropsychology*, 22:5, pp.605-613

Gorby, H.E., Brownawell, A.M. and Falk, M.C. (2010) 'Do specific dietary constituents and supplements affect mental energy? Review of the evidence'. *Nutrition Reviews*, 68:12, pp.697-718

Graham, N.L., Emery, T. and Hodges, J.R. (2004) 'Distinctive cognitive profiles in Alzheimer's disease and subcortical vascular dementia'. *Journal of Neurology, Neurosurgery and Psychiatry*, 75:1, pp.61-71

Gray, S.L., Lai, K.V. and Larson, E.B. (1999) 'Drug-induced cognition disorders in the elderly: incidence, prevention and management'. *Drug Safety*, 21:2, pp.101-122

Green, R.C., Cupples, L.A., Go, R., Benke, K.S., Edeki, T., Griffith, P.A., Williams, M., Hipps, Y., Graff-Radford, N., Bachman, D., Farrer. and L.A MIRAGE Study Group. (2002) 'Risk of dementia among white and African American relatives of patients with Alzheimer's disease. *Journal of the American Medical Association*, 287:3, pp.329-336

Greene, J.D., Hodges, J.R. and Baddeley, A.D. (1995) 'Autobiographical memory and executive function in early dementia of Alzheimer type'. *Neuropsychologia*, 33:12, pp.1647-1670

Greenleaf, W.J. and Tovar, M.A. (1994) 'Augmenting reality in rehabilitation medicine'. *Artificial Intelligence in Medicine*, 6:4, pp.289-299

Gritton, H.J., Stasiak, A.M., Sarter, M. and Lee, T.M. (2013) 'Cognitive performance as a zeitgeber: cognitive oscillators and cholinergic modulation of the SCN entrain circadian rhythms. *PLoS One*, 8:2, e56206

Grothe, M., Zsaborszky, L., Atienza, M., Gil-Neciga, E., Rodriguez-Romero, R., Teipel, S.J., Amunts, K. Suarez-Gonzalez, A. and Cantero, J.L. (2010) 'Reduction of Basal Forebrain Cholinergic System Parallels Cognitive Impairment in Patients at High Risk of Developing Alzheimer's Disease'. *Cerebral Cortex*, 20:7, pp.1685-1695

Grundman, M., Sencakova, D., Jack, C.R., Jr., Petersen, R.C., Kim, H.T., Schultz, A., Weiner, M.F., DeCarli, C., DeKosky, S.T., van Dyck, C., Thomas, R.G. and Thal, L.J. Alzheimer's Disease Cooperative Study'. (2002) 'Brain MRI hippocampal volume and prediction of clinical status in a mild cognitive impairment trial'. *Journal of Molecular Neuroscience*, 19:1-2, pp.23-27

Guerrero-Berroa, E., Luo, X., Schmeidler, J., Rapp, M.A., Dahlman, K., Haroutunian, V. and Beeri, M.S. (2009) 'The MMSE orientation for time domain is a strong predictor of subsequent decline in the elderly'. *International Journal of Geriatric Psychiatry*, 24:12, pp.1429-1437

Guillozet, A.L., Weintraub, S., Mash, D.C. and Mesulam, M.M. (2003) 'Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment'. *Archives of Neurology*, 60:5, pp.729-736

Guo, Q.H., Zhou, B., Zhao, Q.H., Wang, B. and Hong, Z. (2012) 'Memory and Executive Screening (MES): a brief cognitive test for detecting mild cognitive impairment'. *BMC Neurology*, 12:119

Hämäläinen, A., Pihlajamäki, M., Tanila, H., Hänninen, T., Niskanen, E., Tervo, S., Karjalainen, P.A., Vanninen, R.L. and Soininen, H. (2007) 'Increased fMRI responses during encoding in mild cognitive impairment'. *Neurobiology of Aging*, 28:12, pp.1889-1903

Hammar, A. and Ardal, G. (2009) 'Cognitive functioning in major depression - a summary'. *Frontiers in Human Neuroscience*, 3:26

Hansen, R.A., Gartlehner, G., Webb, A.P., Morgan, L.C., Moore, C.G. and Jonas, D.E. (2008) 'Efficacy and safety of donepezil, galantamine, and

rivastigmine for the treatment of Alzheimer's disease: a systematic review and meta-analysis'. *Clinical Interventions in Aging*, 3:2, pp. 211-225

Hansson, O., Zetterberg, H., Buchhave, P., Londos, E., Blennow, K. and Minthon, L. (2006) 'Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment a follow-up study'. *Lancet Neurology*, 5:3, pp.228-234

Hardy, J.A. and Higgins, G.A. (1992) 'Alzheimer's disease: the amyloid cascade hypothesis'. *Science*, 256:5054, pp.184-185

Harms, C, Lautenschlager, M., Bergk, A., Katchhanov, J., Freyer, D., Kapiniya, K., Herwig, U., Megow, D., Dirnagl, U., Weber, J.R. and Hörtnagl, H. (2001) 'Differential mechanisms of neuroprotection by 17 beta-estradiol in apoptotic versus necrotic neurodegeneration'. *The Journal of Neuroscience*, 21:8, pp. 2600-2609

Hasher, L. and Zacks, R.T. (1988) 'Working memory, comprehension, and aging: a review and a new view'. In Bower, G.H. ed, *The Psychology of Learning and Motivation*. New York: Academic Press, pp.193-225

Hasher, L., Zacks, R.T. and Rahhal, T.A. (1999) 'Timing, instructions, and inhibitory control: some missing factors in the age and memory debate'. *Gerontology*, 45:6, pp.355-357

Hasher, L., Chung, C., May, C.P. and Foong, N. (2002) 'Age, time of testing, and proactive interference'. *Canadian Journal of Experimental Psychology*, 56:3, pp.200-207

Hasher, L., Goldstein, D. and May, C. (2005) 'It's about time: Circadian rhythms, memory and aging. In Izawa, C. and Ohta, N. eds. *Human Learning and memory: Advances in theory and application*. Kansas:Lawrence Erlbaum Associates, pp.199-217.

Hasher, L., Lustig, C. and Zacks, R. T. (2007). Inhibitory mechanisms and the control of attention. *In* Conway, A., Jarrold, C., Kane, M., Miyake, A. and Towse, J. (Eds.), *Variation in working memory*. New York: Oxford University Press, pp. 227-249

Hashimoto, M. and Hossain, S. (2011) 'Neuroprotective and Ameliorative Actions of Polyunsaturated Fatty Acids Against Neuronal Diseases: Beneficial Effect of Docosahexaenoic Acid on Cognitive Decline in Alzheimer's Disease'. *Journal of Pharmacological Sciences*, 116:2, pp.150-162

He, J., Farias, S., Martinez, O., Reed, B., Mungas, D. and Decarli, C. (2009) 'Differences in brain volume, hippocampal volume, cerebrovascular risk factors, and apolipoprotein E4 among mild cognitive impairment subtypes'. *Archives of Neurology*, 66:11, pp.1393-1399

Head, D. and Isom, M. (2010) 'Age effects on wayfinding and route learning skills'. *Behavioural Brain Research*, 209:1, pp.49-58

Hedden, T. and Gabrieli, J.D. (2004) 'Insights into the ageing mind: a view from cognitive neuroscience'. *Nature Reviews Neuroscience*, 5:2, pp.87-96

Henderson, V.W., Mack, W. and Williams, B.W. (1989) 'Spatial disorientation in Alzheimer's disease'. *Archives in Neurology*, 46:4, pp.391-394

Herholz, K., Weisenbach, S. and Kalbe, E. (2008) 'Deficits of the cholinergic system in early AD'. *Neuropsychologia*, 46:6, pp.1642-1647

Herrup, K. (2010) 'Reimagining Alzheimer's disease-an age-based hypothesis'. *The Journal of Neuroscience*, 30:50, pp. 16755-16762

Heyn, P., Abreu, B.C. and Ottenbacher, K.J. (2004) 'The effects of exercise training on elderly persons with cognitive impairment and dementia: a meta-analysis'. *Archives of Physical Medicine and Rehabilitation*, 85:10, pp.1694-1704

Ho, A.J., Raji, C.A., Becker, J.T., Lopez, O.L., Kuller, L.H., Hua, X., Dinov, I.D., Stein, J.L., Rosano, C., Toga, A.W. and Thompson, P.M. (2011) 'The effects of

physical activity, education and body mass index on the aging brain'. *Human Brain Mapping*, 32:9, pp.1371-1382

Hofman, M.A. and Swaab, D.F. (2006) 'Living by the clock: the circadian pacemaker in older people'. *Ageing Research Reviews*, 5:1, pp.33-51

Hogervorst, E., Reidel, W.J., Schmitt, A.J. and Jolles, J. (1998). 'Caffeine Improves Memory Performance During Distraction in Middle-Aged, But Not in Young or Old Subjects'. *Human Psychopharmacology*, 13:4, 277-284

Hogervorst, E., Williams, J., Budge, M., Riedel, W. and Jolles, J. (2000) 'The nature of the effect of female gonadal hormone replacement therapy on cognitive function in post-menopausal women: a meta-analysis'. *Neuroscience*, 101:3, pp.485-512

Hogervorst, E., Combrinck, M., Lapuerta, P., Rue, J., Swales, K. and Budge, M. (2002) 'The Hopkins Verbal Learning Test and screening for dementia'. *Dementia and Geriatric Cognitive Disorders*, 13:1, pp.13-20

Hogervorst, E., Huppert, F., Matthews, F.E. and Brayne, C. (2008) 'Thyroid function and cognitive decline in the MRC Cognitive Function and Ageing Study'. *Psychoneuroendocrinology*, 33:7, pp.1013-1022

Hogervorst, E. and Bandelow, S. (2010) 'Sex steroids to maintain cognitive function in women after the menopause: meta-analyses of treatment trials'. *Maturitas*, 66:1, pp.56-71

Holden, M.K. (2005) 'Virtual environments for motor rehabilitation: review'. *CyberPsychology & Behaviour*, 8:3, pp.187-211

Holmes, C., Wilkinson, D., Dean, C., Vethanayagam, S., Olivieri, S., Langley, A., Pandita-Gunawardena, N.D., Hogg, F., Clare, C. and Damms, J. (2004) 'The efficacy of donepezil in the treatment of neuropsychiatric symptoms in Alzheimer disease'. *Neurology*, 63:2, pp.214-219

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., Jones, R.W., Bullock, R., Love, S., Neal, J.W., Zotova, E. and Nicoll, J.A.R. (2008) 'Long-term effects of A β 42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial'. *Lancet*, 372:9634, pp.216-22

Honea, R.A., Vidoni, E., Harsha, A. and Burns, J.M. (2009) 'Impact of APOE on the healthy aging brain: a voxel-based MRI and DTI study'. *Journal of Alzheimer's disease*, 18:3, pp.553-564

Honea, R.A., Swerdlow, R.H., Vidoni, E.D., Goodwin, J. and Burns, J.M. (2010) 'Reduced gray matter volume in normal adults with a maternal family history of Alzheimer disease'. *Neurology*, 74:2, pp.113-120

Honea, R.A., Swerdlow, R.H., Vidoni, E.D. and Burns, J.M. (2011) 'Progressive regional atrophy in normal adults with a maternal history of Alzheimer disease'. *Neurology*, 76:9, pp.822-829

Horne, J. A., and Ostberg, O. (1976) 'A self-assessment questionnaire to determine morningness–eveningness in human circadian rhythms'. *International Journal of Chronobiology*, 4:2, pp. 97-110

Horton, A.M. and Roberts, C. (2001) 'Sex, ethnicity, age and education effects on the Trail Making test in a sample of cocaine abusers'. *International Journal of Neuroscience*, 108:3-4, pp.281-290

<http://www.liv.ac.uk/researchintelligence/issue30/alzheimers.html>.

Huag, H. and Eggers, R. (1991) 'Morphometry of the human cortex cerebri and corpus striatum during aging'. *Neurobiology of Aging*, 12:4, pp.336-338

Hughes, T.F., Chang, C.C., Vander Bilt, J. and Ganguli, M. (2010) 'Engagement in reading and hobbies and risk of incident dementia: the MoVIES project'. *American Journal of Alzheimer's Disease and other Dementias*, 25:5, pp.432-438

- Hughes, T.F., Snitz, B.E. and Ganguli, M. (2011) 'Should mild cognitive impairment be subtyped'. *Current Opinion in Psychiatry*, 24:3, pp.237-242
- Hutchinson, K.A., Balota, D.A. and Duchek, J.M. (2010) 'The utility of Stroop task switching as a marker for early-stage Alzheimer's disease'. *Psychology of Aging*, 25:3, pp.545-559
- Hüttenbrink, K.B., Hummel, T., Berg, D., Gasser, T. and Hähner, A. (2013) 'Olfactory Dysfunction: Common in Later Life and Early Warning of Neurodegenerative Disease'. *Deutsches Arzteblatt International*, 110:1-2, pp.1-7
- Intons-Peterson, M.J., Rocchi, P., West, T., McLellan, K. and Hackney, A. (1999) 'Age, testing at preferred or non-preferred times (testing optimality), and false memory'. *Journal of Experimental Psychology: Learning, Memory and Cognition*, 25:1, pp.23-40
- Iqbal, K., Alonso Adel, C., Chen, S., Chohan, M. O., El-Akkad, E., Gong, C.X., Khatoon, S., Li, B., Rahman, A., Tanimukai, H. and Grundke-Iqbal, I. (2005) 'Tau pathology in Alzheimer disease and other tauopathies'. *Biochimica and Biophysica Acta*, 1739:2-3, pp.198-210
- Iqbal, K., Liu, F., Gong, C.X. and Grundke-Iqbal, I. (2010) 'Tau in Alzheimer Disease and Related Tauopathies'. *Current Alzheimer Research*, 7:8, pp.656-664
- Jack, C.R. Jr, Petersen, R.C., Xu, Y.C., O'Brien, P.C., Smith, G.E., Ivnik, R.J., Boeve, B.F., Waring, S.C., Tangalos, E.G. and Kokmen, E. (1999) 'Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment'. *Neurology*, 52:7, pp.1397-1403
- Jack, C.R. Jr, Shiung, M.M., Gunter, J.L., O'Brien, P.C., Weigand, S.D., Knopman, D.S., Boeve, B.F., Ivnik, R.J., Smith, G.E., Cha, R.H., Tangalos, E.G. and Petersen, R.C. (2004) 'Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD'. *Neurology*, 62:4, pp.591-600

Jack, C.R. Jr, Lowe, V.J., Senjem, M.L., Weigand, S.D., Kemp, B.J., Shiung, M.M., Knopman, D.S., Boeve, B.F., Klunk, W.E., Mathis, C.A. and Petersen, R.C. (2008) '11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnesic mild cognitive impairment'. *Brain*, 131:pt 3, pp.665-680

Jacova, C., Kertesz, A., Blair, M., Fisk, J.D. and Feldman, H.H. (2007) 'Neuropsychological testing and assessment for dementia'. *Alzheimer's & Dementia*, 3:4, pp.299-317

Jagust, W.J., Landau, S.M., Shaw, L.M., Trojanowski, J.Q., Koeppe, R.A., Reiman, E.M., Foster, N.L., Petersen, R.C., Weiner, M.W., Price, J.C., Mathis, C.A. and the Alzheimer's Disease Neuroimaging Initiative. (2009) 'Relationships between biomarkers in aging and dementia'. *Neurology*, 73:15, pp.1193-1199

Jansen, P., Schmelter, A. and Heil, M. (2010) 'Spatial knowledge acquisition in younger and elderly adults: a study in a virtual environment'. *Experimental Psychology*, 57:1, pp.54-60

Jarvis, M.J. (1993) 'Does caffeine intake enhance absolute levels of cognitive performance?' *Psychopharmacology*, 110:1-2, pp.45-52.

Jellinger, K.A., Paulus, W., Wrocklage, C. and Litvan, I. (2001) 'Traumatic brain injury as a risk factor for Alzheimer disease. Comparison of two retrospective autopsy cohorts with evaluation of ApoE genotype'. *BMC Neurology*, 1:3

Jochemsen, H.M., Kloppenborg, R.P., de Groot, L.C., Kampman, E., Mali, W.P., van der Graaf, Y. and Geerlings, M.I. on behalf of the SMART Study Group (2013) 'Homocysteine, progression of ventricular enlargement, and cognitive decline: The Second Manifestations of ARTerial disease-Magnetic Resonance Study'. *Alzheimer's & Dementia*, 9, pp.302-309

Johnson, D.K., Storandt, M., Morris, J.C and Galvin, J.E. (2009) 'Longitudinal study of the transition from healthy aging to Alzheimer disease'. *Archives of Neurology*, 66:10, pp.1254-1259

Johnson-Kozlow, M., Kritz-Silverstein D., Barrett-Connor, E. and Morton, D. (2002) 'Coffee consumption and cognitive function among older adults'. *American Journal of Epidemiology*, 156:9, pp. 842-850

Jorm, A.F., Mather, K.A., Butterworth, P., Antsey, K.J., Christenden, H. And Easteal, S. (2007) 'APOE Genotype and Cognitive Functioning in a large Age-Stratified Population Sample'. *Neuropsychology*, 21, pp.1-8

Kahlaoui, K., Di Sante, G., Barbeau, J., Maheux, M., Lesage, F., Ska, B. and Joannette, Y. (2012) 'Contribution of NIRS to the study of the prefrontal cortex for verbal fluency in aging'. *Brain and Language*, 121:2, pp.164-173

Kalaria, R.N., Sromek, S., Wilcox, B.J, Unnerstall, J.R. (1990) 'Hippocampal adenosine A1 receptors are decreased in Alzheimer's disease'. *Neuroscience Letters*, 118, pp.257-260

Kalmijn, S., Launer, L.J., Ott, A., Witteman, J.C., Hofman, A. and Breteler, M.M. (1997) 'Dietary fat intake and the risk of incident dementia in the Rotterdam Study'. *Annals of Neurology*, 42:5, pp.776-782

Karantzoulis, S. and Galvin, J.E. (2011) 'Distinguishing Alzheimer's disease from other major forms of dementia'. *Expert review of neurotherapeutics*, 11:11, pp.1579-1591

Karran, E., Mercken, M. and De Strooper, B. (2011) 'The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics'. *Nature Reviews Drug Discovery*, 10:9, pp.698-712

Kennaway, D.J., Boden, M.J. and Varcoe, T.J. (2012) 'Circadian rhythms and fertility'. *Molecular and Cellular Endocrinology*, 349, pp.56-61

Kepe, V., Barrio, J.R., Huang, S.C., Ercoli, L., Siddarth, P., Shoghi-Jadid, K., Cole, G.M., Satyamurthy, N., Cummings, J.L., Small, G.W. and Phelps, M.E. (2006). 'Serotonin 1A receptors in the living brain of Alzheimer's disease patients'. *Proceedings of the National Academy of Sciences of the United States of America*, 103:3, pp.702-707

Kesse-Guyot, E., Andreeva, V.A., Lassale, C., Ferry, M., Jeandel, C., Hercberg, S., Galan, P. (2013) 'Mediterranean diet and cognitive function: a French study'. *American Journal of Clinical Nutrition*, 97:2, pp.36-376

Khalifa, Y.M., Bogorad, D., Gibson, V., Peifer, J. and Nussbaum, J. (2006) 'Virtual reality in ophthalmology training'. *Survey of Ophthalmology*, 51:3, pp.259-273

Khatoun, S., Grundke-Iqbal, I. and Iqbal, K. (1992) 'Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein'. *Journal of Neurochemistry*, 59:2, pp.750-753

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., Helkala, E.L., Tuomilehto, J., Soininen, H. and Nissinen, A. (2005) 'Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease'. *Archives of Neurology*, 62:10, pp.1556-1560

Klein, D.A., Steinburg, M., Galik, E., Steele, C., Sheppard, J.M., Warren, A., Rosenblatt, A. and Lyketsos, C.G. (1999) 'Wandering behaviour in community-residing persons with dementia'. *International Journal of Geriatric Psychiatry*, 14:4, pp.272-279

Klein, E.A, Thompson, I.M., Jr, Tangen, C.M., Crowley, J.J., Lucia, M.S., Goodman, P.J., Minasian, L.M., Ford, L.G., Parnes, H.L., Gaziano, J.M., Karp, D.D., Lieber, M.M., Walther, P.J., Klotz, L., Parsons, J.K., Chin, J.L., Darke, A.K., Lippman, S.M., Goodman, G.E., Meyskens, F.L. Jr and Baker, L.H. (2011) 'Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer

Prevention Trial (SELECT)'. *Journal of the American Medical Association*, 306:14, pp.1549-1556

Klencklen, G., Després, O. and Dufour, A. (2012) 'What do we know about aging and spatial cognition? Reviews and perspectives'. *Ageing Research Reviews*, 11:1, pp.123-135

Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D.P., Bergström, M., Savitcheva, I., Huang, G.F., Estrada, S., Ausén, B., Debnath, M.L., Barletta, J., Price, J.C., Sandell, J., Lopresti, B.J., Wall, A., Koivisto, P., Antoni, G., Mathis, C.A. and Längström, B. (2004) 'Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B'. *Annals of Neurology*, 55:3, pp.306-319

Knapp, M. and Prince, M. (2007) '*Dementia UK - A report into the prevalence and cost of dementia*' prepared by the Personal Social Services Research Unit (PSSRU) at the London School of Economics and the Institute of Psychiatry at King's College London, for the Alzheimer's Society. London: The Alzheimer's Society

Knierim, J.J., Kudrimoti, H.S. and McNaughton, B.L. (1995) 'Place cells, head direction cells, and the learning of landmark stability'. *Journal of Neuroscience*, 15:3, pt 1, pp.1649-1659

Korff, A., Liu, C., Ghingina, C., Shi, M. and Zhang, J. (2013) ' α -Synuclein in Cerebrospinal Fluid of Alzheimer's disease and Mild Cognitive Impairment'. *Journal of Alzheimer's Disease*, 36:4, pp.679-688

Lacor, P.N. (2007) 'Advances on the understanding of the origins of synaptic pathology in AD'. *Current Genomics*, 8:8, pp.486-508

Laczó, J., Vicek, K., Vyhnálek, M., Vajnerová, O., Ort, M., Holmerová, I., Tolar, M., Andel, R., Bojar, M. and Hort, J. (2009) 'Spatial navigation testing discriminates two types of amnesic mild cognitive impairment'. *Behavioural Brain Research*, 202:2, pp.252-259

Lai, M.K., Tsang, S.W., Francis, P.T., Esiri, M.M., Keene, J., Hope, T. and Chen, C.P. (2003). 'Reduced serotonin 5-HT_{1A} receptor binding in the temporal cortex correlates with aggressive behaviour in Alzheimer disease'. *Brain Research*, 974, pp.82-87

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A. and Klein, W.L. (1998) 'Diffusable nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins'. *Proceedings of the National Academy of Sciences of the USA*, 95:11, pp.6448-6453.

Lambon Ralph, M.A., Powell, J., Howard, D., Whitworth, A.B., Garrard, P. and Hodges, J.R. (2001) 'Semantic memory is impaired in both dementia with Lewy bodies and dementia of Alzheimer's type: a comparative neuropsychological study and literature review'. *Journal of Neurology, Neurosurgery and Psychiatry*, 70:2 pp.149-156

Larner, A.J. (2012) 'Screening utility of the Montreal Cognitive Assessment (MoCA): in place of or as well as the MMSE?' *International Psychogeriatrics/IPA*, 24:3, pp.391-396

Larson, E.B., Wang, L., Bowen, J.D., McCormick, W.C., Teri, L., Crane, P. and Kukull, W. (2006) 'Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older'. *Annals of Internal Medicine*, 144:2, pp.73-81

Laurin, D., Verreault, R., Lindsay, J., MacPherson, K. and Rockwood, K. (2001) 'Physical Activity and risk of cognitive impairment and dementia in elderly persons'. *Archives of Neurology*, 58:3, pp.498-504

Laver, K.E., George, S., Thomas, S., Deutsch, J.E. and Crotty, M. (2011) 'Virtual reality for stroke rehabilitation'. *Cochrane Database of Systematic Reviews*, 7:9

Le Bars, P.L., Katz, M.M., Berman, N., Itil, T.M., Freedman, A.M. and Schatzberg, A.F. (1997) 'A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo Biloba for dementia. North American EGb Study Group'. *Journal of the American Medical Association*, 278:16, pp.1327-1332

Legg, C., Puri, A. and Thomas, N. (2000) 'Dietary restraint and self-reported meal sizes: diary studies with differentially informed consent'. *Appetite*, 34:3, pp. 235-243

Lehmann, M., Gottfries, C.G., and Regland, B. (1999) 'Identification of cognitive impairment in the elderly: homocysteine is an early marker'. *Dementia and Geriatric Cognitive Disorders*, 10:1, pp.12-20

Leirer, V.O., Tanke, E.D. and Morrow, D.G. (1994) 'Time of day and naturalistic prospective memory'. *Experimental Aging Research*, 20:2, pp.127-134

Lemere, C.A. and Masliah, E. (2010) 'Can Alzheimer disease be prevented by amyloid- β immunotherapy?' *Nature Reviews Neurology*, 6:2, pp.108-119

Lesk, V.E. and Womble, S.P. (2004) 'Caffeine, priming and tip of the tongue: evidence for plasticity in the phonological system'. *Behavioural Neuroscience*, 118:3, pp.453-461

Lesk, V.E., Womble, S.P. and Rumiati, R I. (2007) 'Testing graceful degradation in a patient with aphasia'. *Neurocase*, 13:4, pp.248-55

Lesk, V.E., Honey, T.E.M. and de Jager C.A. (2009) 'The effect of recent consumption of caffeine-containing foodstuffs on neuropsychological tests in the elderly'. *Dementia and Geriatric Cognitive Disorders*, 27:4, pp.322-328

Lesk, V.E. and Walters, E.R. (2012) 'The effect of prior caffeine consumption on neuropsychological assessment in the over 60s'. In Alzheimer's Research UK Yorkshire Network Annual Meeting. June 18th 2012. University of Bradford, England. (2012) *Proceedings of the ARUK Yorkshire Network meeting*. Bradford: ARUK

Levac, D., Miller, P. and Missiuna, C. (2011) 'Usual and Virtual Reality Video Game-based Physiotherapy for Children and Youth with Acquired Brain Injuries'. *Physical and Occupational Therapy in Paediatrics*, 32:2, pp.180-195

Levin, E.D., (1992) 'Nicotinic systems and cognitive function'. *Psychopharmacology*, 108:4, pp.417-431

Lezak, M.D. (1995) '*Neuropsychological assessment*'. New York: Oxford University Press, pp. 366–68.

Lezak, M. D., Howieson, D.B. and Loring, D.W. (2004) *Neuropsychological assessment* (4th ed.). New York: Oxford University Press

Li, L., Cao, D., Desmond, R., Rahman, A., Lah, J.J., Levey, A.I. and Zamrini, E. (2008) 'Cognitive performance and plasma levels of homocysteine, vitamin B12, folate and lipids in patients with Alzheimer's disease'. *Dementia and Geriatric Cognitive Disorders*, 26:4, pp.384-390

Liang, K.Y., Mintun, M.A., Fagan, A.M., Goate, A.M., Bugg, J.M., Holtzman, D.M., Morris, J.C. and Head, D. (2010) 'Exercise and Alzheimer's disease biomarkers in cognitively normal older adults'. *Annals of Neurology*, 68:3, pp.311-318

Lindsay, J., Laurin, D., Verreault, R., Hébert, R., Helliwell, B., Hill, G.B. and McDowell, I. (2002) 'Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Ageing'. *American Journal of Epidemiology*, 156:5, pp.445-453

Lithfous, S., Dufour, A. and Després, O. (2013) 'Spatial navigation in normal aging and the prodromal stage of Alzheimer's disease: insights from imaging and behavioural studies'. *Ageing research reviews*, 12:1, pp.201-213

Liu, L., Wang, P.P., Roebottom, B., Ryan, A., Tucker, C.S., Colbourne, J., Baker, N., Cotterchio, M., Yi, Y. and Sun, G. (2013) 'Assessing the validity of a

self-administered food-frequency questionnaire (FFQ) in the adult population of Newfoundland and Labrador, Canada'. *Nutrition Journal*, 12:49

Lloret, A., Badía, M.C., Mora, N.J., Pallardó, F.V., Alonso, M.D. and Viña, J. (2009) 'Vitamin E paradox in Alzheimer's disease: it does not prevent loss of cognition and may even be detrimental'. *Journal of Alzheimer's Disease*, 17:1, pp.143-149

Lockhart, I.A., Mitchell, S.A. and Kelly, S. (2009) 'Safety and tolerability of donepezil, rivastigmine and galantamine for patients with Alzheimer's disease: systematic review of the 'real-world evidence'. *Dementia and Geriatric Cognitive Disorders*, 28:5, pp.389-403

Logie, R.H., Cocchini, G., Della Salla, S. and Baddeley, A.D. (2004) 'Is There a Specific Executive Capacity for Dual Task Coordination Evidence from Alzheimer's Disease'. *Neuropsychology*, 18:3, pp.504-513

Lonie, J.A., Herrman, L.L., Donaghey, C.L. and Ebmeier, K.P. (2008) 'Clinical referral patterns and cognitive profile in mild cognitive impairment'. *The British Journal of Psychiatry*, 192, pp.59-64

Lonie, J.A., Tierney, K.M., Herrmann, L.L., Donaghey, C., O'Carroll, R, E., Lee, A. and Ebmeier, K.P. (2009) 'Dual task performance in early Alzheimer's disease, amnesic mild cognitive impairment and depression'. *Psychological Medicine*, 39:1, pp.23-31

Lopez, O.L., Mackell, J.A., Sun, Y., Kassalow, L.M., Xu, Y., McRae, T. and Li, H. (2008) 'Effectiveness and Safety of Donepezil in Hispanic Patients with Alzheimer's disease: a 12-week open-label study'. *Journal of the National Medical Association*, 100:11, pp.1350-1358

Lopez-Pousa, S., Turon-Estrada, A., Garre-Olmo, J., Pericot-Nierga, I., Lozano-Gallego, M., Vilalta-Franch, M., Hernandez-Ferrandiz, M., Morante-Munoz, V., Isern-Vila, A., Gelada-Batlle, E. and Majo-Llopert, J. (2005) 'Differential efficacy of treatment with acetylcholinesterase inhibitors in patients with mild and

moderate Alzheimer's disease over a 6-month period'. *Dementia and Geriatric Cognitive Disorders*, 19, pp. 189-195

Lorist, M.M., Snel, J., Mulder, G. and Kok, G. (1995) 'Aging, caffeine, and information processing: an event-related potential analysis'. *Electroencephalography and Clinical Neuropsychology*, 96:5, pp.453-467

Lorist, M.M. and Tops, M. (2003) 'Caffeine, fatigue and cognition'. *Brain and Cognition*, 53:1, pp.82-94

Losh, M., Adolphs, R., Poe, M.D., Couture, S., Penn, D., Baranek, G.T. and Piven, J. (2009) 'The neuropsychological profile of autism and the broad autism phenotype'. *Archives of General Psychiatry*, 66:5, pp.518-526

Luchsinger, J.A. and Mayeux, R. (2004) 'Dietary factors and Alzheimer's disease'. *Lancet Neurology*, 3:10, pp.579-87

Luis, C.A., Abdullah, L., Paris, D., Quadros, A., Mullan, M., Mouzon, B., Ait-Ghezala, G., Crawford, F. and Mullan, M. (2009) 'Serum beta-amyloid correlates with neuropsychological impairment'. *Aging, Neuropsychology and Cognition*, 16: 2, pp.203-218

Lustig, C., Hasher, L., and Tonev, S. T. (2006) 'Distraction as a determinant of processing speed'. *Psychonomic Bulletin & Review*, 13:4, pp.619-625

MacRae, H. (1999) 'Managing courtesy stigma: the case of Alzheimer's disease'. *Sociology of Health and Illness*, 21:1, pp.54-70

Maguire, E.A., Burgess, N., Donnett, J.G., Frackowiak, R.S., Frith, C.D. and O'Keefe, J. (1998) 'Knowing where and getting there: a human navigation network'. *Science*, 280:5365, pp.921-924

Maguire, E.A., Burgess, N. and O'Keefe, J. (1999) 'Human Spatial Navigation: cognitive maps, sexual dimorphism, and neural substrates'. *Current opinion in Neurobiology*, 9:2, pp.1717-177

Maisto, S.A., Galizio, M. and Connors, G.J. (2011) *Drug Use and Abuse*. 6th ed. Wadsworth: Cengage Learning

Malenka, R.C. and Bear, M.F. (2004) 'LTP and LTD: an embarrassment of riches'. *Neuron*, 44:1, pp.5-21

Man, D. W. K., Chung, J. C. C. and Lee, G. Y. Y. (2011) 'Evaluation of a virtual reality-based memory training programme for Hong Kong Chinese older adults with questionable dementia: a pilot study'. *International Journal of Geriatric Psychiatry*, 27:5, pp.513-520

Mangialasche, F., Xu, W., Kivipelto, M., Costanzi, E., Ercolani, S., Pigliautile, M., Cecchetti, R., Baglioni, M., Simmons, A., Soininen, H., Tsolaki, M., Kloszewska, I., Vellas, B., Lovestone, S., Mecocci, P. and NeuroMed Consortium. (2012) 'Tocopherols and tocotrienols plasma levels are associated with cognitive impairment'. *Neurobiology of Aging*, 33:10, pp.2282-2290

Mapstone, M., Steffenella, T.M. and Duffy, C.J. (2003) 'A visuospatial variant of mild cognitive impairment: getting lost between aging and AD'. *Neurology*, 60:5, pp.802-808

Marioni, R.E., Chatfield, M., Brayne, C. and Matthews, F.E. (2011) 'The reliability of assigning individuals to cognitive states using the Mini Mental State Examination: a population-based prospective cohort study'. *BMC Medical Research Methodology*, 11:127

Markesbery, W.R. (1999) 'The role of oxidative stress in Alzheimer Disease'. *JAMA Neurology*, 56:12, pp.1449-1452

Markesbery, W.R. (2010) 'Neuropathologic Alterations in Mild Cognitive Impairment: A Review'. *Journal of Alzheimer's Disease*, 19:1, pp.221-228

Markesbery, W.R., Schmitt, F.A., Kryscio, R.J., Davis, D.G., Smith, C.D. and Wekstein, D.R. (2006) 'Neuropathologic substrate of mild cognitive impairment'. *Archives of Neurology*, 63:1, pp.38-46

Markou, A., Duka, T. and Prelevic, G.M. (2005) 'Estrogens and Brain Function'. *Hormones*, 4:1, pp.9-17

Markwick, A., Zamboni, G. and de Jager, C.A. (2012) 'Profiles of cognitive subtest impairment in the Montreal Cognitive Assessment (MoCA) in a research cohort with normal Mini-Mental State Examination (MMSE) scores'. *Journal of Clinical and Experimental Neuropsychology*, 34:7, pp.750-757

Martínez-Lapiscina, E.H., Clavero, P., Toledo, E., San Julián, B., Sanchez-Tainta, A., Corella, D., Lamuela-Raventós, R.M., Martínez, J.A. and Martínez-Gonzalez, M.Á. (2013) 'Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial'. *The Journal of Nutrition Health and Aging*, 17:6, pp.544-552

Martorana, A., Esposito, Z. and Koch, G. (2010) 'Beyond the cholinergic hypothesis: do current drugs work in Alzheimer's disease?' *CNS neuroscience and therapeutics*, 16:4, pp.235-245

Mattsson, N., Brax, D. and Zetterberg, H. (2010) 'To Know or Not to Know: Ethical Issues Related to Early Diagnosis of Alzheimer's Disease'. *International Journal of Alzheimers Disease*, 841941

Matus, A. (1988) 'Microtubule-associated proteins: their potential role in determining neuronal morphology'. *Annual Reviews in Neuroscience*, 11, pp.29-44

May, C.P., Hasher, L. and Stolfus, E.R. (1993) 'Optimal Time of day and the magnitude of age differences in memory'. *Psychological Science*, 4, pp.326-330

May, C.P. and Hasher, L. (1998) 'Synchrony Effects in Inhibitory Control Over Thought and Action'. *Journal of Experimental Psychology: Human Perception and Performance*, 24:2, pp.363-379

May, C.P., Hasher, L. and Foong, N. (2005) 'Implicit memory, age, and time of day: paradoxical priming effects'. *Psychological Science*, 16:2, pp.96-100

Mayeux, R., Sano, M., Chen, J., Tatemichi, T. and Stern, Y. (1991) 'Risk of dementia in first-degree relatives of patients with Alzheimer's disease and related disorders'. *Archives of Neurology*, 48:3, pp.269-273

Mayeux, R., Honig, L.S., Tang, M.X., Manly, J., Stern, Y., Schupf, N. and Mehta, P.D. (2003) 'Plasma A β 40 and A β 42 and Alzheimer's disease: relation to age, mortality, and risk'. *Neurology*, 61:9, pp.1185-1190

McCabe, J. and Hartman, M. (2008) 'An analysis of age differences in perceptual speed'. *Memory and Cognition*, 36:8, pp.1495-1508

McDonald, C.R., McEvoy, L.K., Gharapetian, L., Fennema-Notestine, C., Hagler, D.J., Holland, D., Koyama, A., Brewer, J.B. and Dale, A.M. (2009) 'Regional rates of neocortical atrophy from normal aging to early Alzheimer disease'. *Neurology*, 73:6, pp.457-465

McKenna, P. and Warrington, E.K. (1980) 'Testing for nominal dysphasia'. *Journal of Neurology, Neurosurgery and Psychiatry*, 43:9, pp.781-788

McShane, R., Gedling, K., Keene, J., Fairburn, C., Jacoby, R. and Hope, T. (1998) 'Getting lost in dementia: a longitudinal study of a behavioural symptom'. *International Psychogeriatrics/IPA*, 10:3, pp.253-260

Medline Plus. (2011) *Cerebral Spinal Fluid (CSF) collection*. [online] Available at: <<http://www.nlm.nih.gov/medlineplus/ency/article/003428.htm>> [Accessed 30th May 2013]

Meerlo, P., Sgoifo, A. and Suchecki, D. (2008) 'Restricted and disrupted sleep: Effects on autonomic function, neuroendocrine stress systems and stress responsivity'. *Sleep Medicine Reviews*, 12, pp.197-210

Mehdi, Y., Hornick, J.L., Isatasse, L. and Dufrasne, L. (2013) 'Selenium in the environment, metabolism and involvement in body functions'. *Molecules*, 18:3, pp.3292-3311

Meinert, C.L., McCaffrey, M.A. and Breitner, J.C.S. (2009) 'Alzheimer's disease Anti Inflammatory Prevention Trial (ADAPT): Design, methods and baseline results'. *Alzheimer's & Dementia*, 5:2, pp.93-104

Mesulam, M., Shaw, P., Mash, D. and Weintraub, S. (2004) 'Cholinergic Nucleus Basalis Tauopathy Emerges Early in the Aging-MCI-AD Continuum'. *Annals of Neurology*, 55:6, pp.815-828

Meyerbröcker, K., Morina, N., Kerkhof, G. and Emmelkamp, P.M. (2011) 'Virtual reality exposure treatment of agoraphobia: a comparison of computer automatic virtual environment and head-mounted display'. *Studies in Health Technology and Informatics*, 167, pp.51-56

Michikawa, M., Fan, Q.W., Isobe, I. and Yanagisawa, K. (2000) 'Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture'. *Journal of Neurochemistry*, 74:3, pp.1008-1016

Mielke, M.W., Leoutsakos, J., Tschanz, J., Green, R., Tripodis, Y., Corcoran, C.D., Norton, M. and Lyketsos, C. (2011) 'Interaction between vascular factors and the APOE E4 allele predict rate of progression in Alzheimer's dementia'. *Journal of Alzheimer's Disease*, 26:1, pp.127-134

Miller, L.S., Lombardo, T.W. and Fowler, S.C. (1995) 'Caffeine and Time of Day Effects on a Force Discrimination Task in Humans'. *Physiology and Behaviour*, 57:6, pp.1117-1125

Minoshima, S., Giordani, B., Berent, S., Frey, K.A., Foster, N.L. and Kuhl, D.E. (1997) 'Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease'. *Annals of Neurology*, 42:1, pp.85-94

Mintun, M.A., LaRossa, G.N., Sheline, Y.I., Dence, C.S., Lee, S.Y., Mach, R.H., Klunk, W.E., DeKosky, S.T. and Morris, J.C. (2006) '[¹¹C]PIB in a

nondemented population: potential antecedent marker of Alzheimer disease'. *Neurology*, 67:3, pp.446-452

Mitchell, A.J. (2009) 'A meta-analysis of the accuracy of the mini-mental state examination in the detection of dementia and mild cognitive impairment'. *Journal of Psychiatric Research*, 43:4, pp.411-431

Mitchell, P.J. and Redman, J.R. (1992) 'Effects of caffeine, time of day and user history on study-related performance'. *Psychopharmacology*, 109:1-2, pp.121-126

Miyake, A., Friedman, N.P., Emerson, M.J., Witzki, A.H., Howerter, A. and Wager, T.D. (2000) 'The unity and diversity of executive functions and their contributions to complex 'frontal lobe' tasks: a latent variable analysis'. *Cognitive Psychology*, 41:1, pp.49-100

Moffat, S.D., Zonderman, A.B. and Resnick, S.M. (2001) 'Age differences in spatial memory in a virtual environment navigation task'. *Neurobiology of Aging*, 22:5, pp.787-796

Molinuevo, J.L., Berthier, M.L. and Rami, L. (2011) 'Donepezil provides greater benefits in mild compared to moderate Alzheimer's disease: implications for early diagnosis and treatment'. *Archives of Gerontology and Geriatrics*, 52:1, pp.18-22

Morris, J.C. (1993) 'The clinical dementia rating scale (CDR): current version and scoring rules'. *Neurology*, 43:11, pp.2412-2414

Morris, M.C. (2009) 'The role of nutrition in Alzheimer's disease: epidemiological evidence'. *European Journal of Neurology*, 16:suppl1, pp.1-7

Morris, J.C., Roe, C.M., Grant, E.A., Head, D., Storandt, M., Goate, A.M., Fagan, A.M., Holtzman, D.M. and Mintun, M.A. (2009) 'Pittsburgh Compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease'. *Archives in Neurology*, 66:12, pp.1469-1475

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Bennett, D.A., Wilson, R.S., Aggarwal, N. and Schneider, J. (2003) 'Consumption of Fish and n-3 Fatty Acids and Risk of Incident Alzheimer Disease'. *Archives in Neurology*, 60:7, pp. 940-946

Morris, R.G., Garrud, P., Rawlins, J.N. and O'Keefe, J. (1982) 'Place navigation impaired in rats with hippocampal lesions'. *Nature*, 297:5868, pp. 681-683

Morsch, R., Simon, W. and Coleman, P.D. (1999) 'Neurons may live for decades with neurofibrillary tangles'. *Journal of Neuropathology and Experimental Neurology*, 58:2, pp.188-197

Mosconi, L., Pupi, A. and De Leon, M.J. (2008) 'Brain Glucose Hypometabolism and Oxidative Stress in Preclinical Alzheimer's Disease'. *Annals of the New York Academy of Sciences*, 1147, pp.180-195

Mosconi, L., Mistur, R., Switalski, R., Tsui, W.H., Glodzik, L., Li, Y., Pirraglia, E., De Santi, S., Reisberg, B., Wisniewski, T. and de Leon, M.J. (2009) 'FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease'. *European Journal of Nuclear Medicine and Molecular Imaging*, 36:5, pp.811-822

Mufson, E.J., Binder, L., Counts, S.E., DeKosky, S.T., de Toledo-Morrell, L., Ginsberg, S.D., Ikonovic, M.D., Perez, S.E. and Scheff, S.W. (2012) 'Mild Cognitive Impairment: Pathology and Mechanisms'. *Acta Neuropathology*, 123:1, pp.13-30

Mulnard, R.A., Cotman, C.W., Kawas, C., van Dyck, C.H., Sano, M., Doody, R., Koss, E., Pfeiffer, E., Jin, S., Gamst, A., Grundman, M., Thomas, R. and Thal, L.J. (2000) 'Estrogen replacement therapy for treatment of mild to moderate Alzheimer's disease: a randomised controlled trial. Alzheimer's Disease Cooperative Study'. *Journal of the American Medical Association*, 283:8, pp.1007-1015

Murphy, D.D., Cole, N.B., Greenberger, V. and Segal, M. (1998) 'Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons'. *Journal of Neuroscience*, 18:7, pp.2550-2559

Nasreddine, Z.S., Phillips, N.A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L. and Chertkow, M.D. (2005) 'The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment'. *Brief Methodological Reports*, 53, pp.695-699

Nathan, P.J. and Stough, C. (2001) 'Inspection time: a neuropsychophysiological test for measuring the functional integrity of the cholinergic system'. *Medicine Hypotheses*, 57:6, pp.759-760

National Audit Office. (2006-2007) *Improving services and support for people with dementia*. London: the Stationery Office

National Institute for Health and Clinical Excellence (2006a) *Dementia, supporting people with dementia and their carers in health and social care*. London: National Institute for Health and Excellence

National Institute for Health and Clinical Excellence (2006b) *Quick Referencing Guide: Dementia. CG42*. London: National Institute for Health and Clinical Excellence.

National Institute for Health and Care Excellence (2011) *TA217 Donepezil, galantamine, rivastigmine and memantine for the treatment of Alzheimer's disease*'. London: National Institute for Health and Clinical Excellence.

Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A. and Feeley, M. (2003) 'Effects of Caffeine on human health'. *Food additives and contaminants*, 20:1, pp.1-30

Nebes, R.D., Butters, M.A., Houck, P.R., Zmuda, M.D., Aizenstein, H., Pollock, B.G., Mulsant, B.H. and Reynolds, C.F. (2001) 'Dual-task performance in depressed geriatric patients'. *Psychiatry Research*, 102:2, pp.139-151

Nehlig, A. (2010). 'Is Caffeine a cognitive enhancer?' *Journal of Alzheimer's Disease*, 20:(suppl 1), pp. 85-94

Nestor, P.J., Scheltens, P. and Hodges, J.R. (2004) 'Advances in the early detection of Alzheimer's disease'. *Nature Reviews Neuroscience*, 5: S34-41

NHS (2011) *Fish and shellfish* [online] Available at <<http://www.nhs.uk/livewell/goodfood/pages/fish-shellfish.aspx>>[Accessed 9th September 2013]

NHS. (2012) *How long will I have to wait to see a consultant*. [online] Available at<<http://www.nhs.uk/chq/Pages/904.aspx?CategoryID=68&SubCategoryID=153>> [Accessed 30th May 2013]

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E.K., Jones, P.K., Ghanbari, H., Wataya, T., Shimohama, S., Chiba, S., Atwood, C.S., Petersen, R.B. and Smith, M.A. (2001) 'Oxidative damage is the earliest event in Alzheimer disease'. *Journal of Neuropathology and Experimental Neurology*, 60:8, pp.759-767

Nunomura, A., Moreira, P.I., Takeda, A., Smith, M.A. and Perry, G. (2007) 'Oxidative RNA damage and neurodegeneration'. *Current Medicinal Chemistry*, 14:28, pp.2968-2975

Nurk, E., Drevon, C.A., Refsum, H., Solvoll, K., Vollset, S.E., Nygård, O., Nygaard, H.A., Engedal, K., Tell, G.S. and Smith, A.D. (2007) 'Cognitive performance among the elderly and dietary fish intake: the Hordaland Health Study'. *American Journal of Clinical Nutrition*, 86, pp. 1470-1478

O'Brien, R.J. and Wong, P.C. (2011) 'Amyloid Precursor Protein Processing and Alzheimer's Disease'. *Annual Review of Neuroscience*, 34, pp.185-204

O'Brien, H.L., Tetewsky, S., Avery, L.M., Cushman, L.A., Makous, W. and Duffy, C.J. (2001) 'Visual mechanisms of spatial disorientation in Alzheimer's disease'. *Cerebral Cortex*, 11:11, pp.1083-1092

O'Bryant, S.E., Waring, S.C., Hobson, V., Hall, J.R., Moore, C.B., Bottiglieri, T., Mssman, P. and Diaz-Arrastia, R. (2010) 'Decreased C-reactive protein levels in Alzheimer disease'. *Journal of Geriatric Psychiatry and Neurology*, 23:1, pp. 49-53

O'Bryant, S.E., Xiao, G., Barber, R., Huebinger, R., Wilhelmsen, K., Edwards, M., Graff-Radford, N., Doody, R. and Diaz-Arrastia, R. (2011) 'A Blood-Based Screening Tool for Alzheimer's Disease That Spans Serum and Plasma: Findings from TARCC and ADNI'. *PLos One*, 6:12, e28092

O'Claassen, D.O. and Wylie, S.A. (2012) 'Trends and Issues in Characterizing Early Cognitive Changes in Parkinson's Disease'. *Current Neurology and Neuroscience reports*, 12:6, pp.695-702

O'Connor, D.W., Pollitt, P.A., Hyde, J.B., Brook, C.P., Reiss, B.B. and Roth, M. (1988) 'Do general practioners miss dementia in elderly patients'. *British Medical Journal*, 297:6656, pp.1107-1110

Oddo, S., Caccamo, A., Tran, L., Lambert, M.P., Glabe, C.G., Klein, W.L. and LaFerla, F.M. (2006) 'Temporal profile of amyloid- β (A β) oligomerization in an in vivo model of Alzheimer disease. A link between A β and tau pathology'. *The Journal of Biological Chemistry*, 281:3, pp.1599-1604

Ohsugi, H., Ohgi, S., Shigemori, K. and Schneider, E.B. (2013) 'Differences in dual-task performance and prefrontal cortex activation between younger and older adults'. *BMC Neuroscience*, 14:10

O'Keefe, J. and Nadel, L. (1978) '*The hippocampus as a cognitive map*'. Oxford: Oxford University Press

Oken, B.S., Kishiyama, S.S., Kaye, J.A. and Howieson, D.B. (1994) 'Attention deficit in Alzheimer's disease is not stimulated by an anticholinergic/antihistaminergic drug and is distinct from deficits in healthy ageing'. *Neurology*, 44:4, pp.657-662

Okonkwo, O.C., Mielke, M.M., Griffith, H.R., Moghekar, A.R., O'Brien, R.J., Shaw, L.M., Trojanowski, J.Q. and Albert, M.S, Alzheimer's Disease Neuro initiative (2011) 'Cerebrospinal fluid profiles and prospective course and outcome in patients with amnesic mild cognitive impairment'. *Archives of Neurology*, 68:1, pp.113-119

O'Rourke, J.F.F., Beglinger, L.J., Smith, M.M., Mills, J., Moser, D.J., Rowe, K.C., Langbehn, D.R., Duff, K., Stout, J.C., Harrington, D.L., Carlozzi, N., Paulsen, J.S., and the PREDICT-HD Investigators of the Huntington Study Group. (2011) 'The Trail Making Test in Prodromal Huntington Disease: Contributions of Disease Progression to Test Performance'. *Journal of Clinical and Experimental Neuropsychology*, 33:5, pp.567-579

Overshott, R. and Burns, A. (2004) 'Clinical assessment in dementia'. *Psychiatry*, 3:12, pp.13-18

Owen, A.M., Milner, B., Petrides, M. and Evans, A.C. (1996) 'A specific role for the right parahippocampal gyrus in the retrieval of object location: A positron emission tomography study'. *Journal of Cognitive Neuroscience*, 8:6, pp.588-602

Pagel, J.F. (2013) Medications and Sleep. In Kushida, C. '*Encyclopedia of Sleep*'. Waltham: Academic Press, pp.538-542

Pandi-Perumal, S.R., BaHammam, A.S., Brown, G.M., Spence, D.W., Bharti, V.K., Kaur, C., Hardeland, R., and Cardinali, D.P. (2012) 'Melatonin antioxidative defense: therapeutical implications for aging and neurodegenerative processes'. *Neurotoxicity Research*, 23:3, pp.267-300

Pantel, J., Schonknecht, P., Essig, M. and Schroder, J. (2004) 'Distribution of cerebral atrophy assessed by MRI reflects patterns of neuropsychological deficits in Alzheimer's dementia'. *Neuroscience letters*, 361, pp.17-20

Pappas, B.A., Bayley, P.J., Bui, B.K., Hansen, L.A. and Thal, L. (2000) 'Choline acetyltransferase activity and cognitive domain scores of Alzheimer's patients'. *Neurobiology of Aging*, 21, pp.11-17

Pasquier, F. (1999) 'Early diagnosis of dementia: neuropsychology'. *Journal of Neurology*, 246:1, pp.6-15

Passini, R., Rainville, C., Marchland, N. and Joannette, Y. (1995) 'Wayfinding in dementia of the Alzheimer type: planning abilities'. *Journal of Clinical and Experimental Neuropsychology*, 17:6, pp.820-832

Paudel, M.L., Taylor, B.C., Ancoli-Israel, S., Blackwell, T., Stone, K.L., Tranah, G., Redline, S., Cummings, S.R., Ensrud, K.E. and Osteoporotic Fractures in Men (MrOS) Study. (2010) 'Rest/activity rhythms and mortality rates in older men: MrOS Sleep Study'. *Chronobiology International*, 27:2, pp.363-377

Paulsen, J.S. (2011) 'Cognitive Impairment in Huntington Disease: Diagnosis and Treatment'. *Current Neurology and Neuroscience Reports*, 11:5, pp.474-483

Peuhkuri, K., Sihvola, N. and Korpela, R. (2012) 'Diet promotes sleep duration and quality'. *Nutrition Research*, 32, pp.309-319

Pendlebury, S.T., Cuthbertson, F.C., Welch, S.J., Mehta, Z. and Rothwell, P.M. (2010) 'Underestimation of cognitive impairment by Mini-Mental State Examination versus the Montreal Cognitive Assessment in patients with transient ischemic attack and stroke: a population-based study'. *Stroke*, 41:6, pp.1290-1293

Pengas, G., Patterson, K., Arnold, R.J., Bird, C.M., Burgess, N. and Nestor, P.J. (2010) 'Lost and found: Bespoke Memory Testing for Alzheimer's Disease and Semantic Dementia'. *Journal of Alzheimer's Disease*, 21:4, pp.1347-1365

Perry, E.K., Perry, R.H., Blessed, G. and Tomlinson, B.E. (1977) 'Necropsy evidence of central cholinergic deficits in senile dementia'. *Lancet*, 1:8004, pp.189-189

Pesini, P., Pérez-Grijalba, V., Monleón, I., Boada, M., Tárraga, L., Martínez-Lage, P., San-José, I. and Sarasa, M. (2012). 'Reliable Measurements of the β - Amyloid Pool in Blood Could Help in the Early Diagnosis of AD'. *International Journal of Alzheimer's Disease*, 2012:604141

Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G. and Kokmen, E. (1999). 'Mild Cognitive Impairment: Clinical Characterization and Outcome'. *Archives of Neurology*, 56:3, pp.303-308

Petersen, R.C., Thomas, R.G., Grundman, M., Bennett, D., Doody, R., Ferris, S., Galasko, D., Jin, S., Kaye, J., Levey, A., Pfeiffer, E., Sano, M., van Dyck, C.H. and Thal, L.J. Alzheimer's Disease Cooperative Study Group (2005) 'Vitamin E and donepezil for the treatment of mild cognitive impairment'. *New England Journal of Medicine*, 352:23, pp.2379-88

Petersen, R.C. (2009) 'Early Diagnosis of Alzheimer's Disease: Is MCI too Late?' *Current Alzheimers Research*, 6:4, pp.324-330

Petersen, R.C. (2011) 'Mild Cognitive Impairment'. *The New England Journal of Medicine*, 364:23, pp.2227-2234

Petersen, R.C., Aisen, P., Boeve, B.F., Geda, Y.E., Ivnik, R.J., Knopman, D.S., Mielke, M., Pankratz, V.S., Roberts, R., Rocca, W.A., Weigand, S., Weiner, M., Wiste, H. and Jack, C.R. jnr (2013) 'Criteria for mild cognitive impairment due to alzheimer's disease in the community'. *Annals of Neurology*, epub ahead of print, doi 10.1002/ana.23931

Pimplikar, S.W., Nixon, R.A., Robakis, N.K., Shen, J. and Tsai, L.H. (2010) 'Amyloid-independent mechanisms in Alzheimer's disease pathogenesis'. *Journal of Neuroscience*, 30:45, pp.14946-14954

Podewils, L.J., Guallar, E., Kuller, L/H., Lopez, O.L., Carlson, M. and Lyketsos, C.G. (2005) 'Physical activity, APOE genotype, and dementia risk: findings from

the Cardiovascular Health Cognition Study'. *American Journal of Epidemiology*, 161:7, pp.639-651

Porkka-Heiskanen, T. and Kalinchuk, A.V. (2011) 'Adenosine, energy metabolism and sleep'. *Sleep Medicine Reviews*, 15, pp.123-135

Povova, J., Ambroz, P., Bar, M., Pavukova, V., Sery, M., Tomaskova, H. and Janou, V. (2012) 'Epidemiological of and risk factors for Alzheimer's disease: A review'. *Biomedical Papers*, 156:2, pp.108-114

Prasanthi, J.R.P., Dasari, B., Marwarha, G., Larson, T., Chen, X., Geiger, J.D. and Ghibribi, O. (2010) 'Caffeine protects against induced oxidative stress and Alzheimer's disease-like pathology in rabbit hippocampus induced by cholesterol-enriched diet'. *Free Radical Biology and Medicine*, 49:7, pp.1212-1220

Pratico, D., Uryu, K., Leight, S., Trojanowski, J.Q. and Lee, V.M.Y. (2001) 'Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis'. *Journal of Neuroscience*, 21:12, pp.4183-4187

Prestia, A., Caroli, A., Herholz, K., Reiman, E., Chen, K., Jagust, W.J., Frisoni, G.B. and Translational Outpatient Memory Clinic Working Group for the Alzheimer's disease Neuroimaging Initiative (2013) 'Diagnostic accuracy of markers for prodromal Alzheimer's disease in independent clinical series'. *Alzheimer's and Dementia*, article in press, doi 10.1016/j.jalz.2012.09.016

Procter, A.W., Palmer, A.M., Francis, P.T., Lowe, S.L., Neary, D., Murphy, E., Doshi, R. and Bowen, D.M. (1988) 'Evidence of glutamatergic denervation and possible abnormal metabolism in Alzheimer's disease'. *Journal of Neurochemistry*, 50:3, pp.790-802

Pruessner, J.C., Köhler, S., Crane, J., Pruessner, M., Lord, C., Byrne, A., Kabani, N., Collins, D.L. and Evans, A.C. (2002) 'Volumetry of temporopolar, perirhinal, entorhinal and parahippocampal cortex from high-resolution MR

images: considering the variability of the collateral sulcus'. *Cerebral Cortex*, 12:12, pp.1342-1353

Quinn, J.F., Raman, R., Thomas, R.G., Yurko-Mauro, K., Nelson, E.B., Van Dyck, C., Galvin, J.E., Edmond, J., Jack, C.R. JR, Weiner, M., Shinto, L. and Aisen, P.S. (2010) 'Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial'. *Journal of the American Medical Association*, 304:17, pp.1903-1911

Rahman, A. (2009) 'The role adenosine in Alzheimer's Disease'. *Current Neuropharmacology*, 7:3, pp.207-216

Raschetti, R., Albanese, E., Vanacore, N. and Maggini, M. (2007) 'Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials'. *PLoS Medicine*, 4:11, e338

Raz, N., Gunning-Dixon, F., Head, D., Rodrigue, K.M., Williamson, A., and Acker, J.D. (2004) 'Aging, Sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume'. *Neurobiology of Aging*, 25:3, pp.377-396

Reid, A.T. and Evans, A.C. (2013) 'Structural networks in Alzheimer's disease'. *European Neuropsychopharmacology*, 23, pp.63-77

Reisberg, B., Doody, R., Stoffler, A., Schmitt, F., Ferris, S. and Mobius, H.J. (2003) 'Memantine in Moderate to Severe Alzheimers disease'. *The New England Journal of Medicine*, 348:14, pp.1333-1341

Reitan, R. M. and Davison, L. A. (1974) *Clinical Neuropsychology: Current Status and Applications*. New York: John Wiley

Reitz, C., Brayne, C. and Mayeux, R. (2011) 'Epidemiology of Alzheimer disease'. *Nature Reviews Neurology*, 7:3, pp.137-152

Relling, M.V., Lin, J.S., Ayers, G.D. and Evans, W.E. (1992) 'Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities'. *Clinical Pharmacology and Therapeutics*, 52:6, pp.643-658

Resende, R., Moreira, P.I., Proença, T., Deshpande, A., Busciglio, J., Pereira, C., Oliveira, C.R. (2008) 'Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease'. *Free Radical Biology and Medicine*, 44:12, pp. 2051-2057

Resnick, S.M., Pham, D.L., Kraut, M.A., Zonderman, A.B., and Davatzikos, C. (2003) 'Longitudinal Magnetic Resonance Imaging studies of Older Adults: A Shrinking Brain'. *Journal of Neuroscience*, 23:8, pp.3295-3301

Revett, T.J., Baker, G.B., Jhamandas, J. and Kar, S. (2013) 'Glutamate system, amyloid β peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology'. *Journal of Psychiatry and Neuroscience*, 38:1, pp.6-23

Ribeiro, J.A., Sebastião, A.M. and de Mendonça, A. (2002) 'Adenosine receptors in the nervous system: pathophysiological implications'. *Progress in Neurobiology*, 68:6, pp.377-392

Richardson, E. D. and Marottoli, R. A. (1996) 'Education-specific normative data on common neuropsychological indices for individuals older than 75 years'. *The Clinical Neuropsychologist*, 10, pp.375-381

Riedel, W., Hogervorst, E., Leboux, R., Verhey, F., van Praag, H. and Jolles, J. (1995) 'Caffeine attenuates scopolamine-induced memory impairment in humans'. *Psychopharmacology (Berl)*, 122:2, pp.158-168

Ritchie, K., Carrière, I., de Mendonca, A., Portet, F., Dartigues, J.F., Rouaud, O., Barberger-Gateau, P. and Ancelin, M.L. (2007) 'The neuroprotective effects of caffeine: a prospective population study (the Three City Study)'. *Neurology*, 69:6, pp.536-545

Roberts, J.L., Clare, L. and Woods, R.T. (2009) 'Subjective Memory Complaints and Awareness of Memory Functioning in Mild Cognitive Impairment: A

Systematic Review'. *Dementia and Geriatric Cognitive Disorders*, 28:2, pp.95-109

Roberts, R.O., Cerhan, J.R., Geda, Y.E., Knopman, D.S., Cha, R.H., Christianson, T.J.H., Pankratz, V.S., Ivnik, R.J., O'Connor, H.M. and Petersen, R.C. (2010) 'Polyunsaturated Fatty Acids and Reduced Odds of MCI: The Mayo Clinic Study of Aging'. *Journal of Alzheimer's Disease*, 21:3, pp.853-865

Robinson, J.G., Ljioma, N. and Harris, W. (2010) 'Omega-3 fatty acids and cognitive function in women'. *Womens health*, 6:1, pp.119-134

Rodgers, M.K., Sindone, J.A.3rd. and Moffat, S.D. (2010) 'Effects of age on navigation strategy'. *Neurobiology of aging*, 33:1, pp.202.e15-202.e22

Roe, C.M. and Rentz, D.M. (2013) 'Alzheimer disease, before the diagnosis'. *Neurology*, 80:13, e148-e149

Rogers, S.L., Doody, R.S., Pratt, R.D. and Leni, J.R. (2000) 'Long-term efficacy and safety of donepezil in the treatment of Alzheimer's disease: final analysis of a US multicentre open-label study'. *European Neuropsychopharmacology*, 10:3, pp.195-203

Rosenberg, P.B., Mielke, M.M., Tschanz, J., Cook, L., Stat, M., Corcoran, C., Hayden, K.M., Norton, M., Rabins, P.V., Green, R.C., Welsh-Bohmer, K.A., Breitner, J.C.S., Munger, R. and Lyketsos, C.G. (2008) 'Effects of Cardiovascular Medications on Rate of Functional Decline in Alzheimer Disease'. *American Journal of Geriatric Psychiatry*, 16:11, pp.883-892

Roses, A.D., Saunders, A.M., Alberts, M.A., Strittmatter, W.J., Schmechel, D., Gorder, E., Pericak-Vance, M.A. (1995) 'Apolipoprotein E E4 allele and risk of dementia'. *Journal of the American Medical Association*, 273:5, pp.374-375

Rountree, S.D., Atri, A., Lopez, O.L. and Doody, R.S. (2013) 'Effectiveness of antidementia drugs in delaying Alzheimer's disease progression'. *Alzheimer's & Dementia*, 9:3, pp.338-345

Ruthirakuhan, M., Luedke, A.C., Tam, A., Goel, A., Kurji, A. and Garcia, A. (2012) 'Use of physical and intellectual activities and socialization in the management of cognitive decline of aging and in dementia: a review'. *Journal of Aging Research*, 384875

Ryan, J., Scali, J., Carriere, I., Ritchie, K. and Ancelin, M.L. (2008) 'Hormonal treatment, mild cognitive impairment and Alzheimer's disease'. *International Psychogeriatrics*, 20:1, pp.47-56

Sagaspe, P., Taillard, J., Chaumet, G., Moore, N., Bioulac, B. and Philip, P. (2007) 'Aging and Nocturnal Driving: Better with Coffee or a Nap? A Randomized Study'. *Sleep*, 30:12, pp.1808-1813

Sahakian, B.J., Morris, R.G., Evenden, J.L., Heald, A., Levy, R., Philpot, M. and Robbins, T.W (1988). 'A comparative study of visuospatial memory and learning in Alzheimer- type dementia and Parkinson's disease'. *Brain*, 111:3, pp.695-718

Salthouse, T. A. (1996). 'General and specific speed mediation of adult age differences in memory'. *Journals of Gerontology, Series B: Psychological Sciences and Social Sciences*, 51:1, pp.30-42

Salthouse, T.A. and Babcock, R.L. (1991) 'Decomposing Adult age differences in working memory'. *Developmental Psychology*, 27:5, pp.763-776

Salín-Pascual, R.J., Valencia-Flores, M., Campos, R.M., Castano, A. and Shiromani, P.J. (2006) 'Caffeine challenge in insomniac patients after total sleep deprivation'. *Sleep Medicine*, 7:2, pp.141-145

Samtani, M.N., Farnum, M., Lobanov, V., Yang, E., Raghavan, N., Dibernardo, A., Narayan, V. and the Alzheimer's Disease Neuroimaging Initiative (2012) 'An improved model for disease progression in patients from the Alzheimer's disease neuroimaging initiative'. *Journal of Clinical Pharmacology*, 52:5, pp.629-644

Samuels, E.R. and Szabadi, E. (2008) 'Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans'. *Current Neuropharmacology*, 6:3, pp.254-285

Sandstrom, N.J., Kaufman, J. and Huettel, S.A. (1998) 'Males and females use different distal cues in a virtual environment navigation task'. *Cognitive Brain Research*, 6, pp.351-360

Sano, M., Ernesto, C., Thomas, R.G., Klauber, M.R., Schafer, K., Grundman, M., Growdon, J., Cotman, C.W., Pfeiffer, E., Schneider, L.S. and Thal, L.J. (1997) 'A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease'. *New England Journal of Medicine*, 336:17, pp.1216-1222

Santos, C., Luneta, N., Azevedo, A., de Mendonça, A., Ritchie, K. and Barros, H. (2010) 'Caffeine Intake is associated with a Lower Risk of Cognitive Decline: A Cohort Study from Portugal'. *Journal of Alzheimer's Disease*, 20, S175-185

Sanz Simon, S., Yokomizo, J.E. and Bottino, C.M.C. (2012) 'Cognitive Intervention in amnesic Mild Cognitive Impairment: A systematic review'. *Neuroscience and Biobehavioural Reviews*, 36:4, pp.1163-1178

Sarter, M., Hasselmo, M.E., Bruno, J. P., and Givens, B. (2005). 'Unraveling the attentional functions of cortical cholinergic inputs: Interactions between signal-driven and cognitive modulation of signal detection'. *Brain Research Reviews*, 48, pp.98-111

Satlin, A., Volicer, L., Stopa, E.G. and Harper, D. (1995) 'Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease'. *Neurobiology of Aging*, 16:5, pp.765-771

Scarmeas, N., Stern, Y., Tang, M.X., Mayeux, R. and Luchsinger, J.A. (2006) 'Mediterranean diet and risk of Alzheimer's disease'. *Annals of Neurology*, 59, pp.912-921

Scarmeas, N., Luchsinger, J.A., Schupf, N., Brickman, A.M., Cosentino, S., Tang, M.X. and Stern, Y. (2009) 'Physical Activity, Diet and Risk of Alzheimer Disease'. *Journal of the American Medical Association*, 302:6, pp.627-637

Schaub, A., Neubauer, N., Mueser, K.T., Engel, R. and Möller, H.J. (2013) 'Neuropsychological functioning in inpatients with major depression or schizophrenia'. *BMC Psychiatry*, 13:203 doi 10.1186/1471-244x-13-203

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vendevent, C., Walker, S., Wogulis, M., Yednock, T., Games, D. and Seubert, P. (1999) 'Immunization with amyloid- β attenuates Alzheimer-disease-like pathology in the PDAPP mouse'. *Nature*, 400, pp.173-177

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Vitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S. (1996) 'Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease'. *Nature Medicine*, 2:8, pp.864-870

Schmidt, C., Collette, F., Cajochen, C. and Peigneux, P. (2007) 'A time to think: circadian rhythms in human cognition'. *Cognitive Neuropsychology*, 24:7, pp.755-789

Schmidt, C., Peigneux, P. and Cajochen, C. (2012) 'Age-related changes in sleep and circadian rhythms; impact on cognitive performance and underlying neuroanatomical networks'. *Frontiers in Neurology*, 3:118

Schmitt, A.J., Hogervorst, E., Vuurman, E.F., Jolles, J. and Reidel, W.J. (2003). 'Memory functions and focused attention in middle-aged and elderly subjects

are unaffected by a low, acute dose of caffeine'. *Journal of Nutrition, Health and Aging*, 7:5, pp.301-303

Schneider, L.S., DeKosky, S.T., Farlow, M.R., Tariot, P.N., Hoerr, R. and Kieser, M. (2005) 'A randomized, double-blind, placebo-controlled trial of two doses of Ginkgo Biloba extract in Dementia of the Alzheimer's Type'. *Current Alzheimer Research*, 2:5, pp.541-51

Schroeter, M.L., Vogt, B., Frisch, S., Becker, G., Barthel, H., Mueller, K., Villringer, A. and Sabri, O. (2012) 'Executive deficits are related to the inferior frontal junction in early dementia'. *Brain*, 135: part 1, pp.201-215

Schuff, N. and Zhu, X.P. (2007) 'Imaging of Mild Cognitive impairment and early dementia'. *The British Journal of Radiology*, 80:2, pp.S109-114

Schürks, M., Glynn, R.J., Rist, P.M., Tzourio, C. and Kurth, T. (2010) 'Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials'. *British Medical Journal*, 341:c5702. [DOI:10.1136/bmj.c5702]

Scientific Advisory Committee on Nutrition (2004) '*Advice on Fish Consumption: benefits and risks*'. London: Crown Copyright

Sécher, M. and Ritz, P. (2012) 'Hydration and cognitive performance'. *The Journal of Nutrition, Health and Aging*, 16:4, pp.325-329

Selkoe, D.J. (2002) 'Alzheimer's disease is a synaptic failure'. *Science*, 298:5594, pp.789-791

Selkoe, D.J. and Schenk, D. (2003) 'Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics'. *Annual Review of Pharmacology and Toxicology*, 43, pp.545-84

Seltzer, B., Zolnouri, P., Nunez, M., Goldman, R., Kumar, D., Ieni, J. and Richardson, S. (2004) 'Efficacy of Donepezil in Early-Stage Alzheimer Disease: A randomized Placebo-Controlled Trial'. *Archives of Neurology*, 61:12, pp.1852-1856

Serino, S. and Riva, G. (2013) 'Getting lost in Alzheimer's disease: a break in the mental frame syncing'. *Medical Hypotheses*, 80:4, pp.416-421

Seshadri, S., Beiser, A., Selhub, J., Jacques, P.F., Rosenberg, I.H., D'Agostino, R.B., Wilson, P.W. and Wolf, P.A. (2002) 'Plasma Homocysteine as a risk factor for dementia and Alzheimer's Disease'. *New England Journal of Medicine*, 346:7, pp.476-483

Shallice, T. (1982) 'Specific impairments of planning'. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 298:1089, pp.199-209

Shansky, R.M. and Lipps, J. (2013) 'Stress-induced cognitive dysfunction: hormone-neurotransmitter interactions in the prefrontal cortex'. *Frontiers in Human Neuroscience*, 7:123

Sharwood, L.N., Elkington, J., Meuleners, L., Ivers, R., Boufous, S. and Stevenson, M. (2013) 'Use of caffeinated substances and risk of crashes in long distance drivers of commercial vehicles: case-control study'. *British Medical Journal*, 346:f1140

Shaw, L.M., Vanderstichele, H., Knapik-Czajka, M., Clark, C.M., Aisen, P.S., Petersen, R.C., Blennow, K., Soares, H., Simon, A., Lewczuk, P., Dean, R., Siemers, E., Potter, W., Lee, V.M.Y. and Trojanowski, J.Q. and the Alzheimer's Disease Neuroimaging Initiative. (2009) 'Cerebrospinal Fluid Biomarker Signature in Alzheimer's Disease Neuroimaging Initiative Subjects'. *Annals of Neurology*, 65:4, pp.403-413

Sheehan, B., Burton, E. and Mitchell, L. (2006) 'Outdoor wayfinding in dementia'. *Dementia*, 5:2, pp.271-281

Shen, Q., Loewenstein, D.A., Potter, E., Zhao, W., Appel, J., Greig, M.T., Raj, A., Acevedo, A., Schofield, E., Barker, W., Wu, Y., Potter, H. and Duara, R. (2011) 'Volumetric and visual rating of magnetic resonance imaging scans in

the diagnosis of amnesic mild cognitive impairment and Alzheimer's disease'. *Alzheimer's & Dementia*, 7:4, pp.101-108

Silva, E.J., Wang, W., Ronda, J.M., Wyatt, J.K. and Duffy, J.F. (2010) 'Circadian and wake-dependent influences on subjective sleepiness, cognitive throughput, and reaction time performance in older and young adults'. *Sleep*, 33:4, pp.481-490

Slater, M., Pertaub, D.P., Barker, C. and Clark, D.M. (2006) 'An Experimental Study on Fear of Public Speaking Using a Virtual Environment'. *CyberPsychology & Behavior*, 9:5, pp.627-33

Smith, A. (1968). *The Symbol-digit modalities test: a neuropsychological Screening Test*, Odessa, Florida: Psychological Assessment Resources

Smith, A.D., Smith, S.M., de Jager, C.A., Whitbread, P., Johnson, C., Agacinski, G., Oulhaj, A., Brdley, K.M., Jacoby, R. and Refsum, H. (2010) 'Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial'. *PLoS One*, 5:9, e12244

Smith, D. (2002) 'Homocysteine, B vitamins, and cognitive deficit in the elderly'. *American Journal of Clinical Nutrition*, 75:5, pp.785-786

Smith, C., Graham, D.I. and Nicoll, J.A.R: (2001) 'Tau immunohistochemistry in acute head injury'. *Neuropathology and Applied Neurobiology*, 29:5, pp.496-502

Smith, C.D., Chebrolu, H., Wekstein, D.R., Schmitt, F.A., Jicha, G.A., Cooper, G. and Markesbery, W.R. (2007) 'Brain structural alterations before mild cognitive impairment'. *Neurology*, 68:16, pp.1268-73

Smith, J. and Baltes, P.B. (1997) 'Profiles of psychological functioning in the old and oldest old'. *Psychology and Aging*, 12:3, pp.495-472

Smith, M.L. and Milner, B. (1981) 'The role of the right hippocampus in the recall of spatial location'. *Neuropsychologica*, 19:6, pp.781-793

Socci, D.J., Crandall, B.M. and Arendash, G.W. (1995) 'Chronic antioxidant treatment improves the cognitive performance of aged rats'. *Brain Research*, 693:1-2, pp.88-94

Söderberg, M., Edlund, C., Kristensson, K. and Dallner, G. (1991) 'Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease'. *Lipids*, 26:6, pp.421-425

Sperling, R.A., Aisen, P.S., Beckett, D.A., Bennett, D.A., Craft, S., Fagan, A.M., Iwatsubo, T., Jack, C.R.Jr, Kaye, J., Montine, T.J., Park, D.C., Reiman, E.M., Rowe, C.C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M.C., Thies, B., Morrison-Bogorad, M., Wagster, M.V. and Phelps, C.H. (2011) 'Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging and the Alzheimer's Association workgroup'. *Alzheimer's and Dementia*, 7:3, pp.280-292

Spieler, D.H., Balota, D.A. and Faust, M.E. (1996) 'Stroop performance in healthy younger and older adults and in individuals with dementia of the Alzheimer's type'. *Journal of Experimental Psychology: Human Perception and Performance*, 22:2, pp.461-479

Spiers, H.J., Burgess, N., Maguire, E.A., Baxendale, S.A., Hartley, T., Thompson, P. and O'Keefe, J. (2001) 'Unilateral temporal lobectomy patients show lateralised topographical and episodic memory deficits in a virtual town'. *Brain*, 124:pt 12, pp.2476-2489

Spires, T.L., Meyer-Luehmann, Stern, E.A., McLean, P.J., Skoch, J., Nguyen, P.T., Bacskai, B.J. and Hyman, B.T. (2005) 'Dendritic spine abnormalities in APP transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy'. *Journal of Neuroscience*, 25:31, pp.7278-7287

Spreen, O. and Strauss, E. (1998). *A compendium of neuropsychological tests* (2nd ed). New York: Oxford University Press

Starbucks (2011) *Nutrition by the cup* [leaflet]

Stavric, B., Klassen, R., Watkinson, B., Karpinski, K., Stapley, R., and Fried, P. (1988) 'Variability in caffeine consumption from coffee and tea: possible significance for epidemiological studies'. *Food and Chemical Toxicology*, 26:2, pp.111-118

Stavric, B., and Gilbert, S. G. (1990) 'Caffeine metabolism: a problem in extrapolating results from animal studies to humans'. *Acta Pharmaceutica Jugoslavica*, 40, pp.475-489

Stein, J., Luppá, M., Brähler, E., König, H.H. and Riedel-Heller, S. (2010). 'The Assessment of Changes in Cognitive Functioning: Reliable Change Indices for Neuropsychological Instruments in the Elderly – A systematic Review'. *Dementia and Geriatric Cognitive Disorders*, 29:3, pp. 275-286

Stern, Y. (2012) 'Cognitive reserve in ageing and Alzheimer's disease'. *The Lancet Neurology*, 11:11, pp.1006-1012

Strauss, E., Sherman, E.M.S and Spreen, O. (2006) '*A compendium of Neuropsychological tests: administration, norms and commentary*', 3rd edition, Oxford:University Press

Sunyer, B., Patil, S., Höger, H. and Lubec, G. (2007) 'Barnes Maze, a useful task to assess spatial reference memory in the mice'. *Nature Protocol Exchange*, 198, pp.58-68

Suzuki, M., Sugimura, Y., Yamada, S., Omori, Y., Miyamoto, M. and Yamamoto, J. (2013). 'Predicting Recovery of Cognitive Function Soon after Stroke: Differential Modelling of Logarithmic and Linear Regression'. *PLoS one*, 8:1, e53488

Swainson, R., Hodges, J.R., Galton, C.J., Semple, J., Michael, A., Dunn, B.D., Iddon, J.L., Robbins, T.W. and Sahakian, B.J. (2001) 'Early detection and differential diagnosis of Alzheimer's disease and depression with

neuropsychological tasks'. *Dementia and Geriatric Cognitive Disorders*, 12:4, pp.265-280

Sweetman, S.C. (2009) *Martindale the complete drug reference* (36th edition) London: Pharmaceutical Press

Swift, C.G. and Tiplady, B. (1988) 'The effects of age on the response to caffeine'. *Psychopharmacology*, 94:1, pp.29-31

Szekely, C., Thorne, J.E., Zandi, P.P, Messias, E., Breitner, J.C. and Goodman, S.I. (2004) 'Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review'. *Neuroepidemiology*, 23:4, pp.159-169

Tabert, M.H., Manly, J.J., Liu, X., Pelton, G.H., Rosenblum, S., Jacobs, M., Zamora, D., Goodkind, M., Bell, K., Stern, Y. and Devanand, D.P. (2006) 'Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment'. *Archives in Geriatric Psychiatry*, 63:8, pp.916-924

Tankova, I., Adan, A. and Buela-Casal, G. (1994) 'Circadian typology and individual differences. A review'. *Personality and Individual Differences*, 16:5, pp.671-684

Taragano, F.E., Lyketsos, C.G., Mangone, C.A., Allegri, R.F. and Comesana-Diaz, E. (1997) 'A double-blind, randomized, fixed-dose trial of fluoxetine vs. amitriptyline in the treatment of major depression complicating Alzheimer's disease'. *Psychosomatics*, 38:3, 246-252

Tariot, P.N., Farlow, M.R., Grossberg, G.T., Graham, S.M., McDonald, S. and Gergel, I. For the memantine study group. (2004) 'Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil. A randomized controlled trial'. *Journal of the American Medical Association*, 291:3, pp.317-324

Teng, E.L., Wimer, C., Roberts, E., Damasio, A.R., Eslinger, P.J., Folstein, M.F., Tune, L.E., Whitehouse, P.J., Bardolph, E.L. and Chui, H.C. et al. (1989) 'Alzheimer's dementia: performance on parallel forms of the dementia assessment battery'. *Journal of Clinical and Experimental Neuropsychology*, 11:6, pp.899-912

Teri, L., Gibbons, L.E., McCurry, S.M., Logsdon, R.G., Buchner, D.M., Barlow, W.E., Kukull, W.A., LaCroix, A.Z., McCormick, W. and Larson, E.B. (2003) "Exercise plus behavioral management in patients with Alzheimer disease: a randomized controlled trial." *Journal of the American Medical Association*, 290:15, pp.2015-2022

Tetwesky, S.J. and Duffy, C.J. (1999) 'Visual loss and getting lost in Alzheimer's disease'. *Neurology*, 52:5, pp.958-965

Theuns, J., Del-Favero, J., Dermaut, B., van Dulin, C.M., Backhovens, H., Van den Broeck, M.V., Serneels, S., Corsmit, E., Van Broeckhoven, C.V. and Cruts, M. (2000) 'Genetic variability in the regulatory region of Presenilin 1 associated with risk for Alzheimer's disease and variable expression'. *Human Molecular Genetics*, 9:3, pp.325-331

Thompson, J.C., Stopford, C.L., Snowden, J.S. and Neary, D. (2005) 'Qualitative neuropsychological performance characteristics in Frontotemporal dementia and Alzheimer's disease'. *Journal of Neurology, Neurosurgery and Psychiatry*, 76:7, pp.920-927

Tippett, W.J., Lee, J.H., Zakzanis, K.K., Black, S.E., Mraz, R. and Graham, S.J. (2009) 'Visually navigating a virtual world with real-world impairments: A study of visually and spatially guided performance in individuals with mild cognitive impairments'. *Journal of Clinical and Experimental Neuropsychology*, 31:4, pp.447-454

Tombaugh, T.N. (2004) 'Trail Making Test A and B: Normative data stratified by age and education'. *Archives of Clinical Neuropsychology*, 19:2, pp.203-214

Tombaugh, T.N. (2005) 'Test-retest reliable coefficients and 5-year change scores for the MMSE and 3MS'. *Archives of Clinical Neuropsychology*, 20:4, pp.485-503

Tombaugh, T.N. and McIntyre, N.J. (1992) 'The mini-mental state examination: a comprehensive review'. *Journal of the American Geriatrics Society*, 40:9, pp.92-935

Tong, X.K., Lecrux, C., Rosa-Neto, P. and Hamel, E. (2012) 'Age-dependent rescue by simvastatin of Alzheimer's disease cerebrovascular and memory deficits'. *The Journal of Neuroscience*, 32:14, pp.4705-4715

Tranah, G.J., Blackwell, T., Stone, K.L., Ancoli-Israel, S., Paudel, M.L., Ensrud, K.E., Cauley, J. A., Redline, S., Hillier, T.A., Cummings, S.R., Yaffe, K and SOF Research Group. (2011) 'Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women'. *Annals of Neurology*, 70:5, pp.722-732

Treiber, K.A., Carlson, M.C., Corcoran, C., Norton, M.C., Bretiner, J.C., Piercy, K.W., Deberard, M.S., Stein, D., Foley, B., Welsh-Bohmer, K.A., Frye, A., Lyketsos, C.G. and Tschanz, J.T. (2011) 'Cognitive stimulation and cognitive and functional decline in Alzheimer's disease: the Cache County Dementia Progression Study'. *The Journals of Gerontology, Series B Psychological Sciences and Social Sciences*, 66:4, pp.416-425

Trener, M.R., Crosson, B, DeBoe, J. and Leber, W.R. (1989) '*The Stroop Neuropsychological Screening Test*'. Odessa: Florida: Psychological Assessment Resources.

Tseng, Y.L., Chang, Y.Y., Liu, J.S., Su, C.S., Lai, S.L. and Lan, M.Y. (2009) 'Association of plasma homocysteine concentration with cerebral white matter hyper intensity on magnetic resonance images in stroke patients'. *Journal of Neurological Sciences*, 284:1-2, pp 36-39

Turner, S., Liffé, S., Downs, M., Wilcock, J., Bryans, M., Levin, E., Keady, J. and O'Carroll, R. (2004) 'General practitioners knowledge, confidence and attitudes in the diagnosis and management of dementia'. *Age and Ageing*, 33:5, pp.461-467

Uc, E.Y., Rizzo, M., Anderson, S.W., Shi, Q. and Dawson, J.D. (2004) 'Driver route-following and safety errors in early Alzheimer disease'. *Neurology*, 63:5, pp.832-837

Uneri, C., Sari, M., Akboğa, J. and Yüksel M. (2006) 'Vitamin E-coated tympanostomy tube insertion decrease the quantity of free radicals in tympanic membrane'. *Laryngoscope*, 116:1, pp.140–143

Usher, M., Cohen, J.D., Servan-Schreiber, D., Rajkowski, J. and Aston-Jones, G. (1999) 'The role of locus coeruleus in the regulation of cognitive performance'. *Science*, 283:5401, pp.549-554

Uylings, H.B. and de Brabander, J.M. (2002) 'Neuronal changes in normal human aging and Alzheimer's disease'. *Brain and Cognition*, 49:3, pp.268-276

Valcour, V.G., Masaki, K.H., Curb, J.D. and Blanchette, P.L. (2000) 'The detection of dementia in the primary care setting'. *Archives of Internal Medicine* 160, pp.2964-2968

Valenzuela, M.J. and Sachdev, P: (2009) 'Can cognitive exercise prevent the onset of dementia? Systematic review of randomised clinical trials with longitudinal follow-up'. *American Journal of Geriatric Psychiatry*, 17:3, pp.179-187

van de Pol, L.A., Verhey, F., Frisoni, G.B., Tsolaki, M., Papapostolou, P., Nobili, F., Wahlund, L.O., Minthon, L., Frölich, L., Hampel, H., Soininen, H., Knol, D.L., Barkhof, F., Scheltens, P. and Visser, P.J. (2009) 'White matter hyperintensities and medial temporal lobe atrophy in clinical subtypes of mild cognitive impairment: the DESCRIPA study'. *Journal of Neurology, Neurosurgery and Psychiatry*, 80:10, pp.1069-1074

van der Cammen, T.J., Croes, E.A., Dermaut, B., de Jager, M.C., Cruts, M., Van Broeckhoven, C. and van Duijn, C.M. (2004) 'Genetic testing has no place as a routine diagnostic test in sporadic and familial cases of Alzheimer's disease'. *Journal of the American Geriatrics Society*, 52:12, pp.2110-2113

Van der Heijden, K.B., deSonneville, L.M.J. and Althaus, M. (2010) 'Time-of day effects on cognition in preadolescents: a trails study'. *Chronobiology International*, 27(9-10), pp.1870-1894

van de Rest, O., Geleijnse, J.M., Kok, F.J., van Staveren, W.A., Hoefnagels, W.H., Beekman, A.T. and de Groot, L.C. (2008) 'Effect of fish-oil supplementation on mental well-being in older subjects: a randomized, double-blind, placebo-controlled trial'. *American Journal of Clinical Nutrition*, 88:3, pp.706-713

van de Rest, O., Spiro, A., Krall-Kaye, E., Geleijnse, J.M., de Groot, L.C.P.G.M. and Tucker, K.L. (2009) 'Intakes of (n-3) Fatty Acids and Fatty Fish Are Not Associated with Cognitive Performance and 6-Year Cognitive Change in Men Participating in the Veterans Affairs Normative Aging Study'. *The Journal of Nutrition*, 139:12, pp.2329-2336

van Gelder, B., Bruijsse, B., Tijhuis, M., Kalmijn, S., Giampaoli, S., Nissinen, A. and Kromhout, D. (2007) 'Coffee consumption is inversely associated with cognitive decline in elderly European men: the FINE study'. *European Journal of Clinical Nutrition*, 61:2, pp.226-232

Varon, D., Loewenstein, D.A., Potter, E., Greig, M.T., Agron, J., Shen, Q., Zhao, W., Celeste Ramirez, M., Santos, I., Barker, W., Potter, H. and Duara, R. (2011) 'Minimal atrophy of the entorhinal cortex and hippocampus: progression of cognitive impairment'. *Dementia and Geriatric Cognitive Disorders*, 31:4, pp.276-83

Velayudhan, L., Proitsi, P., Westman, E., Muehlboeck, J.S., Mecocci, P., Vellas, B., Tsolaki, M., Kloszewska, I., Soininen, H., Spenger, C., Hodges, A., Powell,

J., Lovestone, S. and Simmons, A. (2012) 'Entorhinal Cortex Thickness Predicts Cognitive Decline in Alzheimer's Disease'. *Journal of Alzheimer's Disease*, 33:3, pp.755-766

Verhaeghen, P., and De Meersman, L. (1998). 'Aging and the Stroop effect: a meta-analysis'. *Psychology and Aging*, 13:1, pp.120-126

Volicer, L., Harper, D.G., Manning, B.C., Goldstein, R. and Satlin, A. (2001) 'Sundowning and Circadian Rhythms in Alzheimer's Disease'. *American Journal of Psychiatry*, 158, pp.704-711

von Arnim, C.A.F., Gola, U. and Biesalski, H.K. (2010) 'More than the sum of its parts? Nutrition in Alzheimer's Disease'. *Nutrition*, 26(7-8), pp.694-700

Voytko, M.L. (1996) 'Cognitive functions of the basal forebrain cholinergic system in monkeys: memory or attention?' *Behavioural Brain Research*, 75:1-2, pp.13-25

Waldorff, F.B., Siersma, V., Vogel, A. and Waldemar, G. (2012) 'Subjective memory complaints in general practice predicts future dementia: a 4-year follow-up study'. *International Journal of Geriatric Psychiatry*, 27:11, pp.1180-1188

Walters, E.R. and Lesk, V.E. 'The effect of prior caffeine consumption on neuropsychological test scores in the elderly'. Article in prep - a

Walters, E.R. and Lesk, V.E. 'The effect of time of day on cognitive performance in the over 60's'. Article in prep – b

Walters, E.R., Womble, S.P. and Lesk, V.E. (2011) The effect of white fish intake on cognitive performance in the over 60s. *In* British Neuropsychological Society Conference. November 2011.

Wang, J-Z. and Liu, F. (2008) 'Microtubule-associated protein tau in development, degeneration and protection of neurons'. *Progress in Neurobiology*, 85:2, pp.148-175

Wan Shamsuddin, S., Ugail, H. and Lesk, V. (2011a) Development and Usability Evaluation of Virtual Environment for Early Diagnosis of Dementia'. Proceedings of the 2nd Visual Informatics: Sustaining Research and Innovations, Malaysia. Springer

Wan Shamsuddin, S., Walters, E., Ugail, H. and Lesk, V. (2011b) Evaluation of Users Acceptance in Virtual Environment for Early Diagnosis of Dementia. (2011b) Paper Presented at World Conference on Information Technology. Antalya Turkey

Ward, S.L., Newcome, N. and Overton, W.F. (1986) 'Turn left at the church, or three miles north: A study of direction giving and sex differences'. *Environment and Behaviour*, 18, pp.192-213

Wechsler, D. (1981) *Manual for the Wechsler Adult Intelligence Scale – Revised*. New York: Psychological Corporation

Weinert, D. (2010) 'Circadian temperature variation and ageing'. *Ageing Research Reviews*, 9:1, pp.51-60

Weintraub, S., Wickland, A.H. and Salmon, D.P. (2012) 'The neuropsychological profile of Alzheimer disease'. *Cold Spring Harbor Perspectives in Medicine*, 2:4, a006171

Weniger, G., Ruhleder, M., Lange, C., Wolf, S. and Irle, E. (2011) 'Egocentric and allocentric memory as assessed by virtual reality in individuals with amnesic mild cognitive impairment'. *Neuropsychologia*, 49, pp.518-527

Wennberg, M., Tornevi, A., Johansson, I., Hörnell, A., Norberg, M. and Bergdahl, I.A. (2012) 'Diet and lifestyle factors associated with fish consumption

in men and women: a study of whether gender differences can result in gender-specific confounding'. *Nutrition Journal*, 11:101

Werner, P., Rabinowitz, S., Klinger, E., Korczyn, A.D. and Josman, N. (2009) 'Use of the Virtual Action Planning Supermarket for the Diagnosis of Mild Cognitive Impairment'. *Dementia and Geriatric Cognitive Disorders*, 27:4, pp.301-09

Werner, P., Karnieli-Miller, O. And Eidelman, S. (2013) 'Current knowledge and future directions about the disclosure of dementia: A systematic review of the first decade of the 21st century'. *Alzheimer's & Dementia*, 9:2, e74-88

West, R., Murphy, K.J., Armilio, M.L., Craik, F.I. and Stuss, D.T. (2002) 'Effects of time of day on age differences in working memory'. *Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, 57, pp.3-10

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T. and Delon, M.R. (1982). 'Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain'. *Science*, 215:4537, pp.1237-1239

Whitwell, J.L., Shiung, M.M., Przybelski, S.A., Weigand, S.D., Knopman, D.S., Boeve, B.F., Petersen, R.C. and Jack, C.R. (2007) 'MRI patterns of atrophy associated with progression to AD in amnesic mild cognitive impairment'. *Neurology*, 70:7, pp.512-520

Widmann, C.N., Beinhoff, U. and Riepe, M.W. (2012) 'Everyday memory deficits in very mild Alzheimer's disease'. *Neurobiology of Aging*, 33:2, pp.297-303

Wilcock, D.M. (2012) 'A Changing Perspective on the Role of Neuroinflammation in Alzheimer's Disease'. *International Journal of Alzheimer's Disease*, 495293

Wilcox, K.C., Lacor, P.N., Pitt, J. and Klein, W.L. (2011) 'A β Oligomer- Induced Synapse Degeneration in Alzheimer's Disease'. *Cell and Molecular Neurobiology*, 31: 6, pp.939-948

Wild, K., Howieson, D., Webbe, F., Seelye, A. and Kaye, J. (2008) 'Status of computerized cognitive testing in aging: A systematic review'. *Alzheimer's & Dementia*, 4, pp. 428-437

Wilson, R.S., Mendes de Leon, C.F., Barnes, L.L., Schneider, J.A., Bienias, J.L., Evans, D.A. and Bennett, D.A. (2002) 'Participation in Cognitively Stimulating Activities and Risk of Incident Alzheimer Disease'. *Journal of the American Medical Association*, 287:6, pp.742-748

Wilson, R.S., Arnold, S.E., Schneider, J.A., Boyle, P.A., Buchman, A.S. and Bennett, D.A. (2009) 'Olfactory impairment in presymptomatic Alzheimer's disease'. *Annals of the New York Academy of Science*, 1170, pp.730-735

Wilson, R.S. Barnes, L.L., Aggarwal, N.T., Boyle, P.A., Hebert, L.E., Mendes de Leon, C.F. and Evans, D.A. (2010) 'Cognitive activity and the cognitive morbidity of Alzheimer disease'. *Neurology*, 75:11, pp.990-996

Winblad, B. and Poritis, N. (1999) 'Memantine in severe dementia: results of the 9M-BEST Study (benefit and efficacy in severely de-mented patients during treatment with memantine)'. *International Journal of Geriatric Psychiatry*, 14:2, pp.135-146

Winblad, B., Gauthier, S., Scinto, L., Feldman, H., Wilcock, G.K., Truyen, L., Mayorga, A.J., Wang, D., Brashear, H.R. and Nye, J.S. MD, J. S. and The GAL-INT-11/18 Study Group (2008) 'Safety and efficacy of galantamine in subjects with mild cognitive impairment'. *Neurology*, 70:22, pp.2024-2035

Winocur, G. and Hasher, L. (2002) 'Circadian Rhythms and Memory in Aged Humans and Animals'. In Squire, L. and Schacter, D., eds, *Neuropsychology of Memory*, 3rd Edition. New York: Guilford Publishers, pp.273-285

Winocur, G. and Hasher, L. (2004) 'Age and time-of-day effects on learning and memory in a non-matching-to-sample test'. *Neurobiology of Aging*, 25:8, pp. 1107-1115

Wnuk, A., Korol, D.L. and Erickson, K.I. (2012) 'Estrogens, hormone therapy, and hippocampal volume in postmenopausal women'. *Maturitas*, 73:3, pp.186-190

Wright, K.P., Lowry, C.A. and LeBourgeois, M.K. (2012) 'Circadian and wakefulness-sleep modulation of cognition in humans'. *Frontiers in Molecular Neuroscience*, 5:50

Wright, K.P. (JR), Bogan, R.K. and Wyatt, J.K. (2013) 'Shift work and the assessment and management of shift work disorder (SWD)'. *Sleep Medicine Reviews*, 17:1, pp.41-54

Wu, Y.H. and Swaab, D.F. (2007) 'Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease'. *Sleep Medicine*, 8:6, pp.623-636

www.mocatest.org.

Yaffe, K., Barnes, D., Nevitt, M., Lui, L.Y. and Covinsky, K. (2001) 'A prospective study of physical activity and cognitive decline in elderly women: Women who walk'. *Archives of International Medicine*, 161:14, pp.1703-1708

Yang, L., Krampe, R.T. and Baltes, P.B. (2006). 'Basic forms of cognitive plasticity extended into the oldest-old: retest learning, age, and cognitive functioning'. *Psychology and Aging*, 21:2, pp.372-378

Yang, L., Hasher, L. and Wilson, D.E. (2007) 'Synchrony effects in automatic and controlled retrieval'. *Psychonomic Bulletin and Review*, 14:1, pp.51-56

Yang, X. (2010) 'A wheel of time: the circadian clock, nuclear receptors and physiology'. *Genes and Development*, 24:8, pp.741-747

Yoon, C., May, C.P. and Hasher, L. (1999) 'Aging, circadian arousal patterns and cognition'. In Park, D and Schwartz, N., eds, *Cognitive aging: A primer*. Psychology Press, pp.151-170

Yu, W. and Lu, B. (2012) 'Synapses and dendritic spines as pathogenic targets in Alzheimer's disease'. *Neural Plasticity*, 247150

Yurko-Mauro, K., McCarthy, D., Rom, D., Nelson, E.B., Ryan, A.S., Blackwell, A., Salem Jr, N., Stedman, M. on behalf of the MIDAS Investigators (2010) 'Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline'. *Alzheimer's and Dementia*, 6:6, pp.456-646

Zacks, R.T. and Hasher, L. (1994) 'Directed ignoring: Inhibitory regulation of working memory. In Dagenbach, D. and Carr, T., eds. *Inhibitory mechanisms in attention, memory, and language*. New York: Academic Press, pp.241-264

Zamrini, E., De Santi, S. and Tolar, M. (2004) 'Imaging is superior to cognitive testing for early diagnosis of Alzheimer's disease'. *Neurobiology of Aging*, 25:5, pp.685–691

Zeitlin, R., Patel, S., Burgess, S., Arendash, G.W. and Echeverria, V. (2011) 'Caffeine induces beneficial changes in PKA signaling and JNK and ERK activities in the striatum and cortex of Alzheimer's transgenic mice'. *Brain Research*, 1414, pp.127-136

Zhou, J.N., Liu, R.Y., Kamphorst, W., Hofman, M.A. and Swaab, D.F. (2003) 'Early neuropathological Alzheimer's changes in aged individuals are accompanied by decreased cerebrospinal fluid melatonin levels'. *Journal of Pineal Research*, 35:2, pp.404-409

Appendix 1: Lifestyle and Dietary Questionnaire

This questionnaire will assess lifestyle and dietary intake of certain foods and drinks. Please answer the following questions by placing a tick in the box or filling in the space provided. If you are uncomfortable with any of the questions and do not wish to answer specific ones you are not obliged to do so. Please complete the section below

Age:

Height:

Gender:

1. Please indicate which of the following applies to you and how many years this applies.

- Married
- Living with partner
- Widowed
- Single
- Divorced

2. Please state your ethnic origin.....

3. What type of diet do you eat?

- Vegetarian (Please state the number of years you have been a vegetarian.....)
- Vegan (no dairy or meat products)
- Meat eater

4. How many years approximately have you spent in the education system?
.....

5. Do you have a family history of any of the following conditions/ have you ever suffered from any of the following?

	Family History?		Suffering from?	
	Yes	No	Yes	No
Depression				
Stroke				
Alzheimer's Disease				
Epilepsy				
High Cholesterol				
Arthritis				
Diabetes				
Heart Disease				
Hypothyroidism				
Food Allergy Please state to what foods.....				

6. Have you ever suffered from a head injury and were you unconscious? If so please state when this occurred.

.....
.....
.....

7. Please state below if you are currently taking any prescribed medication and what medical condition this is for.....

.....

8. Are you currently taking any vitamins, minerals or other natural supplements?

- Yes
- No
- Sometimes

If so please list supplements including brand and dose.....

9. Are you currently taking any antibiotics?

- Yes
- No

10. Are you currently physically active?

- Yes
- No
- Sometimes

If yes please state the type of exercise you carry out and how many hours of this exercise you undertake per week.....

.....

11. How much water, excluding tea and coffee, do you drink each day?

- 1-2 glasses
- 3-5 glasses
- 6 or more glasses

12. How many alcoholic beverages do you consume on a weekly basis?

- More than five
- 3-4
- 2 or fewer
- None

13. How many cigarettes do you smoke per day?

- More than twenty
- Ten to twenty

- Five to Nine
- Less than Five
- None

14. Please indicate which of the following activities you carry out and state whether this is rarely, sometimes or often.

	Rarely	Sometimes	Often
Reading the newspaper			
Reading a book/novel			
Puzzles such as crosswords			
Sewing or Knitting			
Watch Television			
Play games such as cards or bingo			

15. How many portions of fresh, tinned or frozen fruit do you eat each day? One portion is the equivalent of handful of grapes or one apple etc.

- More than four
- One –three
- One or fewer

16. How many portions of fresh or frozen vegetables do you eat each day? One portion is equivalent to a handful of peas or three florettes of broccoli.

- More than six
- 4-6
- 2-4
- 1 or less

17. Please complete the table below stating how many of the following foods you eat per day, month and week.

	Per Day	Per Week	Per Month
Eggs			
Milk in Pints			
Cups of caffeinated coffee			
Cups of Caffeinated Tea			
Cups of caffeinated green tea			
Salmon			

Sardines			
Mackerel			
Trout			
Herring			
Cod/ Haddock			
Tinned Tuna			
River Cobbler/ Basa			
Shell Fish			
Chocolate – including biscuits, cake and bars			
Chicken			
Red Meats – such as beef			
Take away foods/ Processed food			
Turmeric – an ingredient found in curry			
Walnuts			
Brazil Nuts			
Almonds			
Peanuts			
Hazelnuts			

18. What type of cooking oil do you use?

- Supermarket own brand
- Vegetable Oil
- Olive Oil
- Extra virgin olive oil
- Sun Flower Oil

19. Please state in the space provided which cooking method you use when preparing fish

.....

Thank you for taking the time to complete this questionnaire

Appendix 2: Usability Questionnaire

Personal Details.

Age	:	
Gender	:	
Previous Virtual Reality Experience	:	Yes <input type="checkbox"/> No <input type="checkbox"/>

Please indicate with a number from 1 to 5 whether you agree with the following statements. 1 = Completely disagree, 5 = Completely agree

1. I had quickly learned how to move through the virtual application.						
Completely disagree	1	2	3	4	5	Completely agree
2. I found it easy to move myself through the virtual application.						
Completely disagree	1	2	3	4	5	Completely agree
4. The Instructions for the tasks are clear.						
Completely disagree	1	2	3	4	5	Completely agree
5. I liked using the arrow keys to move around the application.						
Completely disagree	1	2	3	4	5	Completely agree
6. I often feel disoriented in using the application.						
Completely disagree	1	2	3	4	5	Completely agree
7. It was easy for me to look around in the virtual application.						
Completely disagree	1	2	3	4	5	Completely agree
8. I would have preferred to have more freedom of movement.						
Completely disagree	1	2	3	4	5	Completely agree
9. The speed of movement was too fast.						
Completely disagree	1	2	3	4	5	Completely agree
10. Overall, I feel comfortable using the application.						
Completely disagree	1	2	3	4	5	Completely agree
11. Overall, I am satisfied with the experience of using the application.						
Completely disagree	1	2	3	4	5	Completely agree