The Diagnostic Potential of the Olfactory Stress Test in Alzheimer's Disease

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Declaration

I certify that the work in this thesis entitled *The Diagnostic Potential of the Olfactory Stress Test in Alzheimer's Disease* has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself has been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. The research presented in this thesis was approved by the Hunter New England Human Research Ethics Committee on 15/07/2010 (reference number: 10/06/16/5.06) and the Macquarie University Human Research Ethics Committee on 08/03/2012 (reference number: 5201200121).

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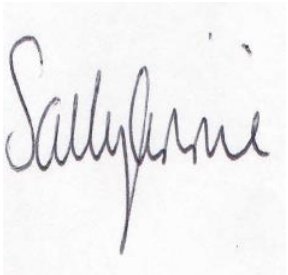

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Abstract

Olfactory deficits are a common symptom of Alzheimer's disease (AD) and have been investigated as a potential biomarker for the early detection of this condition. One limitation has been the ability of olfactory tests to differentiate olfactory impairment associated with AD from those seen in other neurological conditions. The Olfactory Stress Test (OST) utilises the University of Pennsylvania Smell Identification Test (UPSIT) to measure olfactory ability before and after intranasal administration of the anticholinergic drug atropine. Cholinergic deficits are a central feature in emerging AD, and a change in UPSIT score or *atropine effect* stands to represent a sensitive measure of incipient decline. In this study the OST was explored in participants with AD (n=10), Parkinson's disease (PD; n=13), Vascular Cognitive Impairment (VCI; n=11) and cognitively unimpaired older adults (Controls; n=25). Each participant also underwent neuropsychological evaluation, volumetric imaging of the hippocampi and apolipoprotein E genotyping. The AD group demonstrated a greater atropine effect than the control group or PD group, and there was no difference evident between the control, PD, and VCI group. A significant correlation was found between the atropine effect and a non-memory composite neuropsychological score. The differential effect of atropine on olfactory performance in people with AD in comparison with controls and other neurological conditions points to a potential diagnostic advantage of the OST over standard tests of olfaction. However, the significant effect of gender and the variance in the atropine effect seen in each group, makes its utility as a diagnostic tool uncertain. Methodological issues remain and longitudinal studies are required to evaluate whether the OST can be used to detect preclinical AD with adequate specificity.

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1. Chapter One - General Introduction

Alzheimer ’s disease (AD) is a devastating neurodegenerative condition that involves biological changes to the brain long before clinical symptoms appear. Diagnostic tools have been developed to detect AD in its earliest form with the hope that future treatments can be prescribed before brain damage is irreparable. People with AD perform significantly worse on tests of olfaction than age and sex-matched individuals and performance on tests of olfactory function have been shown to be a sensitive measure of early AD. However, their usefulness as a diagnostic tool has been limited given that reduced sense of smell is common amongst older adults and also an early feature of many other neurodegenerative disorders.

In 2012, the Olfactory Stress Test (OST) was introduced as a novel method to unmask biological changes characteristic of AD pathology (Schofield et al., 2012). Rather than measure olfaction per se, the OST measures the effect of a cholinergic challenge on olfactory ability. In developing a biological marker (biomarker), it is essential to understand how the OST works in a sample of cognitively intact older adults, as well as in people with AD. The OST must also be able to differentiate people with AD from those patients with other pathology. As such, the OST will be explored in four groups: cognitively unimpaired older adults, people with a diagnosis of probable AD, people with Parkinson’s disease (PD) and finally people with Vascular Cognitive Impairment (VCI). PD was chosen as a suitable patient comparison group because PD has long been associated with olfactory impairments similar in type and severity to AD. A challenge for researchers has been to develop a measure of olfaction that may be able to differentiate these conditions. VCI was chosen as the second comparison group to investigate the clinical utility of the OST. The differential diagnosis of VCI and clinical

AD can be challenging due to the similarity of clinical symptoms in these conditions and the development of a simple, non-invasive test would hold many benefits.

To set the context for the OST, Chapter Two provides a general introduction to AD, with a focus on the cholinergic hypothesis of Alzheimer's pathophysiology. The importance of early detection of AD and the status of current biomarker research is then discussed. In Chapter Three, the human olfactory system is described, including a review of olfactory impairment, and the olfactory deficits seen during the course of AD as compared with those seen in PD and VCI. Chapter Three also explores the utility of olfactory tests as a diagnostic tool in these neurological conditions. Chapter Four provides a detailed description of the OST including its biological rationale and use in previous research. In Chapter Five, the specific aims of the thesis are presented, followed in Chapter Six by the methodology and characterisation of the study sample. The results are presented in Chapter Seven. In the discussion, in Chapter Eight, the implications of the current study are explored and limitations of the study are set out as well as ideas for future research.

2. Chapter Two – Alzheimer's disease

2.1 Definition and Diagnosis

Dementia is a syndrome, usually chronic, that is characterised by progressive global deterioration of cognitive functions, including memory, learning, orientation, language and judgement, which interfere with social and occupational functioning. It is frequently accompanied by neuropsychiatric symptoms, such as depression, psychosis or behavioural problems (Kelley & Petersen, 2007).

The most common cause of dementia is AD, which was first described by Alois Alzheimer in 1906. AD is a neurodegenerative brain disease, clinically characterised by cognitive deficits in memory, executive functioning and language. There are associated impairments in activities of daily living and a range of behavioural and psychological symptoms. Olfactory impairments are common and constitute an early feature of the disease{Doty, 2001 #82;Serby, 1991 #154}. The majority of AD cases are sporadic, thought to be caused by a complex interplay between genetic and environmental factors (Yan & Feng, 2004).

The most widely used clinical diagnostic criteria for AD are defined in the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision (DSM-IV-TR). Using this system, a diagnosis of *definite* AD can only be made post mortem, when the neuropathological features of the illness can be identified. However, a diagnosis of *probable AD* is made when the patient has measurable memory deficit, plus a deficit in at least one other cognitive domain, which causes significant impairment in social or occupational functioning and represents a significant decline from a previous level of functioning. The cognitive deficit must be characterised by gradual onset and progressive decline and not be accounted for by other central nervous system

conditions, systemic conditions, substance induced conditions, or occur exclusively during the course of a delirium**.**

it is now widely acknowledged that the pathophysiological process of AD begins decades before clinical signs of dementia become apparent and three stages of AD have been identified (Sperling et al., 2011; Villemagne et al., 2013).The International Working Group for new Research Criteria for the Diagnosis of AD and more recently, working groups from the National Institute on Aging (NIA) and Alzheimer's Association (AA) have championed efforts to conceptualise AD as a progressive sequence of pathophysiological stages. The NIA-AA consortium proposed revised diagnostic criteria for clinical diagnosis of *dementia due to AD* (McKhann et al., 2011), MCI (Albert et al., 2011) and research criteria for the preclinical stages of AD (Sperling et al., 2011). These criteria are aimed at detecting evidence of underlying AD pathology in the preclinical phase and rely on evidence from biological markers or a proven AD autosomal dominant mutation. In the *preclinical* stage biological changes can be detected in the absence of any clinical symptoms. In the *prodromal* phase, mild cognitive impairment (MCI) becomes apparent on neuropsychological assessment, in the absence of any changes to functional ability. As the disease progresses, cognitive deficits become more severe, difficulties arise with day-to-day living and behavioural changes such as apathy, restlessness, irritability, agitation, aggression and wandering may develop. At this stage a diagnosis of *dementia* (probable AD) can be made. It has been estimated that patients with MCI progress to dementia at a rate of 10% to 15% per year (Bruscoli & Lovestone, 2004; Luis, Loewenstein, Acevedo, Barker, & Duara, 2003), compared with healthy older adults who develop dementia at a rate of one to two percent (Petersen et al., 2001).

While this three stage model of AD is widely accepted, its clinical utility remains challenging. To some extent, the labels of presymptomatic and prodromal AD are

hypotheses rather than a statement of fact, because some of these individuals will die without ever expressing clinical symptoms (Jack et al., 2010). The hypothetical assumption is that an asymptomatic individual with pathological changes indicative of AD would ultimately become symptomatic should they live long enough. Studying older adults without dementia longitudinally and examining predictors of the onset of AD may help identify individuals at high risk and is an area of abundant research (Ewers, Sperling, Klunk, Weiner, & Hampel, 2011; Modrego, 2006; Rabin et al., 2012; Rowe et al., 2013; Shim & Morris, 2011). Despite the need for evidence based models for predicting AD, there is disagreement regarding the independent and joint contribution of various biological and cognitive assessments (Rabin et al., 2012). Furthermore, the ethics and utility of providing someone with a hypothetical diagnosis is debated, particularly in the absence of disease modifying agents.

2.2 Prevalence

According to the International World Alzheimer's Report in 2009, there are currently 35.6 million people living with dementia worldwide, around 60% of these have AD. This is expected to increase to 115.4 million by 2050. In Australasia, there were 310,000 people living with dementia in 2005 (Brodaty, Sachdev, & Anderson, 2005; Ferri et al., 2005). Given the prevalence and degenerative nature of AD and other dementias they represent a major public health issue.

Prevalence of dementia in adults over the age of 85 is high, ranging from 40% to 60% and advancing age is the single most important risk factor for AD (Bondi et al., 2008). Females carry an increased risk of devloping AD compared to males and show faster decline and greater deterioration of cognition than males (Callahan et al., 2001; Dye, Miller, Singer, & Levine, 2012, Proust-Lima et al., 2008).

2.3 Pathological Features of Alzheimer's Disease

Post mortem investigations described by Alzheimer identified plaques, neurofibrillary tangles, and atherosclerotic changes and these remain the hallmark pathological features of the disease (Maurer, Volk, & Gerbaldo, 1997). In addition to these abnormalities at the cellular level, there is widespread degeneration (atrophy) of neurons in many brain regions including the frontal and temporal cortices, hippocampus and the basal forebrain. These are accompanied by neurochemical changes and synaptic loss (Terry et al., 1991). More recently the role of tau and prion proteins, oxidative stress, inflammation, and mitochondrial function have also been explored (Henry et al., 2013). For the purposes of this thesis, the cholinergic hypothesis of AD will be described.

2.4 The cholinergic hypothesis of Alzheimer's Disease.

One of the first theories of AD pathophysiology was the cholinergic hypothesis (Bartus, Dean, Beer, & Lippa, 1982; Contestabile, 2011). In a functioning system, the enzyme choline acetyltranferase (ChAT) converts choline into acetylcholine (ACh) in presynaptic nerve terminals. ACh is then broken down by the enzyme acetylcholinesterase (AChE) into the inactive metabolites choline and acetate, thus removing ACh from the system.

One of the most fundamental and consistent features of AD is the severe degeneration of cholinergic neurons projecting from the basal forebrain to the cortex and hippocampus (Contestabile, 2011; Small, Michaelson, & Sberna, 1996; Younkin et al., 1986). In contrast with the depletion of ACh, other transmitters such as serotonin, norepinephrine and dopamine, do not show such a significant decrease (Kalaria, Kroon, Grahovac, & Perry, 1992). ChAT, a reliable marker of cholinergic neurons, is also remarkably decreased in pathological samples from the cortex and hippocampus

of AD patients (Bartus et al., 1982; Davies & Maloney, 1976; Perry, Gibson, Blessed, Perry, & Tomlinson, 1977). Further support comes from the finding that cholinergic neurons show neurofibrillary degeneration and cell volume loss in the early stages of AD (Mesulam, Shaw, Mash, & Weintraub, 2004; Sassin et al., 2000).

Central to the cholinergic hypothesis is the role of the cholinergic system in cognitive functioning. Drugs such as scopolamine and atropine that reduce ACh activity can induce Alzheimer-like cognitive deficits in healthy volunteers. In contrast, drugs that increase ACh activity have been shown to reverse scopolamine induced cognitive deficits (Bartus, 1978; Mewaldt & Ghoneim, 1979), and delay progression to AD (Lu et al., 2009; Petersen et al., 2005).

Cholinergic replacement therapy with acetylcholinesterase inhibitors (AChEIs) such as donepezil, galantamine, rivastigmine and tacrine are approved for the *symptomatic* treatment of AD (Ballard et al., 2011). Clinical trials have reported small improvements in cognition and global functioning over a six to twelve month period. These changes are short-lived and patients demonstrate a persistent decline in cognitive functioning over time (Ballard et al., 2011; Doody et al., 2001). Furthermore, not all people with AD show improvements with AChEIs and the differences between 'responders' and 'nonresponders' remain unclear (Craig et al 2011).

There is some discrepancy in reports regarding when cholinergic deficits occur. Some research groups have reported that decreases in cortical cholinergic presynaptic markers occur late in the clinical manifestation of AD (Davis et al., 1999), however, recently cholinergic dysfunction has been detected early in the course of the disease, at the presymptomatic stage (Potter et al., 2011). These researchers found an inverse relationship between cholinergic activity and accumulation of cortical amyloid plaques, with reductions in cholinergic function associated with increased beta amyloid (Aβ)

deposition. This relationship was accentuated in subjects with neuropathologically confirmed AD.

The failure of AChEIs to cure AD and the unclear role of ACh in the aetiology of AD have posed challenges to the cholinergic hypothesis. Recent advocates have proposed a moderating role of cholinergic depletion in the aetiology of AD. For example, cholinergic depletion may lead to a reduction in the ability of the brain to compensate for secondary insults such as stroke or head trauma (Craig, Hong, & McDonald, 2011). Others suggest that cholinergic deficits may be just one of multiple risk factors that can lead to AD, and therefore the exact cause of AD may differ for each individual (McDonald, 2002).

2.5 Early Identification of Alzheimer's Disease Pathology

Early identification of preclinical Alzheimer's disease pathology is of great relevance for the development of disease modifying agents. Historically clinical trials have enrolled participants with a clinical diagnosis of AD, by which stage pathological damage may be irreversible (Albers, 2006). It is hoped that treatment might be more effective in the preclinical and prodromal phases of the disease when there may be more opportunities to reverse pathological changes (Albers, 2006). Recruitment to clinical trials is now being enriched by the use of biomarkers in an attempt to target participants in the preclinical and prodromal stages of the disease.

2.6 Biomarkers for the Early Diagnosis of Alzheimer's Disease

The idea of a *preclinical* phase of AD has resulted in a surge of research to identify biological markers of the disease. Biological markers, or biomarkers, are indicators of specific changes that characterise AD in vivo (Jack et al., 2010), that can be measured objectively (Atkinson et al., 2001). A suitable biomarker must have the ability to detect a fundamental feature of AD neuropathology, have a clear cut-off between normal and

abnormal results and be able to distinguish AD from other dementias. The predictive accuracy of a biomarker must be weighed against costs regarding patient burden and risk (e.g., radioactivity of PET tracers), invasiveness of procedure (lumbar puncture in cerebrospinal fluid analysis) and accessibility of the equipment (Hampel, 2012). Biomarkers must also be practical. Given the epidemiological data on the current and future prevalence of AD, they must be inexpensive and accessible to allow for the potential of mass screening. Biomarkers of AD do not reach abnormal levels simultaneously. Jack et al. (2010) formulated and recently updated a model which outlines the hypothetical temporal sequence of the five most widely studied biomarkers of AD pathology as can be seen in Figure 1. This model has been widely reproduced and serves as a graphical summary of the current status of dynamic biomarker research.

Figure 1 Timeline of the Biomarkers of AD.

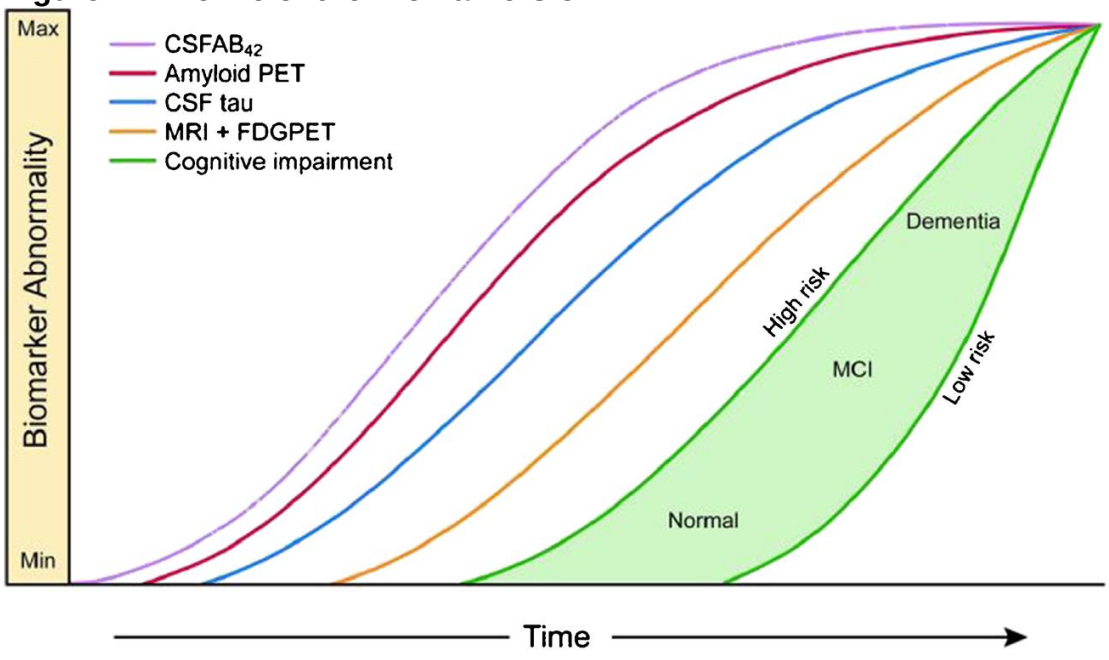


Figure 1 The revised dynamic biomarkers of the AD pathological cascade model – 2012 version. The hypothesised evolution of changes in several different biomarkers over time, relative to clinical disease onset. Source Schofield, Finnie, & Yong (2014). $CSFAB_{42}$ = Amyloid detected in cerebrospinal fluid (CSF) analysis; Amyloid PET = Amyloid detected in Positon Emission Tomography (PET); CSF Tau = Tau levels detected in CSF; FDGPET = flurodeoxyglucose PET.

2.6.1 The amyloid cascade hypothesis.

The amyloid cascade hypothesis suggests that deposition of Aβ triggers neuronal dysfunction in the brain. The amyloid precursor protein (APP) is processed into Aβ, which accumulates inside neuronal cells and extracellularly, and aggregates into neuritic plaques. Accumulation of soluble and insoluble Aβ peptide is implicated in the pathogenesis of AD and is considered by many to be the earliest pathological change, occurring one to two decades prior to cognitive impairment (Jack et al., 2013; Jack et al., 2010; Masters & Beyreuther, 2006; Pike et al., 2007). An imbalance between the production and clearance of Aβ in AD is supported by data from post mortem studies (Price & Morris, 1999) and in vivo assessment. Virtually all individuals with AD have high Aβ burden (Villemagne et al., 2011), compared with 50-60% of individuals with MCI (Wolk et al., 2009) and 30-40% of apparently healthy older adults (Pike et al., 2007; Rowe et al., 2007; Sperling et al., 2009). These figures have been used to predict the level of prodromal AD in the wider population.

In the original hypothesis, neuronal dysfunction was thought to be a result of the toxic effect of the total amyloid load, however it is now thought that specific changes in Aβ processing, such as the cleavage of APP into Aβ peptides may be of more importance (Ballard et al., 2011; Hardy, 1997; Mullan & Crawford, 1993).

The clinical evaluation of Aβ requires analysis of cerebrospinal fluid (CSF) or scanning with positron emission tomography (PET). CSF analysis reflects neurochemical changes arising from Aβ and has been shown to have a sensitivity and specificity of greater than 85% for discriminating between AD and normal ageing (Formichi, Battisti, Radi, & Federico, 2006). CSF is extracted via the process of lumbar puncture which is an invasive procedure and can result in side effects ranging from headache and infection to CSF leakage. Furthermore, the ability of CSF analysis to distinguish AD from dementia with Lewy Bodies, fronto temporal dementia and vascular

dementia is less certain with specificities of approximately 60% reported (Henry et al., 2013).

PET amyloid imaging can also be used to measure Aβ load. The Pittsburgh Compound-B (C-PiB), a carbon-11-labelled derivative of the thioflavin-T amyloid dye, that binds with high affinity and high specificity to neuritic Aβ plaques is the most commonly used (Klunk et al., 2004). Studies have indicated the utility of C-PIB in tracking progression from MCI to AD (Quigley, Colloby, & O'Brien, 2011). PET amyloid scanners are only available in some capital cities in Australia, and referral can be made by a specialist, however the cost is high and it is mainly used as a research tool rather than for clinical diagnosis.

Emerging evidence shows that Aβ has potent actions on the cholinergic system and might contribute directly to cholinergic deficits in AD (Auld et al., 1998; Yan & Feng, 2004). In vitro studies have shown that Aβ can reduce choline uptake, decrease the rate of ACh synthesis, and inhibit ACh release (Dolezal & Kasparova, 2003; Yan & Feng, 2004). Further evidence for the negative impact of Aβ on cholinergic function has come from mouse models. Transgenic animals have been created that overexpress mutant AD-related proteins such as the amyloid precursor protein (APP), presenilin PS1, and PS2. These mice exhibit many of the neuropathological and behavioural features of the human disease (German & Eisch, 2004). Relative to wild-type mice, these platelet-derived amyloid precursor protein *(*PDAPP) mice have significantly lower production of ACh. When injected with scopolamine, wild-type mice show a seven-fold increase in hippocampal ACh production, but this response is blunted in PDAPP mice suggesting levels of soluble Aβ may moderate response to cholinergic therapy (Bales et al., 2006).

2.6.2 Structural changes / atrophy.

Brain atrophy begins in limbic areas of the cerebral cortex, and then spreads in a largely predictable manner across the hippocampus, the neocortex and a number of subcortical nuclei (Braak & Braak, 1995; Hampel, 2012). Atrophy rates of 3-7% per year have been demonstrated in AD and the rate increases with disease progression (Jack et al., 1998; McEvoy et al., 2009). Brain atrophy has been shown to be present at least five years prior to the clinical diagnosis of AD (Jack et al., 2010; McEvoy et al., 2009). Significant increases in atrophy rates have been reported for cognitively normal controls who progress to MCI or AD at three-year follow-up, compared with those who remained stable (Jack et al., 2000).

Hippocampal volume is an established AD biomarker (Hampel et al., 2008). Measured by structural brain imaging, performed with magnetic resonance imaging (MRI) or computed tomography (CT), it is used extensively in clinical diagnosis and tracking of AD. High-resolution MRI allows detailed examination of brain structure in vivo, is widely available in urban centres and is minimally invasive. However, the use of MRI in the diagnosis of AD has been limited as there are no standardised values for brain volume that would establish the significance of a specific amount of atrophy for an individual person cross-sectionally. Therefore the main clinical utility of structural imaging is to assist with differential diagnosis and rule out other conditions such as tumours or hydrocephalus that may cause symptoms similar to AD but would require different treatment (Ballard et al., 2011).

2.6.3 Genetic testing.

Although not strictly a biomarker, the apolipoprotein E epsilon 4 (ApoE Ɛ4) allele on chromosome 19 represents the main genetic risk factor for AD (Michaelson, 2014; Ward et al., 2012). This gene has three allelic variants (2, 3 and 4) and five common

genotypes (2/3, 3/3, 2/4, 3/4, and 4/4). Carriers of two ApoE Ɛ4 alleles (homozygotes, 4/4) have a higher risk and earlier onset of AD than heterozygous carriers (Ahn Jo et al., 2006; Corder et al., 1994; Corder et al., 1993). In a recent meta-analysis, the prevalence of one ApoE Ɛ4 allele in AD was 48.7% (Ward et al., 2012). ApoE status can be assessed through a blood test and has become routine in research settings. However, ApoE Ɛ4 accounts for only part of the genetic risk for AD and does not provide a timeline for when clinical symptoms may occur, which make its clinical interpretation challenging.

2.6.4 Neuropsychological Functioning.

Although not strictly a biomarker, neuropsychological or cognitive markers make an important contribution to prediction models and have been recommended for use as a non-invasive screening method to be followed by biological assessments in those who screen positive (Rabin et al., 2012). Indeed, the ability to identify cognitive changes prior to clinical dementia led to the adaptation of the term MCI (Petersen et al., 2009). Information can be derived from neuropsychological testing, self-reports, and informant reports. A better understanding of the profile of neuropsychological deficits associated with early AD has resulted in earlier and more reliable clinical diagnosis (Salmon et al., 2002) and it has improved the ability to better detect the disease in people who are in a prodromal phase before the manifestation of obvious clinical symptoms (Jacobson, Delis, Bondi, & Salmon, 2002). Episodic memory is usually the first cognitive ability to decline during the prodromal and preclinical stage of AD, followed by decline in executive functioning, processing speed, attention, and semantic knowledge (Bondi et al., 2008). Considerable research suggests that memory performance may be poor but stable a number of years prior to the diagnosis of clinical AD but then decline rapidly in the year or two immediately preceding diagnosis (Smith et al., 2007). Numerous studies have shown cognitive deficits in people at risk of AD.

People with a family history of AD perform significantly worse on tests of episodic memory than people with no family history of AD (Caselli et al., 2004; La Rue, Matsuyama, McPherson, Sherman, & Jarvik, 1992; La Rue, O'Hara, Matsuyama, & Jarvik, 1995). Performance on tests of episodic memory is also sensitive to ApoE status: carriers of one or more ApoE Ɛ4 alleles perform worse than non-carriers (Bondi et al., 1995).

2.6.5 Other markers.

Total tau protein (T-tau) is thought to be a measure of axonal damage and neuronal degeneration associated with AD. T-tau can be measured using CSF analysis. Threefold increases in CSF T-tau in AD patients compared with controls have been reported (Blennow & Hampel, 2003). The combination of CSF T-Tau and Aβ provides 85% sensitivity and 86% specificity for discriminating between AD and controls, although its ability to discriminate AD from other dementias is weaker (Henry et al., 2013) and its utility is limited by the invasive nature of the lumbar puncture procedure.

Finally, functional imaging with flurodeoxyglucose positron emission tomography (FDG-PET) has been used to measure neuronal metabolism, thought to reflect synaptic loss. Studies have distinguished AD from healthy controls with 90% sensitivity and specificity (Bloudek, Spackman, Blankenburg, & Sullivan, 2011) and individuals with MCI who show this pattern are more likely to progress to AD. However, the ability to distinguish AD from other dementias is more variable (Modrego, 2006, Panegyres, Rogers, McCarthy, Campbell, & Wu, 2009).

2.7 Current State of Biomarkers

The detection of AD in its prodromal and preclinical stages remains challenging. Preclinical features of AD are silent and would therefore require mass screening of asymptomatic individuals, perhaps who fall within a high risk group. This is not a novel

concept, screening programmes have been successfully implemented for various conditions such as breast and cervical cancer.

While the field of biomarker research has advanced, there is still no clear test or group of tests, that can be used definitively to calculate the risk of an individual (as opposed to a population) developing AD, let alone provide beneficial information on the likely timeline of symptom onset or severity (Ellis et al., 2009). Even when the proposed marker is pathologically relevant, the sensitivity and specificity must be tested, and the differential diagnostic approach with biomarkers has only just begun (Andreasen et al., 2001; Arai, 1996; Blennow, 2004; Hampel, Goernitz, & Buerger, 2003; Sunderland et al., 2003). The majority of biomarkers are expensive, invasive and difficult to access, primarily available at urban clinical centres or research facilities within Western societies.

Recently, an alternative biomarker has been suggested for the detection of incipient AD. The findings that olfactory impairments are evident in the preclinical stages of AD, in people at risk of AD; and that there is a direct relationship between olfaction and AD pathology, has led researchers to investigate whether olfactory testing could be used as a potential biomarker. This biomarker is very attractive in that it would be non-invasive, low in cost and easily accessible through a GP surgery or memory clinic.

This chapter provided some important background into the nature of AD and its neuropathological features. It reviewed some of the diagnostic issues and challenges involved with early identification of AD and development of biomarkers to assist this process. It highlighted the potential role of olfaction as a biomarker for AD. The next chapter provides introductory information on the olfactory system and its assessment, and reviews the literature on olfactory impairments in AD and the current status of olfaction as a biomarker in this disease.

3. Chapter Three - Olfaction

Our sense of smell is responsible for a number of functions including enjoyment of food and perfumes, as well as recognition of hazards in the environment such as toxins, smoke, and spoiled food. Impairment of olfactory ability therefore has the potential to diminish quality of life (Blomqvist, Bramerson, Stjarne, & Nordin, 2004), affect food choices and may increase the risk for exposure to environmental hazards (Santos, Reiter, DiNardo, & Costanzo, 2004).

3.1 The Olfactory System

As with other senses, olfactory processing represents a hierarchy of processes ranging from simple sensory level processing such as the passive detection of a smell, through to more complex processing at the perceptual level (e.g., odour discrimination), and then more cognitively demanding processes of odour identification and odour memory (Zelano & Sobel, 2005). Studies mapping the olfactory processing network, which utilise electrophysiological techniques and functional neuroimaging, have identified a complex organisation of central brain structures involved in olfactory processing.

Odour detection is a sensory process that occurs when the molecules of an odourant bind to odour-receptors in the olfactory epithelium within the nasal cavity. This can occur passively through eating and drinking or actively through the nose.

The olfactory system is neuroanatomically unique, as the vast majority of projections remain ipsilateral, that is, stimuli presented to the left nostril are processed by the left hemisphere and stimuli presented to the right nostril are processed by the right hemisphere, with only minor contralateral projections via the anterior commissure (Shipley and Ennis 1996; Cinelli & Kauer, 1992).

Odour detection is a sensory process that occurs when the molecules of an odourant bind to odour-receptors in the olfactory epithelium within the nasal cavity. This can occur passively through eating and drinking or actively through the nose. Odour molecules bind to hair-like projections called cilia, which are found on primary olfactory neurons in the olfactory epithelium. The axons of these neurons travel through the cribriform plate to enter the central nervous system. A large multigene family encodes this information and the interaction of the right molecule with the right receptor causes the receptor to change its shape. This ‘conformational change’ gives rise to an electrical signal which is sent to the olfactory bulb, located at the orbital surface of the frontal lobes. Different odours are represented by distinct spatial activity patterns, much like a topographical map.

The olfactory bulbs are paired structures, located at the base of the brain over the cribriform plate. Similar to the retina, in terms of its neural processing, the olfactory bulbs have been viewed as the “olfactory thalamus” as they perform the final stage of sensory processing before information is sent to the cortex (Doty 2010). As a neural circuit, the olfactory bulbs have one source of sensory input (axons from olfactory receptor neurons of the olfactory epithelium), and one output (mitral cell axons) but they are highly connected. The piriform cortex sends projections to the amygdala, entorhinal cortex, dorsomedial prefrontal cortex and the insular cortex (Cinelli, Ferreyra-Moyano, & Barragan, 1987; Weismann et al., 2001). The entorhinal cortex provides most of its outputs to the hippocampus (Albers, 2006). The primary structures involved in olfactory perception are depicted in Figure 2.

The specific roles of each of these regions is still debated, however it is thought that the piriform cortex is probably the area most closely associated with identifying the

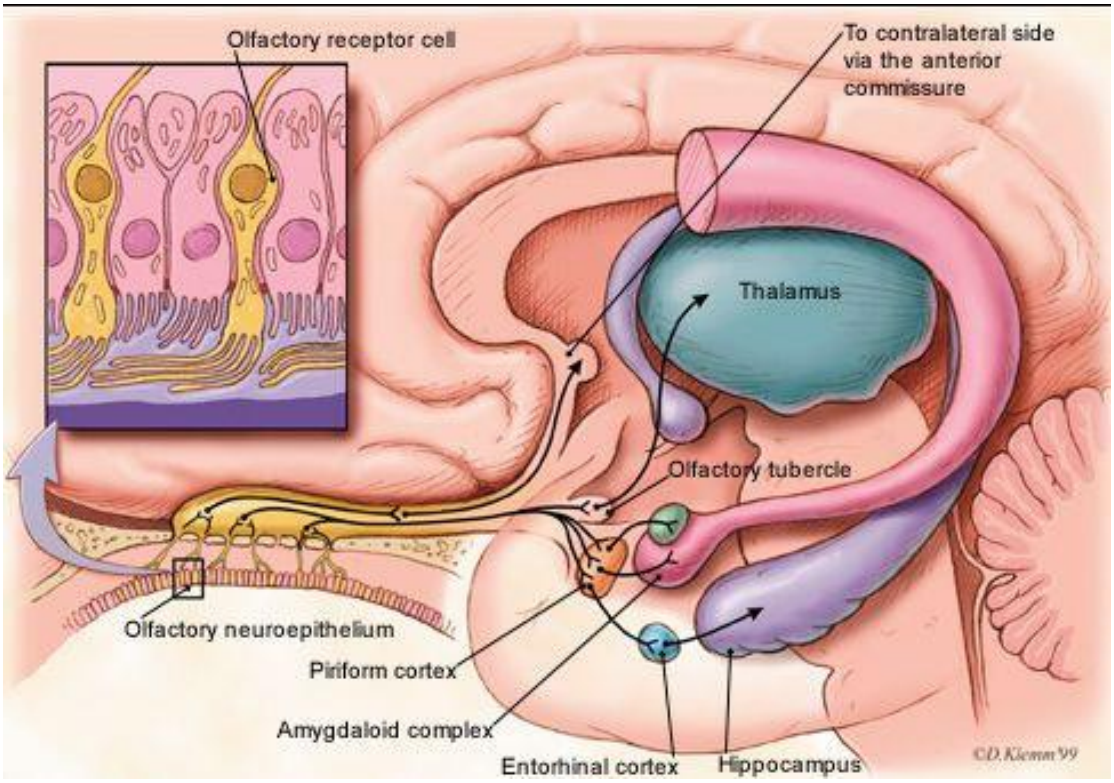
odour, the amygdala is involved in social olfactory functions and the entorhinal cortex is associated with olfactory memory.

3.2 Assessment of Olfactory Function

Many independently designed tests of olfaction exist ranging in scientific merit; however a few olfactory assessment batteries are commercially available. An advantage of commercially available tests is that they include clear administration instructions and normative data to enable comparison with age-matched peers.

Quantitative measurements of olfaction reflect the hierarchical nature of the olfactory system with assessments designed to capture functioning at the basic level of detection through to higher order abilities of odour identification. Several threshold tests are commercially available, including the Smell Threshold Test (Doty, 2000). Typically these tests involve the subject being presented with a series of low concentrate odourant with one or more blanks and being asked to identify which smells strongest. The absolute threshold is the lowest odourant concentration that can be reliable detected, which can then be compared against normative data.

Figure 2 The Olfactory System (Source: David Klemn, 2000)



Odour identification tests measure ability to correctly identify and name an odour. Richard Doty designed the first of these tests, the University of Pennsylvania Smell Identification test (UPSIT), known commercially as the Smell Identification Test (SIT; Doty, Shaman, & Dann, 1984).The UPSIT has become the most widely used odour identification test and is available through Sensonics Incorporated (www.sensonics.com). The UPSIT is a supra-threshold test that consists of 40 items. Each item is presented as a microencapsulated strip that is released by scratching the label with a pencil tip. The examinee is asked to identify the odour from four options available. One point is awarded for each correct answer and the total score (out of 40) can be compared to a normative database comprising nearly 4,000 individuals. Numerous abbreviated and culturally adapted versions of the UPSIT have been developed, including the 12-item Cross-Cultural Smell Identification Test (CC-SIT; Doty, Marcus, & Lee, 1996), the 10-item Brief Smell Identification Test (B-SIT; Tabert et

al., 2005), the 3-item Pocket Smell Test (PST; Duff, McCaffrey, & Solomon, 2002), the 3-item Quick Smell Test (Q-SIT; Jackman & Doty, 2005) and the Culturally Adapted Smell Identification Test (CA-SIT; Conti et al., 2013).

Other odour identification tests include “Sniffin Sticks” (Kobal et al., 1996), the Connecticut Chemosensory Clinical Research Center – Olfactory Test (CCCRC; Toledano, Gonzalez, Rodriguez, & Galindo, 2007), the San Diego Odour Identification Test (SDOIT; Anderson, Maxwell, & Murphy, 1992) and the Scandinavian Odour Identification Test (SOIT; Nordin, Bramerson, Liden, & Bende, 1998). While the presentation of the odours vary from microencapsulated scratch and sniff strips, to pens or opaque bottles containing a liquid, the basic methodology remains the same: examinees are asked to smell the target odour and choose the correct response from options available and correct responses are tallied to obtain a final score.

In Australia, normative data is available for the UPSIT (Mackay-Sim & Doty 2001) and Sniffin’ Sticks (Mackay-Sim et al., 2004). Despite the availabilty of objective tests and norms of smell tests, the sense of smell is still rarely measured in a clinical setting and has been referred to as a neglected sense. This may be due to the finding that people are generally unaware of olfactory impairments. In a population-based epidemiological study, responses to the question ‘do you have a normal sense of smell?’ were compared with the results from standardised olfactory testing using the using the San Diego Odour Identification Test (Murphy et al., 2002). Self-reported impairment was low (9.5%) in comparison with results of standardised assessment (24.5%). Given low rates of awareness of olfactory impairment, it may be rare for individuals to present to the GP with concerns and thus screening would need to be initiated by knowledgeable GPs.

3.3 Olfactory Impairment

The advent of standardised assessment of olfactory ability has brought with it terminology to describe performance. Anosmia refers to total smell loss; hyposmia often rated as mild, moderate or severe, reflects a person with some sense of smell, albeit impaired. It is worthwhile noting that due to the frequency of olfactory impairments in older adults, a person can have moderate hyposmia yet still perform within the normal range for age.

In the general population hyposmia is fairly common (Doty & Kamath, 2014; Landis & Hummel, 2006; Shu et al., 2009) and can occur as a result of damage at any stage of the olfactory system. In a large, population-based study of adults aged 25 to 75 years, (*N* = 1387) the prevalence of pronounced hyposmia was 18% and anosmia 3.6% (Bramerson, Johansson, Ek, Nordin, & Bende, 2004). In a similar study in Sweden (***n*** = 1312), the overall prevalence of hyposmia was 19.1%, comprising 5.8% with anosmia (Vennemann, Hummel, & Berger, 2008). Olfactory dysfunction is more prevalent in men than in women, in smokers than non-smokers, and in people with nasal congestion and upper respiratory tract conditions (Murphy et al., 2002). It is also a feature of numerous neurological conditions such as corticobasal syndromes, Progressive Supranuclear Palsy, Multiple Sclerosis, Down Syndrome, Multiple System Atrophy, Motor Neurone disease and Korsakoff's syndrome (Attems, Walker, & Jellinger, 2014; Bramerson et al., 2004; Doty, 2012; Murphy et al., 2002; Vennemann et al., 2008).

Sex differences in olfaction have been consistently reported, with females outperforming males on tests of odour identification, detection, discrimination and memory (Doty & Cameron, 2009; Mackay-Sim, Grant, Owen, Chant, & Silburn, 2004; Mackay-Sim, Johnston, Owen, & Burne, 2006; Murphy et al., 2002). The basis of this finding is not clear but reproductive hormones and cultural factors are most frequently implicated (Doty & Cameron, 2009).

3.4 Olfaction and Ageing

It is well established that olfactory abilities decrease with increasing age, and that compared with younger adults, older adults exhibit a decline at various levels of olfactory processing {Murphy, 2002 #354}. In the Epidemiology of Hearing Loss Study, olfactory ability of 2491 people aged 53 to 97 years was assessed using the SDOIT (Anderson et al 1992). The overall prevalence of olfactory impairment was 25%. This increased to 29% in 70-79 years-olds and 63% in 80-97 year-olds (Murphy et al., 2002). Deterioration reflects declines at the sensory and higher levels and mechanisms thought to cause age-related olfactory impairment include normal anatomical and physiological changes in aging, environmental factors, medications and disease (Schiffman, 1997), and atrophy of the olfactory bulb (Smith, 1941) and tract (Bhatnagar, Kennedy, Baron, & Greenberg, 1987). Olfactory dysfunction is not an inevitable feature of aging and some people maintain olfactory function well into their seventh and eighth decades. This leads to the possibility that olfactory loss may be due to undiagnosed, age-related pathological conditions in which olfactory function is lost early in the disease process (Mackay-Sim, Grant, Owen, Chant, & Silburn, 2004). In a recent study, olfactory dysfunction was shown to be a stronger predictor of five year mortality than several common causes of death, such as heart failure, lung disease and cancer, indicating that this evolutionarily ancient special sense may signal a key mechanism that affects human longevity (Pinto, Wroblewski, Kern, Schumm, & McClintock, 2014). Olfactory dysfunction is a common feature of many neurodegenerative diseases, including AD, PD with and without dementia, and dementia with Lewy bodies (Attems et al., 2014; Doty, 2012). The literature on olfactory impairments in AD will now be reviewed.

3.5 Olfaction in Alzheimer's Disease

People with AD perform significantly worse on odour identification and threshold tests than age- and sex-matched individuals (Albers, 2006; Attems et al., 2014; Bahar-Fuchs, Chetelat, et al., 2010; Doty, Reyes, & Gregor, 1987; Gray, Staples, Murren, Dhariwal, & Bentham, 2001; C. Hawkes, 2003; Kjelvik, Sando, Aasly, Engedal, & White, 2007; Kovacs, 2004; Mesholam, Moberg, Mahr, & Doty, 1998; Rahayel, Frasnelli, & Joubert, 2012; Seligman, Kamath, Giovannetti, Arnold, & Moberg, 2013; Serby, Larson, & Kalkstein, 1991; Stamps, Bartoshuk, & Heilman, 2013; Sun, Raji, Maceachern, & Burke, 2012; Warner, Peabody, Flattery, & Tinklenberg, 1986; Westervelt, Carvalho, & Duff, 2007). The consistent nature of this impairment has led to olfactory dysfunction being recommended for inclusion in the diagnostic criteria for AD (Foster, Sohrabi, Verdile, & Martins, 2008). There remain a number of issues with using olfaction diagnostically. First, there is no solid olfactory profile for dementia sub-types making olfaction a non-specific diagnostic feature. Second, there is a lack of longitudinal research to definitively associate olfaction with the development of AD (Sun et al., 2012). Due to these limitations, the results of olfactory testing are most effectively used in combination with other risk factors to predict which individuals may progress to AD (Conti et al., 2013).

Olfactory deficits have been reported in the prodromal and preclinical stages of AD (Bahar-Fuchs, Moss, Rowe, & Savage, 2010; Djordjevic, Jones-Gotman, De Sousa, & Chertkow, 2008; Eibenstein et al., 2005; Peters et al., 2003; Royall, Chiodo, Polk, & Jaramillo, 2002; Wang et al., 2002). This has led to the investigation of olfactory testing as a potential biomarker for AD.

3.6 Olfactory impairment as a biomarker for Alzheimer's Disease.

Olfactory impairment is an attractive biomarker for AD, being inexpensive, accessible and non-invasive. Furthermore, testing serves a purpose in itself, as results indicating impoverished olfactory function can lead to counselling regarding potential safety concerns and matters related to quality of life, such as the necessity of a smoke alarm or methods to maximise food choices.

Attempts have been made to develop a clear cut-off between results that indicate normal functioning and those that may indicate the presence of AD. For example, a score of less than 29 out of 40 on the UPSIT has a sensitivity of 83% and specificity of 78% in distinguishing patients with AD from healthy controls (Morgan, Nordin, & Murphy, 1995) and a score of eight or less on the B-SIT has 79% sensitivity and 92% specificity for AD (Kjelvik et al., 2007). These levels of sensitivity and specificity are quite acceptable for a biomarker, with the Biomarkers Definitions Working Group of the National Institutes of Health suggesting 80% as a suitable target for both.

Longitudinal studies have attempted to use performance on olfactory tests to predict conversion to AD, typically in collaboration with other markers. Stanciu et al., (2014) combined performance on the SOIT with self-report of olfactory impairment to predict conversion to dementia. In the sample of 1529 cognitively intact participants, 159 were diagnosed with AD or vascular dementia over a 10-year period. Low scores on the odour identification test and self-report of olfactory loss both predicted transition to dementia and these two effects were additive. In a two-year longitudinal study, Conti et al., (2013) administered the CA-SIT to 88 MCI subjects and 46 healthy control subjects. Within the MCI group, 47% of participants with olfactory impairments progressed to dementia compared with 11% who had normal olfaction. Finally, Devanand et al., (2008) showed that UPSIT score, functional ability, verbal memory performance,

hippocampal volume, and entorhinal cortex volume strongly predicted conversion to AD over a three-year period, with 85% sensitivity and 90% specificity.

The ability of olfactory assessment to predict which cognitively intact individuals might develop MCI has also been investigated. Graves et al. (1999) found that individuals who were anosmic at baseline and who had at least one ApoE Ɛ4 allele had almost five times the risk of cognitive decline than individuals with normal olfactory functioning and no ApoE Ɛ4 allele. Wilson and colleagues (2009) found that B-SIT score was associated with more rapid decline in episodic memory and with increased risk of developing MCI, even after controlling for baseline level of episodic memory and possession of an ApoE Ɛ4 allele. In the same study, in 34 people who died without evidence of cognitive impairment, a lower B-SIT score was associated with a higher level of cortical plaques and neurofibrillary tangles, suggesting that among older people without clinical manifestations of AD or MCI, olfactory dysfunction is related to both the level of AD pathology in the brain and the risk of subsequently developing prodromal symptoms of the disease.

Olfactory deficits have been found in other groups of people at risk of developing AD. Serby et al., (1996) reported that asymtomatic first degree relatives of people with AD have significantly lower UPSIT scores than nonrelatives. Healthy controls with one or more ApoE Ɛ4 alleles have been shown to have lower scores on tests of odour threshold (Bacon, Bondi, Salmon, & Murphy, 1998), the SDOIT (Murphy, Bacon, Bondi, & Salmon, 1998), and the B-SIT (Salerno-Kennedy, Cusack, & Cashman, 2005) than those without an ApoE Ɛ4 allele. However presence of the ApoE Ɛ4 allele status has not consistently been associated with olfactory impairments (Doty, Petersen, Mensah, & Christensen, 2011; Olofsson et al., 2010).

Although there is evidence suggesting an association between decreased olfaction and AD, rigorously designed longitudinal cohort studies are still necessary to clarify the value of olfactory identification testing in predicting the onset of AD (Sun et al., 2012.

3.7 Mechanisms of olfactory dysfunction in Alzheimer's disease

Numerous pathological changes characteristic of AD are present in olfactory-related brain regions early in the course of the disease (Albers, 2006). For the purposes of this review, changes to the cholinergic system and Aβ deposition in olfactory regions will be reviewed.

3.7.1 Cholinergic System.

The olfactory system, and particularly the entorhinal cortex and olfactory bulb, is rich in ACh (Doty, Bagla, & Kim, 1999) and changes in ACh have been associated with changes in olfactory ability (Wilson, Fletcher, & Sullivan, 2004). Skouby and Zilstorff-Pedersen (1954) and Zilstorff-Pedersen (1955) reported a reduction in tasks of olfactory threshold detection following the intranasal administration of small amounts of acetylcholine hydrochloride (0.1-10 μg/ml in saline) and acetyl-beta-methyl-choline hydrochloride (10 μg/ml in saline). Serby et al., (1990) found subcutaneous administration of scopolamine hydrobromide could decrease odour detection sensitivity but they had no effect on odour identification as measured by the UPSIT.

Other than these studies, there is limited research investigating the effect of cholinergic treatment on olfaction in humans. Preclinical investigations suggest that manipulation of the cholinergic system can produce changes to olfactory function. In rats, scopolamine has been shown to reduce performance on a number of olfactory tasks, including habituation (Hunter & Murray, 1989), short term memory (Ravel, Elaagouby, & Gervais, 1994), perceptual learning (Fletcher & Wilson, 2002), and discrimination (Chaudhury, Escanilla, & Linster, 2009). In contrast, physostigmine, a

reversible cholinesterase inhibitor, has been shown to enhance odour discrimination performance in rats in a dose-related manner (Doty et al., 1999). Likewise, in a series of studies, scopolamine impaired performance on a task of odour discrimination, whereas a cholinergic muscarinic receptor agonist (oxotremorine), enhanced performance in rats (Chapuis & Wilson, 2013).

3.7.2 Amyloid.

In the same way as memory impairments have long been accounted for by the presence of plaques and tangles in memory processing systems of the brain, olfactory deficits may be similarly explained by plaques and tangles in areas of the brain essential for olfactory functioning. Accumulation of Aβ occurs in many areas of the olfactory system, including the olfactory bulb (Arnold et al., 2010; Braak & Braak, 1998; Kovacs, Cairns, & Lantos, 2001), olfactory epithelium (Arnold et al., 2010), nasal cavity (Kameshima, Nanjou, Fukuhara, Yanagisawa, & Tooyama, 2012) and entorhinal cortex and hippocampus, regions involved in odour recognition and odour memory (Price, Davis, Morris, & White, 1991).

In a group of community-dwelling older people without evidence of cognitive impairment, lower odour identification scores were associated with higher levels of neurofibrillary pathology in the entorhinal cortex and hippocampus (Wilson et al., 2009; Wilson, Schneider, et al., 2007) as well as central olfactory processing regions (Wilson, Arnold, Schneider, Tang, & Bennett, 2007). In rodent models of AD, both neurofibrillary loss and Aβ pathology have been associated with olfactory loss (Wesson, Levy, Nixon, & Wilson, 2010). For instance, investigations with transgenic PDAPP mice suggest that olfactory dysfunction correlates with the spatiotemporal deposition of Aβ and that Aβ deposition in the olfactory bulb and olfactory cortical areas contributes to olfactory loss.

3.8 The Use of Olfaction in the Differential Diagnosis of AD

The ability of current olfactory tests to distinguish AD from other neurocognitive disorders, and particularly PD, has been the focus of much research. Before reviewing the ability of olfactory testing to differentiate between neurodegenerative diseases, a brief summary of the olfactory deficits in PD and VCI will be described.

3.9 Olfaction in Parkinson's disease.

PD is a common neurodegenerative disorder resulting in motor symptoms such as bradykinesia, rigidity and rest tremor, and non-motor symptoms such as cognitive impairment, neurobehavioural problems, and sensory and sleep difficulties. Olfactory dysfunction is the second most common feature of PD with 70-90% of patients with PD having measurable olfactory deficits. (Double et al., 2003; Hawkes, Shephard, & Daniel, 1997). Olfactory deficits are an early feature of PD, are present in asymptomatic individuals at risk of PD and may have a role in predicting who will develop the disease (Ponsen et al., 2004; Ponsen, Stoffers, Wolters, Booij, & Berendse, 2010; Ross et al., 2008). As with AD, attempts have been made to use olfactory testing as a diagnostic tool and this has been shown to be useful in the differential diagnosis of PD from progressive supra-nuclear palsy, MPTP-induced Parkinsonism, multiple atrophy and essential tremor (Bohnen et al., 2010; Busenbark, Huber, Greer, Pahwa, & Koller, 1992; Doty, 2012; Morley & Duda, 2010).

Despite being well characterised, the cause of olfactory impairments in PD is poorly understood. Doty (2012) provides a review of the possible mechanisms which includes damage to the cholinergic, serotonergic and noradrenergic systems as well as neuronal loss and distribution of Lewy bodies (Pearce, Hawkes, & Daniel, 1995, Daniel & Hawkes, 1992). Olfactory dysfunction in the later stages of PD may also be a result of a behavioural sniffing impairment (Sobel et al., 2001).

Although there is a vast amount of research comparing olfactory performance in healthy populations with AD or PD populations, few studies have directly compared all three groups to determine whether AD and PD populations demonstrate different patterns of impairments. Mesholam and colleagues (1998) address this question in a meta analysis of 26 studies published before 1996. As expected they found severe deficits in odour identification, recognition and threshold for patients with AD and PD, but no discriminating olfactory deficits were found between the groups. A more recent meta-analysis (Rahayel et al., 2012) explored studies published between 1970 and 2011 and yielded a much larger pool of 81 studies. They concluded that while people with both AD and PD are impaired across all measures of olfaction, the PD group was more impaired on low-level perceptual olfactory tasks whereas the AD group was more impaired on higher-order olfactory tasks such as odour identification and odour memory. The discrepancy in these results could be due to a number of factors. Most simply it could reflect the larger amount of data in the 2012 study, which allowed a difference to be statistically meaningful. The huge increase in studies reflects the surge of interest in this area which may also be fuelled by the availability of commercial olfactory tests. While both meta-analyses included studies using standardised and non standardised assessments of olfaction, the later study has a smaller proportion of independently designed olfactory tests. Furthermore, Rahayel and colleagues applied a more thorough selection criteria ensuring that diagnostic criteria for AD or PD were reported and met current diagnostic standards. The earlier study may have included a greater proportion of participants with a mixed diagnosis.

3.10 Olfaction in Vascular Dementia and VCI.

Vascular dementia (formerly known as multi-infarct dementia) has been long recognised as one of the most common types of dementia, second to AD. VCI, like MCI is a term that was introduced to allow for earlier identification and management of

disease before the level of cognitive impairment was of the severity to impact daily functioning (Hachinski & Bowler, 1993, Bowler, 2007). Cognitive impairments may result from single or multiple infarcts, leukoaraiosis, incomplete infarction, haemorrhage, and arteriosclerosis (Bowler, 2007; Groves et al., 2000). Risk factors for vascular damage are extensive and include hypotension, hypertension, diabetes, atrial fibrillation, hyperlipidemia, coronary heart disease, congestive failure, cigarette smoking and alcohol abuse

Olfactory deficits in vascular dementia have been reported on tasks of olfactory threshold and recognition (Knupfer, 1986) and identification (Duff et al., 2002, Gray et al., 2001; Knupfer, 1986) however; the prevalence of olfactory impairments is not well established. A pubmed search for studies investigating olfactory impairments in VCI yielded no results. The most likely explanation for olfactory impairment - damage to areas of the brain involved in olfactory processing - is challenged by the finding that olfactory deficits have been reported in cases of stroke where olfactory circuits have not been affected, pointing to wider neurological involvement (Asai, Udaka, Hirano, & Ueno, 2008; Green, McGregor, & King, 2008; Moo & Wityk, 1999; Rousseaux, Muller, Gahide, Mottin, & Romon, 1996; Sela et al., 2009).

In the differential diagnosis of AD from vascular dementia, Knupfer (1986) found that people with AD performed worse on tasks of olfactory threshold and identification. Unfortunately this study used non-standardised measures of olfactory function which may be unavailable or impractical for clinicians. Duff et al., (2002) used the PST to successfully discriminate patients with AD from vascular dementia and major depression. Patients with AD scored significantly lower than patients with either vascular dementia or major depression and a PST score of zero or one discriminated between patients with and without AD with a sensitivity of 100% and specificity of

92.5%. In the only study comparing UPSIT performance in AD and vascular dementia, Gray et al (2001) found that both groups showed a similar degree of olfactory impairment and it was not possible to differentiate the two.

3.11 Summary

This chapter has attempted to provide an overview of the olfactory system and provide an introduction to methods to assess olfactory function, in particular odour identification. Olfactory impairment has been shown to be a strong predictor of five-year mortality and is a consistent and pervasive feature of many neurodegenerative diseases. However, there is no current test that can provide adequate differential diagnosis of AD from other neurological conditions.

Wesson, Wilson, & Nixon (2010) suggest several further developments that will be necessary before olfactory assessment can be used as a suitable biomarker for AD. First, they recommend that a standardised testing battery that reduces user error and is adapted for different ethnic groups needs to be established as a ‘standard’ measurement. Second, a large-scale investigation using such a ‘standard’ test needs to be performed alongside other biomarkers (i.e., cerebro-spinal fluid, amyloid-β and tau, cognitive testing) to robustly assess the relationship between olfactory loss and other factors implicated in AD. Third, a method that robustly differentiates the origin of olfactory loss as being either due to AD pathology or other pathology is required to allow definitive diagnoses. Finally, longitudinal studies of individuals during the progression of disease, coupled with independent measures of structural or functional deficits in relevant brain regions, will be critical in establishing the utility of olfactory tests as a disease progression or treatment outcome measure. In regard to point three, the OST represents such a new method. By using existing olfactory tests to measure the effect of a cholinergic challenge, the OST may be a measure of cholinergic

functioning. To the extent that cholinergic functioning may also predict amyloid load in AD, the OST may be a proxy measure of amyloid burden. If this is the case, the OST may be able to robustly differentiate AD from other dementias.

4. Chapter Four - The Olfactory Stress Test

The model of a stress test draws upon the idea that presymptomatic disease can be unmasked. This model is used in other areas of health care, most notably in cardiology to detect coronary artery disease. In this model, asymptomatic disease is unmasked by stressing the cardiovascular system beyond usual levels. An electrocardiogram (ECG) is recorded at rest, and then again when the cardiac system is stressed by exercise. At rest the ECG appears normal, however when the cardiac system is under stress, the ECG is able to reveal underlying deficits in functioning.

4.1 Description of the Olfactory Stress Test

Rather than measure olfaction per se, the OST measures the effect of a cholinergic challenge on olfactory ability. The UPSIT is used to assess baseline olfactory functioning. Then, one milligram of atropine sulphate (0.1 ml of 10mg/ml solution) is sprayed high into the left nostril. The patient is then asked to kneel down with their forehead touching the floor for 60 seconds to promote retention of the spray close to the olfactory epithelium from where it may diffuse to the olfactory bulb. After 45 minutes, the patient completes the remaining UPSIT again. A unirhinal testing paradigm is used such that the left nostril is used throughout, with the right nostril occluded with cotton wool. The UPSIT is administered and scored according to the instructions and templates in the manufacturer's manual. The change in UPSIT score is considered to represent the impact of atropine on olfactory functioning or a*tropine effect*. The greater the decline in olfactory function, after atropine, the greater the atropine effect; for example an UPSIT score of 15 at baseline, and 11 after atropine, would result in an atropine effect of -4; which would be a greater atropine effect than an atropine effect of -2.

4.1.1 Atropine.

Atropine is an anticholinergic agent similar to scopolamine but its central nervous system effects are less marked (Eger, 1962; Ostfeld, Machne, & Unna, 1960; Pradhan & Roth, 1968). Atropine sulphate has a slower onset and more prolonged effect than most other anticholinergic drugs. Once in the bloodstream it is distributed throughout the body and crosses the blood-brain barrier. It is rapidly cleared from the blood and completely metabolised in the liver and excreted in the urine and metabolites. A half-life of four hours has been reported (Ellinwood, Nikaido, Gupta, Heatherly, & Nishita, 1990). The OST utilises Atropt eye drops which contain atropine sulphate (1%) and the following inactive ingredients: disodium edetate, benzalkonium chloride, hypromellose, boric acid, and purified water.

4.1.2 Psychometric properties of the UPSIT.

The OST relies on the assumption that a reduction in performance on the UPSIT is a result of the effect of atropine. To make this assumption it is essential to understand the psychometric properties of this test.

The UPSIT consists of four individual booklets each containing 10 items. The performance correlations between the UPSIT booklets range from 0.73 (Booklets 2 and 3) to 0.77 (Booklets 3 and 4). In the original validation studies, Doty (1989) evaluated test retest performance over a period of two weeks. He recruited 69 participants with a range of olfactory ability and the test retest correlation was found to be $r = .95$ ($p < .001$). Other researchers have investigated test retest for periods of between two weeks and six months with correlations from $r = .91$ to .95 (Doty, McKeown, Lee, & Shaman, 1995; Frank, Dulay, & Gesteland, 2003). No data have been published on same-day test retest reliability of the UPSIT.

It is well established that UPSIT scores diminish with age and that females perform better than males at all ages (Doty, Applebaum, Zusho, & Settle, 1985; Doty, Shaman, Applebaum, et al., 1984).

Performance on the UPSIT has been shown to be sensitive to the effects of drug treatment. Twenty-five patients with mild to moderate AD, planned for donepezil treatment, were recruited to an open label study. The UPSIT, as well as other measures of cognition and daily functioning, was completed prior to commencing treatment and again after three months. Those who were treated with donepezil showed improvements to UPSIT scores over a period of six months and UPSIT score best predicted global functioning outcome (Velayudhan & Lovestone, 2009). The extent to whether this was a result of increased cholinergic activity is of interest. In contrast, the UPSIT was not sensitive to the effects of subcutaneous administration of scopolamine hydrobromide in healthy young controls, possible due to ability of young controls to compensate to a cholinergic challenge (Serby et al., 1990).

The UPSIT was originally developed in the US and has been translated and validated for use in other countries. British, Chinese, French, German, Italian, Korean, Portuguese, Spanish and Japanese UPSIT versions are available (Doty, 2007). To date, an Australian version is not available, however, Australian normative data for the UPSIT American version has been published (Mackay and Doty, 2001). In this validation study, 204 Australians aged from 17 to 80 years old (mean age 41), were matched on the basis of race, gender, age and smoking status to a group from the UPSIT database of North American subjects. The results indicated that Australians scored two points lower than the Americans on the UPSIT, with mean scores of 34 (*SD* =0.7) and 36 (0.8) respectively. Cultural rather than biological differences between the groups were considered to be the primary cause of this difference as Australians

performed worse on five items (chutney, fruit punch, liquorice, turpentine and grape) and these items accounted for the difference in scores between the two groups. Similarly, in clinical studies of Australians with schizophrenia, the UPSIT scores for cases and controls were about three points lower than for similar populations in North America (Kopala, Good, Torrey, & Honer, 1998; Striebel, Beyerstein, Remick, Kopala, & Honer, 1999).

In summary, the OST requires accurate measurement of olfactory functioning before and after atropine. The UPSIT was chosen because it has become the gold standard of olfactory testing and has been shown to be sensitive to the effects of cholinergic therapy. The lack of same day test retest data is unfortunate however the psychometric properties of the UPSIT over longer periods are established.

4.1.3 Unirhinal testing.

The OST utilises a unirhinal testing paradigm primarily to minimise the dose of atropine required. In the initial study with the OST, Schofield et al (2012) chose to use the left nostril. This decision was based on some evidence that performance on tasks of odour naming and identification may be better when stimuli are presented to the left nostril/ hemisphere (Broman, Olsson, & Nordin, 2001; Herz, McCall, & Cahill, 1999; Homewood & Stevenson, 2001; Murphy, Jernigan, & Fennema-Notestine, 2003) though this finding has not been replicated in participants with AD or MCI (Bahar-Fuchs, Chetelat, et al., 2010; Bahar-Fuchs, Moss, et al., 2010). The decision was also influenced by the work of Murphy et al. (2003) who found a strong relationship between left hippocampal volume and performance on an odour identification task. The decision to use the left nostril/hemisphere in the original study was also influenced by the inclusion of a task of verbal memory and interest in whether there was a relationship between verbal episodic memory, left-sided odour identification and left hippocampal

volume. In the interest of comparing this study with the pilot study the same testing paradigm was used.

Unirhinal normative data for the 40-item UPSIT are not available, however data for unirhinal administration of split halves of the UPSIT have been investigated. Good, Martzke, Daoud, & Kopala (2003) administered 20 items of the UPSIT to each nostril in a large cohort study of 270 healthy adults aged 15-64. Unirhinal performance was worse than birhinal performance but did not differ according to nostril of presentation.

4.2 Previous Findings with the Olfactory Stress Test

In the initial study, Schofield et al., (2012) administered the OST to participants with probable AD, cognitive impairment–not dementia (CIND) and cognitively normal controls. People with past traumatic brain injury, stroke, active psychiatric illness or major medical illness were excluded. The diagnosis of AD was made using DSM-IV and NINCDS-ADRDA criteria. A diagnosis of CIND was made when performance on any of the cognitive assessments fell more than 1.5 standard deviations below age matched normative means, but the individual did not meet criteria for dementia. Participants were considered ‘cognitively normal’ if their performance on all cognitive tests fell within 1.5 standard deviations from age matched normative means. The final sample consisted of 15 AD patients, 13 with CIND and 29 controls. As expected, the AD group had a significantly lower mean baseline UPSIT score than the control group or CIND group indicating reduced olfactory function. There was no difference in baseline UPSIT score between the control and CIND group.

In the 2012 study, 20 items of the UPSIT were completed before atropine and the remaining 20 items were completed after atropine. UPSIT booklet combinations 1 & 2 and 3 & 4 were used and these were counterbalanced, so that half the sample received Booklets 1 & 2 at baseline, and Booklets 3 & 4 following atropine, while the other half

received Booklets 3 & 4 at baseline and Booklets 1 & 2 after atropine. The correlation of the combined Booklets 1 & 2 and Booklets 3 & 4 is high (0.92; Doty, Frye, & Agrawal, 1989).

The results indicated that atropine reduced UPSIT performance by over two points in the AD group (M = -2.43, SD = 1.45) and CIND group (M = -2.77, SD = 2.71), but had no effect in the control group (M = 0.28, SD = 2.15). Eighty-six percent of the AD group and 92% of the CIND performed worse on the UPSIT following atropine, compared with 31% of the control group. These rates were very similar to the respective proportions of people with AD and healthy older adults who demonstrate high levels of Aβ (Pike et al., 2007; Rowe et al., 2007; Sperling et al., 2009).

To compare the OST with other indicators of AD, all participants underwent an MRI and blood test to determine hippocampal volume and ApoE genotype. In the sample as a whole, the atropine effect correlated strongly with left hippocampal volume (r = .53, p < .001) and the presence of an ApoE Ɛ4 allele was associated with a greater atropine effect (p = .014).

The size of the atropine effect also correlated with episodic memory (r =.57, p < .001) as assessed by the Audio Recorded Cognitive Screen (ARCS), a screening tool for the detection of cognitive impairment or dementia that uses an audio device to administer selected neuropsychological tests to unsupervised individuals (Schofield et al., 2010).

In the second investigation, Schofield et al (2013) modified the OST such that the full 40-item UPSIT was administered before and 45 minutes after atropine. The sample consisted of 64 individuals aged 60-87, who performed within normal range on neuropsychological assessment. ApoE genotype and hippocampal volume was

collected. The mean atropine effect was 0.41 (range -4 to 7). Individuals with at least one Ɛ4 allele had a greater atropine effect (*M* = -0.8, *SD* = 1.8) than those with no Ɛ4 allele (*M* = 0.95, *SD* = 2.4). There was no significant correlation between the size of the atropine effect and neuropsychological test scores which may have reflected the lack of range of scores (all participants were within the normal range). These initial investigations indicate promise for the OST as a possible biomarker for AD. The potential mechanism of action of the OST will be described below.

4.3 A Biological Rationale for the Olfactory Stress Test

The body of work using anticholinergic drugs to alter cognitive functioning is consistent with the model of a stress test and provides a template of strategies with which to develop an OST. Administration of scopolamine has been shown to produce transient memory impairments in normal young participants (Broks et al., 1988; Drachman & Leavitt, 1974; Ebert & Kirch, 1998; Kopelman & Corn, 1988; Nissen, Knopman, & Schacter, 1987; Wesnes & Revell, 1984) and older adults (Huff, Mickel, Corkin, & Growdon, 1988; Snyder, Bednar, Cromer, & Maruff, 2005; Sunderland et al., 1987). Furthermore, scopolamine has been found to cause exaggerated cognitive decline in people with AD and older adults relative to normal controls (Ray et al., 1992; Sunderland, Tariot, Murphy, et al., 1985; Sunderland et al., 1987).

One study has been identified that explored the effects of atropine on cognitive functioning. Inzelberg, Shapira, & Korczyn (1990) randomly assigned participants to receive either 1 mg of atropine or saline solution intravenously. Memory assessment, based on the Wechsler Memroy Scale (Wechsler, 1945), was completed at baseline and again after either atropine or placebo. After atropine, participants with AD or PD with dementia declined on a test of attention, but this decrease was significant for the AD group only. Furthermore, the control group showed an increase in their score on the

logical memory subtest following atropine, as did the AD patients after placebo, which presumably reflected learning or a practice effect; however this effect was not seen in the AD group after atropine. The authors suggested that failure to show a learning effect on a memory task after atropine in patients with dementia associated with AD, PD and vascular changes indicated a blunting effect of atropine rather than ability to produce a deficit.

People at risk of developing AD have been shown to have cognitive sensitivity to a cholinergic challenge. Participants with an ApoE Ɛ4 allele experienced significant impairments on memory tasks after the anticholinergic agent, trihexyphenidyl, compared to non-ApoE Ɛ4 carriers and this impairment was more persistent suggesting that ApoE Ɛ4 may play a significant role in increasing cognitive sensitivity to anticholinergics (Pomara, Willoughby, Wesnes, & Sidtis, 2004).

Previous research suggests that cognitive sensitivity to scopolamine might be predictive of future cognitive decline. Barker (1995; 1998) administered scopolamine to 24 healthy older adults at baseline. Marked decrements in cognitive functioning were observed immediately and participants who demonstrated the greatest scopolamine-induced decrements had higher rates of decline on tasks of working memory and vigilance after three and six years.

Further evidence of a cholinergic hypersensitivity comes from exploration of the side effects of drugs with anticholinergic properties. More than 600 drugs have anticholinergic activity, and previous research estimates that 14 of the 25 most commonly prescribed drugs for older adults have a detectable anticholinergic effect (Tune, Carr, Hoag, & Cooper, 1992). Polypharmacy increases the risk of anticholinergic drug intake in older adults. Cross-sectional studies have found an association between anticholinergic drug use and low cognitive performance (Cancelli, Beltrame, Gigli, &

Valente, 2009; Cancelli et al., 2008; Cao et al., 2008; Lechevallier-Michel, Molimard, Dartigues, Fabrigoule, & Fourrier-Reglat, 2005; Uusvaara et al., 2009). Recently, a transdermal scopolamine patch developed for prophylaxis and treatment of motion sickness has been associated with causing mental confusion or delirium in older adults with undetected incipient dementia or MCI (Seo, Suh, Chin, & Na, 2009). In addition, Nebes, Pollock, Perera, Halligan, & Saxton (2012) found that use of anticholinergic medication is associated with greater decrements on tasks of cognition, executive function, mood, and sleep in older adults who carry one or more ApoE Ɛ4 allele than in those without, however this result is not consistent (Uusvaara et al., 2009). The link between anticholinergic drug intake and cognition has been the subject of two longitudinal studies which concluded that older adults taking anticholinergic drugs were more likely to be classified as MCI but were not at increased risk for dementia suggesting the effects of anticholinergic therapy on cognition were not severe enough to cause dementia (Ancelin et al., 2006; Bottiggi et al., 2006).

The mechanism of cholinergic hypersensitivity is not clear, however it is plausible that healthy young controls are better able to compensate for a challenge to their cholinergic system by increasing ACh release, whereas older adults and people with AD may be less able to modify ACh release, resulting in a larger effect of ACh depletion. The idea of a reduced functional reserve within the cholinergic system may account for exaggerated cognitive decline in people with or at risk of AD.

The differential effects of scopolamine in patients with AD and other neurodegenertaive disorder has also been expolored. Rabey, Neufeld, Treves, Sifris, & Korczyn (1996) explored the effect of scopolamine on cognitive function in people with AD (n = 11), multi-infarct (vascular) dementia (n = 8), PD with dementia (n = 7), and healthy age-matched controls (n = 11). After completing the baseline cognitive

assessment, each patient was randomly assigned to receive either scopolamine 0.5 mg dissolved in 150 ml saline or placebo administered intravenously. Ninety minutes later the cognitive assessment was repeated. One week later the participants returned and completed the alternative treatment (scopolamine or placebo). At baseline the three patient groups demonstrated significant cognitive impairments in comparison to the control group but were statistically comparable to each other. Scopolamine produced a significant decrement in cognitive functioning in all of the groups but the greatest decrement was seen in the control group who supposedly have an intact cholinergic system. There was no distinction between the various diagnostic categories. While the authors also suggested that the integrity of the cholinergic system. They noted that among the healthy control group there appeared to be a subpopulation that was sensitive to the effects of a cholinergic challenge. They hypothesised that this subpopulation may have an incipient cholinergic impairment, and in this group, normal cognitive performance relied on compensatory cholinergic release. If this was the case, they suggest that the administration of scopolamine may have a predictive value in the diagnosis of dementia.

The extensive body of literature on the effects of scopolamine on cognitive function, provides a rich tapestry on which to develop the OST. It is hypothesised that olfactory functioning may also be sensitive to cholinergic agents and that this effect may exaggerated in older adults with or at risk of AD, in contrast to unimpaired controls and people with other neurodegenerative diseases.

Initial work with the OST supports this claim and provides preliminary indication that the OST holds promise as a simple, inexpensive, noninvasive screen for early and preclinical AD. It appears to show sensitivity to early AD pathology. The next stage in developing this biomarker is to replicate these findings and assess specificity for AD

pathology by evaluating the properties of the OST in patients with other neurodegenerative conditions.

5. Chapter Five - Aims and Hypothesis

The primary aim of this study was to compare the performance of the OST in participants with AD, PD and VCI with a control group of cognitively normal older adults. Previous research suggests that the OST can produce a greater effect on olfactory functioning in people with AD, in comparison to controls (Schofield et al., 2012). The first aim of this study was to replicate this finding. The following hypothesis relates to this aim:

Hypothesis 1: The AD group will show a greater atropine effect than the control group

The second aim of this study was to explore the effect of the OST in a group of individuals diagnosed with PD. Olfactory dysfunction is the second most common feature of PD and an early feature of the disease. It is present in asymptomatic individuals at risk of the disease and it may have a role in predicting who develops the disease (Ponsen et al., 2004; Ponsen et al., 2010; Ross et al., 2008). Available olfactory tests have not been able to consistently differentiate between AD and PD. The OST may provide a unique way to measure sense of smell that can differentiate between these conditions and may provide useful insights into the biological underpinning of olfactory impairments in these groups. The following hypotheses relate to this aim:

Hypothesis 2: There will be no difference in atropine effect between the PD group and control group.

Hypothesis 3: The AD group will have a greater atropine effect than the PD group.

The OST will also be explored in a group of individuals with VCI. The differential diagnosis between AD and VCI can be challenging due to similarity of clinical symptoms. Investigation of the OST in this population will serve as a measure of the

clinical utility of the OST as a diagnostic tool. If an atropine effect is found in a sample with very similar levels of cognitive impairment it points to the implication that the OST is targeting the pathology related to AD. Hypotheses Four and Five relate to this aim:

Hypothesis 4: There will be no difference in the atropine effect between the VCI group and control group.

Hypothesis 5: The AD group will have a greater atropine effect than the VCI group.

Finally the relationship between the OST and other potential antecedents or markers of AD will be explored. Schofield et al. (2012) reported that the OST correlated with measures of memory, hippocampal volume and the presence of an ApoE ε4 allele in a sample of patients with AD, cognitive impairment not dementia and controls. These results were partially replicated in a study of cognitively intact older adults with a significant difference seen in atropine effect between carriers and noncarriers of one or more ApoE ε4 alleles but no correlation between atropine effect and cognitive functioning. In this study data from the AD and control group will be pooled to explore the relationship between hippocampal volume, and neuropsychological performance; data from the PD and VCI groups will not be included given these conditions do not fit with the normalcy-AD continuum of potential cholinergic dysfunction. Data from all groups will be pooled to investigate the impact of ApoE genotype on the atropine effect. The following hypothesis relate to these aims.

Hypothesis 6: There will be a correlation between atropine effect and hippocampal volume in the AD and control group.

Hypothesis 7: There will be a correlation between the atropine effect and a memory composite score in the AD and control group.

Hypothesis 8: There will be a correlation between the atropine effect and a non-memory composite score in the AD and control group.

Hypothesis 9: Participants with one or more ApoE Ɛ4 alleles will have a greater atropine effect than people with no ApoE Ɛ4 allele.

5.1 Original Contribution of the Current Study

The OST is under investigation as a potential biomarker for AD. To date there is no clinical marker of cholinergic functioning and the OST represents a novel approach to measure cholinergic integrity. Research with the OST is in its infancy and the development of a biomarker is a complex process. A suitable biomarker must have the ability to detect a fundamental feature of AD neuropathology (it is hypothesised that the OST is a proxy measure of cholinergic function), have a clear cut-off between normal and abnormal results, and be able to distinguish AD from other dementias. This research assesses the ability of the OST to show a clear cut-off between normal and abnormal functioning and to differentiate AD from other dementias. The first contribution is achieved through comparison of the OST in cognitively unimpaired older adults with an AD sample and the latter is achieved through investigation of OST performance in PD and VCI.

Many current biomarkers in use and in development for the detection of AD are expensive, invasive and difficult to access, primarily available only at urban clinical centres or research facilities within Western societies. A biomarker for AD that could be administered quickly and cheaply within a memory clinic or GP setting would be a significant development. The OST is ultimately under investigation for its ability to identify AD in the preclinical stage. Early detection is of great relevance for the development of disease modifying agents which might be more effective in the preclinical and prodromal phases of the disease when there may be opportunities to

reverse pathological changes. Ultimately, and with the arrival of disease modifying agents, clinically relevant biomarkers may be able to provide an opportunity for early diagnosis. Although the exact point of when diagnosis should be made is a matter of ethical debate, early diagnosis may allow patients to actively participate in future planning of their affairs, minimise potential hazards such as driving or mismanagement of medications and permit psycho-education regarding the disease and its implications. This would become more relevant when disease modifying agents are available.

6. Chapter Six – Methodology and Characterisation of Groups

6.1 Ethical Approval

This study was approved by the Human Research Ethics Committees of the Hunter New England Health Service and Macquarie University. The participants were provided with written information on the study and gave written informed consent to participate. Participants were free to withdraw from the study at any time and for any reason, and were assured that this would have no effect on their medical care. Participants were paid $30 to cover travel expenses.

6.2 Analysis

IBM SPSS Statistics version 20 was used to perform statistical analyses. Any *z* score values below -3.00 or above 3.00 were replaced by these cut-off values to preventing skewing of the data. A Type I error rate corresponding to $p < .05$ was adopted for all analysis.

Analysis of variance (ANOVA) was used to compare clinical groups with respect to cognitive, volumetric and olfactory measures, with post hoc Tukey comparisons. This allowed for thorough characterisation of the sample prior to analysis of the OST.

The primary aim of this study was to explore the atropine effect in three neurological conditions in comparison with a control group and compare the atropine effect in the AD group with two other clinical groups.

A priori power analysis using GPower (Faul, Erdfelder, Buchner, & Lang, 2009) based on the atropine effect reported in the Schofield et al (2012) study for controls and AD, indicated that a sample size of 26 (13 controls and 13 with AD) would have 95% power for detecting an effect ($p<.05$).

To this end, five planned comparisons were conducted within an ANOVA framework. The comparisons were as follows: control versus AD, control versus PD, control versus VCI, AD versus PD, and AD versus VCI. The atropine effect was further explored by comparing the difference in the UPSIT scores before and after atropine between each group, using the Fisher Z test procedure.

To determine the association between the atropine effect and neuropsychological performance, and hippocampal volume, partial correlations controlling for age, sex and education were conducted. Finally, to compare the atropine effect in people with and without an ApoE Ɛ4 allele, an independent samples *t*-test was employed.

6.3 Participants

Sixty-four participants were initially recruited via a memory disorders clinic, local support groups and advertising in patient newsletters and patient clinics.

6.4 Inclusion Criteria

Participants were recruited if they were aged 60 years or above, were able to provide informed consent, had a MMSE score of greater than 18/30 and had sufficient skills to complete study procedures, that is, no known impairment to vision or hearing, and ability to understand study procedures and task instructions as evaluated during the clinical interview.

To be included in the AD group, participants needed to demonstrate deficits on two or more tasks of verbal learning and memory plus at least one other domain on a neuropsychological test battery. Deficits were defined as a score of 1.5 standard deviations or more below published normative means. In addition, the individual or a carer needed to report impairment in activities of daily living.

To be included in the PD group participants needed to have a current neurological diagnosis, no report of cognitive or functional impairment was required for this group.

To be included in the VCI group, participants needed to report a history of stroke confirmed by brain imaging *or* have evidence of transient ischaemic attack(s) *or* white matter changes revealed by brain imaging *and* demonstrate a deficit in at least one domain on the neuropsychological test battery. Deficits were defined as a score of 1.5 standard deviations or more below published normative means.

After data collection, each participant was discussed at a consensus conference attended by at least one neurologist and one neuropsychologist in order to confirm suitability for inclusion into the data analysis and confirm diagnostic group assignation. During the consensus conference neuropsychological test data, medical history, data collected during the neurological and physical examination and MRI data were considered. Data from the OST was not scored prior to the consensus conference to ensure that this information did not impact assignment to participant group.

6.5 Exclusion Criteria

To prevent any potential irritation from administration of atropine, participants were excluded if they had any chronic or acute nasal problems (e.g., sinusitis, allergic rhinitis, nasal congestion). Individuals with a history of traumatic brain injury or an uncontrolled mood disorder were excluded to preclude people with cognitive deficits potentially unrelated to dementia. Participants were excluded if they scored 10 or less on the baseline UPSIT. This cut-off level was chosen as it represented chance-level performance in a four-alternative forced choice paradigm employing 40 items. In order to be able to measure a reduction in UPSIT score it was considered necessary for baseline UPSIT scores to be better than chance.

6.6 Sample

Fifty-nine of the initial sixty-four participants were included in the analysis. Four individuals were excluded because they scored 10 or less on the baseline UPSIT and one person was excluded from the PD group because she was being investigated for a diagnosis of primary orthostatic tremor (discovered after she completed the study procedures).

Within the AD group, one participant with a diagnosis of AD and on treatment with donepezil, was referred by a local neurologist to participate in the research. He was considered to have above average premorbid ability based on his educational and occupational history. His caregiver reported increasing difficulties with activities of daily living, such as keeping financial records and remembering appointments and medications. His results on ApoE testing identified one Ɛ4 allele (Ɛ3:Ɛ4). On neuropsychological assessment, his performance on the memory tasks were impaired, however his scores for other cognitive domains were within 1.5 *SD* of the mean. It was agreed at the consensus conference that he was suitable for inclusion in the AD group.

Within the VCI group, nine participants had experienced a stroke and two had evidence of white matter changes.

Table 1 Demographic data

Variable	Controls	AD	PD	VCI
N	25	10	13	11
Mean Age (*SD*)	71.1 (5.5)	75.1 (6.0)	70.9 (6.2)	71.0 (6.6)
Mean years of education (*SD*)	10.8 (2.9)	11.6 (3.0)	11.5 (4.0)	10.7 (2.3)
% male	16	70	62	46

There were no differences between the groups in terms of age, F (3, 55) = 1.30, $p > .05$, or years of education, $F < 1$. The AD group and PD group consisted of a majority of male participants while the control group had a female majority. The percentage of males and females in each group differed significantly, χ^2 (3, N = 59) = 1.59, $p < .01$.

6.7 Procedure

6.7.1 Clinical Interview

A medical history was obtained via a structured interview with the participant and an informant. Demographic information, history of the presenting illness, and family history was obtained. Participants were asked to provide information on current medical conditions and current medications. This information was used to determine eligibility for the study, that is, to determine whether participants had other conditions that may affect olfaction or cognition. Information regarding current medications was not included in the analyses. Given that this thesis was primarily interested in change from baseline on the UPSIT test over a 40-minute period, it was felt unlikely that stable concomitant medication would affect the performance of the OST. The medical history and clinical interview were conducted by a neurologist (supervisor Peter Schofield; PS) and/or the author (SF).

6.7.2 Medical and Neurological Exam

A standard medical and neurological examination was performed by a neurologist (PS). This included, but was not limited to, assessment of height, weight and blood pressure as well as speech, facial expression, tremor, reflexes, tone, posture, gait and kinesia.

6.7.3 Neuropsychological Battery

Neuropsychological functioning was assessed in all participants using standardised tests addressing several cognitive domains. The cognitive battery included a general screening test, a task assessing verbal learning and memory, three tasks of visuospatial functioning, tasks of written and oral information processing speed, a task of attention, two tasks of executive functioning and an object naming task. The assessment was completed in a clinic room that was well lit, comfortable and quiet. During the assessment, noise and visual distractions were kept to a minimum. No clocks or calendars were visible to the participant. The neuropsychological assessments were completed by the author.

The tests comprising the neuropsychological battery are summarised according to cognitive domain in Table 2 and described below.

Table 2 Neuropsychological Test Battery

Domain	Tests	Abbreviation
General cognition	Mini-Mental State Examination	MMSE
Verbal Learning & Memory	Rey Auditory Verbal Learning Test	RAVLT
Attention	WAIS-III Digit Span	DS
Information Processing Speed	Trail Making Test - Part A	TMT-A
	Symbol Digit Modalities Test - Written	SDMT-W
	Symbol Digit Modalities Test - Oral	SDMT-O
	Trail Making Test - Part B	TMT-B
Executive function	Stroop Test	-
	Letter Fluency	-
Language	Boston Naming Test – 15 items	BNT

	Category Fluency	-
Visuospatial function	Drawing a clock	-
	Copying a clock	-
	Benton Judgement of Line Orientation	JLO

6.7.3.1 General cognition.

Mini-Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975): The MMSE is a brief cognitive screening tool that measures orientation to time and place, attention, language, registration, memory, and visuospatial skills. A maximum score of 30 is calculated by totaling correct responses, with a higher score representing better performance.

6.7.3.2 Verbal learning and memory.

Rey Auditory Verbal Learning Test (RAVLT): The RAVLT consists of verbal learning, recall and recognition measures. The administration procedure described by Senior et al. (1999) was used. In the learning phase, the examiner read out a list of 15 unrelated words (List A) at a rate of one word every two seconds. After each trial the participant was asked to recall these words in any order. After five learning trials, the examiner read out a distractor list (List B) and the participant was asked to recall these words. Immediately after recall of the distractor list, the participant was asked to recall the words from List A (immediate recall). Thirty minutes later the participant was asked to recall the words from List A again (delayed recall). Finally the participant was provided with a page showing all 15 words from List A with 15 new distractor words and was asked to identify which of the words belonged to List A (recognition trial).

Analysed data comprised total learning scores (trials 1-5), immediate recall and delayed recall. Each score was converted to a *z* score using age and education matched normative data reported by Senior (1999).

6.7.3.3 Attention.

Wechsler Adult Intelligence Scale-Third edition Digit Span (WAIS-III; Wechsler, 1997): The digit span task is composed of two tasks administered one after the other. For Digits *Forward* the examiner read a series of number sequences and the examinee was required to repeat the number sequence in the same order as presented. For Digits *Backward*, the examinee was required to repeat number sequences in reverse order. The task was administered according to the instructions provided in the WAIS-III manual. The scores for forward and backward administration were summed and normative data from the WAIS-III manual was used to obtain a *z* score.

6.7.3.4 Information processing speed.

Trail Making Test Part A (TMT-A; Reitan, 1955): The participant was presented with an A4 worksheet and asked to draw lines to connect consecutively numbered circles. Standardised instructions were provided and the participant was asked to complete a sample to confirm their understanding. Further instruction was then provided as needed. The participant was instructed to work as quickly as possible. The examiner pointed out any errors as they occurred and encouraged the participant to correct the mistake and continue. Total time taken to complete TMT-A was recorded and a *z* score was obtained using normative data published by Tombaugh (2004).

Symbol Digit Modalities Test - Written & Oral (SDMT-W/SDMT-O; Smith, 1991): The SDMT consists of two trials. The participant was presented with a worksheet containing a key that matched nine symbols to a number from one to nine. Below the key, the symbols were presented in rows with a blank row underneath. Following a practice trial on the first 10 items, the participant was asked to fill in the blank spaces using the number paired to the symbol shown. In the written trial (SDMT-W) the participant was asked to write down their response on the worksheet; in the oral trial

(SDMT-O) they called out their answer. The total score was the number of items completed correctly in 90 seconds on each trial. The total score was converted to a *z* score using age- and education-matched norms published in the manual.

6.7.3.5 Executive functioning.

Trail Making Test - Part B (TMT-B; Reitan, 1955): The participant was asked to connect numbered and lettered circles alternating between the two sequences as quickly as possible. Standardised instructions were provided and the participant was asked to complete a sample to confirm their understanding. Further instruction was then provided as needed. The examiner pointed out any errors as they occurred and encouraged the participant to correct the mistake and continue. Total time taken to complete TMT-B was recorded and a *z* score was obtained using normative data published by Tombaugh (2004)

Stroop test (Golden, 1976): The Stroop task consisted of three trials. In each trial stimuli were presented on a laminated A4 worksheet in five columns and the participant was instructed to read down each column as quickly as possible. In Trial 1 the participant was presented with the words *red*, *green* and *blue* printed in black ink and asked to read the words. In Trial 2 the participant was presented with series of four red, green or blue crosses (e.g., XXXX) and asked to name the colour. In Trial 3 the words *red*, *green* and *blue* were printed in a non-matching colour (e.g., *green*) and the participant was asked to name the ink colour. In each trial if the participant made a mistake, they were stopped and asked to correct the mistake before continuing. The total score was the number of correct responses in 45 seconds on each trial. An interference *T* score was calculated using a table in the manual that predicts performance on Trial 3 based on performance on Trials 1 and 2. The *T* score was then converted to a *z* score.

Letter Fluency: Participants were asked to say as many words as possible beginning with a specified letter during one minute. Three trials were performed (using the letters F, A and S). The participants were instructed not to give words that are proper nouns (e.g., *France*), numbers, or to use the same word with a different ending (e.g., *fast*, *faster*). The total score was number of words produced in the three trials. This was converted to a *z* score based on age-, education- and sex-adjusted norms (Tombaugh, Kozak, & Rees, 1999).

6.7.3.6 Language.

Boston Naming Test-15 Items (BNT-15; Graves, Bezeau, Fogarty, & Blair, 2004) Participants were shown 15 black and white line drawings of objects with names ranging in frequency of occurrence from high to low. Participants were asked to name all the drawings. If there was a misperception or lack of recognition of the object, the participant was provided with a stimulus cue (e.g., for *pretzel* the stimulus cue is 'something you eat'). If the participant failed to respond or provided an incorrect response a phonemic cue was provided (e.g., 'pre-'). The total correct was the sum of correct responses given spontaneously or after a stimulus cue. This score was converted to a *z* score according to normative data published by Graves et al. (2004).

Category Fluency: Participants were asked to produce words according to a specific semantic category (animals) during one minute. The total score was number of words produced. This was converted to a *z* score based on sex-, age-, and education-adjusted norms published by Tombaugh et al. (1999).

6.7.3.7 Visuospatial skills.

Drawing a clock to command: The participant was instructed to draw the face of a clock, placing all numbers in the circle, and set the time to ten past eleven. The drawing was scored using standardised criteria out of 10 with a maximum of two points for clock

face, four points for the presence and sequence of numbers and four points for the presence and placement of hands.

Copying a clock: The participant was presented with a line drawing of a clock showing the time at 10 past 11 and asked to copy it below. The scoring was identical to the command version of this task.

Benton Judgement of Line Orientation (JLO; Benton, Varney, & Hamsher, 1978): This test examined the participant's ability to estimate angular relationships between line segments by visually matching angled line pairs. For each item, the participant was asked to match a pair of angled lines to a multiple choice card below. A short version of was used, consisting of 15 items. The score was the number of items on which the participant's judgement for both lines was correct. The total score out of 15 was converted to a percentile according to the normative data provided in the manual. This score was then converted to a *z* score.

6.7.3.8 Neuropsychological composite scores.

The neuropsychological data was condensed into composite scores in order to characterise the samples and investigate the performance of the OST. The methodology for obtaining composite scores followed the general approach taken by Pike et al. (2007). *A memory composite score* was calculated by taking the average of the *z* scores for the RAVLT total learning, RAVLT immediate recall and RAVLT delayed recall tests. A *non-memory composite* score was calculated by taking the average of the *z* scores for DS, TMT-A, SDMT-W, SDMT-O, TMT-B, Stroop, Letter fluency, BNT, Category fluency, and JLO.

6.7.4 Psychological Functioning

Depression, Anxiety and Stress Scales - 21 (DASS-21; Lovibond & Lovibond, 1995): The DASS-21 consists of three self-report scales designed to measure symptoms consistent with depression, anxiety and stress. Each of the three DASS scales contains seven items. Participants use a four-point severity/frequency scale to respond to each item indicating the extent to which they had experienced each state over the past week. A total score and scores for Depression, Anxiety and Stress subscales were calculated by summing the scores for the relevant items. Normative data for the DASS-21 provide a label to characterise the degree of severity relative to the population as a whole. Scores associated with each severity label are shown below. The labels are used to describe the full range of scores in the population so 'mild' means that the person is above the population mean but does not represent a mild level of the disorder and are likely to be well below the typical severity of somebody seeking help (Lovibond & Lovibond, 1995).

Table 3 DASS Severity labels

Severity	Depression	Anxiety	Stress
Normal	0 - 4	0 - 3	0 - 7
Mild	5 - 6	4 – 5	8 - 9
Moderate	7 - 10	6 - 7	10 - 12
Severe	11 - 13	8 - 9	13 - 16
Extremely Severe	14 +	10 +	17 +

6.7.5 Functional Ability

Information about functional ability was collected using standardised questionnaires administered via interview by the neurologist (PS) or author (SF). The questionnaires were completed in reference to changes from premorbid levels of functioning and the

caregivers were asked for examples and justifications when responding to these items to ensure that the data collected reflected changes to premorbid ability rather than lack of earlier engagement in certain activities.

Activities of Daily Living Scale (ADL; Schwab & England (1969) : The participant (and informant for all clinical groups) was asked to rate their ability to conduct activities of daily living based on a scale of zero to 100 with zero being completely dependent – bedridden, and 100 being completely independent. Where there was a discrepancy between the participant and informant further information was collected and clinical judgement was used to determine a final score, as per the administration guidelines.

Functional Assessment Questionnaire (FAQ; Pferrer, Kurosaki, Harrah, Chance, & Filos (1982): The participant (and an informant for all clinical groups) was asked to complete the FAQ. This scale comprises of 10 items that assess a variety of instrumental activities of daily living and complex cognitive/social functions. These include paying bills, keeping financial records, shopping alone, and playing games of skill. There are six possible responses for each question (normal, has difficulty but does by self, requires assistance, dependent, never did but could now, or never did and would have difficulty now). A total score out of 30 was obtained, with a greater score being indicative of greater impairment.

Clinical Dementia Rating Scale (CDR; Morris, 1993): The CDR was developed for the evaluation of staging severity of dementia. It is based on a structured interview designed to assess changes in memory, orientation, judgement and problem solving, participation in community affairs, home and hobbies and personal care. A score of zero indicates no cognitive impairment, 0.5 indicates questionable or very mild dementia, one indicates mild dementia, two indicates moderate dementia, and three indicates severe dementia.

6.7.6 Clinical data analysis.

Table 3 shows the means and standard deviations for the MMSE and neuropsychological composite scores, psychological functioning and functional ability in each group. Impaired performances in comparison with the control group are indicated.

The control group performed within 1.5 *SD*s of the mean across all tasks of cognitive functioning.

A one-way between subjects analysis of variance was conducted to compare the effect of group on neuropsychological performance (MMSE, memory composite and non-memory composite score), psychological functioning and functional ability.

There was a significant effect of group on MMSE ($F(3,55) = 6.87$, $p < .01$), memory ($F(3,55) = 20.67$, $p < .01$), and non-memory ($F(3,55) = 9.79$, $p < .01$). Post hoc comparisons using the Tukey HSD test can be seen in Table 4. As expected, the AD, PD and VCI groups performed worse on neuropsychological assessment than the control group. Furthermore, the AD group performed worse than the PD group on the MMSE and memory composite score. Taken together, these results suggest that the AD group had the greatest level of cognitive impairment but that all groups were significantly more impaired than the control group. The raw data and *z* scores for the full neuropsychological battery can be found in the Appendix.

There was a significant difference in psychological functioning between the groups (DASS Total score: $F(3,50) = 3.68$, $p < .05$). Further investigation showed a significant difference in the Anxiety subscale, $F(3,51) = 4.27$, $p < .01$. Post hoc comparisons using the Tukey HSD test, see Table 4. The AD group obtained a significantly lower score on the anxiety subscale than the control group and PD group, indicative of less anxiety. This may reflect difficulty completing the questionnaire, as items for this subscale require the patient to recall specific physical symptoms in the past two weeks

and reduced recall of these events would lead to a lower score. The difficulties of the use of self-report questionnaires in people with AD have been well summarised by Frank, Lenderking, Howard, & Cantillon (2011).

Table 4 Clinical Data.

Variable	Controls	AD	PD	VCI
Neuropsychological Test Scores				
Mean MMSE (*SD*)	27.9 (1.7)	24.4 (3.2)*	27.7 (1.6) ˆ	25.5 (3.5) *
Mean memory composite score	0.24 (0.76)	-1.98 (0.42)*	-0.68 (0.93) *ˆ	-1.15 (1.00) *
Mean non-memory composite score	0.01 (0.49)	-0.84 (0.52) *	-0.43 (0.54)	-1.12 (0.91) *
Psychological Test Scores (DASS)				
Mean Depression Score (*SD*)	7.08 (7.85)	4.50 (5.21)	7.17 (5.88)	2.40 (2.46)
Mean Anxiety Score (*SD*)	6.25 (6.55)	0.44 (0.88) *	7.67 (5.18) ˆ	3.00 (3.43)
Mean Stress Score (*SD*)	9.33 (6.09)	6.22 (6.04)	9.83 (5.29)	4.50 (4.79)
Mean DASS Total Score	22.67 (15.79)	11.75 (11.13) *	24.67 (12.37)	9.90 (7.95)
Functional Test Scores				
Mean CDR Score (*SD*)	0.04 (0.14)	0.85 (0.24)*	0.19 (0.33) ˆ	0.55 (0.35)*ˆ
Mean ADL Scale (*SD*)	95.6 (10.0)	84.0 (15.8)	85.0 (16.2)	81.8 (14.7)*
Mean FAQ Total (*SD*)	2.14 (4.39)	12.25 (6.80)*	2.92 (4.58) ˆ	6.64 (5.94)

*indicates significant difference from the control group; ˆ indicates significant difference from the AD group, $p < .05$.

In regard to functional ability, there was a significant effect of group on the CDR score ($F(3,54) = 28.11$, $p < .01$), ADL score ($F(3,54) = 3.88$, $p < .01$), and FQ total score ($F(3,49) = 8.47$, $p < .01$). Post hoc analysis can be seen in Table 4. The AD group was significantly more impaired than all other groups on the CDR, and more impaired than the control group and PD group on the FAQ. Overall the AD group showed the greatest level of impairment. The VCI group was more impaired than the control group on the CDR and ADL scale. The PD group were not significantly more impaired than the control group on any functional measure.

6.7.7 Apolipoprotein E Genotyping

All participants were asked to provide a five millilitre blood sample to allow for the identification of their ApoE genotype. One control passed away prior to blood being

collected, one person from the PD group and two people from the VCI group refused to provide a blood sample, resulting in a total sample size of 58. ApoE genotyping was performed according to standard protocols (Poirier et al., 1993). The data are shown in Table 5.

Table 5 ApoE Genotype

ApoE Type	Controls	AD	PD	VCI
N	24	10	12	9
One or more Ɛ4 allele	7 (29%)	4 (40%)	6 (50%)	3 (33%)
Ɛ4:Ɛ4	1 (4%)	2 (20%)	1 (8%)	1 (11%)
Ɛ2:Ɛ4	2 (8%)	0	0	0
Ɛ3:Ɛ4	4 (17%)	2 (20%)	5 (42%)	2 (22%)
No Ɛ4 allele	17 (71%)	6 (60%)	6 (50%)	6 (67%)

The rates of ApoE Ɛ4 allele presence (one or more Ɛ4 allele) did not differ by group, χ^2 (3, N = 55) = 1.59, $p > .05$. The rate of ApoE Ɛ4 prevalence in the AD group and VCI group was consistent with published rates of 40-50% ((Davidson et al., 2006; Frisoni et al., 1994; Ward et al., 2012) and 30% to 40% respectively (Chuang et al., 2010; Davidson et al., 2006). However, the rate of ApoE Ɛ4 prevalence in the PD group was slightly higher than anticipated, where published rates are 14% to 27% (Whitehead, Bertrandy, Finnan, Butler, Smith & Ben-Shiomo, 1996, Koller, 1995). The rate of ApoE Ɛ4 prevalence in the control group was also slightly higher than the published rates of 14-26% (Federoff, Jimenez-Rolando, Nalls, & Singleton, 2012; Frisoni et al., 1994, Koller, 1995, Whitehead et al., 1996).

6.7.8 Volumetric MRI

All participants were asked to undergo an MRI scan to allow for the measurement of estimated total intracranial volume, and hippocampal volume. Ten participants refused

to have an MRI scan resulting in a total sample of 47. MR acquisitions were performed on a Siemens Avanto 1.5 T MR scanner. A true inversion recovery sequence was used to provide strongly T1-weighted 2 mm coronal slices for manual measurement. Using a PACS image viewer, total HV was obtained by summation of the area measurement, multiplied by the slice thickness. Corrected hippocampal volume was calculated using a validated method that standardizes hippocampal volume for intracranial volume (Buckner et al., 2004; Jack et al., 1989). An independent rater (Dr Houman Ebrahimi, a radiologist) who was blinded to the clinical status of the study participant performed the measurements. The volumetric data are shown in Table 6.

Table 6 Volumetric Data Values are mean and (*SD*) mm^3.

Variable	Controls	AD	PD	VCI
N	21	7	12	9
ICV	1482,228 (196,108)	1681,766 (143,352)	1521,531 (176,052)	1462,764 (202,296)
Corrected Left HV	3,378 (448)	2,125* (346)	3,265 (321) ˆ	3,256 (699) ˆ
Corrected Right HV	3,437 (489)	2,251* (525)	3,385 (344) ˆ	3,295 (653) ˆ
WM hypointensities	4,840 (3,837)	9,318 (1,0282)	6,959 (10,342)	14,510 (14,883)

*indicates significant difference between from the control group, ˆ indicates significant difference from the AD group, $p < .05$.

A one-way between subjects analysis of variance was conducted to compare the effect of group on volumetric data. There was a significant effect of group on corrected left hippocampal volume (F (3,45) = 13.37, $p < .01$), and corrected right hippocampal volume (F (3,45) = 10.69, $p < .01$). Post hoc comparisons using the Tukey HSD test can be seen in Table 6. The AD group had significantly smaller HV than all the other groups. There was no effect of group on ICV. The difference in volume of white matter hypointensities between the VCI group and control group was approaching significance ($p = .06$). Together this data suggests that the AD group differed from the othe groups

in regards to hippocamapl volume, as would be predicted from previous research (Hampel et al., 2008).

6.7.9 Olfactory Stress Test

The OST was administered according to the following procedures. The right nostril was occluded with cotton wool and the participant completed a full 40-item UPSIT. One squirt of atropine was delivered via an atomiser nasal spray bottle into the left nostril. Immediately after the atropine was administered, the participant was asked to either kneel down with their forehead touching the floor or lie face down across a clinic bed with their forehead inclined towards the floor for 60 seconds. After a break of 45 minutes, the full 40-item UPSIT was completed again using the left nostril only. The UPSIT was administered according to the instructions in the booklets. Participants with any motor difficulties (e.g., tremor) were assisted in scratching the microencapsulated strip and marking their answer. The UPSIT was scored using the scoring templates provided by the manufacturers. The atropine effect was calculated by subtracting the baseline UPSIT total score from the post-atropine UPSIT total score.

Two modifications were made to the administration of the OST in this study, in comparison with the 2012 study. In this study, participants completed a full (40-item) UPSIT before and after atropine administration. This modification was made because slight variations in average 10 item test scores have been previously been reported among the booklets which may mask or exaggerate a potential atropine effect (Doty et al., 1989). As there were no perceived time constraints requiring use of an abbreviated UPSIT and on the advice of the developers of the UPSIT (Doty, personal communication) the full 40-item UPSIT was administered before and after atropine.

A second modification concerned the delivery mechanism of atropine. In the 2012 study a nasal spray bottle that delivered a mist spray was used. In this study an

atomising spray bottle was used which delivers smaller particles than the mist. This change was made due to the availability of the atomiser bottle and not because of a perceived advantage of this methodology.

6.8 Baseline UPSIT Data

The baseline unirhinal UPSIT performance will be presented in detail here to establish the appropriateness of unirhinal testing. Based on the literature it was hypothesised that gender and group might contribute to baseline UPSIT scores.

A two-way analysis of variance was conducted to test the difference in baseline UPSIT score based on gender and group. Gender did not have a significant effect on baseline UPSIT score, $F < 1$, see Figure 3. Group had a significant effect on baseline UPSIT score, $F(3,51) = 12.00$, $p < .01$. Post hoc comparisons using the Tukey HSD test can be seen in Table 7. The patient groups had a significantly lower baseline UPSIT score than the control group, but there was no significant difference in the baseline UPSIT score between the patient groups.

Table 7 Baseline UPSIT Scores

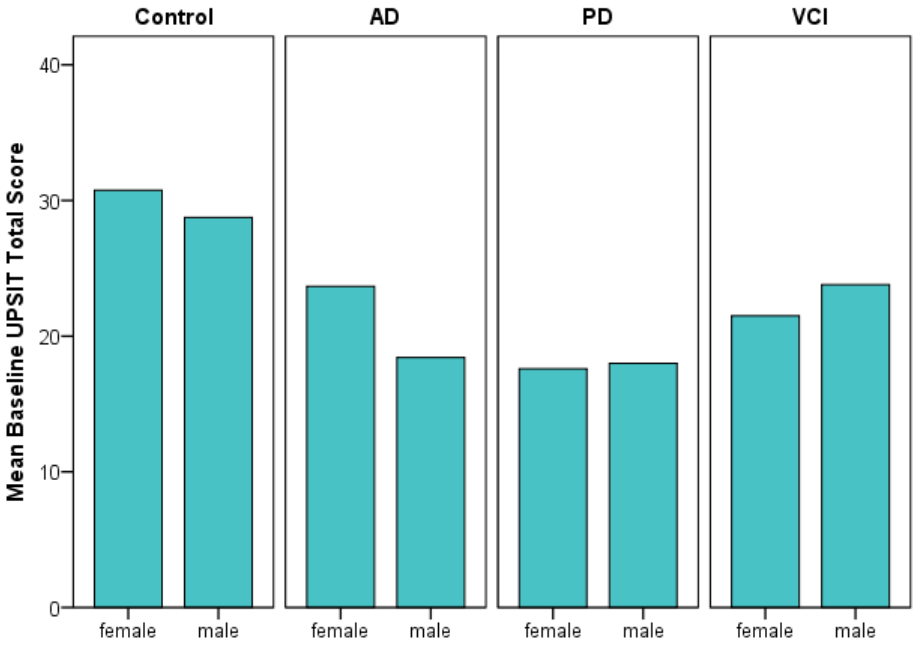
	Controls	AD	PD	VCI
Mean Baseline UPSIT Score (SD)	30.44 (4.23)	20.00 (5.81)*	17.85 (6.18)*	22.55 (5.30)*

*indicates significant difference from the control group, $p < .01$.

Due to the lack of normative data for unirhinal administration of the full 40-item UPSIT, the birhinal cut-off points were used to explore level of olfactory functioning in each group. It is acknowledged that this may underestimate the participants true birhinal olfactory functioning. Figure 3 shows the baseline UPSIT scores for participants in each group with the cut-off points for anosmia, microsmia and normosia indicated. It is apparent that the majority of the control group had some level of microsmia, however their scores were average for age with their mean performance at the 42nd percentile,

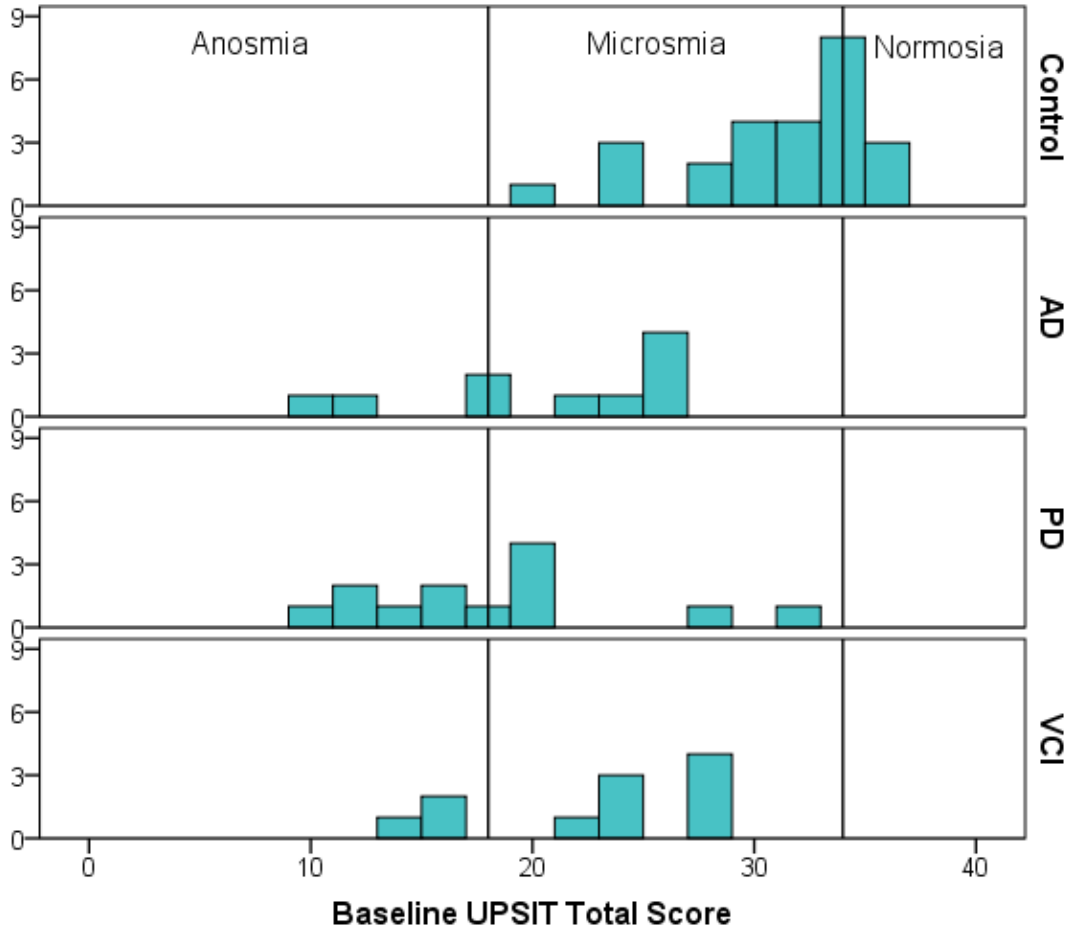
when compared with normative data. All of the participants in the patient groups had some level of olfactory impairment. In the AD and PD groups the majority of participants had severe microsmia or anosmia.

Figure 3 Baseline UPSIT scores



There was no correlation between baseline UPSIT scores and age (r = .02) or education (r = .12), ps > .05, perhaps due to the limited age range seen in this sample. There was a significant correlation between baseline UPSIT score and the memory composite score, r = .50, p < .01 and non-memory composite score, r = .44, p < .01, indicating better cognitive functioning was associated with better odour identification ability. There was no difference in mean baseline UPSIT score in those with an ApoE Ɛ4 allele as compared to those without, t (53) = 0.30, p > .05, suggesting that in this sample presence of an ApoE Ɛ4 allele did not affect UPSIT performance.

Figure 4 Baseline UPSIT scores



When the UPSIT is administered birhinally, AD patients show a high degree of uniformity when tested in different laboratories with scores ranging from 17 to 20.5 (Djordjevic et al., 2008; Doty et al., 1987; Kareken et al., 2001; Koss, Weiffenbach, Haxby, & Friedland, 1987; Li, Howard, & Gottfried, 2010; Moberg et al., 1997; Serby et al., 1991; J. Wang et al., 2010; Warner et al., 1986). The unirhinal UPSIT scores in the AD group were comparable to this literature.

Unirhinal olfactory performance has been evaluated in PD. Doty, Stern, Pfeiffer, Gollomp, & Hurtig (1992) investigated performance in each nostril using split halves of the UPSIT (20 items for each nostril). Scores of 10 to 11 were reported and prorated might indicate a score of 20 to 22 on the UPSIT which is slightly higher than the current study. However, more variation is reported in birhinal administration of the UPSIT in

patients with PD, with scores ranging from 16 to 25 (Bohnen et al., 2007; Bohnen et al., 2010; Bohnen, Studenski, Constantine, & Moore, 2008; Chou & Bohnen, 2009; Kesslak et al., 1988; Marras et al., 2005; McKinnon et al., 2010; Postuma, Gagnon, Vendette, & Montplaisir, 2009; Sobel et al., 2001). In the current study the results were comparable with birhinal data but within the lower range of previous findings. Consistent with previous research unirhinal testing was able to detect a significant difference between older adult controls and people with PD.

Odour identification ability has not been extensively studied in patients with vascular changes. Gray et al. (2001) reported birhinal scores of 12 on the UPSIT, much lower than the current study. It is possible that this represents the difference in samples either in regards to severity of symptoms or cause of vascular damage.

While unirhinal assessment of odour identification using the UPSIT was able to differentiate patient groups from healthy controls, it was unable to differentiate between neurodegenerative diseases. Consistent with previous research, a low score on the UPSIT was not predictive of a particular neurodegenerative disease and therefore not helpful in the differential diagnosis of these conditions.

The baseline UPSIT scores in this study were highly comparable with those reported in the previous OST study (Schofield et al. 2012). They were also higher than the median UPSIT scores reported in the study investigating the effect of donepezil treatment on UPSIT score in which a baseline UPSIT score of 14 is reported (range 6-26; Velayudhan et al., 2009). It was therefore assumed that the UPSIT, when administered unirhinally would be sensitive to the effects of a cholinergic challenge in this sample.

7. Chapter Seven- Results

The previous chapter provided a thorough context in which to explore the properties of the OST in this study. As expected the AD, PD and VCI groups performed worse than a control group on neuropsychological and functional assessment, the AD group showed reduced hippocampal volumes in comparison to controls, and the VCI group had reduced white matter hypointensities. It was determined that unirhinal administration of the UPSIT was able to differentiate between controls and patient groups and is an appropriate baseline to measure the effects of a cholinergic challenge. With this characterisation of the sample and methods complete, the data regarding the OST will now be presented.

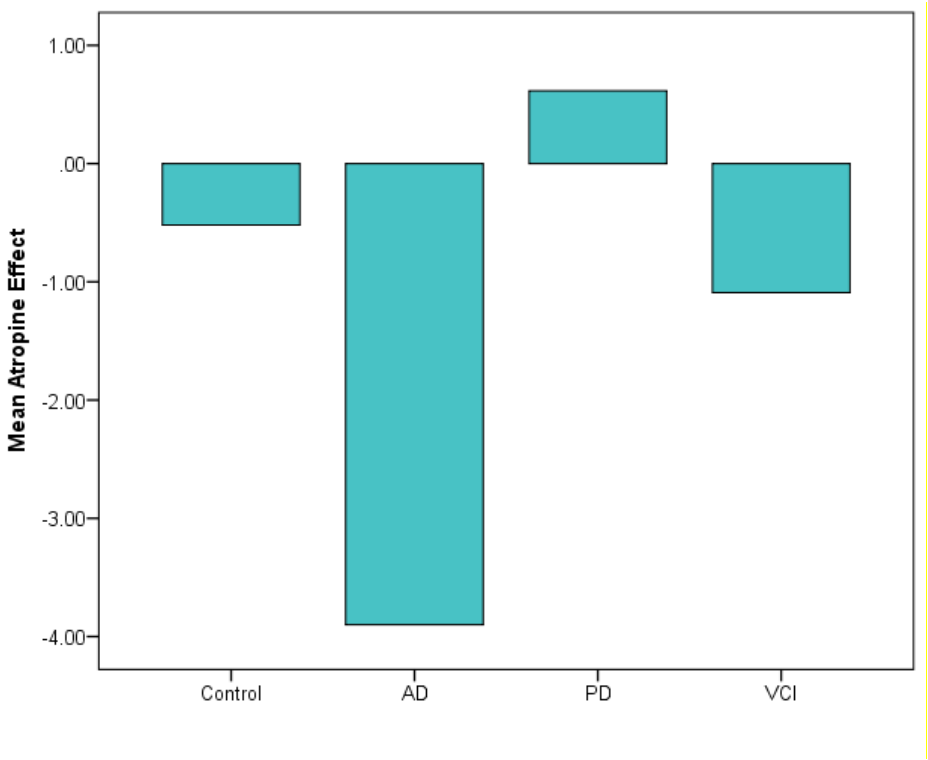
7.1 Atropine Effect

The primary aim of this study was to explore the atropine effect in three neurological conditions in comparison with a control group and compare the atropine effect in the AD group with two other clinical groups. The atropine effect was calculated by subtracting the baseline UPSIT total score from the post-atropine UPSIT total score. The difference is considered to represent the impact of atropine on olfactory functioning. The greater the atropine effect, the more the decline in UPSIT performance following atropine (i.e., an atropine effect of -4 would be greater than an atropine effect of -2). The mean atropine effect in each group is shown in Figure 5. It can be seen that the AD group had the greatest atropine effect, with almost a four point decline on UPSIT following atropine. To compare the atropine effect in the control group with the patient groups, and the atropine effect for the AD group against the PD and VCI group, five planned comparisons were conducted within an ANOVA framework, with post hoc Tukey comparisons.

In support of the hypotheses the AD group had a greater atropine effect than the control group, t (55) = 2.45, $p < .05$, and PD group, t (55) = 2.91, $p < .05$. There was no significant difference between the atropine effect in the AD group and VCI group, t (55) = 1.74, $p > .05$.

In further support of the hypotheses, there was no significant difference between the atropine effect in the control group and PD group, t (55) = 0.90 or control group and VCI group, t (55) = 0.43, both ps > .05.

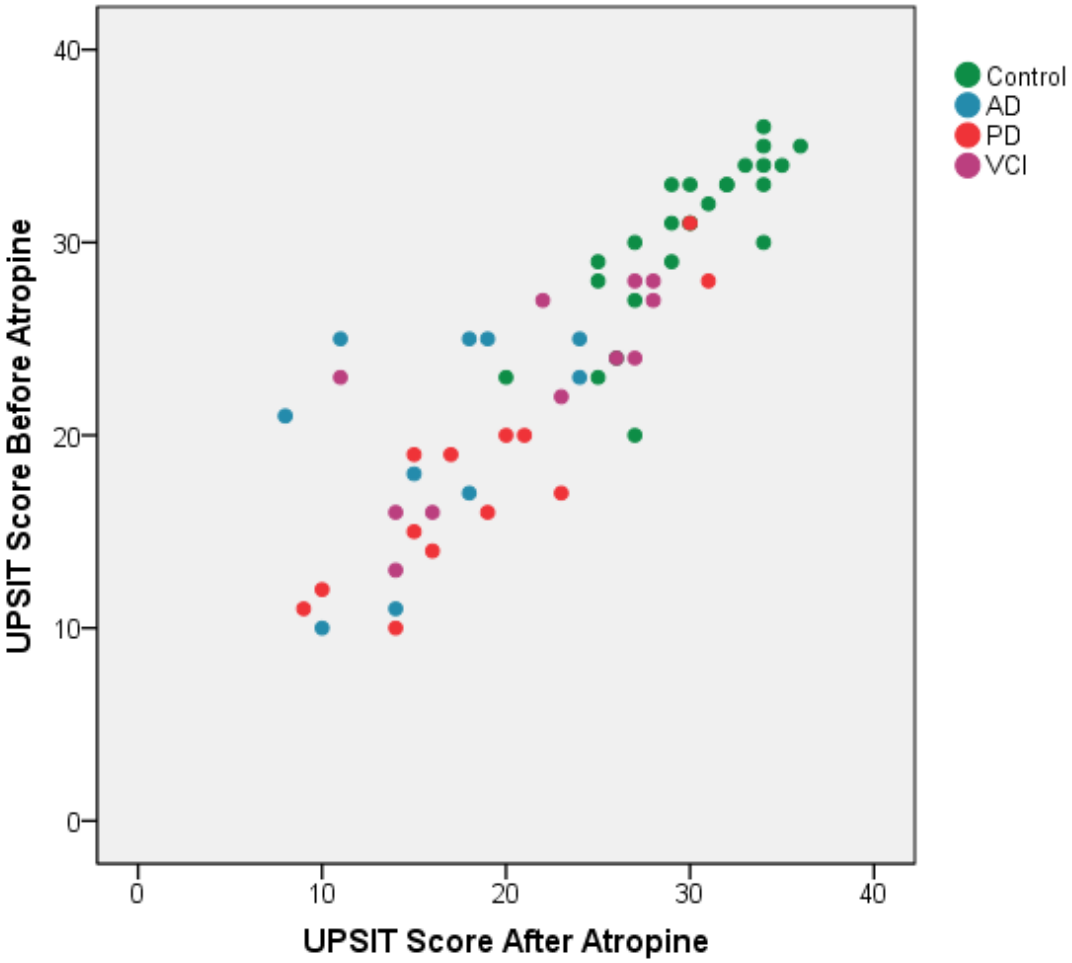
Figure 5 Mean Atropine effect



Further support for the differential effect of the OST in AD was explored by investigating the test retest performance of the UPSIT before and after atropine using paired sample correlations and a paired sample t-test. Figure 5 shows the correlations of the UPSIT score before and after atropine in each group.

In the AD group, there was no correlation between the UPSIT score before and after atropine, (r = .45, p > .05) and the difference between the UPSIT score before and after atropine was approaching significance (t (9) = 2.07, p = .07). In contrast, in the control group, PD group and VCI group there was a moderate to strong positive correlation between the total UPSIT score before and after atropine (controls: r = .81, PD group, r = .90, VCI group r = .76, all ps < .01) and the UPSIT scores before and after atropine did not differ significantly (control group, t (24) = 1.03; PD group, t (12) = .87; VCI group, t (10) = .86, all ps > .05). The difference between the correlations in the AD group and PD group was statistically significant, z = 2.0, p < .05. The differences between the correlations for the AD group and control group (z = 1.48, p = .07) and VCI group (z = 0.99, p = .16) were not significant. These results suggest a differential effect of the OST in the AD group, particularly in relation to the PD group.

Figure 6 UPSIT Scores Before and After Atropine

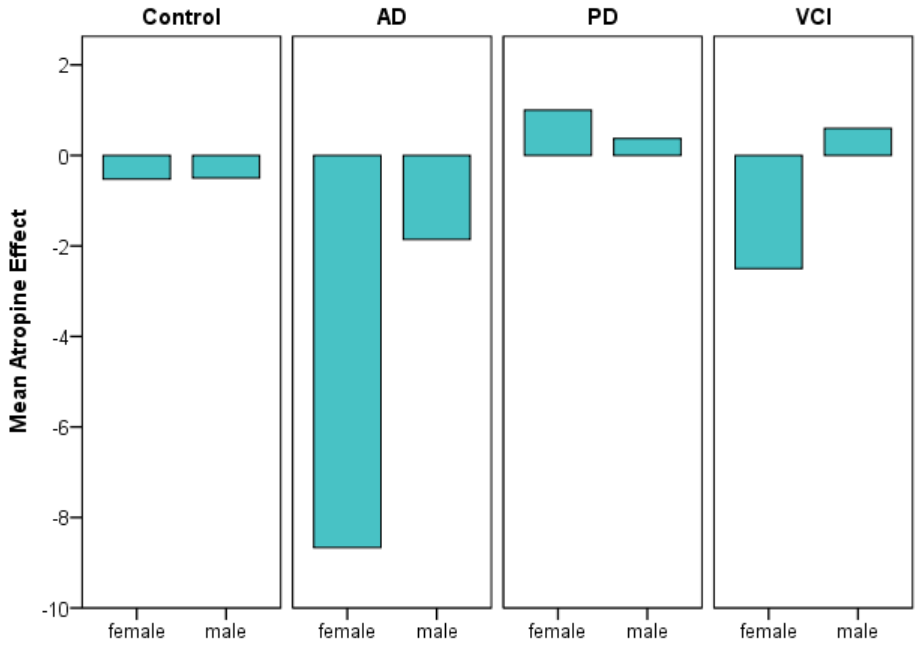


7.2 The Effect of Gender, Age and Education on the OST

There was a significant difference in the proportion of males and females in each group. Due to the well-documented influence of gender on olfactory function, the impact of gender will be explored here. Figure 7 shows the baseline UPSIT scores according to gender in each group. It is apparent that the female participants with AD (n = 3) had the greatest atropine effect, and the individual atropine effect for these participants was -6, -7 and -13. To test the effect of gender and group on atropine effect, a two-way analysis of variance was conducted. The ANOVA showed a significant main effect of gender, F (1, 51) = 4.85, $p < .05$ and group F (3, 51) = 5.18, $p < .01$. The interaction between gender and group was not significant F (3, 51) = 2.35, $p > .05$. The finding that gender had a significant impact on the

atropine effect was not expected and the implication of this will be reviewed in the Discussion. There was no significant correlation between the atropine effect and age (r = .16, p > .05) or education (r = .00, p > .05).

Figure 7 Mean Atropine Effect in Males and Females



7.3 Relationship between the OST and Other Markers of AD

The data from the control group and AD group was pooled to explore the relationship of the OST with hippocampal volume and neuropsychological performance.

7.4 Hippocampal volume

Hippocampal volume is an established AD biomarker. Imaging with brain MRI has been used extensively in clinical diagnosis and tracking of AD and its availability makes it a useful biomarker for comparison with the OST. Corrected hippocampal volume was used in all analysis, see section 6.12.

Partial correlations, controlling for age, sex, education and baseline UPSIT score, showed no significant correlation between atropine effect and corrected left

hippocampal volume, r = .25, p > .05, or corrected right hippocampal volume, r = .37, p >.05. Figures 6 and 7 show the relationship between atropine effect and corrected hippocampal volume, all participant groups are included.

Figure 8 Atropine Effect and Corrected Left Hippocampal Volume

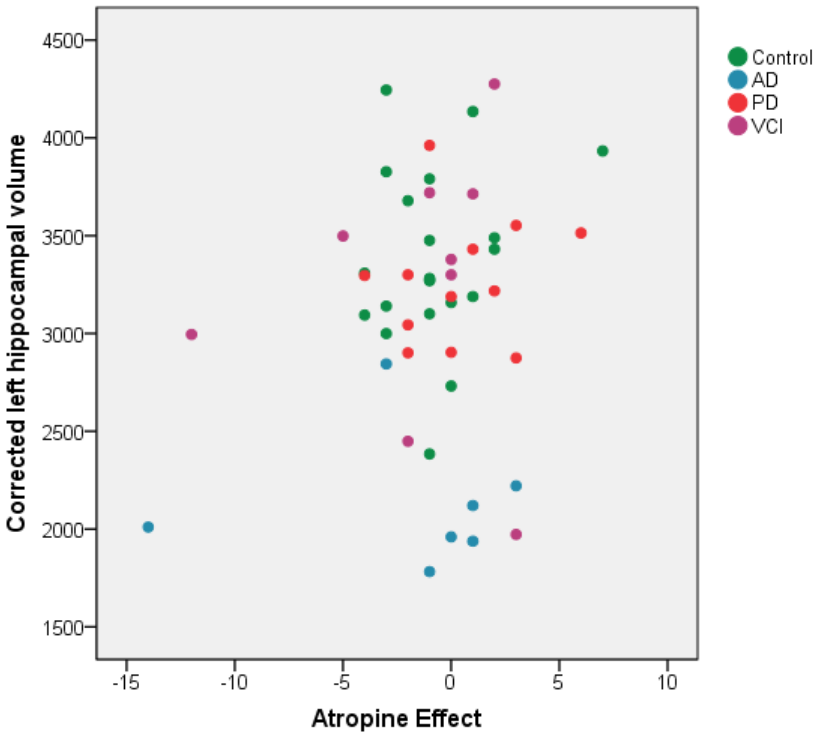
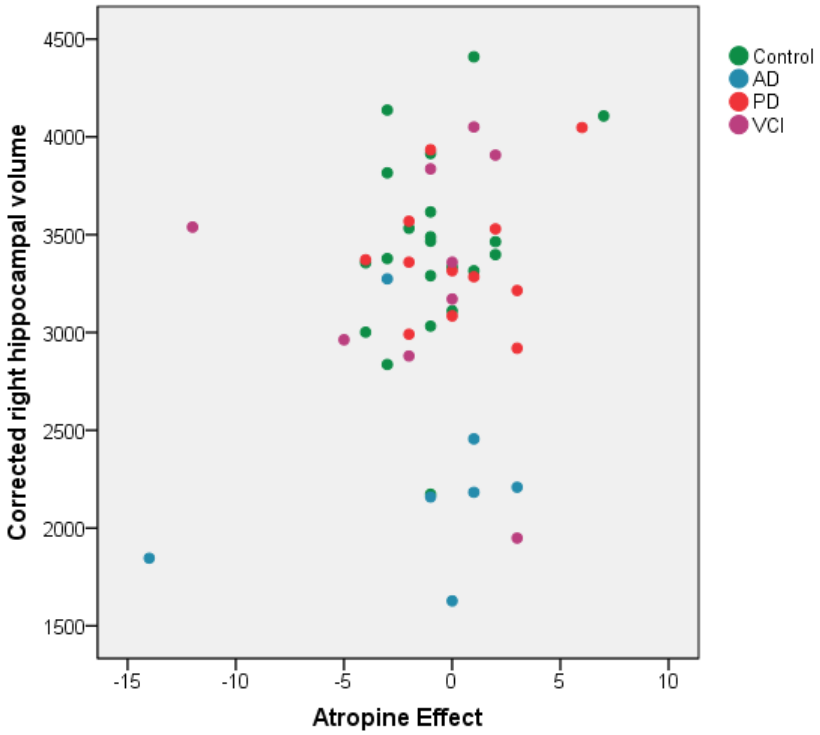


Figure 9 Atropine Effect and Corrected Right Hippocampal Volume



7.5 Neuropsychological test scores

It is well established that performance on neuropsychological assessment deteriorates with the clinical progression of AD and it was hypothesised that there would be a correlation between the atropine effect and a memory and non-memory composite score. The current sample consisted of a range of performance on neuropsychological assessment with the AD group showed the greatest impairment on the memory composite score.

There was no correlation, controlling for age, sex, education, and baseline UPSIT score, between atropine effect and MMSE score (r = -.10, p < .05), see Figure 10, or between atropine effect and the non-memory composite score (r = .14, p < .05), Figure 11.

Figure 10 Atropine Effect and MMSE Score

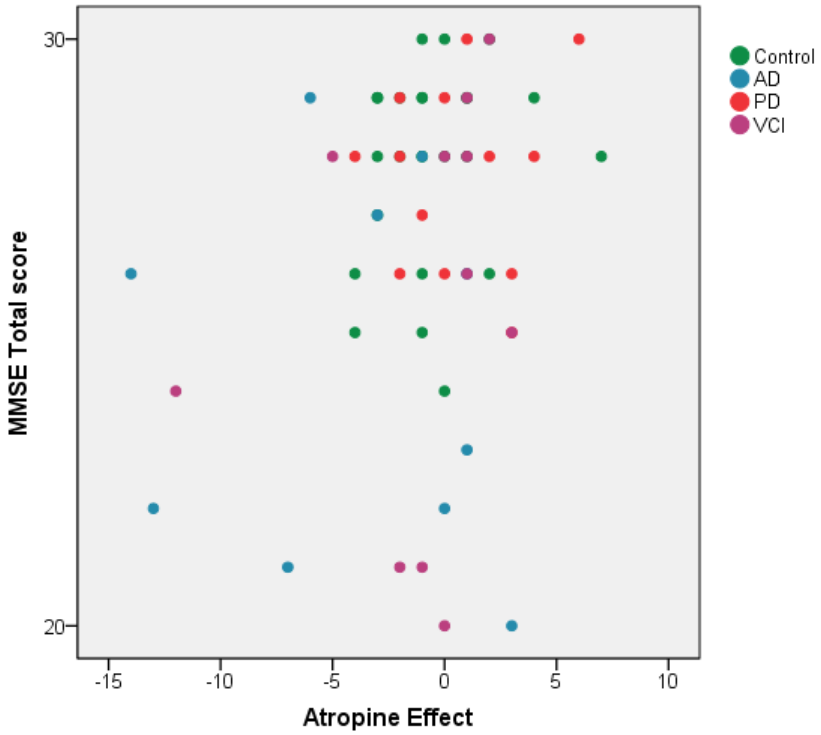
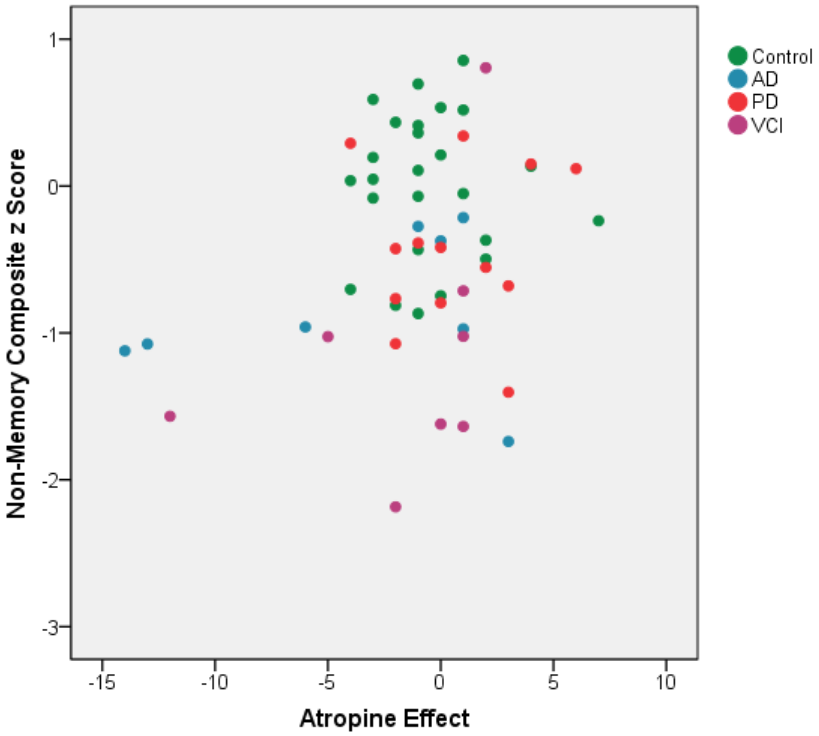
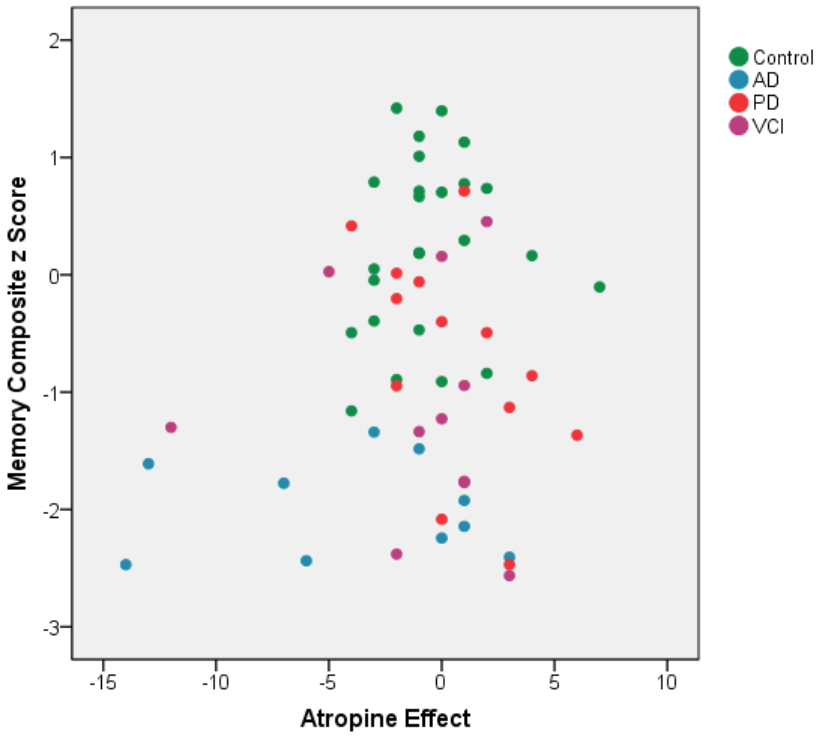


Figure 11 Atropine Effect and Memory Composite Score



There was a correlation, controlling for age, sex, education and baseline UPSIT score, between the atropine effect and the memory composite score (r = .36) however this did not reach statistical significance (p = .09), see Figure 12.

Figure 12 Atropine Effect and Non-memory Composite Score



When the data was pooled to include all patient groups, there was a significant partial correlation between atropine effect and MMSE score (r = .28) and atropine effect and the non-memory composite score (r = .34), both ps < .05. There was no correlation between the atropine effect and the MMSE score (r = .26), non-memory composite score (r = .36) or memory composite score (r = -.19) when the PD and VCI groups were combined.

7.6 ApoE Ɛ4 status.

Presence of an ApoE Ɛ4 allele represents the main genetic risk factor for AD. To investigate the hypothesis that participants with one or more ApoE Ɛ4 alleles will have a greater atropine effect than people with no ApoE Ɛ4 allele the participants were

grouped according to ApoE status. The mean atropine effect in participants with no Ɛ4 allele one and two Ɛ4 alleles is shown in Figure 8.

The mean atropine effect in participants with one or more Ɛ4 alleles was not significantly greater than the mean atropine effect in participants with no Ɛ4 alleles, t (53) = 1.40 p >.05. This finding did not support the hypothesis. The trend shown in Figure 8 may be largely accounted for by the fact that one of the five participants with two Ɛ4 alleles obtained the greatest atropine effect of -14. However three participants with Ɛ4/Ɛ4 alleles showed no change or improved on the UPSIT following atropine, as can be seen in Figure 14.

Figure 13 Mean Atropine Effect and ApoE status

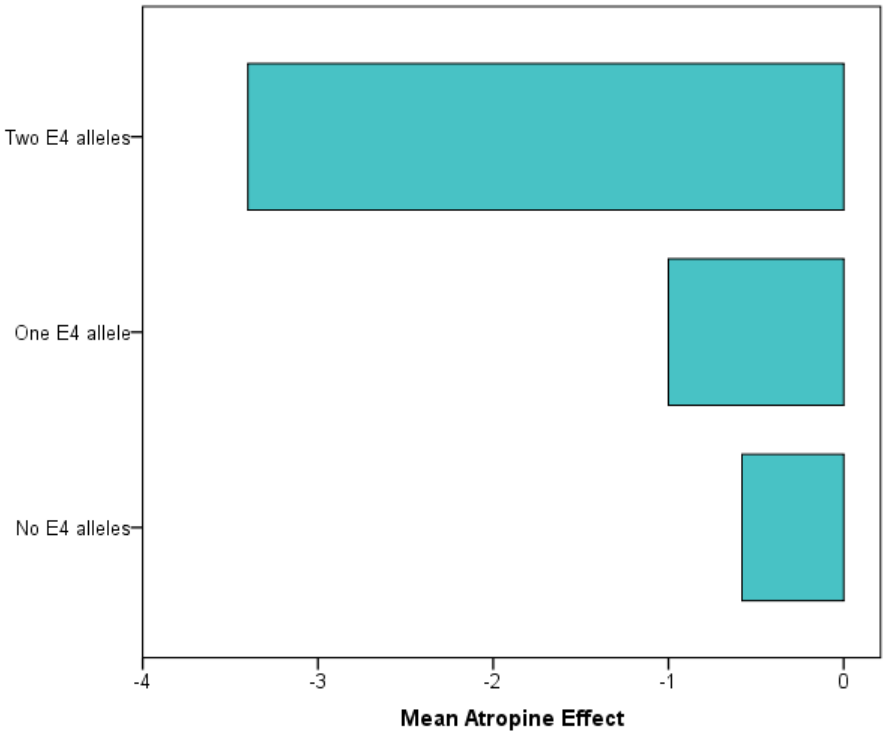
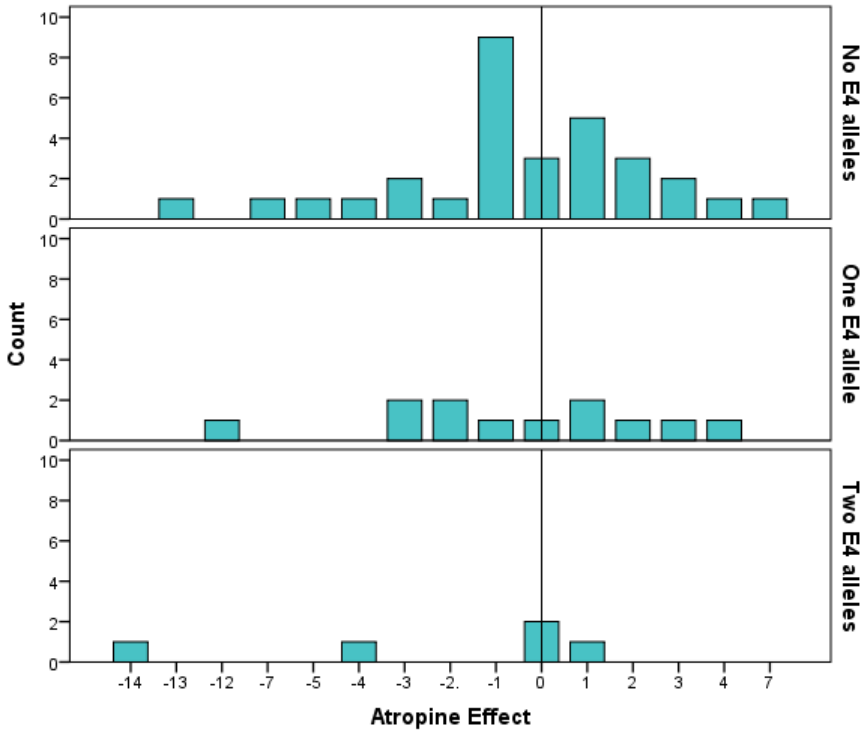


Figure 14 Individual Atropine Effect in Participants According to ApoE status

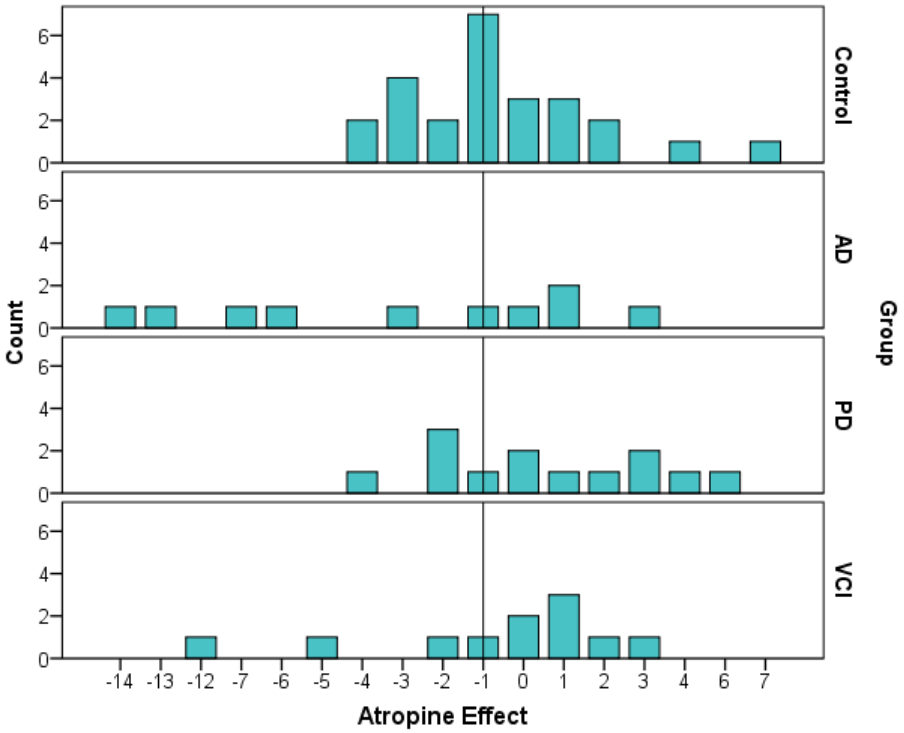


7.7 Clinical Utility of the OST

When looking at group differences, the OST appears to have differential properties within an AD sample. In order to be a useful biomarker in a clinical setting, the OST would require a clear cut-off score such that the administrator could easily classify an individual's score on the OST as being indicative of AD or not. In order to investigate a potential cut-off score, it is necessary to review the atropine effect of each individual within the groups.

Figure 15 shows the atropine effect for each individual. As can be seen, participants from the AD group had the greatest atropine effect (-14 and -13) followed by the VCI group (-12). No participants in the control group or PD group obtained an atropine effect stronger than -4. The largest increase in UPSIT score was found in the control group (+7), followed by the PD group (+6).

Figure 15 Individual Atropine Effect in Participants According to Patient Group



In the initial study, Schofield et al. (2012) found that the control group had a mean atropine effect of 0.28 and the percentages of participants with an atropine effect of less than zero in the control group (31%) and AD group (86%) were very similar to the respective rates of AD pathology at autopsy in cognitively normal samples and clinically diagnosed AD. For that reason, an atropine effect of less than zero was previously suggested as a cut-off indicative of underlying AD pathology.

In the current study the control group obtained a mean atropine effect of -0.52, with a 99% confidence interval of -0.89 to 1.92. Using these data, it might be reasonable to assume a one to two point change in UPSIT score (positive or negative) in older adults is due to inherent variability in the test rather than the impact of atropine.

Table 8 shows the proportion of participants in each group who obtained an atropine effect of less than zero, less than -1 and less than -2.

Table 8 Olfactory Stress Test Results

Cut-off Score	Controls	AD	PD	VCI
Mean atropine effect, *M* (*SD*)	-0.52 (2.52)	-3.9 (5.91)	0.69 (2.87)	-1.09 (4.21)
Atropine effect < 0, n (%)	15 (60%)	6 (60%)	5 (39%)	4 (36%)
Atropine effect < -1, n (%)	8 (32%)	5 (50%)	4 (31%)	3 (27%)
Atropine effect < -2 n, (%)	6 (24%)	5 (50%)	1 (8%)	2 (18%)

It is evident that an atropine effect of less than zero is more likely for a person with AD than PD or VCI, but could also indicate the performance of a non-impaired control. Looking at the proportion of participants in each group who obtained an atropine effect of less than -1, it is apparent that half of the AD group had this level of decline in comparison to only a third for the other groups. An atropine effect of -2 further differentiates the groups however a chi-square analysis determined that the percentage of participants obtaining an atropine effect of less than zero (χ^2 (3, N = 59) = 2.89), less than one (χ^2 (3, N = 59) = 1.47) or less than two (χ^2 (3, N = 59) = 5.85) was not significantly different in each group, *p*s > .05.

8. Chapter Eight – Discussion and Conclusion

The OST is under investigation as a suitable biomarker for AD. The primary aim of this study was to compare the performance of the OST in participants with AD, PD, and VCI with a control sample of cognitively unimpaired older adults. Overall, the results showed a difference in the performance of the OST in the AD group as compared to the other groups.

8.1 The OST in AD Compared to Controls

As hypothesised, the AD group was sensitive to a cholinergic challenge as shown by a 3.8 point reduction in performance on the UPSIT, following atropine, and a lack of correlation between the UPSIT scores before and after atropine. In contrast, the mean atropine effect in cognitively unimpaired older adults was -0.52 and there was a high correlation between baseline and post-atropine UPSIT scores. This is consistent with previous research (Schofield et al., 2012; Schofield, et al., 2013). This finding is also consistent with earlier work by Serby et al. (1990) who found that subcutaneous administration of scopolamine in healthy young controls did not significantly affect UPSIT performance.

The exaggerated effect of atropine on olfactory performance in people with AD in comparison with controls is consistent with the large body of research into the effects of anticholinergic drugs on cognitive functioning which was prominent in the 1980s (Ray et al., 1992; Sunderland, Tariot, Murphy, et al., 1985; Sunderland et al., 1987; Sunderland, Tariot, Mueller, et al., 1985; Sunderland et al., 1986). Our work and this previous work has found that in response to a cholinergic challenge, people with AD have an exaggerated deficit on functions vulnerable to cholinergic functioning such as cognition and olfaction. This may be accounted for by the theory of cholinergic reserve. A functioning cholinergic system is able to compensate for a cholinergic challenge by

increasing ACh release, whereas a compromised, vulnerable system (such as in AD) may be less able to modify ACh release, thus resulting in a larger detrimental effect.

In the development of a biomarker it is essential to have a clear cut-off point indicative of pathology so that results can be clearly interpreted. In the pilot study (2012), an atropine effect of less than zero was used for exploratory analysis to indicate possible AD pathology. Therefore *any* decline in performance was considered to be the result of atropine. This cut-off point was chosen as the percentages of participants with an atropine effect of less than zero in the control group (31%) and AD group (86%) were very similar to the respective rates of AD pathology at autopsy in cognitively normal samples and clinically diagnosed AD. In the current study, 60% of the participants with AD had an atropine effect of less than zero, with the remaining 40% showing an improvement or no change on UPSIT scores after atropine. A cut-off score of zero in this study would have failed detect cases of AD in 40% of cases.

The discrepancy in the results is difficult to explain. As shown in Table 9, the samples in both studies were highly comparable in terms of age, gender, education, MMSE and baseline UPSIT score. A difference in HV is evident however this may result from methodological differences in calculating this figure: in the 2012 study HV was calculated manually however, in the current study computer software was used. It is possible that the difference in results is due to modifications in the OST and the impact of the method of atropine delivery on the results will be discussed in the limitations section.

Table 9 Sample Characteristics from the 2012 study and Current study

	Controls		AD	
Variable	2012	Current study	2012	Current Study
N	29	25	14	10
Mean Age (*SD*)	74.0 (6.6)	71.1 (5.5)	75.3 (4.6)	75.1 (6.0)
% female (*n*)	20 (69%)	21 (84%)	4 (29%)	3 (30%)
Mean Education (*SD*)	10.6 (3.1)	10.8 (2.9)	11.1 (2.6)	11.6 (3.0)
Mean MMSE (*SD*)	29.0 (1.6)	27.9 (1.7)	23.6 (4.0)	24.4 (3.2)
Mean Left HV	1819 (370)	3377 (447)	1385 (293)	2125 (346)
% with 1 or more ε4 allele	31%	29%	50%	40%
Mean UPSIT (*SD*)	14 / 20 (2.6)	30 / 40 (4.2)	10 / 20 (2.7)	20 / 40 (5.8)
Mean Atropine Effect (*SD*)	0.3 (2.2)	-.5 (2.5)	-2.4 (1.5)	-3.8 (6.1)
% with Atropine Effect < 0	31%	40%	86%	60%

A cut-off score of zero suggests that any reduction in UPSIT score is a result of atropine and does not account for any inherent variability in performance on the UPSIT on repeat testing. The UPSIT is a widely used assessment tool that has been well validated for use in research and has excellent test retest data. It is considered the gold standard in olfactory testing and it is often used to assess the validity of new olfactory assessments (Frank et al., 2003; Toledano et al., 2009). However, when any test is given on successive occasions, a participant will usually not earn identical scores at each session. The fluctuation in test score is attributable to changes in the participant's motivation, fatigue, emotional state, alertness, luck in guessing, as well as changes in conditions of testing, such as time of day. Fluctuations in test score that occur for such reasons are usually described as reflecting errors of measurement (Chapman & Chapman, 1978).

As a result of measurement error, some fluctuation in UPSIT performance may be expected regardless of atropine administration. The OST requires a participant to repeat the UPSIT approximately 45 minutes after the initial test. No guidance or corrective feedback was provided. It might be anticipated that practice effects, familiarisation with testing procedures, and to a certain extent recall of previous answers may improve performance on repeat testing, however fatigue after a reasonably long afternoon of study procedures may reduce performance.

To a certain degree, the control group permitted an investigation of the extent to which UPSIT performance may fluctuate in a cognitively intact group of older adults. The difference in UPSIT performance before and after atropine was -0.52, with a 99% confidence interval of -0.89 to 1.92. A one to two point change in UPSIT score (positive or negative) in unimpaired older adults may be due to practice effects or measurement error, rather than the impact of atropine.

Test-retest data for the UPSIT have not been published in impaired samples. It is possible that test retest properties for participants with olfactory impairments are lower given the inherent difficulty of reliably measuring an impaired sense. In people with impaired UPSIT scores, indicative of anosmia, it may be possible that floor effects mask a potential atropine effect. In other words an individual was scoring so low at baseline it was not possible to reduce their performance further with atropine. Participants were excluded from the study if they scored 10 or less on the baseline UPSIT. This cut-off level was chosen as it represented chance-level performance in a four-alternative forced choice paradigm (40 items). In order to be able to measure a change on the UPSIT, it was considered necessary for baseline UPSIT scores to be better than chance. However, even amongst participants who met this criterion, some

commented that they were unable to smell anything and therefore were guessing or using deduction to make their responses (as is encouraged in the manual).

Another consideration in this regard is the test retest reliability of the UPSIT when administered unirhinally. Unirhinal administration has been associated with poorer olfactory performance (Good et al., 2003) and may therefore reduce reliability. The *nasal cycle* may impact performance on olfactory tests ability when assessment is unirhinal. Nasal cycling refers to the finding that most individuals have a unilateral nasal obstruction due to the normal physiological phenomenon of turbinate engorgement. The obstruction changes in a cyclical manner from side to side throughout the day and night, with the individual typically unaware of this occurrence (Principato & Ozenberger, 1970). These side-to-side fluctuations have been reported to have periods ranging in length from one to seven hours in adults (Lang, Grutzenmacher et al 2003). Although this nasal cycle was originally presumed to be present in 80% of adults, recent studies suggest that this may be an overestimate (Flanagan & Eccles, 1997) and the proportion of subjects exhibiting the alternating rhythmicity associated with the classic nasal cycle decreases with age (Mirza, Kroger, & Doty, 1997). Although unirhinal testing may be affected by the presence of a nasal cycle (if the nostril engorged is the one which you are testing), it seems unlikely that this would be able to account for the pattern of results seen in this study but it may have increased variance in UPSIT scores.

It is prudent to explore factors, other than atropine that may reduce UPSIT performance in an AD sample. Completing the UPSIT twice in one day may create fatigue and worsen performance over time. Indeed, Warner et al. (1986) reported that people with AD may have more difficulty with items towards the end of the UPSIT due to a fatigue effect. They noted that AD patients took approximately 35 minutes to complete the UPSIT compared with 20 minutes for healthy controls. In the current

study, a time difference was also observed, with the control group completing the UPSIT within 15 to 20 minutes, and the patient groups taking up to 45 minutes. The PD group were observed to have the most difficulty and many needed physical help to complete the UPSIT (i.e., to scratch the microencapsulated strip, raise the UPSIT to the nostril and/or mark their answer). There is some support for this in the data. The range of scores on the UPSIT before and after atropine were identical for the control group, however the patient groups did have a lower range as can be seen in Table 10. This may have also been the effect of sampling, as participants who scored less than 10 at baseline were excluded from the study. Attempts were made to manage fatigue - all participants were offered a hot drink and encouraged to have a rest in the waiting room between UPSIT assessments.

Table 10 Range of UPSIT Scores Before and After Atropine

UPSIT Range	Controls	AD	PD	VCI
Before atropine	20 - 36	10 - 25	10 - 31	13 - 28
After Atropine	20 - 36	8 – 24	9 - 31	11 - 28

If fatigue was causing the reduction in performance on the UPSIT after atropine, it would seem likely that this would occur in all of the patient groups. The PD and VCI had similar levels of olfactory impairment at baseline yet had minor differences in UPSIT scores before and after atropine and these scores were highly correlated. This suggests that fatigue is unlikely to account for the reduction in UPSIT score following atropine seen in the AD group.

Another possibility is that greater cognitive impairment in the AD group led to an exaggerated atropine effect, perhaps due to a mediating factor of attention or memory. If this were the case, the greatest atropine effect would be expected in the AD group,

followed by the VCI group, as this group demonstrated similar levels of deficits in neuropsychological functioning. Indeed this pattern of results did occur with the VCI group obtaining a mean atropine effect of -1. The atropine effect in the VCI group was not statistically significant from the control group but neither was it significantly different from the AD group.

It is noted that the baseline UPSIT score was significantly correlated with the memory and non-memory composite score, indicating better cognitive functioning was associated with better odour identification ability. This supports previous research. Westervelt, Ruffolo, and Tremont (2005) found that odour identification ability measured with the B-SIT was moderately correlated with general cognitive functioning, language skills, and memory. Verbal retrieval difficulties, poor working memory and slow cognitive speed have also been found to significantly affect performance on the UPSIT {Dulay, 2008 #1924}. While it was anticipated that intranasal administration of atropine would result in effects localised to the olfactory bulb, atropine may have been absorbed and crossed the blood-brain barrier resulting in a more systemic effect. In this case, previous research would predict that atropine may reduce cognitive performance in the AD group (Inzelberg, 1990) and therefore a reduction in UPSIT score may have been mediated by a reduction in cognitive functioning. It may have been useful to include a measure of cognitive function after the administration of atropine to explore this possibility further. Another way to assess the moderator of cognitive function in atropine effect would be to compare the correlation between the atropine effect and cognition within each individual group. If it could be shown that the atropine effect correlated in the AD group but not the PD or VCI group this would support the role of cholinergic sensitivity in the atropine effect.

Gender played a significant role in predicting atropine effect and there was an additive effect of gender and patient group. Females with AD showed the greatest decline in UPSIT score following atropine, with a mean atropine effect of -8. However, due to the small sample sizes when looking at gender and group, caution must be exercised in interpreting these results. In a review of sex differences in cognition and AD, Li and Singh (2014) reported that the differences in learning and memory between male and female brains are evident throughout the lifespan, that females carry an increased risk of developing AD pathology compared to males, even when controlling for increased lifespan (Callahan et al., 2001; Dye, Miller, Singer, & Levine, 2012), and that females show faster decline and greater deterioration in cognition than males (Proust-Lima et al., 2008). In a meta-analysis of data from 15 studies (*n* = 828 men; 1,238 women), Irvine, Laws, Gale, and Kondel (2012) found that cognitive function in AD is both more severely and more widely affected in women than men. Although clear gender differences have been reported in AD, the reasons for these differences are still unclear. The major biological hypothesis concentrates on age-related sex hormone reduction, genetic risks, impact of risks from other diseases and differences in brain anatomy such as atrophy and glucose metabolism (Li & Singh, 2014). It is also possible that differences in cognition and AD between the sexes reflects differences in cholinergic functioning.

As detailed in the introduction, the cholinergic system is strongly implicated in cognitive processes. Treatments that increase cholinergic functioning improve performance on cognitive tasks, whereas treatments that decrease cholinergic function impair performance. Despite the known sex differences in cognitive and olfactory functioning, sex differences in the cognitive response to scopolamine have not been reported in clinical studies. However, Berger-Sweeney, Arnold, Gabeau, and Mills (1995) investigated the differential effect of scopolamine on learning and memory in

male and female mice. They found that females were more sensitive than males to the effects of scopolamine and implicated a role for the cholinergic system in these differences. They reported that the organization of the cholinergic system is sexually dimorphic and females show reduced cholinergic functioning across a number of measures of choline uptake (Miller, 1983), AChE activity (Luine, Renner, Heady, & Jones, 1986) and ChAT activity (Hortnagl et al., 1993). It is hypothesised that the OST unmasks cholinergic dyfunction, and it therefore follows that if females have a vulnerable cholinergic system, the atropine effect would be more pronounced in this group.

The finding that some participants with a diagnosis of AD improved on the UPSIT after atropine does not support the concept of a universally vulnerable cholinergic system in AD. Theorists suggest that there are many risk factors that contribute to AD with the exact cause differing for each individual (McDonald, 2002). it is possible that in some people cholinergic functioning may be the primary causative factor in AD while in others alternative risk factors are more influential in the expression of AD. The finding that some people respond to AChEIs while others do not also support this theory (Craig et al., 2011).

8.2 The Olfactory Stress Test in Other Neurological Conditions

This study was the first study to investigate the OST in PD and VCI. As hypothesised, there was no significant difference between the mean atropine effect in the control group, PD group or VCI group and the baseline and post-atropine UPSIT correlated highly in these groups. This was an important finding with respect to the aim of characterising the specificity of the OST. Olfactory impairments are common in patients with PD and, to date, olfactory tests have not been able to successfully differentiate between PD and AD. This result points to an advantage of the OST over

standard tests of olfaction. This finding also supports the idea that olfactory impairments in these conditions are related to different biological causes and may provide further information on the functioning of the cholinergic system in these conditions. The cholinergic system has been implicated in the aetiology of PD and Doty (2012) has suggested that olfactory deficits in PD may be related to cholinergic functioning. The nucleus basalis, a major cholinergic nucleus with projections to olfaction-related brain regions, is significantly damaged in PD, with autopsy studies reporting decreases in cell numbers ranging from 54% to 77% (Arendt 1983). An association between Ach and olfactory function in PD has been found in a PET study (Bohnen et al., 2010). Furthermore, scopolamine has also been shown to induce cognitive impairments in a group of cognitively intact parkinsonian patients. However, if a vulnerable cholinergic system is responsible for change in UPSIT score, the same result might be expected in AD and PD and therefore this study does not support the suggestion that cholinergic function is central to olfactory processes in PD.

The finding that 38% of the PD group and 36% of the VCI group obtained an atropine effect of less than zero was not unexpected. It is slightly more unusual that such a high proportion (56%) of the control group had an atropine effect of less zero, however this is not necessarily inconsistent with the literature. As detailed in Chapter Two, AD has a long preclinical period in which cognitively asymptomatic individuals show considerable amounts of AD pathology at autopsy as well as neurofibrillary degeneration and cell volume loss in cholinergic neurons (Davis, Schmitt, Wekstein, & Markesbery, 1999; Iacono et al., 2014; Knopman et al., 2003; Mesulam et al., 2004; Sassin et al., 2000). It is therefore likely that the control group, PD and VCI group contained participants with some level of preclinical AD pathology. In a large retrospective autopsy study of demented patients with the clinical diagnosis of probable AD, 83.2 % revealed AD-related pathology, but only 41.1 % were diagnosed with

‘‘pure’’ AD without concomitant pathologies; 24.2 % showed additional cerebrovascular lesions, 9.1 % additional Lewy pathology, and 2.6 % various other concomitant brain lesions, while 10.7 % were diagnosed as ‘‘pure’’ vascular dementia without AD pathology (Jellinger & Attems, 2008). It is possible that the participants in this study who obtained an atropine effect of less than zero were the ones with underlying AD pathology, regardless of their clinical diagnosis. Future research using longitudinal designs and post mortem verification of diagnosis are needed to test this hypothesis.

8.3 The Relationship Between the OST and Other Markers of AD

A further aim of this study was to compare the performance of the OST with other validated markers of AD. This aim assumes that atropine effect has a linear value, such that an atropine effect of -14 represents more AD symptomatology or pathology than an atropine effect of -3. It further assumes a relationship between cholinergic functioning and the degree of clinical symptoms (cognition), structural changes (hippocampal volume) and genetic disposition (ApoE status). Work with the OST so far been inconsistent. In the first published study, the atropine effect correlated strongly with episodic memory (r = .57, p < .001; Schofield et al., 2012). In the second study of cognitively intact older adults, there was no significant correlation between the atropine effect and MMSE or delayed recall. This may have been a result of lack of range in cognitive performance to show a correlation. In this study, the original hypothesis anticipated a correlation between the atropine effect and neuropsychological scores in the control and AD group. This was not supported, though there was a trend for this relationship between atropine effect and the memory composite score. In the sample as a whole there was a significant correlation between the atropine effect and two measures of neuropsychological functioning (the non-memory composite score and MMSE); with poorer cognitive functioning associated with a greater atropine effect. However there was no correlation between the atropine effect and a composite

memory score. This finding was somewhat unexpected as cognitive functioning in the VCI group, and to a lesser extent the PD group, may not have been associated with cholinergic functioning but may have been a result of other underlying biological causes (i.e., the stroke). There was no correlation between the atropine effect and neuropsychological composite scores in the PD and VCI group.

Results in regards to the effects of ApoE on atropine effect have been more consistent with both studies showing that individuals with one or more Ɛ4 alleles have a significantly greater atropine effect than people with no Ɛ4 allele (Schofield et al., 2012, Schofield et al., 2013). This finding was not replicated in this study, however the sample was unusual in regards to the rate of ApoE Ɛ4 allele carriers in the PD group.

In contrast with previous research, there was no correlation between atropine effect and left hippocampal volume in the AD and control group or in the sample as a whole. This may be due to the method of volume measurement which differed from the previous study or sample sizes. Lack of correlation in the sample as a whole was not expected due to the inclusion of patients with PD and VCI where hippocampal atrophy may be related to factors other than AD pathology (Kandiah et al., 2014; Kim, Moon, & Han, 2013).

8.4 Implications of the current study.

The OST is a simple, noninvasive, inexpensive tool that shows some suggestion of sensitivity and specificity for AD pathology. The implications of having a widely available tool to assist in diagnosis of AD are numerous. AD is a growing worldwide concern and there is evidence that early diagnosis is beneficial economically and socially. The limited success of treatments thus far has been attributed, in part, to the fact that current treatments are only available at the late stage of the disease when pathological damage has become irreversible (Albers, 2006). Treatment might be more

effective in the preclinical and prodromal phases of the disease when there may be more opportunities to reverse pathological changes (Albers, 2006). A staged procedure in which individuals undergo initial screening with olfactory testing to see who will go on to more expensive and definitive biomarker analysis may be appropriate (Schofield et al., 2014). Given the hypothesis that the OST is a proxy measure of cholinergic functioning, and by association to amyloid load, it may also be used to evaluate the efficacy of cholinergic drug therapy in a similar way that standard olfactory testing has been evaluated for its ability to predict response to cholinergic treatment for AD (Velayudhan & Lovestone, 2009).

Early diagnosis is also of great interest in the development of disease modifying agents. Inclusion of study participants who do not have underlying AD pathology, and are therefore unlikely to decline or show differential responses to placebo or investigational drugs, diminishes study power and adds to costs and time. Clinical trial recruitment is now being enriched by the use of biomarkers in an attempt to target therapy more appropriately (Ahmed et al., 2014; Lorenzi et al., 2010; Macklin, Blacker, Hyman, & Betensky, 2013). Biomarkers aid our understanding of the presymptomatic stages of the disease and enable the identification of individuals with early disease who, by participating in clinical trials of investigational treatments with disease-modifying potential, contribute unique and vital information necessary to evaluate novel therapies (Schofield, Finnie, & Yong, 2014). Obtaining participants for clinical trials can be challenging and the inclusion of biomarkers in the sampling process is expensive and places further demands on participants in terms of time and exposure to invasive procedures. The development of biomarkers that are easily administered may allow for the enrichment of clinical trial samples without the detriment to patient recruitment.

The OST utilises the known olfactory impairments seen in AD and other neurodegenerative diseases but goes one step further in attempting to use the differential cause of these deficits to unmask AD pathology. The OST may be useful in the diagnosis of AD, particularly when combined with other markers. The OST may also be used to track the progression of the disease if it is successfully shown that the atropine effect correlates with disease severity.

8.5 Strengths of the current study

The current study adds to the development of the OST, by investigating the differential properties in other neurological conditions. The AD, PD and VCI groups showed a similar level of olfactory impairment at baseline and therefore the OST does not seem to cause a reduction in UPSIT performance due a nonspecific deficit in olfactory function. Patients with PD and VCI have a compromised olfactory system that may have been susceptible to pharmacological stress. However this research showed that the OST did not cause a decline in UPSIT performance beyond that seen in controls. If the OST acted on a general olfactory vulnerability, an atropine effect would have been expected in these groups.

The fact that an atropine effect was not found in the VCI group is also clinically important. Differential diagnosis between VCI or vascular dementia and AD can be a clinical challenge, particularly in the absence of a clearly identifiable vascular event. The fact that an atropine effect was not found in a sample with very similar levels of cognitive impairment points to the implication that the OST is targeting the pathology specific to AD.

Indirectly, the current study provides new insights into unirhinal performance of the UPSIT in unimpaired older adults and three neurodegenerative groups. This is

particularly helpful as there are no published normative data for unirhinal testing with the full 40-Item UPSIT.

8.6 Limitations of the current study

In this study, atropine was administered using an ‘atomising’ nasal spray bottle. The atomiser bottle aerates the liquid and dispenses smaller particles than the ‘misting bottle’ that was used by Schofield et al. (2012). A limitation of intranasal administration of pharmaceutical products is the ability to determine their dose and distribution within the olfactory system. While it was hypothesised that atropine would concentrate in the olfactory bulb, it is possible that the atropine was delivered to the anterior nasal cavity or absorbed by the nasal mucosa. The delivery and distribution of drugs in the nasal cavity depends on droplet size, velocity of the droplets (speed at which they leave the pump) and spray angle (Cheng et al., 2001). Larger droplets, faster spray, and wider spray angles have been found to increase deposition of the drug on the anterior region of the nasal airway which prevents deposits reaching the target region (i.e., the olfactory bulb; Cheng et al., 2001; Frank, Kimbell, Pawar, & Rhee, 2011). Scheibe, Bethge, Witt, and Hummel (2008) compared the efficacy of a nasal spray, drops applied with a pipette, and a system producing squirts. Blue food dye was used to visualise the intranasal distribution of the liquid and the investigation was performed using nasal endoscopy. The nasal spray distributed the dye into the nasal mucosa, but most of it was intercepted by the middle turbinate and did not reach the olfactory cleft effectively. In summary, drug delivery with nasal sprays is generally suboptimal and has been blamed for the historic failure of intranasal drugs (Bateman, Whymark, Clifton, & Woolford, 2002; Scheibe et al., 2008).

Another limitation of the use of intranasal delivery is the reliability of the dose administered. During the course of the study, the neurologist administering the nasal

spray (PS) noted that on some occasions the pump mechanism did not seem to deploy a full spray. Occasionally the participants themselves denied feeling a spray sensation. It was impossible to measure the volume and distribution of spray effectively as the spray nozzle was opaque and hidden within the nasal cavity. Each participant was administered one spray and, to prevent any potential side effects, the spray was never readministered. An informal investigation of the spray bottle by deploying the nasal spray into the air 20 times, with a primer between each spray, indicated variation in the spray. On approximately 25% of trials the nozzle seemed to deliver a less than full spray as indicated by the height and breadth of the spray discharged.

In conclusion, the amount of atropine administered and then absorbed by the olfactory bulb is unknown. The importance of this cannot be overstated. If the participants were not receiving the same dose of atropine, or if the atropine was not reaching the olfactory bulb in each participant equally, variation in the atropine effect would be expected. It may be possible that the participant who obtained an atropine effect of -14 received the highest dose of atropine, or indeed, that those participants with AD who improved after the spray had not received a (full) dose. Measurement of the distribution of atropine via the atomiser nasal spray was beyond the scope of this study, however, it raises important questions for further research.

Another limitation, inherent to most studies investigating a mixture of neurodegenerative diseases, was the difficulty recruiting participants with a pure diagnosis of AD, PD or VCI. It is possible that a mixed aetiology could have been present in some of the participants. An unusual feature of the sample was relatively high rate of ApoE Ɛ4 alleles in the PD group in comparison to published rates. This may point to incorrect diagnosis or may be a sampling error due to fairly low sample numbers.

A significant weakness of this study was the lack of a measure of olfactory sensitivity (threshold). It is possible that changes in odour identification in the AD group, were related to the impact of atropine at a more basic perceptual level. If atropine reduced olfactory sensitivity it would follow that performance on a measure of identification would also be reduced - one needs to be able to detect an odour before it can be identified. There is prior evidence for an effect of an anticholinergic agent on olfactory threshold (Skouby, 1954; Zilstorff-Pedersen, 1955 Serby, 1990). Serby et al. (1996) found that subcutaneous administration of scopolamine in healthy young volunteers induced a deficit in olfactory threshold. It is possible that atropine was affecting the more basic olfactory ability of detection in the AD sample, thus giving the perception of a decline in odour identification score. Although this is of interest in understanding the mechanism of action of the OST, a differential effect of atropine at any stage of olfactory processing remains useful in the early detection of this disease.

While the sample was well-matched in regards to education, a proxy measure of IQ, further information regarding premorbid and current IQ would have been useful and such measures should be included in future research. Given that the baseline UPSIT score was significantly correlated with the memory and non-memory composite score it may be hypothesised that general intellectual ability would have been associated with higher scores on the UPSIT. There was no significant correlation between atropine effect and the memory or non-memory composite score; therefore it would not be hypothesised that general intellectual function would affect response to the OST. Inclusion of a premorbid measure of IQ would be included to better characterise the sample rather than to explore effect of IQ on the OST.

As noted there was a significant sampling bias in regards to the proportion of males and females in each group. The sample was largely opportunistic and participants were

approached as part of their routine care at a memory clinic. Participants in the AD, PD and VCI groups were required to have an informant to provide information on functional ability. The control group consisted of partners of participants and volunteers from local community groups. This sampling method and the inclusion criteria may have led to a bias. Female carers appeared to be keen to get involved in research, both in being a willing informant and participating as a control. In contrast, the female participants with AD, PD or VCI did not always attend clinic appointments with male carers, instead being accompanied by their offspring who could not participant as a control due to age restrictions and refused to participate as an informant. This resulted in fewer female patients participating and fewer available male carers to become controls. It is noted that there was a similar gender ratio in the 2012 study (see Table 9) and this may have been due to similar factors. Given the sex differences in olfaction and the potential sex differences in the cholinergic system this was a significant limitation. Due to the sample sizes (i.e., there were only three female AD participants), the current study cannot reliably address these questions and future research with the OST should aim to match groups according to sex.

8.7 Future research

The development of a biomarker is a lengthy process, with this research representing a very small part of this challenge. This research has been particularly useful in highlighting several areas that require additional focus.

One of the next steps in developing the OST is to perform longitudinal studies in a sample of older adults with prodromal and preclinical AD to determine whether the OST can predict the development of symptomatic AD. It may also be fruitful to explore the properties of the OST in risk groups such as relatives of people with AD, or people with a genetic variant of AD.

Further investigation of some of the methodological issues surrounding the use of the UPSIT to measure olfaction before and after atropine is also essential. Test-retest data for the UPSIT when administered after a 45 minute interval without administration of atropine would be helpful to understand what level of change might be expected. Like-wise, a blinded, placebo-controlled trial in which half of the participants receive atropine and the other a placebo would be useful to further validate the action of atropine as opposed to a more general effect of nasal spray administration. Following the strategies use for research in cognition, these studies may begin in unimpaired controls, however ultimately they would be needed in an AD sample and would serve to provide test-retest data for the UPSIT in participants who are known to have an impaired sense of smell. This would further the understanding of expected measurement error in the administration of the UPSIT and determine the ability of the UPSIT to measure change in response to a cholinergic stressor.

Serious concerns were raised during this research about the mechanism of the delivery of atropine. Imaging studies in which atropine is administered with a dye may be useful to accurately determine dose and distribution of atropine delivery and its relationship to atropine effect.

Finally, to fully determine the utility of the OST as a marker of cholinergic functioning and amyloid deposition, it would be necessary to include the OST in a large scale biomarker study. In particular, comparison of the OST against PET amyloid imaging would allow for a gold standard validation of the utility of the OST. Plans for this study have been initiated.

9. References

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10. Appendices

10.1 Appendix 1: Table 11 Neuropsychological Data, Values and Means and (*SD*s)

	Controls		AD		PD		VCI	
	Raw	Z score	Raw	Z score	Raw	Z score	Raw	Z score
MMSE	27.9 (1.7)	-	24.4 (3.2)*	-	27.7 (1.6)	-	25.5 (3.5)	-
RAVLT Trials 1-5	45.2 (10.2)	0.25 (0.85)	25.4 (5.3)	-1.57 (0.54)	37.3 (11.6)	-0.54 (1.04)	30.2 (11.0)	-1.07 (1.18)
RAVLT Immediate Recall	9.3 (2.8)	0.27 (0.87)	1.1 (1.2)	-2.48 (0.49)	6.0 (3.3)	-0.92 (1.15)	4.5 (2.7)	-1.36 (1.03)
RAVLT Delayed Recall	8.6 (2.8)	0.21 (0.75)	0.8 (1.6)	-1.90 (0.44)*	5.8 (3.1)	-0.59 (0.83)	4.4 (3.9)	-1.02 (1.06)
DS	15.7 (3.1)	0.1 (0.7)	13.5 (3.0)	-0.4 (0.7)	14.7 (4.3)	-0.1 (1.0)	13.0 (4.0)	-0.5 (1.0)
TMT-A	42.8 (16.5)	0.0 (1.0)	74.6 (38.0)	-1.4 (1.3)	44.3 (20.2)	-0.6 (1.2)	63.4 (26.5)	-1.3 (1.4)
SDMT-W	40.0 (9.5)	0.5 (1.0)	24.9 (13.3)	-1.3 (1.2)	31.9 (10.8)	-0.6 (1.1)	28.1 (10.1)	-1.0 (1.3)
SDMT-O	47.8 (10.3)	0.3 (0.9)	29.3 (13.9)	-1.4 (1.0)	38.2 (10.3)	-7.0 (0.8)	32.9 (11.8)	-1.2 (1.2)
TMT-B	131.6 (122.8)	-0.38 (1.33)	191.0 (194.2)	-0.79 (1.10)	154.9 (80.5)	-1.14 (1.10)	172.5 (104.7)	-1.79 (1.49)
Stroop	-	-0.4 (0.6)	-	-0.7 (1.0)	-	-0.4 (0.8)	-	-0.8 (0.8)
Letter Fluency	40.8 (13.9)	0.5 (1.0)	29.8 (7.6)	-0.6 (0.4)*	36.9 (14.4)	0.1 (1.0)	19.8 (9.6)	-1.3 (0.9)
BNT	12.8 (1.5)	-0.29 (0.66)	8.0 (4.8)	-1.26 (0.82)	12.2 (2.1)	-0.28 (0.96)	9.4 (2.9)	-1.09 (0.72)
Category fluency	18.2 (3.6)	0.47 (0.79)	11.1 (4.5)	-1.41 (1.00)	17.0 (6.6)	0.00 (1.25)	12.7 (5.9)	-0.80 (1.63)
Drawing a clock raw	9.0 (1.2)	-	7.6 (2.5)	-	9.4 (0.7)	-	8.3 (2.2)	-
Copying a clock raw	9.7 (0.6)	-	9.2 (1.1)	-	9.7 (0.5)	-	9.4 (1.0)	-
JLO	22.6 (4.6)	-0.63 (1.02)	20.7 (7.0)	-0.82 (1.10)	22.1 (5.8)	-0.58 (1.04)	21.1 (6.5)	-0.80 (1.16)

*indicates significant difference from the control group, $p < .05$.

10.2 Appendix 2: Hunter New England Human Research Ethics Committee - Ethics Approval Letter

HUNTER NEW ENGLAND
NSW HEALTH

15 July 2010

Associate Professor P Schofield
Neuropsychiatry Service
Calvary Mater Newcastle

Dear Professor Schofield,

Re: Olfactory Stress Test performance in several neurological disorders (10/06/16/5.06)

HNEHREC Reference No: 10/06/16/5.06
NSW HREC Reference No: HREC/10/HNE/141

Thank you for submitting the above protocol for single ethical review. This project was first considered by the Hunter New England Human Research Ethics Committee at its meeting held on **16 June 2010.** This Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research (2007)* (National Statement) and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. Further, this Committee has been accredited by the NSW Department of Health as a lead HREC under the model for single ethical and scientific review. The Committee's Terms of Reference are available from the Hunter New England Area Health Service website: http://www.hnehealth.nsw.gov.au/Human_Research_Ethics.

I am pleased to advise that following acceptance under delegated authority of the requested clarifications and revised information statements and consent forms by Dr Nicole Gerrand, Manager, Research Ethics & Governance in consultation with members of the Hunter New England Human Ethics Committee, the Hunter New England Human Research Ethics Committee has granted ethical approval of the above project.

The following documentation has been reviewed and approved by the Hunter New England Human Research Ethics Committee:

- For the Patient Information Sheet (Version dated 18 June 2010);
- For the Control Information Sheet (Version dated 18 June 2010); and
- For the Patient and Control Consent Form (Version dated 18 June 2010)

For the protocol: **Olfactory Stress Test performance in several neurological disorders**

Approval from the Hunter New England Human Research Ethics Committee for the above protocol is given for a maximum of **3** years from the date of this letter, after which a renewal application will be required if the protocol has not been completed.

The *National Statement on Ethical Conduct in Human Research (2007)*, which the Committee is obliged to adhere to, include the requirement that the committee monitors the research protocols it has approved. In order for the Committee to fulfil this function, it requires:

Hunter New England Research Ethics & Governance Unit

(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 850 Facsimile (02) 49214 816
Email: hnehrec@hnehealth.nsw.gov.au
http://www.hnehealth.nsw.gov.au/Human_Research_Ethics

10.3 Appendix 3: Macquarie University Human Research Ethics Committee - Ethics Approval Email

From: **Ethics Secretariat** ethics.secretariat@mq.edu.au
Subject: External Approval Noted- Savage (Ethics Ref: 5201200121)
Date: 8 March 2012 1:55 pm
To: A/Prof Greg Savage greg.savage@mq.edu.au
Cc: Mrs Sally Deborah Finnie sally.scott@students.mq.edu.au

Dear A/Prof Savage

Re: "Olfactory stress test performance in several neurological disorders"

The above application was considered by the Executive of the Human Research Ethics Committee. In accordance with section 5.5 of the National Statement on Ethical Conduct in Human Research (2007) the Executive noted the final approval from the Hunter New England and your right to proceed under their authority.

Please do not hesitate to contact the Ethics Secretariat if you have any questions or concerns.

Please do not hesitate to contact the Ethics Secretariat at the address below, if you require a hard copy letter of the above notification.

Please retain a copy of this email as this is your official notification of external approval being noted.

Yours sincerely

Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee