MEASURING MEMORY IN AN ALZHEIMER'S TREATMENT TRIAL USING A VISUAL SEARCH TASK

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A THESIS SUBMITTED TO
THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGY
YORK UNIVERSITY
TORONTO, ONTARIO

JUNE 2014

ABSTRACT

Alzheimer's Disease (AD) is characterized by episodic memory deficits attributed to damage to the hippocampal formation. AD therapies specifically targeting hippocampal function may be best evaluated through the use of selective hippocampal tasks. I used a nonverbal hippocampal-dependent target-in-scene detection task to determine if task performance shows age-related decline and/or AD-related impairments. Participants located objects ('targets') that appeared/disappeared in flickering natural scenes, yielding faster search times for remembered targets than for forgotten ones. AD patients took longer and required more fixations to detect targets, indicating impaired memory. Furthermore, the AD and aged populations exhibited slower pupillary responses. As part of a clinical trial, I next asked whether deep-brain stimulation of the extended hippocampal circuit would modify memory performance in patients with early AD. The double-blind treatment trial is still underway, thus treatment efficacy is yet to be evaluated, however, trial participants showed a measurable, progressive memory impairment in this task.

DEDICATION

“No influence is as powerful as that of the mother.” Mom, this is for you.

ACKNOWLEDGEMENTS

The biggest thank you goes to my supervisor for her undeniable patience, encouragement, and guidance throughout this journey. From the beginning, she saw the potential in me, and continuously believed in me, even in the moments I doubted myself. Words cannot nearly express how grateful I am for having offered me this opportunity, and for having helped me to see it through.

Thank you to the greatest lab mates anyone could ask for: Benjamin Cassidy (for his inspiration), Sonya Chand (for her cheerfulness), Roman Skybin (for his optimism), Rodrigo Montefusco-Siegmund (for always offering his help without expecting anything in return – minus the occasional beer), Jonathan Mikkila (for offering his MATLAB expertise in times of need), Timothy Leonard (for his statistics and MATLAB advice), Omri Arbiv (for MALTAB help and words of encouragement), Andrea Gomez Palacio (for her positivity), Patricia Sayegh (for helping ground me and providing me with great feedback!), and Athena Ko (for putting things into perspective – which I needed at times!).

I am extremely grateful for my 'European family', for unconditionally loving me, even in my most stressful times: Marina Kishliansky, Jovana Vojnovic, and Iva Mandic. You guys are what I am most grateful to graduate school for!

Thank you to my friends for supplying me with an endless amount of love and support: My don family, particularly Alexandra C, Fraser D, DerrBear K, Mandy Y, my co-don Alex, Ryan, Annette, Tim, Daniel F, and Apoorva B.

Thank you also goes to the senior don clan. Your influential positivity, alongside the many laughs we shared, made this journey as wonderful as it was: ‘Bekah’ Carlsson, ‘Cass’ Brennan, ‘Di’ Fernando, as well as my twin, Patrick Hazzard.

A special shout out goes to my dear friend Corinne Babiolakis for her constant support and encouragement, in sending me daily photos of ***our dream*** to keep me motivated, and for always reminding me that there is someone out there rooting for me!

Thank you to my editorial board, particularly Adriano Nacarato (especially for the last minute edits!), Paula Di Noto, Denise Murphy, Leanna Taggio, Graeme Mc Dowell and Timothy Leonard.

Thanks is also due to my graduate friends for keeping me motivated and inspired, and for being uplifting during trying times: Mariann O, Gaby L, Paula D, Nev, Mike, Alex R, David, Saam, Lily, Diane, Andrea, Ben V, YGO, Stefie, Chen, Larissa.

A noteworthy thank you goes to my residence supervisor, Stephanie Francis, for her selflessness. She is much to humble to say it, but I know she ***always*** tried to help me in any way she could. She has been an exceptional mentor to me, and has provided me with a wealth of life lessons, while nourishing my personal growth throughout this time.

And the biggest thank you goes to my mother and sister, Denise, for all of the heart-to-heart talks and motivational speeches, for helping me with the many times I had to pack and move, for helping me with the cooking and cleaning… and thank you to my extended family the Murphy-Walkers (for always giving me hugs)… but mostly, for being the best family anyone could ever ask for.

Statement of contribution

For my Master's thesis work, I applied a memory task that had previously been created in the lab to test 2 new populations: older adults and individuals with probable Alzheimer's Disease. I created majority of the stimuli I used, and arranged the balanced stimulus sets used in the experiments outlined in chapters 2 and 3. My supervisor and I came up with the experimental design, both in the ordering of the image sets and the time points for testing. I was responsible for data collection from the Alzheimer's patients, older adults and younger adults, with assistance from Jordana Wynn and my supervisor. I administered the Montreal Cognitive Assessment (MoCA) to a majority of the older adults, with help from Jordana Wynn. I applied existing code to convert data into MATLAB for eye movements and trial events. I wrote the majority of the code to analyze the group-by-condition effects of search time, scene familiarity and fixation data, while only writing parts of the code to analyze entropy. My supervisor analyzed the pupil data by modifying the code I wrote for scan path analysis. I generated the figures and tables reflecting the analyses of the Master's work. Finally, I wrote the manuscript (**chapter 2** of the thesis) together with my supervisor, and was sole author on the remaining thesis chapters.

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Introduction

INTRODUCTION

The hippocampus and memory

Memory is a term used to describe the mechanisms involved in the encoding, storage, and retrieval of information over time. Memory is undeniably useful, and we rely on it heavily to perform basic daily functions. Memory allows us to recall the locations of misplaced items, and helps us navigate the route we use to get from home to work; it can also help us respond appropriately to loved ones compared to the way we behave around a sworn enemy or a complete stranger. The significance of memory in almost every aspect of life has led us to pursue a better understanding of the mechanisms responsible for its function and dysfunction. The discovery of amnesia in the famous patient H.M. further illuminated the significant role the hippocampus – located in the medial temporal lobe - plays in memory (Scoville, & Milner, 1957; Brown & Schafer, 1888; Penfield & Boldrey, 1937; Bechterew, 1900). H.M.'s surgery, a desperate attempt to remove the brain tissue causing his seizures, included the removal of large sections of the medial temporal lobe, which left him unable to remember who he had been talking to just 10 minutes prior. His inability to recall personal experiences, and events that occurred after the surgery, affected what researchers now commonly refer to as episodic memory. This incidental finding would be the first to elucidate the possibility of studying memory separate from other cognitive abilities, as well as reveal a more detailed depiction of the specific role of the hippocampus. A few years later, O'Keefe and Dostrovsky (1971) discovered the existence of place cells in the hippocampus, neurons that selectively fired for preferred

places in space. O'Keefe and Nadel (1978) postulated that together these place cells aided in creating 'cognitive maps' of our environments, which we could use for navigation. It was later posited that perhaps place cells were not only limited to combining information about space and time, but rather may also have the ability to create other non-spatial relational links (Konkel & Cohen, 2009; Cohen & Eichenbaum, 1993). This is the fundamental idea behind relational memory, where the hippocampus is thought to create associations among items.

It is widely accepted that as a person ages, hippocampal synaptic numbers decrease and neurons die; this atrophy has been observed through several different imaging techniques (Anderton, 1997; Driscoll et al., 2009; Raz et al., 2004; Raz, 2005). Accordingly, older adults begin to exhibit difficulties with hippocampal-dependent memory. Although some degree of hippocampal atrophy may be expected, aging is also accompanied by increased risk for developing cognitive impairments beyond that predicted by age. Many older adults, particularly after the age of 65, are diagnosed with having mild cognitive impairment (MCI) (Gauthier et al., 2006). They exhibit noticeable cognitive deficits, yet their difficulties are not severe enough to hinder their everyday activities, not qualifying as full blown dementia. Although MCI can plateau or even reverse, it is far more common for those originally diagnosed with MCI (>80%) to experience a conversion into dementia of the Alzheimer's type (Anderson et al., 2012).

Alzheimer's Disease

Alzheimer's Disease (AD) is a progressive degenerative disease characterized by irreversible neuron loss in brain regions that affect cognition. It is the most common form of dementia, a set of symptoms often describing memory decline accompanied with at least one other cognitive deficit as listed in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*), causing an interference with day-to-day function (Mckhann et al., 2011). One of the first areas affected in AD is the hippocampal formation, including the entorhinal cortex, resulting in memory deficits one of the preliminary symptoms (Padurariu et al., 2012). Although the cause of AD remains elusive, pathological characteristics of the disease, common in most individuals with AD, have been identified. Dr. Alois Alzheimer was the first to discover plaques and tangles in the post-mortem brains of demented individuals, leading researchers to believe that these plaques and tangles played a crucial role in the development of AD pathology (Hippius & Neundörfer, 2003). It is now generally accepted that atypical cleavage of a regular transmembrane protein is what leads to the smaller, more sticky protein fragments that aggregate into the first of the pathological hallmarks of AD, the 'plaques' (Minati et al., 2009). The plaques are then thought to interfere with neuronal communication, contribute to tangle formation, initiate inflammatory responses, and eventually result in cell death (Oddo, 2003; Goedert, & Spillantini, 2006; Eikelenboom et al., 1991). The extent to which plaques play a causal role in tangle formation is unknown. Debates about which of the two occurs first and results in the other, the plaques or the tangles, are ongoing and remain controversial (Oddo, 2003; Goedert & Spillantini, 2006; Eikelenboom et al., 1991; Minati et al.,

2009). Tangles, formed by the hyperphosphorylation of an important cytoskeletal protein ‘tau’, have been tightly associated with cognitive status (Ghoshal, 2002; Taniguchi et al., 2005). Under normal conditions, the tau protein assists the transport of nutrients and waste products from the cell body to the axon terminal. The hyperphosphorylation of tau results in it having a disfigured conformation, destroying its function in the process (Minati et al., 2009). Contrary to the hypothesis that both of these disease hallmarks may play a causal role, emerging evidence reveals that some individuals never develop dementia despite large deposits of plaques and tangles (Price & Morris, 1999), meanwhile, others that exhibit profound dementia maintain low levels of these histopathological markers (Pimplikar et al., 2012). Thus, some researchers now suspect that these characteristics may not be causal agents, but merely byproducts of the disease (Struble et al., 2010).

In addition to the amyloid plaques and neurofibrillary tangles, persons with AD have lower levels of choline acetyltransferase (ChAT) protein, an enzyme responsible for producing the neurotransmitter achetylcholine (Ach), in cholinergic neurons (Wilcock et al., 1982). Ach plays a crucial role in learning and memory processes, particularly in the hippocampal formation. Interestingly, ChAT levels have been associated with the amount of cognitive damage observed in individuals with AD (Wilcock et al., 1982). The putative link between cognitive loss and ChAT, however, has been disputed with reports of some individuals with AD showing no reductions in ChAT (Davis et al., 1999; Palmer et al., 1986). Moreover, other studies have shown that persons with different diseases/conditions may exhibit similar degrees of ChAT activity, yet experience no cognitive issues (Kish et al., 1989). Taken together, these

biochemical foundations thought to be behind the pathogenesis of AD, instead, may be factors or by-products from the preceding events that result in the disturbed system.

Over the last few decades, researchers have found that individuals with AD appear to have a higher risk for developing seizures or displaying epileptiform brain activity (Amatniek et al., 2006). Epilepsy is a neurological disorder most commonly observed as uncharacteristic synchronous brain activity initiated in the medial temporal lobe, overlapping the regions primarily affected in AD. Recent evidence suggests that amyloid clusters, which are thought to invade neuronal networks in the hippocampal formation in the earliest stages in AD, are what cause the cognitive decline and epileptiform brain activity observed (Yan et al., 2012; Palop & Mucke, 2011; Palop & Mucke, 2009; Palop et al., 2007; Palop et al., 2006). Other studies argue that the brain activity typical of epilepsy may be what underpins the cognitive problems experienced (Uhlhaas, & Singer, 2006; Griffith et al., 2006). In addition, one study found that under normal conditions mice that had theta (3-8 Hz) and gamma (30-90 Hz) frequency coupling - important for normal cognitive function – began to show alterations in oscillatory coupling. These frequency coupling changes observed led to impairments on spatial memory tasks, even before any amyloid or plaques were formed (Goutagny et al., 2013). Furthermore, cognitively impaired rodents with hippocampal hyperactivity, thought to result from amyloid clusters, were treated with commonly used antiepileptic drugs and experienced improvements in spatial memory tasks (Koh et al., 2010). Despite all the research that has been done in attempts to uncover the etiological root of the disease, many questions regarding the pathogenesis of AD remain unanswered.

While a cure for AD remains elusive, and as the prevalence of the disease is on the rise, effective treatment strategies are of increasing importance.

Current treatments for AD mainly consist of pharmacological agents. In the early to intermediate stages of AD, physicians often prescribe cholinesterase inhibitors as treatment (Winblad & Jelic, 2004). As the name suggests, these drugs inhibit the enzymatic activity of the protein that breaks down acetylcholine, leading to increased levels of the neurotransmitter at the synapses where learning and memory processes occur. Although it has been documented on several accounts that cholinesterase inhibitors are able to delay disease-related symptoms, the drug's effects appear to be temporary, usually lasting up to one year from initial use. In the intermediate to severe stages, doctors will often refer patients to 'memantine', an NMDA antagonist. In healthy individuals, the mechanisms underlying learning and memory depend on the binding of the neurotransmitter glutamate to its receptor NMDA. However, individuals with AD have elevated levels of glutamate, which can lead to the over-excitation of neurons, resulting in their death (Francis, 2003). Memantine works by blocking NMDA receptors from glutamate, regulating its activity. The effects of this drug are controversial: some studies show benefits in the earlier stages of AD (Bakchine & Loft, 2008); others claim is do not (Dysken et al., 2014). Even in the later stages, however, it is often recommended to be used alongside cholinesterase inhibitors for increased efficiency (Tariot et al., 2004). Vitamin E, a powerful antioxidant, has also been used in high doses as a potential treatment. However, the effects of one study that showed its possible benefit in AD have yet to be replicated (Dysken et al., 2014). The effects of the aforementioned drugs are not only limited, but their benefits appear to be lost with

time. Considering the limited efficacy of current pharmacological treatments, and the recent discovery of physiological/circuit dysfunction at early stages of the disease, treatments that target the excitability of cell populations may be useful options. Additionally, researchers are also stressing the importance of earlier diagnosis for optimal treatment results, and this may depend on selective tests that are able to detect changes in brain areas that are primary targets of the disease.

In my Master's work, I set out to investigate the following hypotheses:

1. Memory diminishes as a function of age and AD, as measured by longer repeated trial search times

2. The Autonomic Nervous System (ANS), which controls the change in pupil size, experiences dysfunction in Alzheimer's Disease; thus, diminished autonomic responses in AD we would be able to measure via pupillary responses

3. The AD patients receiving the DBS-f treatment would experience a reduction in memory decline

Chapter 2:

Pupillary response and visual search performance identify and separate age-related from Alzheimer's-related memory decline

INTRODUCTION

Episodic memory is known to decline with aging, but even more so in progressive dementias such as Alzheimer's Disease (AD), where it constitutes one principal criterion for diagnosis. These memory deficits generally follow the course of damage to the hippocampus and interconnected medial-temporal lobe (MTL) structures in AD (Braak and Braak, 1991; van Hoesen et al., 1991), consistent with the suggested role of the MTL in forming spatiotemporal associations that support episodic memory (Burgess et al., 2002, Cohen and Eichenbaum, 1993). Some of the most commonly used clinical measures of cognitive decline in AD, such as the Alzheimer's Disease Assessment Scale – Cognitive test (ADAS-cog) and the Mini Mental State Examination (MMSE), emphasize an array of cognitive functions and use verbal report, constraining their specificity to memory for spatiotemporal associations that may index MTL degeneration with AD. In addition, the sensitivity and specificity of these tests have been questioned due to practice effects and variation in experimenter scoring (Galasko et al., 1993; De Jager et al, 2003). (Elfgrenetal.,1994; Chenetal., 2000; Simons et al., 2002; Thompson et al., 2005; Tierney et al., 2005).

In response to this, several memory tests have been conducted in AD patients that require or correlate with some type of hippocampal or MTL activity, including sequential spatial route-taking from virtual navigation (Belassen et al., 2012), and recognition memory that requires pattern separation among similar objects (Stark et al., 2013). Whereas these tasks include spatial memory and pattern separation/recollection memory requirements, respectively, both thought to depend on hippocampal function, a

distinction has been made between these abilities and those necessary for episodic memory (Aggleton et al., 1999; Burgess et al., 2002; Moscovitch et al., 2005), thus there may be grounds for tests of complementary, episodic-like, MTL-dependent processes. In addition, measures that are diagnostic at one stage of disease progression may not be optimal at other stages, necessitating different tasks or different task versions. Finally, for treatments that target specific neural structures, the tasks best-suited to determine their efficacy are presumably those most sensitive and specific to the function of those structures.

Here, we present a scene search task that measures hippocampal-dependent single-trial memory for objects-in-context, while avoiding confounds with language or visuomotor skills which have been shown to be impaired in the AD population (Tippett & Sergio, 2006; Tippett et al., 2007). In this task, rapid detection of the embedded object on repeated trials indicates explicit memory for the object in that scene (Chau et al., 2013). We hypothesized that memory would decline with age and additionally with AD status, measured as an increase in repeated-trial search times. Furthermore, memory for scenes alters the pupillary response (Naber et al., 2013), which is regulated by the autonomic system (Bitsios et al., 1998; Samuels & Szabadi, 2008; Gilzenrat et al., 2010). Because the autonomic system is known to be dysregulated in MCI and early AD (Collins et al., 2012; Femminella et al., 2014), we also measured pupillary responses to these scenes to determine if memory-related autonomic responses are diminished in the AD population.

MATERIALS AND METHODS

Participants. Seventeen university students (5 males, ages 19 – 32 years, mean(SD) age 22.8(3.1) years), 21 older adults (5 males, mean(SD) age 67.3(8.5) years) and 9 older adults diagnosed with probable early AD (5 males, mean(SD) age 69.1(7.8) years), participated in the study. Older adults completed the Montreal Cognitive Assessment (MoCA) a brief neuropsychological test shown to be sensitive to mild cognitive impairment (MCI; Nasreddine et al, 2005; Damian et al., 2011; Marwick et al., 2012) to conversion from MCI to AD (Julayanont et al., 2014) and to individuals at risk for developing MCI (Newsome et al., 2013; Nasreddine et al., 2005). Ten older adults scored a 26 or higher (range: 26 – 31) on the MoCA and therefore were categorized as healthy older adults; seven scored 24 or lower (range: 21-24) and were categorized as at-risk for developing MCI (Damian et al., 2011). Three individuals scored a 25 and could not be placed in either category, thus they were excluded from further analysis. One older adult participant was excluded due to difficulty obtaining consistent eye tracker signal. The probable early AD designation was given according to the National Institute of Aging Alzheimer's Association criteria (Jack Jr. et al., 2011). These participants were recruited as part of a clinical trial involving deep brain stimulation (DBS) at Toronto Western Hospital. To enroll in the clinical trial, patients must have scored between 12-24 on the Alzheimer's Disease Assessment Scale –cognitive test 11 (ADAS-cog11; Cano et al. 2010), and either 0.5 or 1 on the Clinical Dementia Rating (Morris, 1997). The main experiment described here took place after device

implantation but before initiation of DBS treatment (or placebo); pre-operative results from a subset of participants are included, for comparison.

All participants had normal or corrected- to-normal vision. Participants were informed about the purpose of the experiment and its risks, and written informed consent was obtained. Younger adult and older AD participants volunteered without monetary compensation; older adults received $10/hr in accordance with our ethical guidelines. Experimental procedures for all participants were approved by the York Human Participants Review Subcommittee; older adults recruited from Rotman Research Institute database (N=7) additionally followed the guidelines approved at the Rotman Research Institute; early AD participants were selected for and participated in the clinical trial in accordance with the ethical guidelines set by the research ethics board (REB) of the University Health Network and the Center for Addiction and Mental Health.

Stimuli. We selected a range of natural scenes, including wildlife, city, rural, and indoor scenes, that could be displayed at 1280 x 1024 pixel resolution (full screen), as described previously (Chau et al., 2011; Hoffman et al., 2013). One object per scene was modified (color change or disappearance, Figure 1A) using Adobe Photoshop (San Jose, CA). To discourage bias in search strategies, sets of images were balanced for target location (quadrant on screen) and category (animate/inanimate).

Experimental apparatus and session design. Participants used a chin rest to minimize head movements throughout the study. A 38.0 x 30.5cm monitor displaying the task stimuli was placed 51cm away from young participants, and 61-63cm away from all other participants. Eye gaze and pupil diameter were tracked using the iView X infrared eye tracking system at 60 Hz sampling rate (SensoMotoric Instruments, SMI, Berlin, Germany), following 13-point calibration and validation. Stimulus presentation software (Presentation, Neurobehavioral Systems, CA, USA), received online gaze position information from iView enabling gaze-contingent experimental control and sent event codes to the iView data acquisition stream for alignment of eye position data to trial events. Image selection, presentation timing, and response buttons were also controlled in Presentation. After calibration, three example trials were given to ensure that participants understood the task. Participants then began with Experiment 1: flicker change detection followed by Experiment 2: target detection.

Experiment 1: Flicker change detection memory task (Figure 1B). Each trial consisted of the 500ms presentation of an original image (Image A) which alternated with the 500ms presentation of a modified version of that same image lacking the target object (Image A'). Critical to the appearance of a 'flicker', a gray screen lasting 50ms was inserted between each image alternation (as shown in Figure 1B). This visual interruption makes the changed object difficult to detect; however, once detected, the change is difficult to ignore (Rensink et al., 1997; Simons & Levin, 1997). Trials ended when the target object was detected, i.e. fixated for 1 second, or after 45 seconds of cumulative on-screen viewing time, whichever came first. At the end of each trial,

whether the target object was detected or not, the gray screen between scenes was removed, revealing the target object to the participant as a single blinking object on an apparently static background. Immediately following the reveal of each trial, a verbal report screen would appear asking the participant, "Was this the first time you saw this picture?" Participants gave yes/no answers that were logged by button press for later analysis of scene familiarity. The flicker task consisted of 2 sets of 20 trials. Each set contained 10 novel images, and those same 10 images repeated after 2-4 intervening trials, therefore each participant could have remembered a maximum of 20 targets in the flicker task.

Experiment 2: Uncued target detection memory task (Figure 1c). This task is described in Hoffman et al., (2013). Briefly, in each target detection trial the original image (including the target object) was presented for five seconds, or until the participant fixated on the target object for one second, whichever came first. A reveal followed each trial end, whereby the target object appeared to blink, exactly like the reveal in the flicker task. Because the target object was uncued during the trial, participants would simply scan novel images for the maximum trial length until the reveal instructed them on which object was that scene's 'target', which participants were asked to remember. Each participant saw a series of 10 images, and this series was repeated 4 times, allowing 3 chances to demonstrate memory for a given image. Only a subset of the AD group (N=3) participated in Experiment 2, and some of these participants had been exposed to the images approximately two weeks prior to the testing date of this study; however, their search times on the first image presentations in this session were

comparable to the search time performances of individuals who were never before exposed to the images.

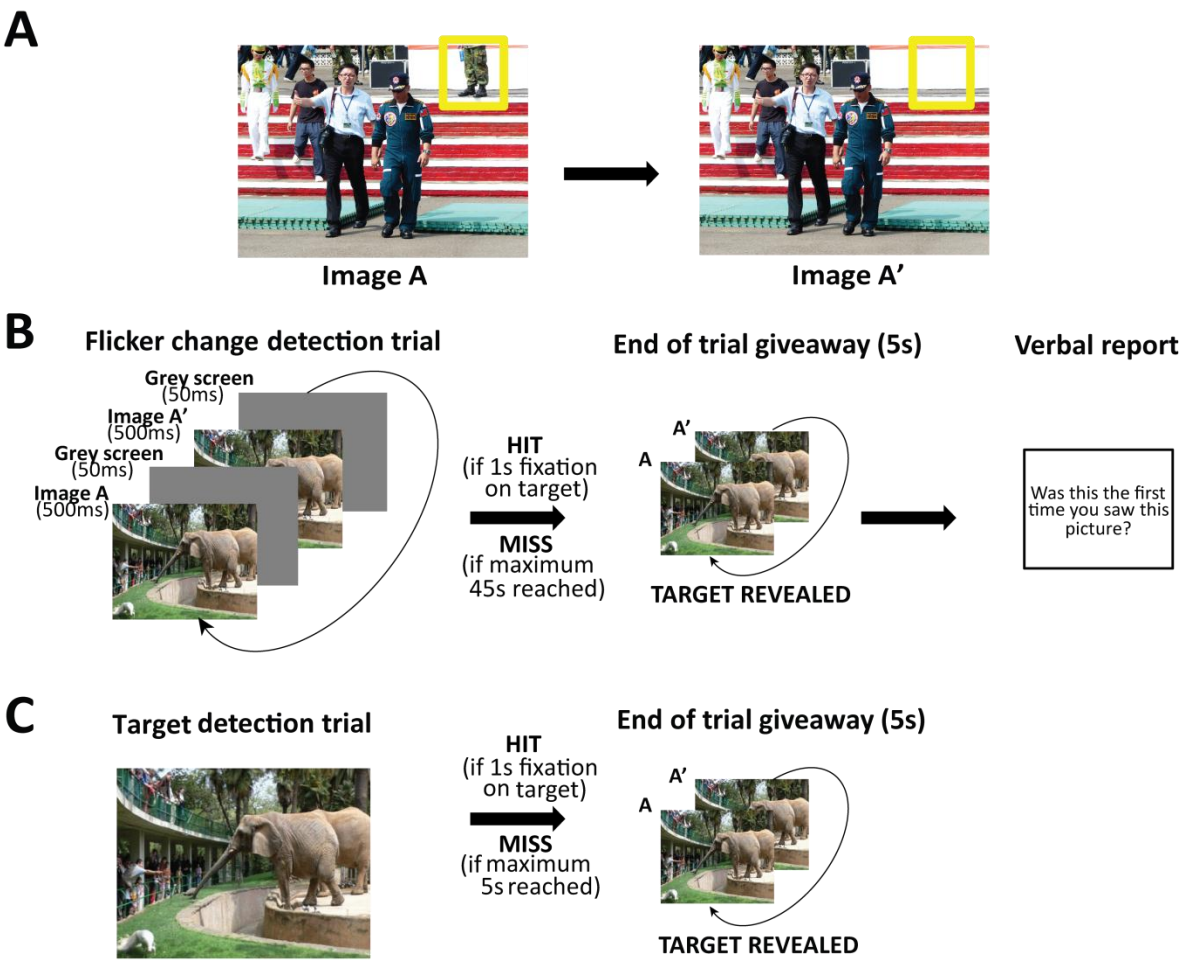

Figure 1. **Experimental design.** A) Example stimulus. Image A is the original image containing the target object outlined in yellow, where Image A' is a modified version of Image A with the target object absent. The yellow outline is for illustrative purposes only and was absent during the task. B) Flicker change detection trial sequence on testing sessions. At the end of each trial, a verbal report screen was shown prompting

the participant for their recognition of the scene. C) Target detection trial sequence on testing sessions.

Data analysis.

Fixation times and locations were calculated with iView X iTools IDF Event Detector, using a dispersion based algorithm with a minimum fixation duration of 80ms and maximum dispersion of 100 pixels (Salvucci & Goldberg, 2000). Fixation times and locations, and pupil diameter was then analyzed using MATLAB (Natick, MA). Search measures were calculated from trial onset to the time of target detection. The algorithm for detection selected the first fixation in a given trial's 'target' area of interest (AOI) that led to one second of fixating in the AOI with no more than one fixation outside of the AOI. Drift correction was applied between trials, to ensure accuracy of the AOI. Search times for each trial excluded the times that participants spent looking outside of the screen dimensions.

To quantify the spatial dispersion of fixations, we calculated entropy from the center of the scan path: $H=\ln(\sigma_1 \sigma_2)$, where σ_1 and σ_2 represent orthogonal directions of maximal search variance around the search centroid ('H_{path}' in Maei et al., 2009). This measure was shown to be a sensitive measure of memory-guided "search", expressed as changes in the swim path of mice with hippocampal damage and APP-mutant mice modeling Alzheimer's pathology. For each participant we calculated the average repeated-trial

entropy, search time (time from onset to detection) number of fixations per trial, and scene familiarity (correctly remembered rate – falsely remembered rate) then group differences were statistically evaluated. We compared group responses with the Kruskal-Wallis test (non-parametric ANOVA) and, if significant at a two-tailed alpha level of 0.01, pairwise post-hoc tests (Wilcoxan rank sum) were run to test for 1) Alzheimer's-related performance differences, (comparing AD patients to healthy and at-risk age-matched older adults) and 2) aging effects (comparing young adults to healthy and at-risk older adults).

Horizontal pupil diameter was sampled from each trial at 60 Hz. For each trial, values were mean-subtracted from the baseline levels taken from a 500ms window leading up to trial onset. Pupil response velocity (first derivative of diameter samples) was calculated around the time of trial onset and averaged for a given trial type (novel/repeated) for each participant. The time of maximal velocity of repeated trials across all participants was used as the reference time point to compare peak velocities across groups using an ANOVA, followed by post-hoc paired tests to determine differences between groups. The repeated-trial effect was measured as the difference in velocity (repeated – novel trials) for each participant as a function of time from trial onset. Group averages were calculated and for each group, differences from the null hypothesis (no difference between novel and repeat velocities) was tested and Benjamini-Hochberg FDR corrected to account for multiple comparisons (Benjamini & Hochberg, 1995).

RESULTS

Experiment 1: Flicker Change Detection Memory Task

Visual scan paths.

The dispersion of repeated-trial scan paths (Figure 2A, light blue lines) differed across groups ($H_{(3,\ 44)}$=29.25, p=1.9 x 10^{-6}). They were more focused (e.g. less diffuse) in healthy younger adults than in healthy or at-risk older adults (younger – healthy older: $W_{(n1=17,n2=10)}$=190, p=0.013; younger – at-risk: $W_{(n1=17,\ n2=8)}$=163, p=6.5 x 10^{-4}). Within the aging populations, AD patients displayed the most diffuse repeated-trial search, compared to healthy ($W_{(n1=9,\ n2=10)}$=133, p=8.7 x 10^{-5}) and at-risk ($W_{(n1=9,\ n2=8)}$=40, p=9.8 x 10^{-4}) older adults, and there was a trend for at-risk adults to also show more diffuse search compared to healthy older adults ($W_{(n1=10,\ n2=8)}$=96, p=0.068). The degree of dispersion can be influenced by the duration of scan paths, e.g. when objects are remembered and therefore located quickly. To address this, we evaluated the number of fixations and the search times on repeated trials.

The average number of fixations on repeated trials showed group effects similar to those seen with the entropy measure ($H_{(3,\ 44)}$=36.27, p= 6.5 x 10^{-8}), including an age-related increase in average number of fixations before target detection (younger adults v. healthy older adults: $W_{(n1=17,\ n2=10)}$=222, p= 4.2 x 10^{-5}; younger adults v. at-risk older adults: $W_{(n1=17,\ n2=8)}$=169, p=1.7 x 10^{-4}). AD patients showed a clear impairment, with

more fixations than healthy adults ($W_{(n1=9,\ n2=10)}$=135, p=2.1 x 10^{-5}) and at-risk older adults ($W_{(n1=9,\ n2=8)}$=36, p=8.2 x 10^{-5}).

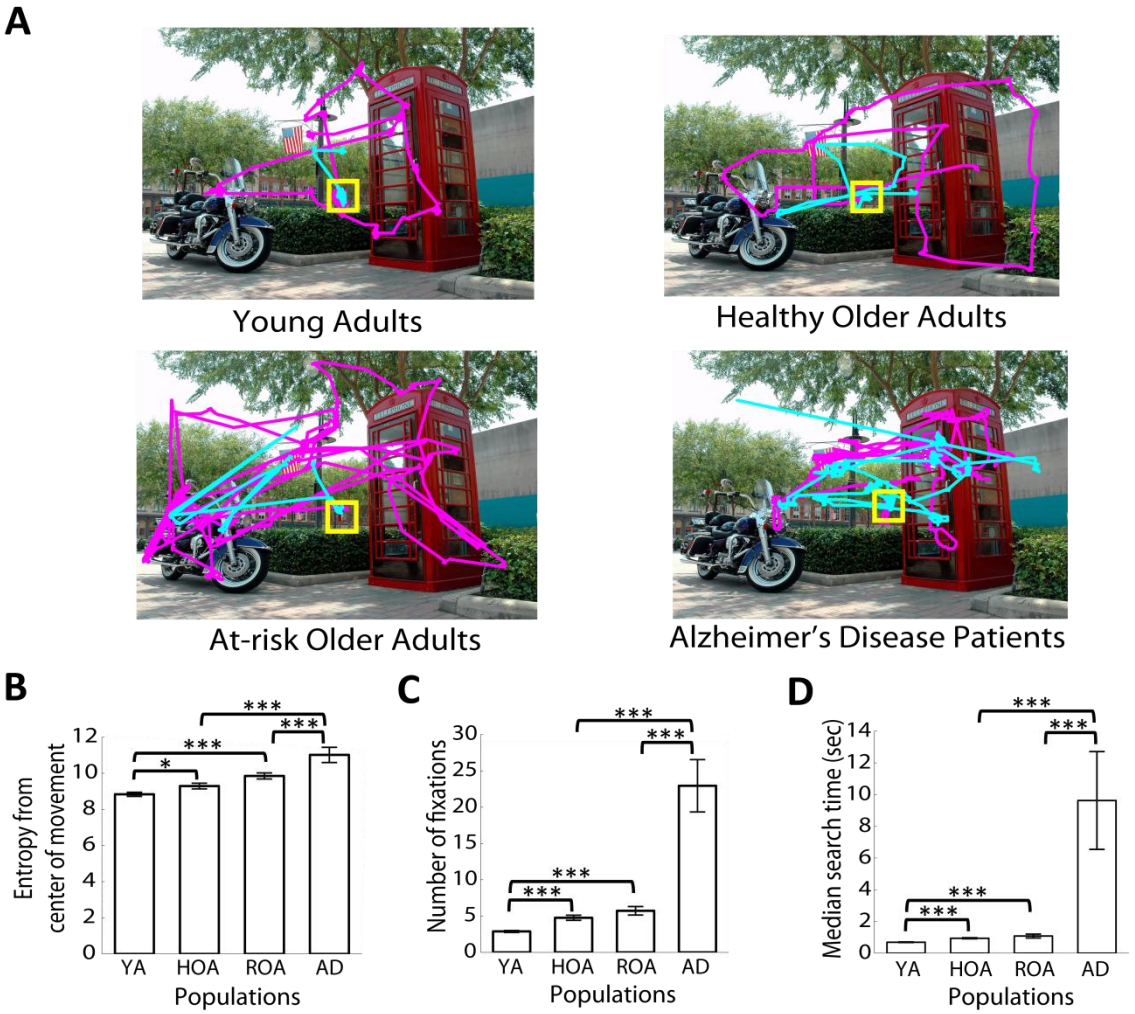

Figure 2. **Memory-guided visual search during flicker change detection.** *A) Superimposed scan paths during novel and repeated flicker trials.* Scan paths for an example scene were taken from a single participant from each group. Scan paths for novel trials are shown in purple, while scan paths for repeated trials are shown in light blue. *B) Entropy from center of movement in repeated trials across populations.* (YA = Young Adults (N=17), HOA = Healthy Older Adults (N=10), ROA = at-Risk Older

Adults (N=8), AD = Alzheimer's Disease patients (N=9)). The mean entropy calculated relative to the center of the eye scan path measured for each group, where higher values indicate greater movement disarray. Error bars represent the standard error of the mean. *C) Number of fixations in repeated trials across populations.* The mean number of fixations displayed as bars for repeated trials for each group. *D) Median search time for target detection in repeated trials across populations.* The averaged median search time in seconds for detecting target objects in repeated trials for each group. Error bars represent the standard error of the mean.

As expected, more fixations in aged and particularly AD- groups corresponded to longer search times for target detection in repeated trials ($H_{(3, 44)}$=34.72, p= 1.4 x 10^{-7}). Younger adults found the repeated target faster than healthy ($W_{(n1=17, n2=10)}$=219, p=7.9 x 10^{-5}) and at-risk ($W_{(n1=17, n2=8)}$=165.5, p=7.3 x 10^{-4}) older adults, and AD patients took significantly longer to find repeated target objects than their healthy ($W_{(n1=9, n2=10)}$=135, p=2.2 x 10^{-5}) and at-risk ($W_{(n1=9, n2=8)}$=36, p=8.2 x 10^{-5}) age-matched counterparts, demonstrating both age and AD-related memory impairments for the objects in the scenes.

Familiarity (old/new) judgments for the scenes were worse for AD patients than all other groups (younger adults: $W_{(n1=9, n2=17)}$=60.5, p=4.8 x 10^{-4}, healthy older adults: $W_{(n1=9, n2=10)}$=50, p=2.4 x 10^{-4}, at-risk older adults: $W_{(n1=9, n2=8)}$, p=7.4 x 10^{-4}) indicating that the AD patients were impaired on this measure; however, no differences in scene

familiarity among the young adults and other older adults were detected, and two of the AD patients were at ceiling .

AD group controls.

The memory performance of the AD patients was measured before any clinical treatment was applied, but after a surgical procedure that took place approximately two weeks prior to their participation in this study. To rule out the impact of the procedure on their performance, we analyzed the performance of eight of the AD patients on the same experimental design but with a unique set of scenes, prior to any surgical procedure. Their performance, before and after surgery, did not differ for any of the measures (entropy from center of movement, the number of fixations in repeated trials, and median search time for repeated targets).

Pupillary responses.

Based on previous literature showing memory-based changes in pupillary responses in young adults, we analyzed pupillary responses to novel and repeated images for all groups. Both novel and repeated images elicited rapid constriction of the pupil, though the response velocity differed across groups. Younger adults had higher peak velocity than any older group (v. HOA $t(25) = 3.69$, $p = 0.001$; v. ROA $t(22) = 3.76$, $p = 0.011$; v. AD $t(24) = 7.07$, $p = 2.61 \times 10^{-7}$), and healthy older adults had higher peak velocity than did the AD group ($t(15) = 3.03$, $p = 0.008$). Furthermore, repeated trials evoked faster velocities than novel trials, but only in healthy populations, i.e. in younger ($t_{(16)} =$

4.71, p = 2.4 x 10^-4) and older adults ($t_{(9)}$ = -3.26, p = 0.01), but not in the at-risk or AD groups (Figure 3b). Our results indicate that image onset evokes a general pupillary response that weakens with age and dementia, and that memory-dependent differences in pupillary responses could segregate healthy from at-risk and already-impacted populations.

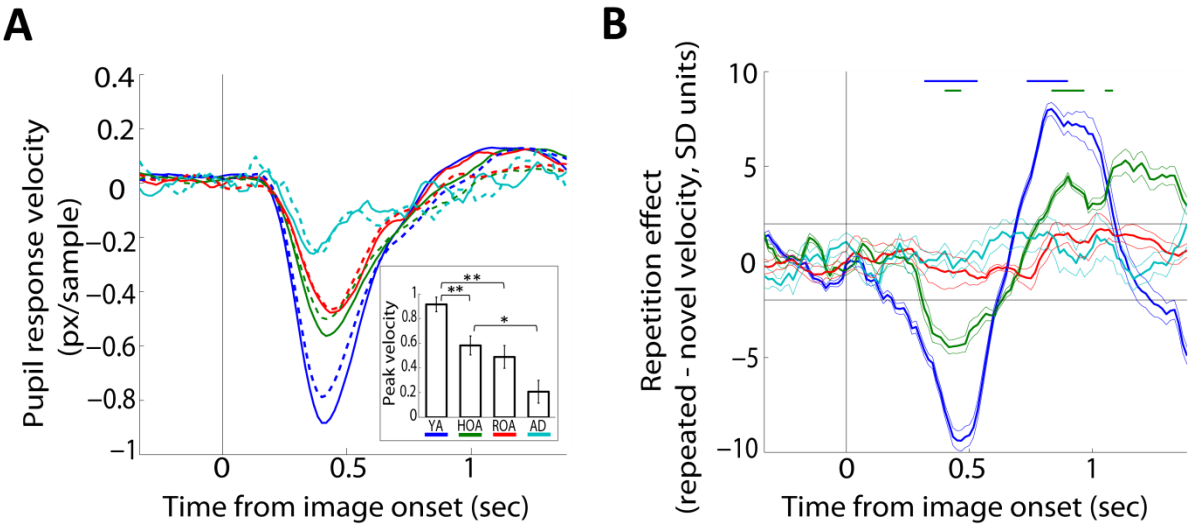

Figure 3. Pupillary responses following image onset. A) *Average pupil velocity following image onset in novel and repeated trials across groups.* Novel-trial velocity indicated with dotted lines; repeated-trial velocity with solid lines. Color conventions describe in inset. *Inset.* Average repeated-trial peak velocity for each group. Error bars = SEM. YA = Younger Adults (N=17), HOA = Healthy Older Adults (N=10), ROA = at-Risk Older Adults (N=8), AD = Alzheimer's Disease patients (N=9). **B)** *Memory-related pupillary response following image onset.* Shown are the average within-subject

differences between novel- and repeated-trial pupil velocity, for each group. Color conventions are the same groups as in Figure 3A. The times of significant differences between novel and repeated velocities are plotted at the top of the plot, in colors corresponding to the groups: younger adults in blue ($p<0.001$) and healthy older adults in green ($p<0.01$), both FDR corrected for multiple comparisons.

Experiment 2: Target Detection Task

Target detection search times.

In the target detection task, the image remains on and only after 5 seconds is the target object 'revealed'. Whereas all groups detected the targets after several repetitions, there were group differences in the rate of learning, as measured by the search times on the first repetition of each image ($H=22.68$ (2, $N=38$), $p= 4.7 \times 10^{-5}$). AD patients were impaired in their detection of target objects compared to their healthy ($W(n1=4, n2=10)=46$, $p= 0.0028$) and at-risk ($W(n1=4, n2=8)=42$, $p= 0.004$) age-matched counterparts. We also found age-related differences between younger adults and healthy older adults ($W(n1=17, n2=10)=183$, $p=0.001$) and with those in the at-risk group ($W(n1=17, n2=8)=155.5$, $p=0.003$).

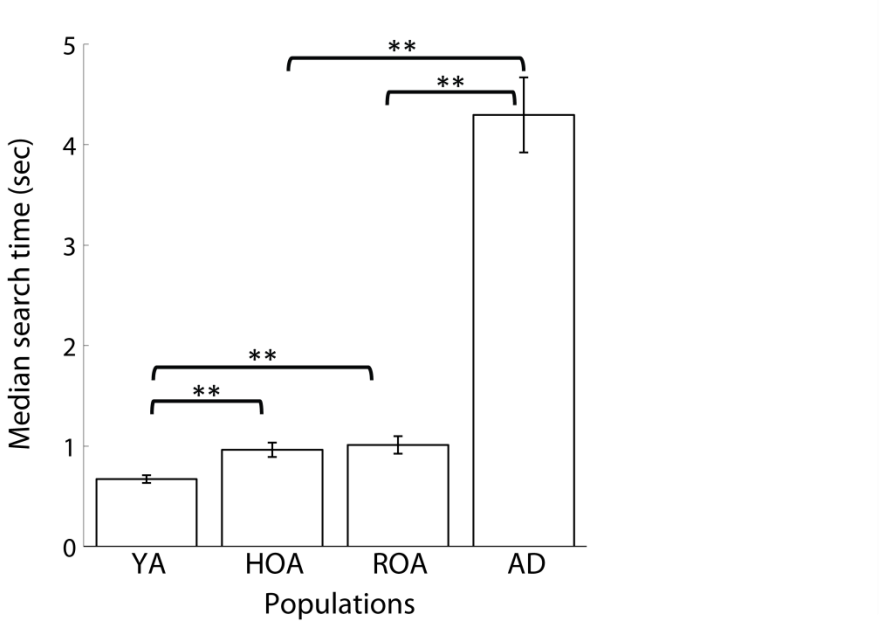

Figure 4. Search times to detect target on the first repetition. The average median search times for detecting target objects per group (YA = Young Adults (N=17), HOA = Healthy Older Adults (N=10), ROA = at-Risk Older Adults (N=8), AD = Alzheimer's Disease patients (N=4)) on the first repetition of the non-cued images. Errors bars represent the standard error of the mean. ****** = $p<0.005$**.**

DISCUSSION

Changed objects in natural scenes are detected using contextual cues (Brockmole et al., 2006; Hollingworth, 2006; Torralba et al., 2006; Becker and Rasmussen, 2008) and this process is thought to require MTL function (Chau et al., 2013; Smith and Squire 2008;

Ryan and Cohen, 2004). Here, we use two variations of search-related target detection to reveal performance differences in

It has been widely documented that in the earliest stages of Alzheimer's disease, the hippocampus is one of the primary areas affected resulting in memory deficits. Earlier treatment administration has been shown to produce better treatment outcomes, emphasizing the importance of early diagnosis. In line with this view, it is crucial to have the most sensitive instruments for detecting performance changes resulting from damage to those primarily affected brain regions. Traditional cognitive batteries used with the Alzheimer's population include the ADAS-cog and the MMSE. These tests are comprised of multiple subcomponents that test several cognitive domains including memory, as measured by word recall. Performance on these tests may be confounded by language ability, rendering an individual with intact memory, but impaired language skills, to be incorrectly labelled as memory impaired. These possible confounds in language ability have made non-verbal measures of memory increasingly appealing.

Few studies have implemented the use of non-verbal virtual environments in detecting dementia, however, many of the actual memory test measures used in these studies require verbal responses (deIpolyi et al., 2007; Cushman et al.,2008; Zakzanis et al., 2009). Other studies that manage to employ non-verbal virtual environments for detecting dementia, require the use of joysticks or mice for navigation (Bellassen et al., 2012). Yet, the implementation of joystick usage to navigate about the environment may pose a problem with various cognitively impaired populations. The ability to

integrate visual and motor information appears to be compromised in individuals with MCI and AD (Tippett & Sergio, Tippett et al., 2007; 2006; Salek et al., 2011). Thus, tests involving the use of joysticks or mice that aim to measure cognition may be confounded by deficits in visual-motor integration.

A human analogue of the spatial-temporal Morris Water Maze test is an example of another nonverbal memory test that has been used with AD patients (Laczo et al., 2010). However, older individuals may find this test to be lengthy and tiresome. As well, safety of such a test may be put to question as issues regarding motor abilities and balance increase with aging populations.

The use of computerized cognitive batteries, such as the CANTAB, is becoming a preferred method of cognitive testing with researchers and clinicians. Computerized versions of cognitive tests minimize scoring errors, variations in administration, and can reduce practice effects observed with the pencil and paper versions of these tests (Zygouris & Tsolaki, 2014). In particular, paired associates learning (PAL) – a test that measures the ability to remember associations among elements of an experience – has been argued to be an ideal paradigm for detecting AD impairments (Lowndes & Savage, 2007). The PAL of the CANTAB requires individuals to form object-location associations with different patterns inside boxes appearing in different locations on the screen. Although this version of PAL has been shown to distinguish between groups of older adults from those with MCI and AD (Lee et al., 2003; Swainson et al., 2001; Fowler et al., 2007), it does not deliver a naturalistic experience of everyday life.

Scenes from our day-to-day life exhibit ‘crowding’, that is, the many complex features from various objects are combined to create our perception of the multifaceted environment we perceive as a whole. However, the unique characteristics of objects that help us to identify them seem to be lost with the characteristics of other objects immediately surrounding them, making object recognition in this ‘crowded’ view more effortful (Pelli & Tillman, 2008). Unlike the PAL of the CANTAB, where crowding is virtually diminished, the natural scenes in the change detection task provide a similar intensity of ‘crowding’ as expected in real-life environments. Similarly, an additional benefit of the change detection task is that it provides for a more realistic simulation of daily life events in which we depend on our memory, such as having to remember where in the house we last had our keys. Our task also provides a natural method of eye tracking, an activity we continuously take part in, as compared to having to tap parts of a screen.

Eye tracking provides a wealth of information about several different eye measures, reflecting even our subconscious efforts in cognitive tasks. Our paradigm revealed that AD patients fixate much more in repeated trials than their age-matched counterparts, lending support to previously reported data in line with these results. Similarly, researchers have postulated that pupillary responses may be a window into the neurophysiological processes that underlie cognitive functions (Laeung et al. 2012). It is well known that pupil dilation directly reflects norepinephrine (NE) release from the locus coeruleus (LC), a brain structure that has also been implicated in memory consolidation and other cognitive processes (Beatty & Kahneman, 1966; Laeng et al.

2012; Verney et al., 2004; Privitera et al., 2010). The simultaneous activation of the LC-NE system and the presence of the pupillary response led to the belief that pupillary dilations may be a reflection of LC-NE engagemement (Koss, 1986). Thus, researchers had discovered a potential noninvasive, continuous physiological measure of LC activity. In fact, pupillary dilations have been observed in novelty-recognition tasks, as well as tasks measuring memory load with varying lengths of numbers (Verney et al. 2004; Beatty & Kahneman, 1966). Moreover, pupillary dilations have been positively correlated with target detection in complex natural scenes, even when participants did not report awareness of the target presence (Privitera et al. 2010). Our results showed that AD patients exhibited a decreased pupillary response to target detection in repeated trials compared to all other groups. Strikingly, this measure showed significant differences in pupillary response between healthy older adults and those in the at-risk group prior to target detection, with the at-risk group responding similarly as the AD patients.

In our second experiment, we tested the use of a non-flicker version of the task with the added benefit of rapid employment to assess its sensitivity in detecting mild AD. A substantial burden of cognitive testing for clinicians and researchers comes from the time needed to administer such tests. Many of the cognitive instruments that rely on nonverbal measures of memory are time consuming, increasing the likelihood that participants will quit or become fatigued, rendering results questionable in terms of accuracy. The AD population continued to exhibit impairments on the shorter memory assay, which also lacked the flicker as a potential perceptive compound. Importantly,

this version of the task may be rapidly administered with a maximum six minute time period, and continues to be sensitive in detecting impairments from mild AD.

One future direction to supplement the findings of this paper could include testing individuals with MCI. This unique non-verbal hippocampal-dependent naturalistic memory test is capable of detecting memory impairments in those at-risk for MCI and individuals with mild AD. Thus, this test can be expected to be able to detect intermediate level memory impairments of those with diagnosable MCI. In addition, comparing measures of this test to hippocampal volumetrics would lend support to the task's ability of detecting behavioral changes as a consequence of altered hippocampal volume.

Chapter 3:

Assessing the effects of Deep Brain Stimulation to the fornix (DBS-f) in mild AD using the change detection task

INTRODUCTION

The brain is unquestionably the most complex organ in the human body. For years researchers have dedicated themselves to try to understand the ways in which its structure and function collectively go on to produce our experience of reality. In the early 1900's, Dr. Wilder Penfield probed parts of the cerebral cortex in an attempt to find the seizure-causing brain tissue in a patient with untreatable epilepsy. This experimental stimulation of the cortex led to the first understanding of cortical function in motor and sensory processes, and granted stimulation value in studying the brain function (Penfield & Boldrey, 1937). It was not until the introduction of stereotactic surgeries, which introduced an effective head restraining method allowing controlled and accurate electrode placement, that researchers could begin to elucidate the functional role of deeper brain structures, such as the hippocampal formation (Elias, & Lozano, 2010). The hippocampal formation is essential for memory, including input from the entorhinal cortex (EC), and outputting information through the subiculum (Amaral & Witter, 1989). This stimulation procedure, referred to as Deep Brain Stimulation (DBS), acts like a 'pacemaker' for the brain. Through the production of electrical pulses that target specific brain areas, DBS ultimately aids in altering irregular brain activity (Elias, & Lozano, 2010).

Over the last few decades, DBS has grown in popularity as a surgical treatment for several neurological conditions. In addition to its ability to manage the symptoms for a variety of movement disorders, such as Parkinson's disease and dystonia, DBS has been shown to have surprisingly therapeutic benefits for various mood disorders

including treatment-resistant depression (Elias, & Lozano, 2010). It became clear to researchers that by altering the structures and circuits targeted, DBS demonstrated the potential to aid in the management of a number of neurologically-derived conditions that had previously proven difficult to treat using existing interventions. An important association would be uncovered linking the potential benefits of DBS to Alzheimer's Disease (AD) when one such study was conducted by Lozano and colleagues (Hamani et al., 2008). In their initial experiment, they hypothesized that DBS may help manage obesity via stimulation of specific hypothalamic sub-regions known to be core structures responsible for appetite and satiety. However, when they performed electrode stimulation on a morbidly obese patient, they unexpectedly observed that the patient was suddenly able to recall very vividly an event that occurred several decades prior. Lozano and colleagues proceeded to test the patient on numerous recall and recognition tasks while alternating the stimulation from on to off without the patient's awareness. They observed that the patient had memory enhancements that accompanied the stimulation, leading Lozano and colleagues to speculate that these changes may have resulted from accidental excitation of the fornix, a structure located slightly anterior to the hypothalamus. Furthermore, studies have shown that lesioning the fornix, which acts as one of the main input/output structures of the hippocampus, creates similar impairments as those attributed to hippocampal damage (Cassel et al., 1998; Fletcher et al., 2006).

There have been a few studies that have reported memory enhancements from stimulating hippocampal inputs in both animals and humans. Previous literature has shown that DBS to the Entorhinal Cortex (EC) - another input structure to the

hippocampus - in mice, leads to increased neurogenesis in the hippocampus (Stone et al., 2011). Consequently, those newly created neurons grow and take on a similar morphology as mature neurons in the hippocampal network, later interconnecting with the surrounding circuitry. The addition of these neurons to the memory circuitry is thought to underlie the resulting improvements observed in the Morris water maze spatial memory task. Moreover, stimulating the EC in epilepsy patients leads to improved memory for navigating in a virtual environment (Suthana et al., 2012). Both of these studies suggest that DBS targeting hippocampal function may benefit memory, which may prove to be especially useful for individuals with impaired memory (Hamani et al, 2008). In fact, rodent studies have shown that fornix stimulation increases co-modulatory activity in the hippocampus that predicts successful memory retrieval, and in this way can help to improve spatial memory performance in otherwise amnestic animals (Shirivalkar et al., 2010).

Persons with AD, who experience difficulties with memory as a result of damage to the hippocampal formation, are an example of one such group that may benefit from this type of treatment. After the previous experiment revealed the potential benefit of DBS on memory, Lozano and colleagues investigated the effects of DBS on AD. They hypothesized DBS may lessen or even possibly reverse the memory decline presented in AD. They ran a Phase I safety clinical trial delivering stimulation to the fornix in 6 mild AD patients and assessed memory performance using the mini mental state examination (MMSE) (Laxton et al., 2010). The MMSE is a brief screening questionnaire that measures cognitive impairment (Tombaugh & McIntyre, 1992). They compared the rate of change in performance in an 11 month period before having

surgery, to the rate of change in performance in the 11 month period after having surgery, while receiving stimulation. Stimulation was able to lessen the decline in performance in majority of the patients, and even reversed performance impairments in 2 of the patients. The results of this study, which mainly assessed the safety of using DBS in the AD population, led to the ongoing phase II clinical treatment trial that focuses on assessing its effects with comparisons to a non-treatment group.

The Phase II longitudinal double-blind study (ADvance study) currently being conducted uses DBS-f in patients with mild AD. The hypothesis is that the stimulation treatment will alleviate memory deficits in the patients, as compared to those whose stimulation is turned off, throughout the one-year trial. The flicker change detection task that our lab previously developed (Chau et al, 2011) may prove to be a critical test of memory for this study.

Atrophy of brain regions occurs variably across patients and over time, making it difficult for some of the already existing diagnostic tests to accurately report changes in memory. In addition, tests currently used with this population require verbal responses which may be confounded by age- or disease-related language deficits. Our change detection task is unique in that it relies on the eyes to be able to tell us about memory. Patients are shown flickering images where targets appear/disappear in alternation with the flicker, and are later repeated to test for target memory. Previously, our lab has shown that explicit recall for target objects in repeated trials yielded in faster detection of remembered targets than for forgotten ones. This eliminates the need for any verbal report from the patient and provides us with a more objective memory measure. DBS-f is a novel method of therapy for AD that targets hippocampal function,

hence evaluating its efficacy may be done best through the use of specific and selective hippocampal tasks. Consequently, we hypothesized that our nonverbal hippocampal-dependent episodic target-in-scene detection task would perhaps prove to be more a sensitive assay in detecting memory changes over time in the ADvance study patients. To attempt to validate the use of this task with the AD population, we compared the measures of the task to some of the standardized tests currently being used in AD.

MATERIALS AND METHODS

Participants.

Older adults with mild Alzheimer's disease. A total of 12 subjects (7 males and 5 females; mean age) were selected from an already existing phase II clinical trial patient pool (the ADvance study). The ADvance study patients were selected based on the ADvance trial inclusion criteria as shown in **table 1**. The age range of patients were between 53-78 years, with an average age of 67.9 (SD=6.8). Written informed consent was obtained, and the study was conducted in accordance with protocols approved by University Health Network Research Ethics Board and the ethical guidelines set by the York Human Participants Review Subcommittee.

	INCLUSION CRITERIA
1.	Informed consent signed by the subject AND a reliable caregiver.
2.	55-80 years of age (inclusive)
3.	Probable Alzheimer's disease according to the National Institute of Aging Alzheimer's disease Association criteria.
4.	Clinical Dementia Rating (CDR) global rating of 0.5 or 1 at screen.
5.	ADAS-Cog-11 score of 12-24 inclusive at screening AND baseline (with minimum score ≥ 4 on item 1).
6.	If female, subjects need to be post-menopausal or surgically sterile or willing to use birth control methods for the duration of the study.
7.	The subject has an available caregiver or appropriate informant who can reliably report on daily activities and function.
8.	Subject is living at home and likely to remain at home for the study duration.
9.	General Medical Health Rating (GMHR) ≥ 3 (good or excellent general health).
10.	Subjects must be a good surgical candidate for placement of a deep brain stimulator as judged by the DBS surgical team.
11.	Fluency (oral and written) in the language in which the standardized tests will be administered.
12.	The subject is currently taking a stable dose of cholinesterase inhibitor (AChEI) medication (donepezil, galantamine, or rivastigmine) for at least 60 days prior to signing the informed consent form (NOTE: These medications may NOT be initiated, discontinued or modified after the study initiation for the length of study participation).

Table 1. Inclusion criteria for enrolling in the Advance study. Individuals chosen to participate in the Advance study must have met all 12 of the criteria listed in the table above.

Task Design.

Stimuli. The images used in this study were of natural scenes, selected and adjusted as described in **Chapter 2**. An example stimulus is presented in **figure 1**.

Behavioral tasks. The change detection and target detection trial paradigms used were the same as described in **Chapter 2**. The design of the paradigms is shown in **figure 1**.

Final change detection task. The final change detection task was comprised of 20 'remembered' trials taken from previous sets, ranging from the Pre-op baseline to those

shown at the 9M testing time point. Remembered trials were defined as the repeated trials in which the target objects were found under the 'remembered' search time threshold (described further in the **data analysis** section). Occasionally, there were patients who had less than 20 remembered trials from the beginning of the study, thus to complete the final sets we added trials with the next fastest search times from previous sessions until the sets were complete.

Behavioral testing procedure.

Behavioral testing. Patients were tested at various time points throughout the one year study (**figure 6**). Two baseline measurements were taken; one baseline was taken one day before surgery (Pre-Op baseline), while the second baseline measurement was taken two weeks after surgery (Post-Op baseline), immediately before the treatment condition was applied. Each time point, or session, contained both flicker change and non-flicker target detection test sets. Change detection sets encompassed two blocks of 20 images; within one block were 10 novel images, and then those same 10 images were repeated within the block in a non-specific order. Novel-repeat images were presented with lags of 1-6, meaning a novel image and its corresponding repeated image could be separated with 1-6 different images between them. A long-term target detection set was made up of the 10 images taken from the very first session (the Pre-Op baseline), which were repeated sequentially to total 20 trials. Target detection sets were shown twice allowing patients to see each image 4 different times. The target detection set was shown to patients twice at the end of each session, after the two

session-specific flicker change detection sets were shown. **Table 3** illustrates the data collected for each patient from the start of this study to the present day.

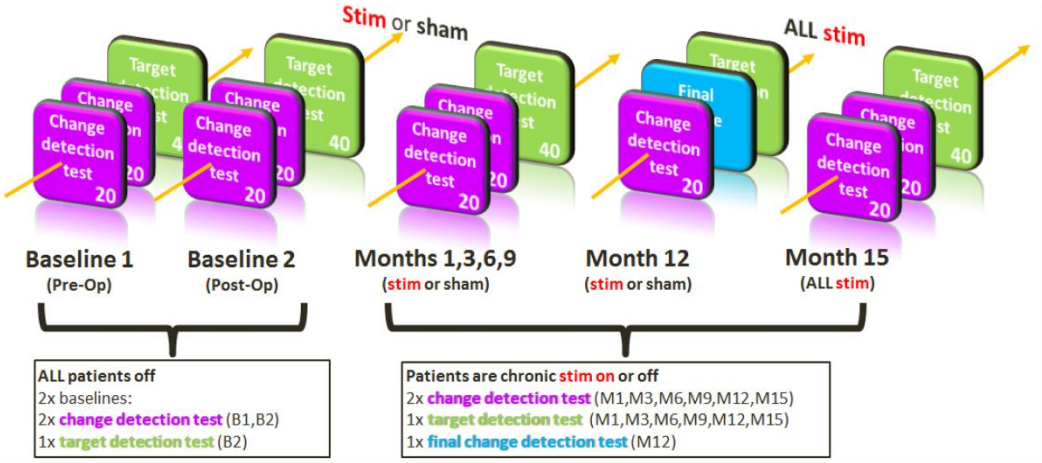

Figure 5. *Experimental testing paradigm.* Patients were tested one day before undergoing surgery for electrode implantation (Baseline 1, Pre-Op) and again two weeks later (Baseline 2, Post-Op) prior to treatment assignment either receiving stimulation (stim) or not (sham). After treatment assignment, patients were tested at time points counted as one, three, six, nine, twelve, and fifteen months after the initial surgery. At the end of the twelve month visit, all patients had their devices turned on to receive chronic stimulation, whose effects could be monitored at the fifteen month time point. All sessions, except for the twelve month time point, included testing on two separate sets of the change detection test (20 novel trials; 20 repeated trials), followed by testing on the target detection test (10 images, repeated 3 times each). The twelve month testing time point included testing on one change detection set, followed by a final change detection set comprised of the most memorable images from prior change detection sets as defined in the task description.

Patient	Pre-Op	Post-Op	1M	3M	6M	9M	12M	15M
1	X			X	X	X	X	
2	X	X	X	X	X	X	X	X
3				X	X	X	X	X
4				X	X	X	X	X
5	X	X	X	X	X	X	X	
6	X	X	X		X	X		
7	X	X	X	X				
8	X	X		X	X		X	
9	X	X	X	X				
10	X	X	X	X				
11	X	X	X					
12		X						

Table 2. Patient data collected across time. This table provides an overview of the data (N=56) that has been collected from the 12 patients over a 15 month time period, where each 'X' signifies the session in which data was obtained. Occasionally, patients were unavailable for testing (empty blocks) due to medical complications or personal hardships. Baseline data is missing for 2 individuals (patients 2 and 3) due the start of this collaboration, as those patients had already begun the treatment prior to when we joined.

ADvance study procedure. The ADvance study was designed to be a 12-month double-blind, randomized, controlled study to assess the efficacy of a deep brain stimulation treatment to the fornix (DBS-f) in mild Alzheimer's disease. After meeting inclusion criteria (**table 1**), patients underwent surgery to receive electrodes targeting the post-commissural fornix. The study was randomized for treatment condition among the patients; one half of the subjects had the stimulation 'turned on' and set between 1-10V

(the highest voltage that did not elicit autonomic related symptoms to a maximum upper limit of 10V, tested per individual) two weeks post-surgery, while the other half had the stimulation turned on but set to an amplitude of 0 V (this treatment condition will be referred to as the 'stimulation off' group). Stimulation was set to a frequency of 130Hz with a 90 microsecond pulse width. All study participants and experimenters of the study were blinded to the treatment assignment (stim or sham) throughout the entire study until after the last day of testing, which was done at the 12 month (12M) visit. After testing at the 12M visit, the devices from all the patients were turned on for chronic stimulation. My supervisor, Dr. Kari Hoffman, and I have, and will continue, to remain blind to all patient treatment conditions until all of the data analysis for the project is complete. A series of neuropsychological tests were planned for administration at various time points throughout the study, as outlined in **table 2**.

	Pre-Op	Post-Op	1M	3M	6M	9M	12M	15M
ADAS-cog	X		X	X	X	X	X	X
CVLT	X			X	X	X	X	X
MRI Scan	X	X					X	
PET Scan	X		X		X		X	

Table 3. Advance study data collection. The table provides an overview of the data planned to be collected at each time point ranging from the pre-op (one day before surgery) and post-op (two weeks after surgery before the treatment condition is applied) baselines, to the 15 month time point. ADAS-cog = Alzheimer's Disease Assessment

Scale – cognitive test; CVLT = California Verbal Learning Test; MRI = Magnetic Resonance Imaging; PET = Positron Emission Tomography.

Data analysis.

Data analysis. Search time and scene familiarity data analysis was done using MATLAB (version R2012a). A few trials displayed calibration inaccuracies, and were corrected in the same manner as described in **chapter 2**. Search times for each trial were corrected to exclude the time that participants spent looking outside of the screen dimensions. Trials were separated into novel and repeated categories, and the median search time for repeated trials was calculated for each session of each patient. 'Remembered' trials were determined by plotting a smoothed kernel density plot of repeated trial search times for all sessions per patient, and then taking the inflection point of the first curve from the repeated trials, as described in Chau et al. (2011). Scene familiarity was calculated by subtracting the False Positive Rate (FPR), defined as the novel trials reported as repeated, from the True Positive Rate (TPR), where participants correctly reported seeing a repeated image. Scene familiarity was given as an index, ranging from -1 to 1. The Pearson P correlation was calculated on the ADAS-cog-11 scores of all patients from all sessions, and their respective median repeated trial search times for those sessions, and was considered significant at an alpha of 0.05.

RESULTS

Behavioral results.

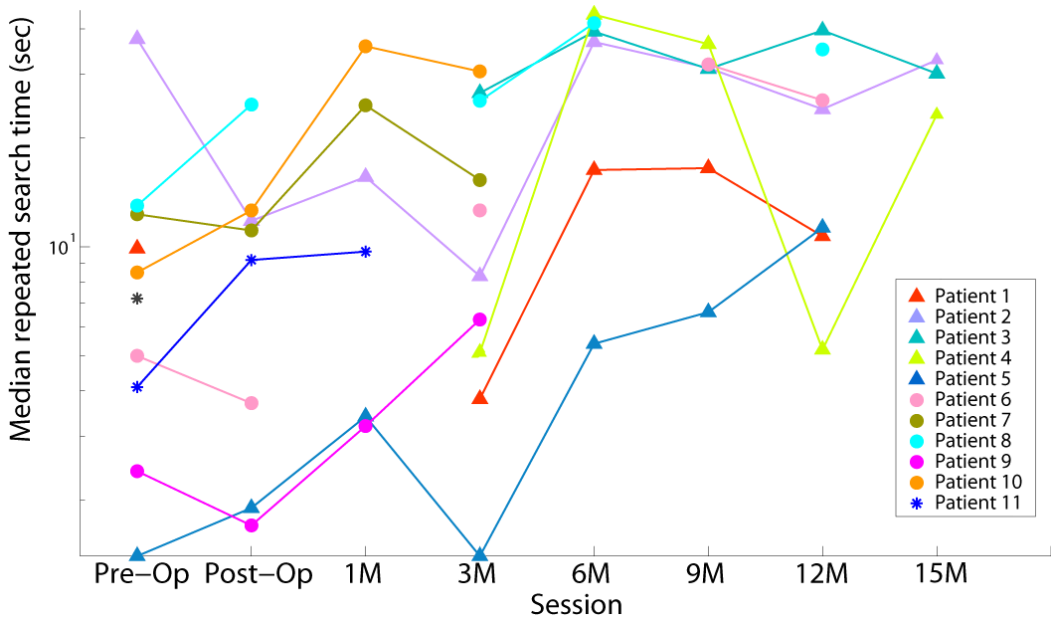

Figure 6. *Median repeated search time across sessions.* Median search time in seconds for target object detection in repeated trials plotted logarithmically for each patient over time. This transformation reveals the individual variability and progression over time, where shorter search times indicate better recall, and overall performance is seen to deteriorate across sessions.

Median repeated search time for targets over time. Although this was the first time this task was used with this clinical population, patients were able to do the task, and did not hit ceiling or floor with their median search time for targets in repeated trials. Though the data displays some degree of individual variability, the range for an

individual's performance does not appear to fluctuate much over time. That is, high performers on this task seem to remain high performers over time and vice versa, with the exception of a few patients.

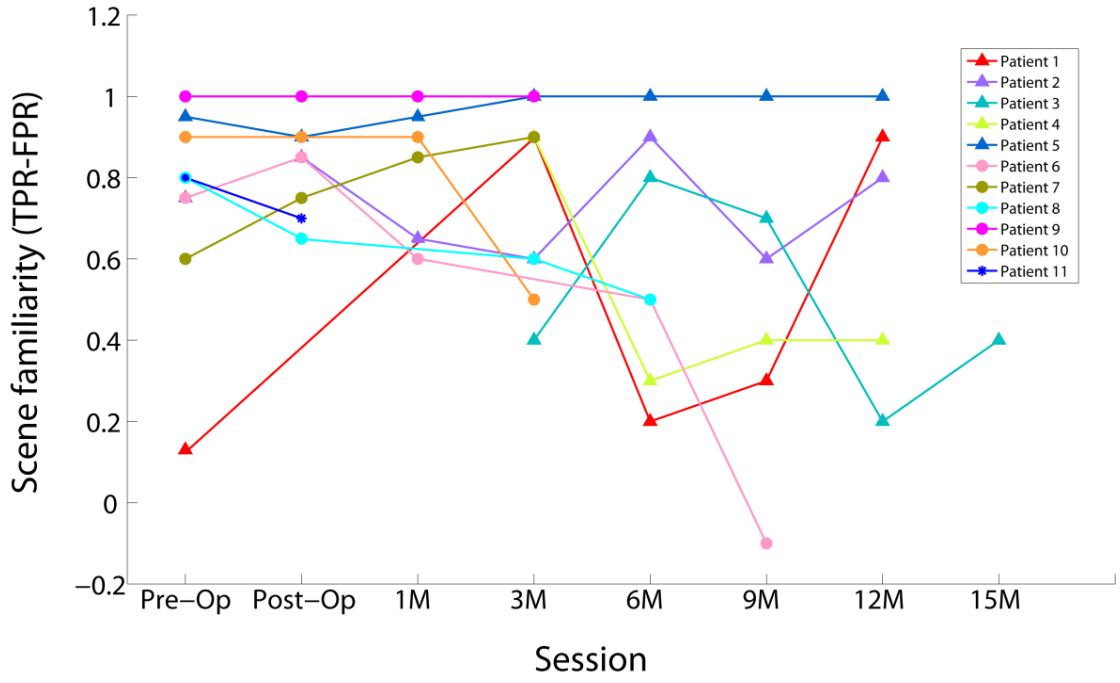

Figure 7. *Scene recognition from all patients across time.* Familiarity of the scenes for all patients, defined as the difference between the False Positive Rate (novel trials reported as repeated) and the True Positive Rate (repeated images correctly reported as repeated), displayed as an index.

Scene familiarity. Predictably, image recognition appeared to be easier for patients than the recollection of scene-specific targets, where majority of the scene familiarity rates were high. In fact, two patients even hit ceiling on this measure. However, this allows

us an opportunity to detect the expected declines in performance over time with disease progression.

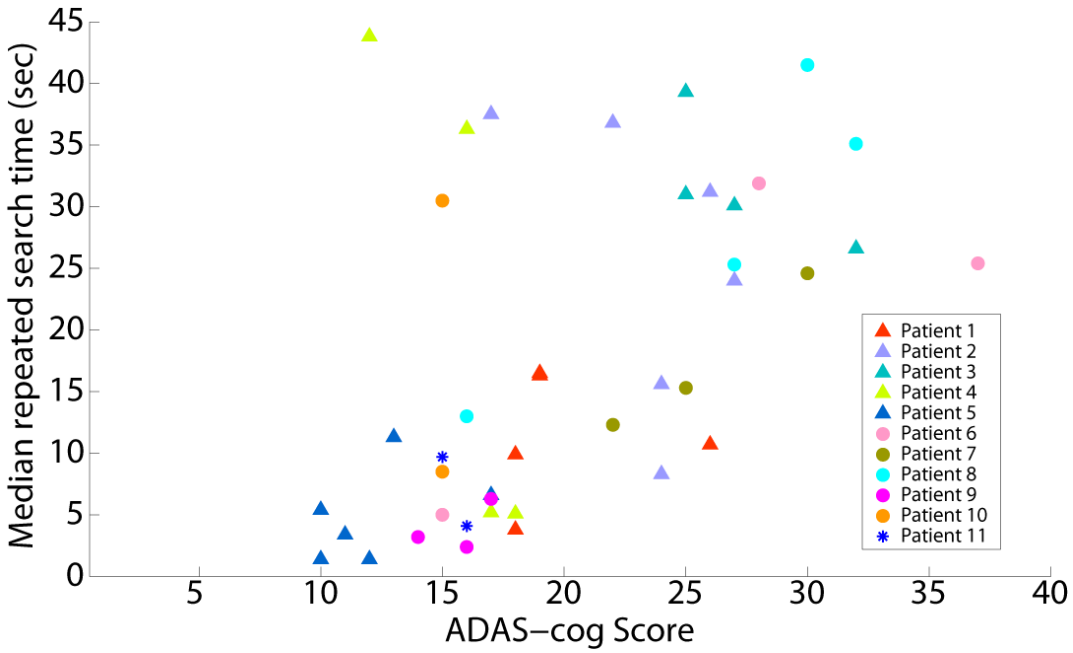

Figure 8. *Median search time comparison with standardized measures (ADAS-cog).* Median search time for target objects in repeated trials was compared to the ADAS-cog, where lower scores on the ADAS-cog mean a better performance, and higher scores mean a worse performance.

Median search time comparison with standardized measures (ADAS-cog). To aid in the validation of the change detection task with the Alzheimer's population, median search time for target objects in repeated trials was compared to a standardized cognitive test commonly used with this clinical population, the ADAS-cog. Overall, there was a general trend for lower ADAS-cog scores corresponding to faster median search times. However, patients' performances on the on the change detection task were quite variable beyond 25 seconds. As previously mentioned, the ADAS-cog is comprised of

multiple different subcomponents that test the function of various brain regions. These results suggest that this modified change detection task may be a sensitive measure of memory/decline in this particular clinical population.

DISCUSSION

We have previously shown that the change detection task requires hippocampal integrity, making any observed task performance changes partially dependent on changes in hippocampal function. Contrary to this, the ADAS-cog is a combination of mini-tasks that measure the function of various brain areas, thus it may not be the most optimal assay for measuring changes in hippocampal function specifically. The variability that we observed in median search time that was above 25 seconds when correlated with the ADAS-cog, where on the ADAS-cog scores did not differ by much, suggests that the change detection task may be detecting performance changes the ADAS-cog may not be. Our rationale for this correlation was to attempt to validate the task with AD, which has never before been used with AD before, however doing so creates a dilemma. On the one hand, we have a task that measures hippocampal-dependent memory, and so we think that in patients undergoing a stimulation treatment targeting hippocampal function, our task may be sensitive to detecting those changes. Attempting to validate this should be achievable by comparing it to a standardized measure already being used with the population, like the ADAS-cog. But on the other hand, we feel our task may be more sensitive to detecting changes in hippocampal function than the more general ADAS-cog, so we would not expect an extremely tight

correlation. Unblinding will help to shed more light on the observed results, and based on the sensitivity of this task, comparisons with other stages of AD and with other dementias may prove useful.

Additional validation of this task could come from correlating time-dependent changes in task performance and hippocampal volume. As mentioned earlier, the hippocampus is one of the primary targets of AD, leading to atrophy of the structure. If change detection task performance were more correlated with hippocampal volume than performance on the ADAS-cog, it would be a convincing piece of evidence for illuminating the sensitivity of our task to changes in hippocampal function compared to the ADAS-cog.

Chapter 4: General conclusion and discussion

CONCLUSION

Earlier diagnosis of AD may prove critical for better treatment results. Episodic/relational memory is one of the primary cognitive domains affected in individuals with AD, and yet the current tests used with the population assess more global declines. In addition, the memory subcomponents of current tests rely on verbal report for assessment, and may be confounded in those with language deficits whose memory is unaffected. In my Masters work, I set out to assess whether our non-verbal hippocampal-dependent flicker change detection task was sufficiently sensitive to identify memory decline attributed to normal aging, and more extensively affected in AD dementia. Whereas the task revealed that non-demented older adults (both healthy and at-risk) took longer to find target objects in repeated trials compared to a younger group, the search time impairment was greatest in those with AD dementia. The task also revealed that older individuals tend to make more fixations in repeated trials, with AD individuals fixating the most. Additionally, older adults have a more widespread fixation pattern than observed in younger adults, but were distinguished from those with AD whose fixations were even more dispersed. Whereas fixation and search time data could classify individuals with AD from an age-matched cohort, and those older adults from a younger group, these measures were unable to categorize the older adults into healthy and at-risk groups. Interestingly, we found that a memory-related pupillary response to image onset was only seen in healthy groups, not in the AD population or in at-risk older adults, suggesting that autonomic responses including the pupillary response may be a valuable diagnostic tool.

While the cause of AD is still being investigated, and no cure is in sight, researchers are aiming to develop effective treatment options for those diagnosed with the disease. The treatments now available for individuals with AD appear to be stage-specific and elicit only temporary results. It is known that persons with AD exhibit irregular epilepsy-like brain activity with increased risk for developing seizures (Amatniek et al., 2006; Yan et al., 2012; Palop & Mucke, 2011; Palop et al., 2007). In light of these findings, therapies that aim to modulate the activity of brain networks, then, may prove to be more effective in treating or reversing symptomology. After determining that the flicker change detection task was effective in detecting differences in memory performance between different groups, I sought to use the task in measuring memory performance in mild AD patients undergoing a novel AD therapy using stimulation. In an attempt to validate the task for the clinical population, I compared target detection search time performance in repeated trials to ADAS-cog scores, and found a significant correlation between both measures. Although qualitatively the data showed a close association between lower ADAS-cog scores and faster search times, we observed an interesting dispersion of search time data beyond 25 seconds on the flicker change detection task. The longer search times that appeared scattered may be an indication of the task's sensitivity overcoming that of the ADAS-cog. The non-verbal measures of memory in this task did not show floor or ceiling effects, and trended with search times, where faster search times usually corresponded to higher scene familiarity rates. These measures were much easier, making it valuable for detecting declines expected over time with disease progression. Only after study

completion and treatment condition unblinding will I be able to comment on the efficacy of this new potential therapy on AD.

LIMITATIONS

Eye tracking. Many researchers are keen to use eye tracking to detail eye movements and provide gaze information, with the unique aspect that it may detect changes that may sometimes reflect subconscious efforts. While this collecting method has its advantages, it also has its drawbacks, particularly when used with older populations. It is fact that with normal aging, the eye changes. Older individuals have smaller pupils and experience trouble with focusing objects, leading to slight impairments in vision (Koretz et al., 1997). Many older adults rely on glasses for vision correction, and with thicker lens, the eye tracker we used was sometimes fooled into recognizing a glare spot. In turn, this led to difficulties in collecting eye tracking data, but was usually corrected with adjustments to camera settings and diode position.

Older adults also become susceptible to developing age-related eye diseases, such as developing cataracts (Hodge et al., 1995). A cataract occurs when proteins in the eye lens begin to break down and create a clouded area as a result of the protein buildup. One of the fundamental factors for determining a good eye signal through the use of eye trackers is by identification of the pupil (Nixon, 2003). The eye tracker system we used measures the positions of 2 corneal reflectance spots relative to the pupil that are matched to specific locations on a screen. A great amount of contrast between the pupil

and the iris needs to exist to be able to identify the pupil accurately. In AD patients who reported to have previous surgical correction for cataracts, I experienced difficulty with getting the tracker to outline the pupil, possibly due to the cloudy buildup making the pupil appear lighter and harder to distinguish from the rest of the eye. In patients with eye issues or vision problems, using touch screens or button press systems to identify detected target objects in repeated trials may prove to be a good alternative.

Current neuropsychological tests used in AD. Current tests used measuring cognition claim to be sensitive and specific in identifying AD dementia and its prodromal stages. Studies on the Montreal Cognitive Assessment (MoCA) have shown that it is sensitive to detecting individuals with MCI using a threshold of 26 (out of a total of 30). However, in using this test with the older adults in the chapter 2 experiments, the limitations of this test became obvious. The MoCA consists of 8 different domains including a naming section (where participants are expected to name the animals they see pictured), a language section (where participants repeat an entire sentence after hearing it), and an abstraction section (where participants are asked to provide the commonality between two listed items). While the purpose of the naming section is to reveal any issues regarding object-naming, one version of the test that pictured a rhinoceros, was consistently mistaken for a hippopotamus. The rapidness in naming the animals mirrored the patient's confidence in their ability to accurately label what they thought they saw. The participants that mistook the animals always correctly identified the other images just as quickly and confidently, lending support to probable failure of this section in classifying object-naming dysfunction in participants. Though losing one point may not seem like much, with only a small point range for individuals to remain

in the ‘healthy’ category (scoring 26-30), a one point deduction reflects a 25% decrease towards the ‘impaired’ range. Put in this way, the faulty image may have a significant difference on total scores, incorrectly labelling individuals as impaired when, in the absence of the image, they would have scored a 26.

In addition to this error, we encountered a few problems with the abstraction section of the test. For the abstraction, participants were given pairs of items and asked to list something that the items had in common with each other (i.e. watch and ruler, were measurement tools). The MoCA scoring instructions detail the points assigned to only a few, very specific answers, which yielded in scoring errors. For example, the accepted answers given for the word pair ‘diamond-ruby’ were ‘gemstones’, ‘jewels’ and ‘precious stones’, while other answers were not accepted. One participant whose second language was English, provided ‘stone’ as an answer. Later analysis showed that the direct translation of ‘gemstone’ in the individual’s native tongue was, in fact, ‘stone’. The language barrier for this particular word-pair resulted in an inaccurate immediate score, which we later accepted upon this realization. The participant clearly could connect the two items, and verbalize their commonality, yet the selectivity of acceptable answers made the participant appear as if they could not according to their score. In fact, it was not uncommon for language to affect a person’s total score, where when repeating the sentences in the language section, ESL participants often dropped ‘a’ or ‘the’ reflecting the lack of these words in their native tongue. In conversing with these individuals before, after, and during breaks, it was quite obvious that these participants often forgot to include ‘a’ or ‘the’ in their speech. Having formed bad habits of not including these sorts of words in everyday speech, these individuals were

prime targets for failing the language parts of the test, however, not as a result of an actual cognitive impairment. These issues give rise to questions regarding the accuracy of detecting the milder abnormalities in cognitive function using tests such as the MoCA, due to some of the inconsistencies I listed. Furthermore, the MoCA, which is comprised of many different cognitive domains, may be a good indicator of global cognitive decline. Although, in diseases where memory seems to be the one of the first cognitive abilities impaired, such as in AD, the MoCA may not be the most optimal assay for differentiating those in the earliest stages of the disease. In fact, subtests more specific for memory are currently being designed (Julayanont et al., 2012).

FUTURE DIRECTIONS

One avenue to continue on for future analysis may include testing individuals with MCI. We showed on our task that several test measures reflected a performance difference between those at-risk for developing the precursor of AD and those with AD, thus an appropriate speculation may be that those with MCI would show an intermediate impairment on the task compared to the other two groups. Additionally, several different types of MCI have been identified, only one of which shows clear impairments on memory (amnestic MCI). In knowing that the change detection task measures hippocampal-dependent memory, while those with amnestic MCI exhibit hippocampal atrophy and memory impairments, one would hypothesize that the task may potentially be able to separate the different MCI subtypes. Thus, testing individuals with different kind of MCI may help to validate the use of this task in

assessing memory. Although time-consuming, longitudinal testing of this paradigm with MCI individuals may prove the task to be a good predictor of susceptibility to the development of dementia, helping to identify those in need of early intervention. To further our findings regarding pupil responses, perhaps pupillary dynamics differ in the different stages of Alzheimer's, and if so may be used to classify them. Finally, we observed other differences among the groups in their pupil responses to the flicker throughout the trial. Qualitatively, this could be seen in the pupils of healthy younger and older adults that reflected the alternating flicker, despite no mean luminance change. These pupil changes in response to the flicker appeared to be diminished in the at-risk and AD groups, in addition to the memory-related changes in pupil velocity. Future work may involve analyzing this aspect of pupil dynamics in different populations, including those with MCI.

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