A crossover atropine titration study in the Olfactory Stress Test for the diagnosis of Alzheimer’s Disease.

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September, 2013
Statement of Originality

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**Unless an Embargo has been approved for a determined period.
Suddenly you were gone, from the all the lives you left your mark upon

Neal Peart

In Memoriam

This thesis is dedicated to the memory of very important men who experienced dementia—some of the Alzheimer’s type; some not—and to their beloved families.

Salvador “Papapa” Levy Z”L
Benjamin “el Zeide” Mankevich Z”L
“Grandpa” Harry Bricker Z”L

Thanks to Associate Professor Peter Schofield for affording me the opportunity to take part in some very important work: the early detection of Alzheimer’s. I hope this thesis has done it justice.

I would also like to show my gratitude to Tammie Moore and Anna Suraev at the Neuropsychiatric Department, Calvary Mater Hospital, for their support.
Also thanks to Megan Valentine and Kim Colyvas at the University of Newcastle Statistical Support Service for being (tremendously) patient with me and my statistics allergy.

To Family - Thanks to Mami y Papi for your love and for always being there when I need them to, come hell or high water. I can only show my immense gratitude by paying it forward to the next gen. Y gracias por el libro de experiencias que llevo escribiendo en mi mente desde los ocho años, más o menos. Los extraño y quiero muchísimo.

Thanks to my dear Suegrita, Dr. Geraldine Bricker-Katz for her love and advice throughout this mad however many years it’s been. And my admiration because she is one of the few mothers in law out there who can appreciate the meaning of \( \alpha<.05, \quad F=15.63, \quad p=.004 \).

Thanks to my fathers-in-law, Dr. Allan Shafer and Arie Van Der Merwe and all the Shafer clan for welcoming me into their family and country with love and open arms
To my three beautiful sisters, Nora, Paloma and Rina and brothers-in-law for looking after me and watching my back for so long.

Last but not least, not by a long shot

How is it that the chorus to that song you like go? If you’re lost you can look, and you will find me?... If you fall I will catch you, I’ll be waiting, time after time. Yeah, that’s my wife Joanne Benhamu for me. She’s pulled me up and pushed me forward every single time. She’s been strong for both of us during all these years of me trying to get it together. I’m lucky. I love you beyond measure. Your turn now, Ms. Shafer! It’s my turn to catch you.

...I catch a break, then a punch to the head; I smile big with a toothless grin (P.J.)
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Abstract
Scope: The Olfactory Stress Test (OST) is a potentially new method to screen for Alzheimer’s disease (AD) at its prodromal stage. There is pressure to find methods of early AD detection in preparation for a time when disease altering drugs become available. Presently, the use of early detection could assist prompt intervention, which could allow patients to start taking acetylcholinesterase inhibitors early for maximum effect. Early intervention has also been shown to improve the quality of life of patients and their caregivers. The OST relies on the cholinergic hypothesis, which posits that AD is driven by a decline of cholinergic activity in the brain. It is understood that cholinergic decline happens before cognitive deficits associated with AD appear. Cholinergic activity in the olfactory bulbs (OBs) influences the relay of environmental cues to the olfactory processing centre of the brain. As such, olfaction also declines during AD even before the emergence of cognitive problems: a fact that is yet to be harnessed in clinical practice for early disease detection. The OST uses an atropine anticholinergic solution intranasally to exacerbate the already compromised cholinergic activity in the OB. This effect is measured by subtracting the score of the University of Pennsylvania Smell Identification Test (UPSIT) administered before the atropine intake from that of an UPSIT administered after the atropine. This difference yields the atropine effect (AE), which is hypothesized to reflect the presence of Alzheimer’s disease pathology in the OB. Healthy individuals should yield a positive and negligible AE reading. A previous OST study has shown promise for this technique. This study was part of an effort to further develop and refine the OST.
Purpose: The aim of this study was to titrate atropine doses. Researchers looked for a dose lower than the 1 mg concentration dose currently used in OST research. The
purpose of this titration study was to determine if a lower atropine dose would elicit a meaningful AE while reducing the risk of potential side effects.

Methodology: Ten participants (six women) over the age of 65 volunteered for this study. Three of the participants responded to a recruitment drive aimed at organizations serving seniors in the Lake Macquarie Municipality. The remainder of the participants had volunteered in previous OST studies and agreed to partake in this titration study after being contacted by a research trial coordinator assisting with the administrative aspects of the study. The methodology followed a repeated measures cross-over design. The researchers administered three concentration doses of atropine to each study participant: 0.1 mg, 0.5 mg and 1 mg. Doses were administered one week apart from each other. During the first session, researchers took a medical history of the participants and administered the Audio Recorded Cognitive Screen (ARCS). ARCS composite scores were used to divide participants between MCI/AD and Control (i.e. those with normal cognitive function). Participants were screened for the presence of the Apolipoprotein ε4 allele, which has been associated with increased risk of developing Alzheimer’s. Genotyping was used to separate participants between Risk and No Risk groups.

Results: Two linear mixed models were conducted: The first linear mixed model looked at the data according to participants divided by ARCS performance and the second one according to group genotype risk. Both linear mixed models explored the fixed effects of research group (i.e. neuropsychological performance and genotype risk respectively), atropine dose and their interaction. The linear mixed model conducted on ARCS-score groups yielded no significant effects. The linear mixed model conducted on genotype risk groups yielded a significant effect for genotype risk for the .5mg atropine dose at $\alpha<.05$, $F=15.634$, $p=.004$. The No Risk group yielded an AE that was positive, $M=.5$. 


95% CI [-1.83, 2.83], whereas the Risk group yielded a negative AE, $M=-4.67$, 95% CI [-7.96, -1.38] at the .5 dose. These results were consistent with OST theory.

Conclusions and implications: Although some significant effects were found, the results need to be addressed with a degree of caution considering the small sample size. Other biases may have been introduced by the crossover design. Further research with larger samples is in order to find a lower, effective dose of atropine in the OST.
Alzheimer’s Disease (AD) rates in Australia are expected to increase in the coming decades due to an ageing population and the concurrent increase in the risk of developing the disease that comes with age (Lindsay et al., 2002). According to the Australian Bureau of Statistics (2011), life expectancy in Australia has increased in the 10 year period to 2011. This follows the trend identified by the Australian Institute of Health and Welfare, which has seen life expectancy in Australia increase steadily between 1900 and 2005 (Australian Institute of Health and Welfare, 2009). In light of this, the prevalence of AD in Australia is projected to increase to approximately 1.13 million people by 2050; that is nearly quadruple the 2009 figure of 245,400 (Access Economics, 2009). AD has the highest prevalence of the neurodegenerative diseases leading to dementia (Barlett, Gray, Byrne, Travers, & Lui, 2006), with Australia, in 2009, being the first country to recognise the disease as a national health priority (Alzheimer's Disease International, 2009). The impact of the disease on individuals and their families is profound, with sufferers requiring increasing care and supervision as the disease progresses. With this in mind, Alzheimer’s Disease International, in their 2013 Report, highlight the potential benefits of early diagnosis as a means for future care planning (Alzheimers Disease International, 2013).

Given the projected increase in rates of diagnosis of AD, there is substantial pressure to find better tools to aid early diagnosis as well as treatment. With the future potential for disease modifying therapies, the application of such therapies early in the course of the pathology will be paramount (Montine, 2011). The Olfactory Stress Test (OST, described later in this paper) provides the possibility of an early and inexpensive screening tool for AD.
Study Aims

The OST is a potential new AD screening method that relies on smell identification to reveal latent pathology occurring at the olfactory bulb (OB) and cortical levels. The OST relies on the use of the anticholinergic drug atropine to assess effects on olfactory identification that might be indicative of AD. As such, the study presented in this thesis aimed to find the optimal atropine dose necessary to elicit such an effect. In Schofield, Ebrahimi, Jones, Bateman, & Murray (2012), the atropine dose administered as part of the OST was 1 mg. This dose level was used because it was considered the maximal safe dose that could be administered intranasally in clinical practice. However, the aim of this study was to titrate the atropine at 0.1 mg, 0.5 mg and 1 mg concentration doses and find the minimal, optimal dose to elicit an Atropine Effect (AE). The atropine titration methodology of this study is described in detail in the journal article manuscript submitted as part of this thesis.

This literature review will begin by providing a description of AD as a disability. It will then present the potential benefits of early detection in financial and clinical terms, taking into account the fact that early diagnosis will become important as disease-altering treatments become available. The literature review will then provide a brief account of the biological changes in AD that are of particular relevance to the OST. Lastly, the OST will be described in detail.

**AD Is a Debilitating Condition for Patients and Their Families; Early Intervention Could Decrease Burden Level and Disability Costs**

The Australian Institute of Health and Welfare (2004) highlight the fact that dementia is the foremost contributor to disability in the elderly. AD itself is characterised by memory deficits, impairing the ability to both learn new information and recall previously learned material; difficulties in orientation to time, place and
person; decline of visuospatial skills; aphasia (i.e. a language disturbance where the ability to remember words and comprehend and formulate language is compromised); apraxia (i.e. inability to execute motor activities despite unimpaired motor function); agnosia (i.e. inability to identify everyday objects) and impaired executive function, resulting in the inability to plan, organize, abstract or initiate tasks (American Psychiatric Association, 2000; Muò et al., 2005). Moreover, the disorder is progressive, so memory or cognitive decline can start with seemingly innocuous forgetfulness (losing one’s keys or wallet) and culminate in the loss of key personal information, such as the name of loved ones or one’s former occupation (American Psychiatric Association, 2000).

**AD causes disability.** Muo et al. (2005) found that AD meets the International Classification of Functioning, Disability and Health (ICF) criteria for disability. The ICF World Health Organization disability classification scheme takes into account environmental (e.g. access to services) and personal (e.g. socio-economic status) factors as determinants for disability (Muò et al., 2005). Muo et al. (2005) found that the following areas of functioning are particularly affected: self, mobility, domestic life, communication and social interaction. ICF codes correlated highly with the Mini-Mental State Examination (MMSE) and the Global Deterioration Scale (GDS) in people with AD (Muò et al., 2005). In a study investigating the medical, environmental and personal factors determining disability among the elderly in Spain, Virués-Ortega et al. (2011) found AD to be the only “mental and behavioural disorder with the strongest and only statistically significant effect over every disability domain in the ICF, namely: “Understanding and Communication”, “Getting Along with People”, “Getting Around”, “Life Activities”, “Participation in Society” and “Self-Care”. In terms of external factors to disability, Virués-Ortega et al. (2011) also found that the absence or scarcity
of services that could facilitate quality of life improvements for patients and their carers can further contribute to the disability status of an individual. Similarly, the lack of availability of medication, social security, services and personal care providers could perpetuate disability for people with AD (Muò et al., 2005).

Another factor that could be either a facilitator or barrier towards better quality of life among AD patients is caregiver’s burden (CB). Looking after a dementing family member has been associated with high levels of stress, depression and decreased quality of life for the carer, which could in turn result in poor quality of life for patients and increase the likelihood of early nursing home placement (Etters, Goodall, & Harrison, 2008; Gaugler, Yu, Krichbaum, & Wyman, 2009). The attitudes and beliefs held by caregivers and family members about AD are another factor contributing towards disability (Muò et al., 2005).

However, the aforementioned disability aspects of AD could be mitigated by putting in place the necessary resources to aid caregivers and improve patients’ quality of life (Etters et al., 2008), and the assertion that doing so earlier in disease progression should stand to reason. For example, there are practical benefits in early detection, such as minimizing hazards at home or providing training to caregivers that might allow them to better prepare for disease progression (Leifer, 2003). However, according to The World Alzheimer’s Report 2011 (Prince, Bryce, & Ferri, 2011), there is a paucity of data in terms of the benefits of early AD diagnosis. The World Alzheimer’s Report 2011 further found that the push for early AD detection comes from interest groups and official health guidelines worldwide rather than from empirical research (Prince et al., 2011). Yet, there are recorded benefits; early intervention with acetylcholinesterase inhibitors (AChEI) (e.g. Donepezil, Rivastigmine and Galantamine) is understood to improve the chances of prolonging a degree of normal functioning if administered in the
earlier stages of AD (Leifer, 2003). Providing adequate support to caregivers can also improve the quality of life of patients and delay nursing home admission (Etters et al., 2008). Additionally, projection studies have found that early AD detection and intervention could bring about substantial fiscal savings for individuals and society as a whole (Serge Gauthier & Poirier, 2008; Getsios, Blume, Ishak, Maclaine, & Hernández, 2012). These issues are explored below.

**The benefits of early administration of acetylcholinesterase inhibitors.**

Despite randomised controlled trials demonstrating that the average effect size of cholinesterase inhibitors is modest, their efficacy is consistently better than placebo, and global changes in cognition, behaviour and functioning have been noted by individual physicians and carers (Doody et al., 2001). A 2004 meta-analysis (reported in Gauthier (2005) of Phase II and III randomized controlled trials (RCTs) of Donepezil (an AChEI), found that there was cognitive improvement in the treatment group lasting at least six months as measured by the Alzheimer’s Disease Assessment Scale - Cognitive Subscale.

Gauthier (2005) also reported on a Nordic study involving 286 participants with mild to moderate AD. The participants were first randomly assigned to a placebo or treatment group, double-blind. At the end of the first year, all participants started receiving open-label Donepezil. The cognitive performance (measured via MMSE) of both groups was compared to the projected performance of the placebo group had it continued to receive the placebo at the end of the first year of the study. Even though participants demonstrated cognitive decline across the board during the study, the treatment group’s MMSE performance during the double-blind stage remained close to baseline while that of the control group fell approximately 2.5 points below. Moreover, during the open-label portion of the study, the group that had constantly received
Donepezil maintained a cognitive performance above that of the group that had started out as placebo, with a statistically significant difference between the two (SG Gauthier, 2005).

However, pharmacotherapy is as yet unable to halt or reverse the degenerative progress of AD, and at best can only delay the appearance of the worst symptoms of neurocognitive decline (Doody et al., 2001; Leifer, 2003).

Projected benefits of early AD detection and intervention. The long-term care needed to manage dementia of the Alzheimer’s type (DAT) can impose a financial burden on taxpayers, the state and care-giving families (Weimer & Sager, 2009). Weimer and Sager (2009) conducted a cost-benefit analysis to explore their hypothesis that early diagnosis and intervention would significantly decrease fiscal burden on caregivers and taxpayers. The researchers followed a theorised cohort of AD patients in the state of Wisconsin, United States, to examine the financial impact of early interventions funded through state and federal medical aid. The authors modelled how early intervention might affect different domains of AD care, including the pharmacotherapy expenditures, early nursing home admission likelihood, family care-giving and support services usage. Employing MMSE scores at time of diagnosis, Weimer and Sager (2009) extrapolated from that point to gauge whether early diagnosis and intervention decreased the use of the aforementioned services. These costs were compared against the costs of diagnosis to identify any fiscal benefits. The authors found that the early recognition and management of AD patients would generate cost savings, especially when the disease was identified in its earlier stages (e.g. MMSE score of 28) and pharmacotherapy was combined with caregiver intervention program (i.e. the provision of support to caregivers) (Weimer & Sager, 2009).
Another modelling study was conducted in the United States to examine the economic implications of earlier diagnosis and treatment and the effect of treatment timing on risk of institutionalization of Medicaid patients (Geldmacher et al., 2013). The authors found that initiation of existing therapies would delay institutionalization by 91 days, which in turn would reduce Medicaid costs by US$19,108 per institutionalized patient (Geldmacher et al., 2013).

A similar study was conducted in the United Kingdom, where prescription of Donepezil is restricted to the moderate stage of DAT (Getsios et al., 2012). Getsios et al. (2012) modelled the direct and indirect costs of AD care over a period of 10 years. Their model assumed that Donepezil would be administered at the time of diagnosis. They found that early treatment reduced costs and led to significant savings both to the health-care system and taxpayers even when considering the high upfront costs of diagnosis (Getsios et al., 2012). It is worth noting that this scenario is unlikely in Australia because Donepezil is listed on the Pharmaceutical Benefits Scheme and is available for prescription for mild to moderately severe AD. Other local factors, such as prevalence of undiagnosed AD, renders imprecise the extrapolation of findings outside the UK (Getsios et al., 2012). However, based on the findings reported above, a similar study evaluating costs and benefits of earlier diagnosis in Australia would be appropriate.

As such, there appear to be potential benefits to early diagnosis, even in the absence of disease-altering medication and relying on the combination of acetylcholinesterase inhibitors and psychosocial interventions. This places screening techniques such as the OST in an ideal position to facilitate early intervention, when there is suspicion that the patient might develop AD or is manifesting MCI, which could potentially convert into fully-fledged AD. However, Wiemer and Sager (2009) did not
recommend early cognitive screening based on their findings. Instead, they suggested that limited financial resources be spent in the development of better pharmacological treatment and those shown to reduce long-term costs of AD care such as caregiver interventions. However, there is a caveat: Weimer and Sager’s (2009) recommendations are based on the American health care system, and again, may not be suitable for extrapolation to other countries, including Australia.

Surveying patient and caregiver’s views, however, might generate a more comprehensive picture as to how early diagnosis might be of use beyond the potential financial benefits discussed above. For example, a participant of an interpretative phenomenological analysis (IPA) study into how people cope with the onset of AD said that she had made a decision to donate her brain to science (Clare, 2002). Based on the data gathered for this IPA, the author found that newly-diagnosed AD patients might find solace in engaging in activities they can still enjoy, identifying ways of contributing to society and accessing information that is relevant to individual needs (Clare, 2002). Such goals can best be accomplished at the prodromal stage of the disease. The findings of this IPA need to be explored quantitatively to ascertain their generalisability to the wider population, as it appears that individual patients and caregivers might find unique benefit from early screening. Therefore, the potential of early diagnosis demands closer consideration. The recently released *World Alzheimer’s Report 2013* does however highlight the importance of early diagnosis in allowing the individual to put in place advanced care directives to facilitate care and medical intervention that fulfils their wishes at the time of diagnosis and prior to significant cognitive decline (Alzheimers Disease International, 2013).

The pathological changes that occur during AD make it possible for diagnosis to occur at its earlier stages. At least, it is plausible to consider the detection of AD
pathology in the brain at a prodromal stage. The sections that follow explore the nature of the disease and how it might allow for screening through changes in olfaction.

**Olfactory Changes Occur in Alzheimer’s Disease and May Precede Obvious Cognitive Impairment**

Olfaction has been found to decline along with cognitive function in AD. Evidence suggests that olfactory decline might precede the emergence of the putative clinical DAT symptoms. A longitudinal study into changes in olfactory threshold in AD found statistically poorer olfactory detection performance among initially healthy participants who became AD-positive during the course of the study (Bacon, Bondi, Salmon, & Murphy, 1998). Another example is the longitudinal study conducted by Tabert et al. (2005), who found that poorer performance in a brief version of the University of Pennsylvania Smell Test (UPSIT) correlated with a higher risk of developing AD or converting to it from mild cognitive impairment (MCI). Similarly, a multi ethnic study found that elderly participants without MCI performed significantly better in the UPSIT than their non-amnesic and amnesic MCI counterparts (Devanand et al., 2010). An epidemiological study found a significant association between the combined baseline olfactory impairment and the 5-year incidence of MCI among healthy, community-dwelling older adults (Schubert et al., 2008). A magnetic resonance imaging (MRI) study found a significant association between olfactory bulb (OB) and tract volume and cognitive performance; the difference in OB and tract volume between control and AD participants, in fact, was significant (Thomann et al., 2009).

AD pathology first appears at the transenthorhinal cortex level, which includes brain areas implicated in memory, emotional and olfactory processing, and later spreading to temporal areas of the brain (Barresi et al., 2012). Even though olfactory deficit is a recognized feature of AD, olfactory problems can subtly emerge before the
onset of clinical AD symptoms (Nordin & Murphy, 1996). However, olfaction is rarely assessed in clinical settings, earning its dubious title, “the neglected sense” (Kovács, 2004).

**Pathological Changes of AD Affect Cholinergic Neurotransmission and Are Related to Olfactory Decline**

**Olfaction and cholinergic neurotransmission.** Cholinergic refers to activity in the brain involving the neurotransmitter acetylcholine (ACh). The olfactory bulbs (OBs) and limbic system are structures that rely on cholinergic neurotransmission to relay information.

It has been recognised that neurons which release the neurochemical ACh are particularly susceptible to damage due to AD (Kovács, 2004). The limbic system handles cognitive functions that are affected by the disease. The hippocampus controls normal learning, retention of information and spatial memory, while the amygdala manages the emotional aspect of learning by associating memories with pleasure and pain (Lezak, Howieson, & Loring, 2004). At the cerebrocortical level, ACh is understood to be involved in the consolidation of memories. This function has been demonstrated through the adverse effect of anti-cholinergic drugs (i.e. muscarinic receptor antagonists) on the acquisition and retention of learned tasks in animal models (Terry & Buccafusco, 2003). This anti-cholinergic effect was first observed in humans during the 1970s (Terry & Buccafusco, 2003). It was found then that anticholinergic agents, such as atropine and scopolamine, had an effect on healthy young adults that mimicked the cognitive symptoms of AD: attention deficits, and impaired acquisition of new information and memory consolidation. Subsequently, the effect has been studied extensively on rats, demonstrating the capacity of anti-cholinergic agents to disrupt learning tasks, conditioning tasks, spatial learning tasks and attention shifting tasks.
ATROPINE TITRATION IN THE OST

(Terry & Buccafusco, 2003). The animal and human models of cognitive decline mentioned above gave rise to the cholinergic hypothesis, which posits that “a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age and AD” (Terry & Buccafusco, 2003, The Cholinergic Hypothesis, para. 2).

Resting atop the cribiform plate and below the brain’s frontal lobe, the OBs are nests of glomeruli that contain synaptic connections between bundles of olfactory nerves and mitral cells. Olfactory cues from the environment reach the olfactory receptor cells. This elicits a neural signal that travels to the olfactory nerves and over the synaptic cleft to the mitral cells. The mitral cells then relay the information to the olfactory tract, which in turn connects with the anterior olfactory nucleus, the amygdala and the entorhinal cortex (Lezak et al., 2004; Van Groen, Kadish, Verhoef, & Wyss, 2008).

**AD Pathology affecting cholinergic activity and impact on behaviour.** There are two types of histological pathology in AD that interfere with cholinergic activity: β amyloid (Aβ) and neurofibrillary tangles (NFTs). Post-mortem studies and animal models have found a link between these pathologies and cognitive and behavioural deficits found in AD. Aβ depositions appear in the basal forebrain during AD concomitantly with decreased cholinergic neurotransmission and cholinergic neuron degeneration. Aβ is understood to disrupt cholinergic neurotransmission (see Auld, Kornecook, Bastianetto & Quirion (2002) for a review). Along with Aβ, tau positive NFTs have been found in the OB during AD, which also increase with disease progression and diminished cholinergic activity (Kovács, 2004). Experiments on animal models have demonstrated that Aβ plays a role in the pathophysiology of AD. For example, Wesson, Levy, Nixon and Wilson (2010) demonstrated that mice genetically
modified to over-express the Aβ precursor protein (APP) manifested behavioural problems consistent with aspects of AD. Mutated and regular mice were exposed to new odours. After a period of no exposure, the mice were exposed to the same odours and the time they spent investigating the new odour was measured. The mutated mice manifested impaired odour habituation, demonstrated by the fact that they took longer to investigate odours to which they had already been exposed. Similarly, mutated mice displayed problems discriminating between odours and took longer to examine new ones. At face value, these behavioural changes pointed to aspects of AD: the apparent difficulty habituating to new odours may have been construed as a diminished ability to incorporate new information and the impaired odour discrimination may have been suggestive of cortical deficits. This is further corroborated by the histological analysis conducted on the animals. The researchers found that “deficits in initial odour investigation” correlated with Aβ deposits within the OB, whereas discrimination deficits correlated with same within the piriform cortex, whose role is to process olfactory stimuli (Suzuki & Bekkers, 2006). Chronologically, Wesson et al. (2010) found that the first olfactory deficits in the mutated mice emerged concomitantly with the appearance of Aβ deposition in the OB. Inversely, but also demonstrative of Aβ plaques’ influence in AD, Bales et al. (2006) showed that anti-Aβ antibody m266 could reverse habituation problems in mutated mice. The researchers suggested that the m266 treatment allows for increased hippocampal cholinergic activity, which facilitates habituation to new environments.

As such, AD’s toll on cholinergic activity has an adverse impact on olfaction, memory and learning because the means of sharing information—cholinergic neurotransmission—is compromised, degenerating further as the disease progresses.
Association between genetic predisposition and pathology of AD. At the genetic level, people with an over-representation of the Apolipoprotein E (ApoE) ε4 allele are at increased risk of developing AD and Aβ pathophysiology (Wolf, Caselli, Reiman, & Valla, 2013). In a study exploring the role of ApoE ε4 in early changes in olfactory function due to AD, Bacon et al., (Bacon et al., 1998) found that MCI participants with poorer odour detection carried at least one ε4 allele. The results of a 2011 meta-analysis indicated that the presence of an ApoE ε4 allele is a moderately strong predictor of conversion from MCI status to DAT (Elias-Sonnenschein, Viechtbauer, Ramakers, Verhey, & Visser, 2011). Elias-Sonnensheim et al. (2011) found that individuals with a double expression of the ApoE ε4 allele were at four times the risk of progressing from MCI to AD compared to non-carriers.

The ε4 allele is the gene responsible for the manufacture of ApoE, which binds with the βA4 peptides—the main ingredient of senile plaques present in the AD brain (Bacon et al., 1998; Strittmatter et al., 1993). Normally, the lipoprotein ApoE is understood to be involved in the growth and repair of myelin and axonal membranes (Namba, Tomonaga, Kawasaki, Otomo, & Ikeda, 1991). However, Namba et al. (1991) found that NFTs and Aβ depositions presented immunoreactivity against ApoE in the brain of AD patients.

Despite the consistency of the effect ApoE ε4 allele has on AD, Elias-Sonnensheim, et al. (2011) warned that genotyping might be of limited clinical benefit due to its low sensitivity and low positive predictive value. Elias-Sonnenshiem et al. (2011) further found that 50% of the participants who developed DAT studied in their meta-analysis were not ApoE ε4 carriers. Similarly, Henderson et al. (1995) found that the prevalence of AD by age 90 among ApoE ε4 homozygous participants was 50%,
Despite finding a linear association between carrying at least one ApoE ε4 and cognitive impairment/AD.

According to Elias-Sonnensheim (2011), the value of AD genotyping lies in research recruitment. In the case of OST research, for example knowing the genotype is valuable as an indicator for the likely presence of AD pathology in otherwise healthy participants.

Lastly, post-mortem studies have suggested that 40% of non-demented elderly persons carry enough AD histological pathology as described above to have warranted a clinical diagnosis (Price et al., 2009). Tau pathology, for example, has been found in brains at Braak Stage II of AD pathology, which corresponds to mild to no cognitive impairment (Attems, Lintner, & Jellinger, 2005). Tsuboi, Wszolek, Graff-Radford, Cookson and Dickson (2003) also found that ApoE ε4, Braak staging and Tau pathology correlated in a linear regression analysis. Tsuboi et al (2003) also discovered Tau pathology in the anterior olfactory nucleus at Braak Stage II; virtually prior to the emergence of clinically significant symptoms. Schofield et al. (2012) postulated that there may have been a likelihood of clinical AD symptoms emerging had such individuals lived longer. The aim of the OST, therefore, is to screen for cholinergic decline described above before it manifests clinically or at the MCI stage.

The Olfactory Stress Test (OST): Rationale and Results (Adapted from Schofield et al. [2012])

Schofield and his team hypothesized that the anti-cholinergic solution atropine should exacerbate impaired neurotransmission among already compromised cholinergic pathways. Taking into account that intranasally administered drugs concentrate at the OB (Graff, Zhao, & Pollack, 2005; Illum, 2000)—where AD histological pathology is also known to accumulate—it was further hypothesized that the atropine should
accumulate there, impairing olfaction as a result. The expectation was that performance on olfactory identification tasks should worsen after the intranasal application of atropine among elderly individuals with underlying AD pathophysiology. Schofield et al., proceeded to test this hypothesis and explore any associations between changes in olfactory performance and known AD markers (i.e. ApoE genotype, memory performance and hippocampal volume).

To test the impact of atropine on olfactory performance, Schofield et al. (2012) devised the Olfactory Stress Test. This consists of administering the University of Pennsylvania Smell Identification Test (UPSIT) to establish a baseline followed by the intranasal administration of atropine. The UPSIT is a 40-item scratch-and-sniff test where the examinee is asked to identify an odour from four different options; details of this test are offered in the manuscript portion of this thesis. After a 45-minute wait, examinees take the UPSIT a second time. The atropine effect (AE); i.e. the impact of the anti-cholinergic on UPSIT performance, is measured by subtracting the first UPSIT, pre-atropine score from the second, post-atropine score (i.e. post-atropine UPSIT – pre-atropine UPSIT = AE). A negative AE (i.e. a negative figure) would indicate a worsening in performance and was hypothesized to point to the likely presence of AD pathophysiology.

In the only study published on the OST at this time (Schofield et al., 2012), Schofield and his associates found that the majority of AE<0 occurred among MCI and potentially AD participants (92% and 86% respectively). Memory scores were highly associated with AE (r=.57, p<0.0001). In fact, AE explained more variance in memory performance (24%) than did hippocampal volume (14%). Similarly, lower mean AE was associated with the presence of ApoE ε4 relative to no ApoE ε4 being present. Further to being a potential diagnostic tool, the OST could also be used as a proxy for
biological markers of AD treatment success (Kovács, 2013). Valyudhan and Lovestone (2009) found that UPSIT scores correlated with improvements in global functioning after treatment with the cholinesterase inhibitor Donepezil. Moreover, changes in UPSIT scores over time were the best predictors of global functioning improvements. It is plausible that the OST could fulfil such a monitoring function in the future.

**Olfactory changes occur in other dementia conditions.** Notwithstanding the well-established association between hyposmia and AD, the notion of predicting the latter using olfaction has been brought into question. This has implications for the OST considering that it revolves around the fact that olfaction is affected in AD. In a systematic review of olfaction as an AD prognostic tool, Sun, Raji, MacEachern, & Burke, (2012) pointed out that an association is insufficient to deem olfaction a viable diagnostic marker. These authors called for longitudinal, randomised control trials to assess the merits of olfaction as an AD diagnostic measure. However, they only found two longitudinal, non-randomised control trials that met their inclusion criteria and only one of these found a statistically significant association between baseline hyposmia and risk of developing AD.

An additional problem, according to Sun et al. (2012), is that Alzheimer’s pathophysiology may not necessarily be the only explanation for decreased olfaction in the elderly. Namely, hyposmia increases in likelihood with age independent of AD clinical symptoms. Other potential confounds in the elderly include decreased hydration; decreased mucosal secretion in the olfactory cleft; thinning of the olfactory mucosa; replacement of olfactory epithelium with respiratory epithelium and prolonged exposure to toxic environmental agents (Lafreniere & Mann, 2009; Sun et al., 2012).

Inasmuch as those factors are concerned, the OST has the advantage of atropine over the studies cited by Sun et al. (2012) and those earlier in this paper. Based on the
theory described above, atropine interacts with deficits in cholinergic activity particular to AD, acting as a control. As such, the OST should be able to discern between ordinary olfactory deficits of aging and those resulting from prodromal AD. However, there are other neurodegenerative diseases that are characterised by dementia and diminished cholinergic activity with concomitant olfactory decline. Two ailments are prominent in the literature to that effect: Parkinson’s disease (PD), dementia with Lewy bodies (LBD) or both simultaneously (e.g. (Baba et al., 2012; Tiraboschi et al., 2006; Whitwell et al., 2007). The fact that these diseases share similar pathological declines (i.e. diminished cholinergic activity and poor olfaction) poses questions about the specificity of the OST. Although this is an important matter, it is beyond the scope of this paper, and Schofield et al. are addressing this question empirically. AD. Following Sun et al.’s (2012) suggestions, however, prospective studies into the OST might be a beneficial step in future research because it would better establish the predictive validity of the OST as a screening test for AD.
A crossover atropine titration study in the Olfactory Stress Test for the diagnosis of Alzheimer’s Disease.

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Abstract

Background: The Olfactory Stress Test (OST) is a potentially new method to screen for Alzheimer’s disease (AD) at its prodromal stage, operating on the fact that cholinergic neurotransmission in the olfactory bulb (OB) is affected in the earlier stages of the disease. The OST uses atropine to gauge a change in olfactory identification performance. Previous research has demonstrated that a drop in olfactory performance is likely to be associated with incipient AD. The aim of this study was to investigate whether atropine doses lower than the 1 mg concentration would still elicit a drop in olfactory identification performance.

Methods: Ten participants (six women) over the age of 65 volunteered to participate in this repeated measures cross-over design study. Participants underwent the OST at three concentration doses of atropine: 0.1 mg, 0.5 mg and 1 mg.Researchers took a medical history of the participants, measured their neuropsychological performance and issued pathology request forms for genotyping.

Results: Two linear mixed models were conducted: The first linear mixed model looked at the data according to participants divided by ARCS performance and the second one according to group genotype risk. Both linear mixed models explored the effects of research group (i.e. neuropsychological performance and genotype risk respectively), atropine dose and a combination of both. The linear mixed model conducted on ARCS-score groups yielded no significant effects. The linear mixed model conducted on genotype risk groups yielded a significant effect for genotype risk at $\alpha<.05$, $F=15.63, p=.004$. Researchers calculated the mean for each genotype group regardless of dose level ($N=9$). The results were consistent with what might be expected from previous OST research. The participants at risk yielded a substantial, negative
mean atropine effect (AE), $M = -3.88$, 95% CI [-6.24, -1.53], while the no risk genotype group yielded a negligible and positive AE, $M = .89$, 95% CI [-.62, 2.40].

**Conclusions:** only the 0.5mg atropine dose was able to distinguish significant differences in AE between the groups with and without the ε4 gene allele. No effects were found of different atropine dosages on AE. However, it is likely that the sample size was too small to detect any differences in AE and make any inferences about the results. Further research with larger samples is desirable in order to find an effective lower dose of atropine in the OST.

**Keywords**

Alzheimer’s disease, dementia, screening, atropine, Olfactory Stress Test

**Background**

Alzheimer’s Disease (AD) rates in Australia are expected to increase in the coming decades due to an ageing population and the concurrent increase in the risk of developing the disease that comes with age [1]. According to the Australian Bureau of Statistics [2], life expectancy in Australia has increased in the 10 year period to 2011. This follows the trend identified by the Australian Institute of Health and Welfare, which has seen life expectancy in Australia increase steadily between 1900 and 2005 [3]. In light of this, the prevalence of AD in Australia is projected to increase to approximately 1.13 million people by 2050; that is nearly quadruple the 2009 figure of 245,400 [4]. AD has the highest prevalence of the neurodegenerative diseases leading to dementia [5], with Australia, in 2009, being the first country to recognise the disease as a national health priority [6]. The impact of the disease on individuals and their families is profound, with sufferers requiring increasing care and supervision as the disease progresses. With this in mind, Alzheimer’s disease International, in their 2013 Report,
highlight the potential benefits of early diagnosis as a means for future care planning [7].

Given the projected increase in rates of diagnosis of AD, there is substantial pressure to find better tools to aid early diagnosis as well as treatment. With the future potential for disease modifying therapies, the application of such therapies early in the course of the pathology will be paramount [8]. Moreover, projection studies in health economics have shown that early treatment facilitated by a prompt diagnosis could represent substantial financial savings for the state and the individual [9-12]. Similarly, acetylcholinesterase inhibitors treatment has been found to yield better results if administered at the moderate stage of the disease [9]. The Olfactory Stress Test (OST) provides the possibility of an early and inexpensive screening tool for AD.

The OST is a potential new AD screening method that relies on smell identification to reveal latent pathology occurring at the olfactory bulb (OB) and cortical levels. The OST relies on the use of the anticholinergic drug atropine to assess effects on olfactory identification that might be indicative of AD.

**Study aims**

In Schofield et al., [13], the atropine dose administered as part of the OST was 1 mg. This dose level was considered the maximal safe dose that could be administered intranasally based on the fact that it is the amount administered intravenously in routine clinical practice. As such, the aim of this study was to titrate the atropine at 0.1 mg, 0.5 mg and 1 mg concentration doses to find the minimal, optimal dose to elicit an Atropine Effect (AE) while keeping potential side effects to a minimum.

Before proceeding into the details of the study and its hypotheses presented in this report, it is important to understand the mechanisms at work in AD in order to
appreciate the rationale for the OST. The remainder of this introduction will explain these mechanisms and describe the OST properties in that context.
Olfactory changes occur in Alzheimer’s Disease and may precede obvious cognitive impairment

Olfaction has been found to decline along with cognitive function in AD. Evidence suggests that olfactory decline might precede the emergence of the putative clinical Dementia of the Alzheimer’s Type (DAT) symptoms. A longitudinal study into changes in olfactory threshold in AD found statistically poorer olfactory detection performance among initially healthy participants who became AD-positive during the course of the study [14]. Another example is the longitudinal study conducted by Tabert et al. [15], who found that poorer performance in a brief version of the University of Pennsylvania Smell Test (UPSIT) correlated with a higher risk of developing AD or converting to it from mild cognitive impairment (MCI). Similarly, a multi ethnic study found that elderly participants without MCI performed significantly better in the UPSIT than their non-amnesic and amnesic MCI counterparts [16]. An epidemiological study found a significant association between the combined baseline olfactory impairment and the 5-year incidence of MCI among healthy, community-dwelling older adults [17]. A magnetic resonance imaging (MRI) study found a significant association between olfactory bulb (OB) and tract volume and cognitive performance; the difference in OB and tract volume between control and AD participants, in fact, was significant [18].

AD pathology first appears at the transenthorhinal cortex level, which includes brain areas implicated in memory, emotional and olfactory processing, and later spreading to temporal areas of the brain [19]. Even though olfactory deficit is a recognized feature of AD, olfactory problems can subtly emerge before the onset of clinical AD symptoms [20]. However, olfaction is rarely assessed in clinical settings, earning its dubious title, “the neglected sense” [21].
Pathological changes of AD affect cholinergic neurotransmission and are related to olfactory decline

**Olfaction and cholinergic neurotransmission.** Cholinergic refers to activity in the brain involving the neurotransmitter acetylcholine (ACh). The olfactory bulbs (OBs) and limbic system are structures that rely on cholinergic neurotransmission to relay information. It has been recognised that neurons which release the neurochemical ACh are particularly susceptible to damage due to AD [21]. Some of the tissues relying on ACh are responsible for cognitive functions affected by AD. At the cerebrocortical level, ACh is understood to be involved in the consolidation of memories. Organs within the limbic system, for example, are in charge of the acquisition and retention of new information [22]. This function has been demonstrated through the adverse effect of anti-cholinergic drugs (i.e. muscarinic receptor antagonists) on the acquisition and retention of learned tasks in animal models [23]. This anti-cholinergic effect was first observed in humans during the 1970s [23]. It was found then that anticholinergic agents, such as atropine and scopolamine, had an effect on healthy young adults that mimicked the cognitive symptoms of AD: attention deficits, and impaired acquisition of new information and memory consolidation. Subsequently, the effect has been studied extensively in rats, demonstrating the capacity of anti-cholinergic agents to disrupt learning tasks, conditioning tasks, spatial learning tasks and attention shifting tasks [23].

The animal and human models of cognitive decline mentioned above gave rise to the cholinergic hypothesis, which posits that “a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age and AD” [23, The Cholinergic Hypothesis, para. 2].

Similarly, the glomeruli inside the OB contain synaptic connections between bundles of olfactory nerves and mitral cells. Olfactory cues from the environment travel
over the synaptic cleft to the mitral cells. ACh released from basal forebrain cells modulates the output of mitral cells, and this information passes to the olfactory tract and to its final destination in the anterior olfactory nucleus, the amygdala and the entorhinal cortex [22, 24].

**AD pathology affecting cholinergic activity and impact on behaviour.** There are two types of histological pathology in AD that interfere with cholinergic activity: β amyloid (Aβ) and neurofibrillary tangles (NFTs). Post-mortem studies and animal models have found a link between these pathologies and cognitive and behavioural deficits found in AD. Aβ depositions appear in the basal forebrain during AD concomitantly with decreased cholinergic neurotransmission and cholinergic neuron degeneration. Aβ is understood to disrupt cholinergic neurotransmission (see Auld et al., [25] for a review). Along with Aβ, tau positive NFTs have been found in the OB during AD, which also increase with disease progression and diminished cholinergic activity [21]. Experiments on animal models have demonstrated that Aβ plays a role in the pathophysiology of AD. For example, Wesson, Levy, Nixon and Wilson [26] demonstrated that mice genetically modified to over-express the Aβ precursor protein (APP) manifested behavioural problems consistent with aspects of AD. Mutated and regular mice were exposed to new odours. After a period of no exposure, the mice were exposed to the same odours and the time they spent investigating the new odour was measured. The mutated mice manifested impaired odour habituation, demonstrated by the fact that they took longer to investigate odours to which they had already been exposed. Similarly, mutated mice displayed problems discriminating between odours and took longer to examine new ones. At face value, these behavioural changes pointed to aspects of AD: the apparent difficulty habituating to new odours may have been construed as a diminished ability to incorporate new information and the impaired
odour discrimination may have been suggestive of cortical deficits. This is further corroborated by the histological analysis conducted on the animals. The researchers found that “deficits in initial odour investigation” correlated with Aβ deposits within the OB, whereas discrimination deficits correlated with same within the piriform cortex, whose role is to process olfactory stimuli [27]. Chronologically, Wesson et al. [26] found that the first olfactory deficits in the mutated mice emerged concomitantly with the appearance of Aβ deposition in the OB. Inversely, but also demonstrative of Aβ plaques’ influence in AD, Bales et al. [28] showed that anti-Aβ antibody m266 could reverse habituation problems in mutated mice. The researchers suggested that the m266 treatment allows for increased hippocampal cholinergic activity, which facilitates habituation to new environments.

As such, AD’s toll on cholinergic activity has an adverse impact on olfaction, memory and learning because the means of sharing information—cholinergic neurotransmission—is compromised, degenerating further as the disease progresses.

Lastly, post-mortem studies have suggested that 40% of non-demented elderly persons carry enough AD histological pathology as described above to have warranted a clinical diagnosis [29]. Tau pathology, for example, has been found in brains at Braak Stage II of AD pathology, which corresponds to mild to no cognitive impairment [30]. Tsuboi, Wszolek, Graff-Radford, Cookson and Dickson [31] also found that ApoE ε4, Braak staging and Tau pathology correlated in a linear regression analysis. Tsuboi et al [31] also discovered Tau pathology in the anterior olfactory nucleus at Braak Stage II; virtually prior to the emergence of clinically significant symptoms.

**Association between genetic predisposition and pathology of AD**

At the genetic level, people with the Apolipoprotein E (ApoE) ε4 allele are at increased risk of developing AD and Aβ pathophysiology [32]. In a study exploring the...
role of ApoE ε4 in early changes in olfactory function due to AD, Bacon et al., [14] found that MCI participants with poorer odour detection carried at least one ε4 allele. The results of a 2011 meta-analysis indicated that the presence of an ApoE ε4 allele is a moderately strong predictor of conversion from MCI status to DAT [33]. Elias-Sonnensheim et al. [33] found that individuals with a double expression of the ApoE ε4 allele were at four times the risk of progressing from MCI to AD compared to non-carriers.

The ε4 allele is the gene responsible for the manufacture of ApoE, which binds with the βA4 peptides—the main ingredient of senile plaques present in the AD brain [14, 34]. Normally, the lipoprotein ApoE is understood to be involved in the growth and repair of myelin and axonal membranes [35]. However, Namba et al. [35] found that NFTs and Aβ depositions presented immunoreactivity against ApoE in the brain of AD patients.

Despite the consistency of the effect ApoE ε4 allele has on AD, Elias-Sonnensheim et al. [33] warned that genotyping might be of limited clinical benefit due to its low sensitivity and low positive predictive value. Elias-Sonnenshiem et al. [33] further found that 50% of the participants who developed DAT studied in their meta-analysis were not ApoE ε4 carriers. Similarly, Henderson et al. [36] found that the prevalence of AD by age 90 among ApoE ε4 homozygous participants was 50%, despite finding a linear association between carrying at least one ApoE ε4 and cognitive impairment/AD.

According to Elias-Sonnensheim [33], the value of AD genotyping lies in research recruitment. In the case of OST research, for example knowing the genotype is valuable as an indicator for the likely presence of AD pathology in otherwise healthy participants.
ATROPINE TITRATION IN THE OST

Schofield et al. [13] postulated that there may have been a likelihood of clinical AD symptoms emerging had such individuals lived longer. The aim of the OST, therefore, is to screen for cholinergic decline described above before it manifests clinically or at the MCI stage.

The Olfactory Stress Test (OST): Rationale and Results (Adapted from Schofield et al. [13])

Schofield and his team hypothesized that the anti-cholinergic solution atropine should exacerbate impaired neurotransmission among already compromised cholinergic pathways. Taking into account that intranasally administered drugs concentrate at the OB—where AD histological pathology is also known to accumulate—it was further hypothesized that the atropine should accumulate there, impairing olfaction as a result. The expectation was that performance on olfactory identification tasks should worsen after the intranasal application of atropine among elderly individuals with underlying AD pathophysiology. Schofield et al., proceeded to test this hypothesis and explore any associations between changes in olfactory performance and known AD markers (i.e. ApoE genotype, memory performance and hippocampal volume).

To test the impact of atropine on olfactory performance, Schofield et al. [13] devised the Olfactory Stress Test (OST). This consists of administering the University of Pennsylvania Smell Identification Test (UPSIT) to establish a baseline. This is followed by the intranasal administration of and finished by a second administration of the UPSIT. The UPSIT is a 40-item scratch-and-sniff test where the examinee is asked to identify an odour from four different options. The first UPSIT reading is then subtracted from the second, yielding an atropine effect (AE). A negative AE indicates a drop in UPSIT performance following the atropine. Further details about the UPSIT and the OST are offered in the methods section. In the only study published on the OST at
this time [13], Schofield and his associates found that the majority of AE<0 occurred among MCI and potentially AD participants (92% and 86% respectively). Memory scores were highly associated with AE (r=.57, p<0.0001). In fact, AE explained more variance in memory performance (24%) than did hippocampal volume (14%). Similarly, lower mean AE was associated with the presence of ApoE ε4 relative to no ApoE ε4 being present. Further to being a potential diagnostic tool, the OST could also be used as a proxy for biological markers of AD treatment success [37]. Valyudhan and Lovestone [38] found that UPSIT scores correlated with improvements in global functioning after treatment with the cholinesterase inhibitor Donepezil. Moreover, changes in UPSIT scores over time were the best predictors of global functioning improvements. It is plausible that the OST could fulfil such a monitoring function in the future.

The objective of this study was to find a lower dose of atropine to the 1mg used by Schofield et al. [13]. This was pursued in the context of two hypotheses based on Schofield et al.’s findings, and genetic theory behind AD: (1) participants considered to have MCI or deemed to be in the early stages of AD according to neuropsychological scores would yield a negative AE and (2) participants at genetic risk of AD would yield a negative AE.

Methods

Sample and Participant Selection

Ten participants (six female) above the age of 65 were recruited for this study. Participants were sourced from community organisations serving seniors within the Lake Macquarie Municipality, word of mouth and previous studies conducted by the Neuropsychiatry Department at the Calvary Mater Hospital (CMH) in Newcastle, NSW. Recruitment was conducted between September 2012 and April 2013. Officials within
the seniors’ organisations targeted were contacted via phone and subsequently sent a flyer advertising the study. Where possible, an associate involved in the research (MB) would attend one of the organisation’s meetings to discuss the study with potential participants. The flyer directed people interested in participating in this study to contact the Neuropsychiatry Department at the CMH. The researcher (MB) then called potential participants to provide further information about the study and inquire whether the interested individuals had any pre-existing conditions (described below) that might exclude them from the study. If participants met the inclusion criteria, and none of the exclusion criteria, they were sent detailed information about the research with the contact details of the researchers (MB and PS). During this initial telephone conversation, potential participants were also informed about the 15$AU stipend for each session attended. Participants recruited from previous studies had already performed the OST at the 1 mg concentration dose; this dose was considered to be their first dose for the current study.

**Inclusion and exclusion criteria.** Participants sought for this study included people who reported memory complaints consistent with MCI, people who had been diagnosed with AD and people who were cognitively healthy. Potential participants were excluded if they had any condition that could be mistaken for AD dementia or that might also account for cognitive impairments, such as: a history of stroke; severe traumatic brain injury associated with loss of consciousness or psychiatric conditions that could affect cognition. Individuals who were highly dependent on medical care, who were not mobile or whose native language was not English were also excluded from the study.

Four participants had responded from seniors’ organisations in the Lake Macquarie Municipality, and all met the inclusion criteria and none of the exclusion criteria. One of these participants withdrew interest after the initial telephone
conversation, so only three took part in the study. The remaining seven participants were recruited from the previous OST study [13]. None of the participants dropped out from the study. Additionally, due to recruitment difficulties and time constraints, only five out of the total 10 participants took all three doses. The remaining participants only took the 0.5mg and 1mg concentration doses. Figure 1 presents a flowchart of the participants’ recruitment.

The study was approved by the Hunter New England Human Research Ethics Committee. The Ethics Committee’s approval was subsequently registered with the University of Newcastle’s Human Research Ethics Committee due to this paper being submitted as an academic research thesis.

**Assessment and Measures**

**Audio Recorded Cognitive Screen (ARCS).** ARCS is an unsupervised neuropsychological assessment instrument for the screening of dementia. It is administered through an audio device, and examinees are provided with a special booklet to write down their responses. ARCS covers memory, verbal fluency, visuospatial functioning, language and attention/executive function. This assessment tool has been tested for its validity and reliability, showing evidence to yield consistent results in discriminating individuals with cognitive impairment, dementia or healthy cognitive functioning. ARCS is also sensitive to differences in cognitive function based on age, gender and education (for a full review and description of the instrument, see [39, 40].

The ARCS score is scaled to a population mean of 100 with an SD of 15 [40]. The ARCS was used to split participants into different research groups: Mild Cognitive Impairment/Alzheimer’s disease (MCI/AD; i.e. participants with mild cognitive impairment or AD) or Control (i.e. participants who appeared cognitively healthy
according to the ARCS). Participants were allocated to the MCI/AD group if they scored less than 78 (i.e. 1.5 SDs below the normal performance score of 100) in the composite scaled score or the same in any of the scaled domain scores even if the composite score reached above 78 [39].

Genotyping. ApoE genotyping was conducted to detect the presence of three alleles: ε4, ε3 and ε2. Participants were considered at risk of AD in the presence of at least one ε4 allele and separated into two groups: risk and no risk. This second grouping of participants was used in a second statistical analysis. The description and rationale of these statistical analyses are provided later in this section.

University of Pennsylvania Smell Identification Test (UPSIT). The UPSIT is a scratch-and-sniff olfactory test that consists of 40 items divided among four booklets with 10 items each. Each page contains an odorant sticker and a multiple choice question providing four options. Examinees scratch the odorant, which releases the stimuli, sniff it, match the smell with its corresponding option above and mark their answers in the area provided on the last page of each booklet.

The reliability of the UPSIT has been established. In a test-retest trial, the developers of the test found that the scores of two test administrations six months apart remained stable (Pearson r=.918; p<0.001) [41]. The UPSIT has been demonstrated to be sensitive to olfactory deficits associated with a number of pathologies including AD [42]. In fact, Doty [41] found the UPSIT to be able to discern among individuals with normal olfaction and those suffering from an olfactory dysfunction.

Because the OST is expected to yield a pre-post difference in UPSIT scores due to the interaction between atropine and underlying pathology, this instrument’s sensitivity to identify olfactory dysfunction is important in detecting subtle physiological changes that might affect performance.
**Atropine Solution.** This is an anti-cholinergic agent and is central to the OST. By stressing cholinergic neurotransmission in the OB, the atropine solution should help expose underlying neuropathology by exacerbating performance in olfactory identification tasks. As such, participants with incipient AD (i.e. those in the MCI/AD group or with a positive readout of ApoE ε4 allele) were more likely to perform significantly lower than healthy participants according to Schofield et al. [13].

The original dose used to test the OST as reported by Schofield et al. [13] was 1mg. The 1mg dose because had been chosen because it is already administered intravenously at that dose routinely in clinical practice. Therefore, it was considered safe to be administered intranasally.

Participants were informed of the side-effects of atropine prior to consenting to the experiment. Possible side effects included nasal dryness that could linger for several hours. Less-likely side effects were eye redness or pain, blurred vision, dizziness, irregular heartbeat, pain or cramping in the abdomen and painful or difficult urination. Participants were assured that medical assistance was at hand should any of the side effects occur.

**Experimental procedures and study design**

The study followed a cross-over design. All participants were requested to attend three sessions originally and undertake the OST at the three doses described above. Participants were encouraged to undertake the OST at each dose one week apart. The reason for the week-long gap between measures revolved around room availability at the CMH, where the study was conducted, and allowing enough time for the atropine’s washout period. The study calendar was laid out based on these constraints and on participant availability.
Steps were taken to keep aspects of the cross-over study design from biasing the true treatment (i.e. atropine) effect [43]. In order to avoid a learning effect (i.e. a period effect), participants were provided no feedback about their UPSIT results, and the four UPSIT booklets were scrambled with each administration (i.e. before and after each atropine dose). With an atropine plasma half-life of 2-3 hours, a minimum seven day washout period was more than sufficient to establish complete elimination from the system between dose administrations [44]. This helped avert a carry-over effect between measures over time [43]. However, the period effect was not computed with the model due to the small sample size [43]. Latin squares were set up to randomize the administration of each dose to participants.

**Participant induction.** Participants were given a consent form at the first session and advised that they could withdraw from the study at any point. Participants were given the opportunity to ask questions about the study and once it was established that they understood the requirements, they signed the consent form. A medical history was taken and the ARCS was administered. The participants were also provided with a pathology request form for genotyping. The ARCS was used to divide participants between MCI/AD and Control groups. The genotyping was employed to divide participants between risk and no-risk groups.

**Olfactory Stress Test (OST).** The OST was administered in three steps. Firstly, the participants took a baseline UPSIT measure using the left nostril, with the right nostril occluded with a cotton ball. With the right nostril still occluded, the researcher administered the atropine solution via nasal spray deep into the left nostril. Secondly, after receiving the atropine, the participants adopted the “Mecca position”, which consists of kneeling with the head resting upside-down on the floor in a praying-like posture. Participants remained in that position for one minute. If participants were
unable to kneel, other positions were used (e.g. laying down on a table with their head hanging down off the side or bending down while standing up so that their heads rested upside down on a table). The “Mecca position” helped retain the atropine spray adjacent to the olfactory mucosa and down the cribriform plate. After a 45-minute wait, examinees took the UPSIT a second time with the right nostril still occluded. All 40 items of the UPSIT are administered at baseline and post treatment. The AE was then calculated from the before and after UPSIT measures. The AE is the impact of the atropine on UPSIT performance and is calculated by subtracting the pre-atropine UPSIT score from the post-atropine UPSIT score (i.e. post-atropine UPSIT – pre-atropine UPSIT = AE). A negative AE (i.e. a negative figure) would indicate a decline in performance.

**Statistical analysis**

Data was entered into MS Excel and analysed in SPSS (versions 19 and 21). Descriptive data was analysed separately for each grouping distribution (i.e. MCI/AD or Control and [genotype] Risk or No Risk) using the same tests. T-tests were used to compare ARCS scores and age differences between groups. Chi Squares using the Monte Carlo method were employed to compare categorical data.

Linear mixed model analyses were conducted to explore the effect of the interaction between experimental groups and atropine dose on AE. Linear mixed model analysis can compute the overall treatment effect and the effect for each dose of atropine, and it can compute the standard error at all the time points, yielding a more robust statistic [45, 46]. The linear mixed model allows for missing data provided it meets the missing at random definition (i.e. data missing can be explained by chance) [46]. The linear mixed model can also handle uneven spaces of the different doses. [47]. Significance levels were placed at p<.05 for all tests.
The linear mixed models were tested using two covariance matrices: compound symmetry and unstructured. Covariance matrices reflect how covariances interact in the model. A compound symmetry covariance structure is restrictive on the data, and it assumes that all covariances are equal, namely: the variances for each dose are equal and covariances between each pair of doses are equal [45]. The unstructured covariance matrix allows these variances at each dose and covariance between pairs of doses to differ, but it is a more complicated model. These were then compared using the Akaike Information Criterion (AIC) and Bayesian-Schwartz Criterion (BIC) for better fit. The model with the lowest Akaike Information Criterion (AIC) and Bayesian-Schwartz Criterion (BIC) were considered to represent the best fit (i.e. the most accurate representation of the data). Lastly, the matrix with the best fit was utilized in the final linear mixed model analysis.

Results

It was hypothesized that (1) people in the MCI/AD group would yield a negative, larger AE than control participants (i.e. those with normal cognitive abilities) and (2) that participants in the Genotype Risk group would yield a negative, larger AE.

A total of ten participants volunteered to the study (six female). Their mean age was 72.15 (SD=4.96). According to the ARCS results, five participants fell within the normal range of cognitive function and five had cognitive impairment, a proportion of whom might be anticipated to have underlying AD as the cause (‘MCI/AD’ group). Only nine participants were included in the statistical analysis of genotype groups due to a missing pathology lab result. The participant for whom the genotyping result was unavailable belonged to the MCI/AD group. Due to time constraints, only five participants took all three doses, and the other five took the OST at 0.5 mg and 1 mg of atropine concentration. However, the missing data (i.e. participants without a 0.1mg
dose) met the missing at random definition. The missing at random assumption was tested by conducting Chi Squares using the Monte Carlo method and independent T-Tests to examine whether there were any differences between the sets of participants who took all three or only two doses. No significant differences were found in the demographics, neuropsychological performance and genotype risk of AD among participants who took all three doses compared to those who only took two (Table 1).

Whereas the researchers had originally aimed to administer each dose at one-week intervals, some participants could not commit to such schedule. This resulted in a break between doses longer than a week. With regards to randomisation, the researchers had organized the administration of each dose to participants according to Latin Squares. However, due to the need for the use of certain atropine doses in parallel studies, the doses were administered according to availability and not the Latin Squares.

With regards to side effects, several participants experienced minor nasal dryness. No other side effects were reported.

The remaining results have been divided into two parts. The first part focuses on the analyses, including the linear mixed modelling, conducted on the data according to MCI/AD and Control groups. The second section focuses on the analysis based on genotype Risk and No Risk groups. Each section contains its own set of descriptive statistics. Table 2 presents the raw UPSITS scores before and after the administration of atropine.

**Results according to groups divided by neuropsychological ability**

The descriptive statistics are provided in Table 3, the mean estimates for the AEs are displayed on Table 5 and the AEs for MCI/AD and Control groups are plotted in Figure 2.
No significant differences were found in terms of age and ratio of genotype risk and gender between the two groups. A significant difference was found in ARCS total scaled scores between MCI/AD and Control groups, *t*(8)=2.86, *p*=.021. Significant differences were found between groups in domain scaled scores, which was as expected considering that each group was comprised of cognitively impaired and healthy individuals respectively (Table 3).

For assessment of AE in Hypothesis 1, two linear mixed model covariance matrices were explored: unstructured and compound symmetry. The model explored the fixed effects of group (i.e. MCI/AD and Control), atropine dose and the interaction of group and atropine dose on AE. The unstructured and compound symmetry matrices were compared using the AIC and BIC. The compound symmetry model proved to be a better fit for the data. However, the model yielded no statistically significant effects (Table 6).

**Results according to Groups divided by Genotype**

Only nine (five female) participants were included in the groups by genotype because the genotyping results of one participant were unavailable. Table 4 shows the demographics according to groups divided by genotype: Risk (i.e. ApoE ε4 positive) and No Risk (i.e. ApoE ε4 negative). There were no differences in ARCS total scaled scores or age between groups. The AE mean estimates are presented in Table 5, and the AE scores are plotted in Figure 3.

The unstructured symmetry matrix in the linear mixed model did not converge, so the fixed effects were explored based on the compound symmetry structure. As such, a significant effect was found only for genotype, *F*=15.63, *p*=.004. This means that even though genetic risk for AD seemed to affect AE, neither atropine dose nor the interaction of genotype and dose exerted any influence on AE. Pairwise comparisons
between the AE of the risk and No Risk groups revealed a significant difference only for the .5mg atropine dose, \( P=0.015, 95\% \text{ CI} (1.14, 9.11), p<.05, \) two-tailed. The AE for the No Risk group at the .5mg atropine dose was positive and smaller (\( M=.50, SE=1.07 \)) compared to that of the Risk group which was negative and larger (\( M=-4.67, SE=1.55 \)). However, follow up pairwise comparisons showed that the difference in AE between the No Risk group (\( M=.33, SE=1.53 \)) and the Risk group (\( M=-5.31, SE=2.63 \)) at the .1mg dose trended toward significance, \( p=.083 \). Similarly, the difference at the 1mg dose between the no risk (\( m=1.83, SE=1.10 \)) and the Risk group (\( M=-1.68, SE=1.55 \)) trended toward significance, \( p=.084 \) (Table 7).

The overall AE for the Risk group (regardless of dose) was negative, \( M=-3.88, 95\% \text{ CI} (-6.24, -1.53) \), while that of the no risk group was positive and close to 1, \( M=.89, 95\% \text{ CI} (-.62, 2.40) \).

Model mean estimate plots show that the AE of the ApoE ε4 No Risk group (i.e. ApoE negative) remained positive (Figure 3). The AEs from ApoE ε4 positive group (i.e. at risk) remained below zero at each dose. However, the dose effect was not significant.

**Discussion**

The OST is a potential new method to screen for AD. The OST relies on atropine to exacerbate any pre-existing cholinergic deficit in the OB. A previous study on the OST used a 1 mg concentration dose of atropine, which is an amount administered routinely in clinical practice and known to be safe. This study sought to find a smaller atropine dose by titrating 1 mg, 0.5 mg and 0.1 mg concentrated doses. The rationale was to find a smaller than 1 mg dose that could still elicit an AE while keeping side effects at a minimum. Concomitantly, two hypothesis were tested based on OST theory [13]: (1) participants suffering from MCI or in the early stages of AD
would yield a negative AE and (2) participants considered at genetic risk of AD (i.e. carriers of at least one ε4 allele) would yield a negative AE.

Two linear mixed models were conducted to analyse the data. The first model tested the first hypothesis and employed data from experimental groups divided by neuropsychological performance (i.e. ARCS performance): MCI/AD and Control groups. This first model explored the effects of group, atropine dose and their interaction on AE. No statistically significant effects were found. Such results were inconsistent with Schofield et al.’s original findings [13].

The second linear mixed model was conducted with the participants separated into groups according to genotype risk: Risk and No Risk. This linear mixed model explored the effects of group, atropine dose and their interaction on AE. A significant effect for genotype risk found at the .5mg dose of atropine. Pairwise comparisons between the Risk and No Risk groups showed that the AE at the 1mg and .1mg doses trended towards significance. The mean group AEs, regardless of atropine dose, showed that the AE of the genotype risk group was negative and substantially distant from zero. Comparatively, the no-risk group’s AE was positive and very close to zero. This second set of results were as expected based on the genetic theory behind AD, namely: People carrying at least one ε4 allele are at risk of developing AD pathology without tangible cognitive complaints. However, these results were insufficient to support hypothesis 2.

Similarly, the present results did not allow the researchers to identify and optimal, minimum dose atropine dose for the OST.

The results of this study need to be considered with caution. Due to the small sample, it is impossible to ascertain whether the non-significant readout of the first linear mixed model (i.e. on participants divided by neuropsychological results) was due to chance or the true absence of interaction between atropine and cholinergic
neurotransmission. Based on the results of Schofield et al. and what is understood about atropine and its effect on cholinergic neurotransmission, it is not unreasonable to consider that the small sample size obscured a true effect. Additionally, the participants in the MCI/AD group, while probably mildly cognitively impaired according to the ARCS, did not have an AD diagnosis. Similarly, the significant results of the second linear mixed model (i.e. groups divided by genotype risk) could have been an artefact of changing the group definitions for the second hypothesis, and the small sample size provided no statistical robustness to draw any meaningful conclusions. Lastly, even though some of the results of the linear mixed model based on genotype risk groups were significant, there was no difference between doses. Therefore, it cannot be concluded whether any of the doses lower than 1 mg was sufficient in eliciting an AE.

The researchers encountered some difficulties during recruitment, which explains the small sample size. The recruitment drive for new participants with cognitive impairment or early AD led to few positive responses. Similarly, some prospective participants from previous studies did not wish to participate in more research.

There were other sources of bias that warrant caution in interpreting the results. Steps were taken to reduce the chance of practice effect: the UPSIT booklets were scrambled before each administration (pre and post atropine) and participants were never provided feedback on their performance. However, the participants responded to the same UPSIT questions in each administration. Even though the participants’ performance was expected to drop after the atropine intake, there is nothing to suggest that they may have relied on other aspects of the test to strategize their answers. For example, each page of each booklet in the UPSIT is distinctively coloured and a different length. Speculatively, participants could have used these cues to inform their
post-atropine responses, thus compensating for their inability to identify a scent. Future OST research might benefit from dividing the four booklets into two sets of two, with each set to be administered before and after the atropine intake. Such strategy is already employed in other OST research currently underway. In the case of the titration study, which required participants to take the UPSIT six times, dividing the test into four booklets for the first two doses may have been more appropriate (e.g. 10 items before and after the first dose, 10 items before and after the second dose—that constitutes one UPSIT [40 items]—and 20 items before and after the final dose).

Additionally, the repeated measures design employed in this study introduced an unnecessary time variable, which increased the risk for trend, subject and carry-over effects [43, 49]. Because the OST would be a one-off measure to screen for AD in clinical practice, a between subject design would have been more suitable to eliminate the aforementioned biases [50].

In addition to a practice effect, the weekly gaps between measures could have introduced random effects beyond the awareness of the researchers that could have distorted the results (e.g. a participant having nasal congestion that already prevents from detecting odours at baseline) [43]. Such effects could have been countered by adequate randomisation or a balanced distribution of participants across treatments [43].

Originally, doses were to be administered following a Latin Squares matrix to ensure that new participants would start on a different dose and follow a different dose schedule each week. However, due to circumstances beyond the researchers’ control, following the Latin Square matrix became impractical; that strategy was therefore abandoned. Although needing to allocate dosages on a session-to-session basis introduced randomness to the process, the approach did not provide the balance Latin
Squares would have afforded. It is impossible to be certain whether this predicament may have not introduced bias.

Lastly, the spray bottle used to administer the atropine delivered a weak spray. It must be questioned whether an entire atropine dose in fact reached the olfactory bulb.

**Conclusion**

More research with larger sample sizes is needed to determine if a lower dose of atropine would yield valid, meaningful results when administered as part of the Olfactory Stress Test.

**Abbreviations**

Alzheimer’s Disease: AD; Olfactory Stress Test: OST; olfactory bulb: OB; Atropine Effect: AE; University of Pennsylvania Smell Test: UPSIT; mild cognitive impairment: MCI; acetylcholine: ACh; β amyloid: Aβ; neurofibrillary tangles: NFTs; Aβ precursor protein: APP; Apolipoprotein E: ApoE; Calvary Mater Hospital: CMH; Audio Recorded Cognitive Screen: ARCS; Bayesian-Schwartz Criterion: BIC

**Competing interests**

Dr Schofield is the inventor of the Olfactory Stress Test for which a provisional patent has been lodged. Dr Schofield is also an inventor of the Audio Recorded Cognitive Screen used in this study. The remaining authors report no financial interests or potential conflicts of interest.

**Authors’ contributions**

The study design was conceived by PS. This manuscript was conceived and drafted by MB. Critical review was provided by PS & MV. Statistical analysis was conceived by MV. MB and MV carried out the statistical analysis. All authors have read and approved this manuscript.
Authors' information

MB is a postgraduate student completing a Masters (Clinical Psychology) in the School of Psychology, Faculty of Science and Information Technology, University of Newcastle. This manuscript forms the research component of this degree.

Acknowledgements

We thank the study participants for their time and valuable contribution to the study. We also thank the seniors’ organisations in Newcastle for their assistance in recruiting patients for the study. We thank Tammie Moore for assistance with the administration of the olfactory stress test and assistance with participant recruitment, Kim Colyvas at the Statistical Support Service, University of Newcastle for assistance with additional statistical analysis.

References


14. Bacon AW, Bondi MW, Salmon DP, Murphy C: Very Early Changes in Olfactory Functioning Due to Alzheimer's Disease and the Role of


36. Henderson AS, Jorm AF, Korten AE, Christensen H, Jacomb PA, Easteal S, Croft L, Mackinnon AJ: Apolipoprotein E allele ε4, dementia, and


44. Atropine Sulphate. 2013.


Table 1  Demographic differences between participants with three and two doses

<table>
<thead>
<tr>
<th></th>
<th>Three Doses (n = 5)</th>
<th>Two Doses (n = 5)</th>
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<td>Mean Age (SD)</td>
<td>70.6 (4.50)</td>
<td>73.71 (5.39)</td>
<td>0.35</td>
</tr>
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<td>ARCS Scaled Total (SD)</td>
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<td>95.60 (36.01)</td>
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</tr>
<tr>
<td>Memory (SD)</td>
<td>91 (18.60)</td>
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<td>0.69</td>
</tr>
<tr>
<td>Fluency (SD)</td>
<td>92.20 (13.55)</td>
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<td>0.40</td>
</tr>
<tr>
<td>Visual (SD)</td>
<td>94.60 (14.15)</td>
<td>79 (36.70)</td>
<td>0.40</td>
</tr>
<tr>
<td>Language (SD)</td>
<td>93.80 (27.25)</td>
<td>100 (23.05)</td>
<td>0.71</td>
</tr>
<tr>
<td>Attention (SD)</td>
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</tr>
<tr>
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<td>ApoE ε4 Genotype</td>
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<td>Positive/Negative</td>
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</table>

*Note.* Three doses: 0.1 mg, 0.5 mg and 1 mg; Two Doses: 0.5 mg and 1 mg. ApoE ε4 Positive = at risk; ApoE ε4 negative = no risk

*One genotyping pathology request was unavailable by the time figures were analyzed; figures pertaining to genotype risk are therefore based on n = 9.*
<table>
<thead>
<tr>
<th>Participant</th>
<th>Genetic Risk</th>
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<th>Pre-Atropine</th>
<th>Post-Atropine</th>
<th>2nd Atropine Dose</th>
<th>Pre-Atropine</th>
<th>Post-Atropine</th>
<th>3rd Atropine Dose</th>
<th>Pre-Atropine</th>
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<td>1</td>
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<td>3</td>
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<td>24</td>
<td></td>
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<td>35</td>
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<td>31</td>
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<td>31</td>
<td>0.5</td>
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<td>31</td>
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<td>39</td>
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<td>33</td>
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<td>0.1</td>
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<td>31</td>
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<td>8</td>
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<td>23</td>
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<td>9</td>
<td>Risk</td>
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<td>25</td>
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<td>22</td>
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<td>10</td>
<td>No Risk</td>
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<td>30</td>
<td>32</td>
<td>0.5</td>
<td>31</td>
<td>31</td>
<td></td>
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</tbody>
</table>

Note. Risk = ApoE ε4 Positive; No Risk = ApoE ε4 Positive. MCI/AD participants scored below 78 in the scaled ARCS total or in any of the subdomains even if the scaled total was above 78.

*Blood tests results unavailable at the time of Statistical Analysis
Table 3 Participant characteristics according to ARCS scores

<table>
<thead>
<tr>
<th></th>
<th>MCI/AD</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>ARCS Scaled Total</td>
<td>73.80 (26.56)</td>
<td>110.40 (10.81)</td>
<td>.021*</td>
</tr>
<tr>
<td>Memory (SD)</td>
<td>79.60 (32.17)</td>
<td>110.20 (10.09)</td>
<td>.077</td>
</tr>
<tr>
<td>Fluency (SD)</td>
<td>94.80 (16.38)</td>
<td>98.20 (15.43)</td>
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</tr>
<tr>
<td>Visual (SD)</td>
<td>67 (26.05)</td>
<td>106.60 (6.43)</td>
<td>0.011*</td>
</tr>
<tr>
<td>Language (SD)</td>
<td>89 (31.2)</td>
<td>104.80 (12.99)</td>
<td>0.33</td>
</tr>
<tr>
<td>Attention (SD)</td>
<td>85.60 (7.3)</td>
<td>111.40 (20.95)</td>
<td>0.049*</td>
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<td>Speed of Writing (SD)</td>
<td>100.80 (20.75)</td>
<td>103.20 (11.60)</td>
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<tr>
<td>Mean Age (SD)</td>
<td>71.68 (4.03)</td>
<td>72.63 (6.2)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

| Gender               |               |             |      |
|                      | Male/Female   | 1/4         | 0.52 |
| ApoE ε4 Genotype     | (n = 4)³      | (n = 5)     |      |
| Positive/Negative    | 2/2           | 1/4         | 0.52 |

*Note.* MCI/AD participants scored below 78 in the scaled ARCS total or in any of the subdomains even if the scaled total was above 78. ApoE ε4 Positive = at risk; ApoE ε4 Negative = no risk

³Only nine genotyping results were available at the time of statistical analysis. All calculations pertaining to genotype figures are based on n = 9.

*p<.05, two-tailed
### Table 4 Participant characteristics according to Genotype

#### ApoE Group

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<tr>
<th></th>
<th>ApoE Positive (N=3)</th>
<th>ApoE Negative (N=6)</th>
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<tbody>
<tr>
<td>ARCS Scaled Total</td>
<td>70 (42.30)</td>
<td>104.17 (11.69)</td>
<td>0.09</td>
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<tr>
<td>Memory (SD)</td>
<td>71.67 (37.50)</td>
<td>110 (11.40)</td>
<td>0.05</td>
</tr>
<tr>
<td>Fluency (SD)</td>
<td>94.67 (25.42)</td>
<td>98.50 (11.71)</td>
<td>0.76</td>
</tr>
<tr>
<td>Visual (SD)</td>
<td>75.33 (39.07)</td>
<td>93.17 (24.88)</td>
<td>0.42</td>
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<tr>
<td>Language (SD)</td>
<td>74.67 (34.30)</td>
<td>105.17 (11.44)</td>
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<tr>
<td>Attention (SD)</td>
<td>88 (4.58)</td>
<td>104.67 (24.5)</td>
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</tr>
<tr>
<td>Speed of Writing (SD)</td>
<td>104 (17.44)</td>
<td>104.33 (15.64)</td>
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<tr>
<td>Mean Age (SD)</td>
<td>70.56 (3.89)</td>
<td>73.88 (5.19)</td>
<td>3.28</td>
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#### Gender

<table>
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<tr>
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<td>ApoE Negative (N=6)</td>
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#### ARCS Grouping

<table>
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<td>ApoE Negative (N=6)</td>
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</table>

*Note. ApoE ε4 Positive = at risk; ApoE ε4 Negative = no risk. MCI/AD participants scored below 78 in the scaled ARCS total or in any of the subdomains even if the scaled total was above 78.*

*aOnly nine genotyping results were available at the time of statistical analysis. All figures pertaining to ApoE ε4 genotyping are based on N = 9.*
Table 5 Atropine Effect Mean Estimates

Atropine Effect according to ARCS performance

<table>
<thead>
<tr>
<th>Dose</th>
<th>MCI/AD M(SE)</th>
<th>95%CI</th>
<th>Control M(SE)</th>
<th>95%CI</th>
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</thead>
<tbody>
<tr>
<td>.1mg</td>
<td>-1.47(1.93)</td>
<td>[-5.51, 2.58]</td>
<td>.10(2.33)</td>
<td>[-4.77, 4.97]</td>
</tr>
<tr>
<td>.5mg</td>
<td>-1.40(1.55)</td>
<td>[-4.68, 1.88]</td>
<td>-.60(1.55)</td>
<td>[-3.88, 2.68]</td>
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<tr>
<td>1mg</td>
<td>1.20(1.55)</td>
<td>[-2.08, 4.48]</td>
<td>1.00(1.55)</td>
<td>[-2.28, 4.28]</td>
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</table>

Atropine Effect according to AD genotype risk

<table>
<thead>
<tr>
<th>Dose</th>
<th>Risk M(SE)</th>
<th>95%CI</th>
<th>No Risk M(SE)</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1mg</td>
<td>-5.308(2.63)</td>
<td>[-10.90, .286]</td>
<td>0.33 (1.53)</td>
<td>[-2.91, 3.57]</td>
</tr>
<tr>
<td>.5mg</td>
<td>-4.667(1.55)</td>
<td>[-7.96, -1.38]</td>
<td>0.50 (1.07)</td>
<td>[-1.83, 2.83]</td>
</tr>
<tr>
<td>1mg</td>
<td>-1.667(1.55)</td>
<td>[-4.96, 1.62]</td>
<td>1.83 (1.10)</td>
<td>[-.49, 4.16]</td>
</tr>
</tbody>
</table>
Table 6 Atropine Effect Pairwise Comparisons for MCI/AD and Control Groups by Atropine Dose

<table>
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<tr>
<th>Mean Difference (Control - MCI/AD)</th>
<th>SE</th>
<th>df</th>
<th>F</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.1mg</td>
<td>1.56</td>
<td>1.56</td>
<td>18.99</td>
<td>0.27</td>
</tr>
<tr>
<td>.5mg</td>
<td>0.80</td>
<td>2.19</td>
<td>16.48</td>
<td>0.13</td>
</tr>
<tr>
<td>1mg</td>
<td>-0.20</td>
<td>2.19</td>
<td>16.48</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*p<.05, two tailed
Table 7 Atropine Effect Pairwise Comparisons for Genotype Groups by Atropine Dose

<table>
<thead>
<tr>
<th>Dose</th>
<th>Mean Difference (No Risk - Risk)</th>
<th>SE</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1mg</td>
<td>5.64</td>
<td>3.04</td>
<td>15.60</td>
<td>3.44</td>
<td>0.083</td>
</tr>
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<td>.5mg</td>
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<td>1.90</td>
<td>15.68</td>
<td>7.41</td>
<td>0.015*</td>
</tr>
<tr>
<td>1mg</td>
<td>3.50</td>
<td>1.90</td>
<td>15.68</td>
<td>3.40</td>
<td>0.084</td>
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</tbody>
</table>

*p < .05, two tailed
Ten Participants in present study

Participants set breakdown

- 5 Participants took three doses.
- 5 participants took only 0.5mg and 1mg concentration doses

4 Participants recruited from senior organisations

1 participant withdrew before first session

7 Participants recruited from previous OST study

Figure 1. Flowchart of participants’ recruitment and breakdown according to number of atropine doses received.
Figure 2. Atropine effects plotted according to groups divided by neuropsychological performance (i.e. ARCS composite scores).
Figure 3. Atropine effects plotted according to group divided by genotype risk (No Risk: ApoE ε4 negative; Risk: ApoE ε4 positive).
References
Bacon, A. W., Bondi, M. W., Salmon, D. P., & Murphy, C. (1998). Very Early Changes in Olfactory Functioning Due to Alzheimer's Disease and the Role of


Clare, L. (2002). We'll fight it as long as we can: Coping with the onset of Alzheimer's disease. *Aging & Mental Health, 6*(2), 139-148.


APPENDIX A

About BMC Neurology

Aims and scope | Editorial team | Open access | Article-processing charges | Indexing services | Publication and peer review process | Editorial policies | Citing articles in BMC Neurology | Why publish your article in BMC Neurology?

This page includes information about the aims and scope of BMC Neurology, editorial policies, open access and article-processing charges, the peer review process and other information. For details of how to prepare and submit a manuscript through the online submission system, please see the instructions for authors.

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Appendix B

BMC Neurology, Instructions for Authors

Instructions for authors

Research articles

Criteria | Submission process | Preparing main manuscript text | Preparing illustrations and figures | Preparing tables | Preparing additional files | Style and language

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