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Research report

Pupillary responses and memory-guided visual search reveal age-related and Alzheimer's-related memory decline

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HIGHLIGHTS

- Nonverbal, episodic-like memory shown in a goal-directed natural scene search task.
- Age and AD predict deficits in target detection time and scanpath efficiency.
- Pupil dynamics diminished with aging and further in Alzheimer's disease.
- Memory increased pupil velocity in healthy adults, but less so in at-risk groups.

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ABSTRACT

Episodic memory – composed of memory for unique spatiotemporal experiences – is known to decline with aging, and even more severely in Alzheimer 's disease (AD). Memory for trial-unique objects in spatial scenes depends on the integrity of the hippocampus and interconnected structures that are among the first areas affected in AD. We reasoned that memory for objects-in-scenes would be impaired with aging, and that further impairments would be observed in AD. We asked younger adults, healthy older adults, older adults at-risk for developing cognitive impairments, and older adults with probable early AD to find changing items ('targets') within images of natural scenes, measuring repeated-trial changes in search efficiency and pupil diameter. Compared to younger adults, older adults took longer to detect target objects in repeated scenes, they required more fixations and those fixations were more dispersed. Whereas individuals with AD showed some benefit of memory in this task, they had substantially longer detection times, and more numerous, dispersed fixations on repeated scenes compared to age-matched older adults. Correspondingly, pupillary responses to novel and repeated scenes were diminished with aging and further in AD, and the memory-related changes were weaker with aging and absent in AD. Our results suggest that several nonverbal measures from memory-guided visual search tasks can index aging and Alzheimer's disease status, including pupillary dynamics. The task measurements are sensitive to the integrity of brain structures that are associated with Alzheimer's-related neurodegeneration, the task is well tolerated across a range of abilities, and thus, it may prove useful in early diagnostics and longitudinal tracking of memory decline.

1. Introduction

Episodic memory decline is one of the principal clinical signs of Alzheimer's disease (AD [54]) and amnestic mild cognitive impairment (aMCI [33]), above and beyond the decline observed with healthy aging [19]. This memory decline is thought to be due in large part to pathological changes in the hippocampal formation (HC), its connected fiber tracts, and adjacent entorhinal cortex (EC).

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The best assays of changes in function to this circuit with aging and progressive dementia must optimize task sensitivity and selectivity [9,24,1], accommodate the abilities of impaired individuals to be tested, and maximize ecological validity, here, inasmuch as it would apply to real-world contextual and episodic memory impairments.

Some tests of hippocampal function – e.g. pattern separation, navigation – are difficult to test in impaired populations, who show floor effects or are unable to properly execute the task, though such tests may be ideal for differential early diagnostics in mildly impaired individuals [61,78]. Other tests that are considered to be sensitive to extended hippocampal system integrity are not necessarily linked to episodic memory, e.g. eye blink conditioning, old/new item discrimination, virtual-reality navigation [57,77,9]. The hippocampus is thought to support episodic memory by 'binding' the relations among items within a given spatiotemporal context, and, indeed, rats with hippocampal lesions are impaired on tests of memory for objects ('what') placed at different locations in an environment ('where'), within a given spatial context ('which') [20,41,23,45]. Similarly, disconnection of a major hippocampal fiber tract in monkeys impairs memory for a visual feature embedded in an abstract scene [30]. Moreover, humans with medial-temporal lobe damage and episodic memory deficits are also impaired at remembering the location of objects in photorealistic spatial scenes [65,75,12]. The flicker change detection and learned target detection versions of these object-location-in-context tasks use memory for a concealed or uncued object to speed/enable detection. We predicted that performance in this task would decline with age, with even greater deficits predicted for individuals with Alzheimer's disease.

The primary performance difference expected in this task across populations is speeded target detection time with repetition, which has previously been associated with explicit memory for the scene-embedded target [12]). Among the changes in search underlying rapid target detection are fewer fixations, and a more directed scan path, deviating from the initial, protracted search when the target was concealed and/or unknown [87,14,15]. In addition to changes in visual search, pupillary responses have been linked to memory for scenes [56] objects [40] and objects in scenes [63]. These memory-related pupillary responses were reported for healthy young adults; they have not yet been tested in aging populations. The pupil's size and motility, on the other hand, has been documented to decrease with Alzheimer's disease and in prodromal cognitive impairment, accompanied by hyper-sensitive pupillary responses in the presence of cholinergic antagonists [28,71,72]. We therefore predicted that the pupillary response to natural scenes would weaken as a function of age and additionally with Alzheimer's disease status, and that the greatest effects of scene repetition on the pupillary response would be observed in the populations with the best memory performance.

2. Materials and methods

2.1. 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 adults diagnosed with probable early AD (5 males, mean(SD) age 69.1(7.8) years), participated in the study (Table 1). All young adults and 14 of the older adults were recruited from the Toronto community; the remaining 7 older adults were recruited from the Rotman Research database. Older adults completed the Montreal Cognitive Assessment (MoCA), a brief neuropsychological test shown to be sensitive to mild cognitive impairment (MCI [18,58,53];) and to conversion from MCI to AD [39] and to individuals at risk for developing MCI [59,58]. Ten older adults scored a 26 or higher (range: 26–31) on the MoCA and therefore were categorized as healthy older adults (HOA); eight scored 24 or lower (range: 21–24) and were categorized as at-risk (ROA) for MCI [18]. Three individuals scored a 25 and could not be placed in either category, thus they were excluded from further analysis. The probable early AD designation was given according to the National Institute of Aging Alzheimer's Association criteria [38,54]. 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 and 24 on the Alzheimer's Disease Assessment Scale – cognitive test 11 (ADAS-cog11 [11]), and either 0.5 or 1 on the Clinical Dementia Rating [55]. The main experiment described here took place after device implantation, but before initiation of DBS treatment. See Table 1 for post-admission performance scores that occurred prior to double-blind assignment into treatment or placebo groups. We compared performance in those tested pre-operatively and found no change to post-operative performance on any of the memory measures we tested (see Table 1; entropy: $t = 0.64$ $p = 0.54$; median search time: $t = 0.38$ $p = 0.72$; fixation number: $t = 2.19$ $p = 0.07$).

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. Participants from the younger adult and older AD groups volunteered without monetary compensation; older adults received $10/h in accordance with our ethical guidelines. Experimental procedures for all participants were approved by the York Human Participants Review Subcommittee; older adults who were recruited from the Rotman Research Institute database (N = 7) additionally followed the guidelines approved by the Rotman Research Institute; early AD participants were selected for and participated in a 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.

2.2. Stimuli

We selected a range of natural scenes, including wildlife, city, rural, and indoor scenes, that could be displayed at a 1280×1024 pixel resolution (full screen), as described previously [12,36,48]. One object per scene was modified (color change or disappearance, Fig. 1A) using Adobe Photoshop (San Jose, CA). Target sizes i.e. maximal horizontal and vertical extent varied between 40 and 224 pixels horizontally and between 48 and 280 pixels vertically, occupying roughly 5–25% of the scene horizontally or vertically. To discourage bias in search strategies, sets of images were balanced for target location (quadrant on screen) and category (animate/inanimate).

2.3. Experimental apparatus and session design

Participants used a chin rest to minimize head movements throughout the study. A 38.0×30.5 cm monitor displaying the task stimuli was placed 51 cm away from young participants ($41 \times 33^\circ$ visual angle, dva), and 61 cm away from all other participants (35×28 dva). Eye gaze and pupil diameter were tracked using the iView X infrared eye tracking system at a 60 Hz sampling rate (SensoMotoric Instruments, SMI, Berlin, Germany), following a 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

Table 1
Demographics of participants and average scores on the neuropsychological batteries and memory tasks. For the pre/post op comparisons, no comparisons were significant; only Fixation Number reached a trend level ($t = 2.28$; $p = 0.07$). Fixation durations were taken from Experiment 1. There was no overall effect of median fixation durations across group; however, there was a repetition effect on fixation durations, with shorter fixations on repeated trials for Young Adults ($W = 153$, $^{***}p = 2.9 \times 10^{-4}$) and Healthy Older Adults ($W = 55$, $^{**}p = 0.0021$).

	Young Adults	Older Adults			
	HYAs (N = 17)	HOAs (N = 10)	NC (N = 3)	ROAs (N = 8)	AD (N = 9)
Age (years)	22.8	66.4	62.7	69	69.1
Sex (M/F)	(5/12)	(2/9)	(0/3)	(3/5)	(5/4)
Education (years)	16.7	14.9	16.5	17.3	–
Bachelor (N=)	10				
Graduate (N=)	7				
MoCA					
Total (/30)	–	28.1	25	23.1	–
CDR					
Total (/1)	–	–	–	–	0.61
ADAS-cog11					
Mem sub (/22)	–	–	–	–	11.4
Total (/75)	–	–	–	–	16.3
AD pre/post-op					
Entropy $^{n.s}$	–	–	–	–	10.7/11.0
Fixation Number $^{n.s}$	–	–	–	–	28.8/22.95
Med. Search Time $^{n.s.}$	–	–	–	–	11.4/9.6
Fixation durations, in ms (SD)					
Novel trials	365 (71)	342 (48)	–	265 (56)	256 (74)
Repeated trials	244 (50)***	245 (25)**		222 (57)	228 (75)

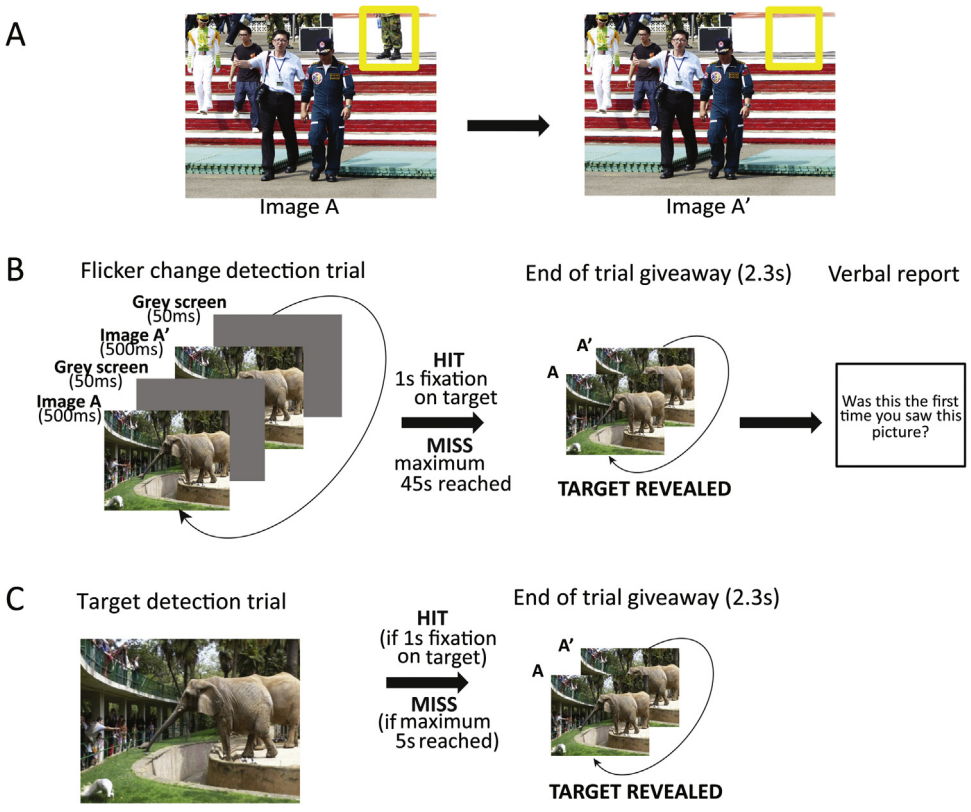

Fig. 1. Experimental design. (A) Example stimulus. Image A is the original image containing the target object outlined in yellow, where Image A' is a modified version of Image A with the target object absent. The yellow outline is for illustrative purposes only and was absent during the task. (B) Flicker 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

then began with Experiment 1: flicker change detection, followed by Experiment 2: target detection. After completion of these two experiments, young and old adults, but not the AD group, continued with additional sets of flicker change detection stimuli, previously reported [87] and not described in the present study.

2.3.1. Experiment 1: flicker change detection memory task (Fig. 1B)

Each trial consisted of the 500 ms presentation of an original image (Image A) which alternated with the 500 ms 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 last-

ing 50 ms was inserted between each image alternation (as shown in Fig. 1B). This visual interruption makes the changed object difficult to detect; however, once detected, the change is difficult to ignore [73,64]. Trials ended when the target object was detected, i.e. fixated for 1 s, or after 45 s 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, lasting 2.3s. 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 old/new judgments. One set of flicker detection trials contained 10 novel images, and those same 10 images repeated after 2–4 intervening trials. Participants performed 2 such sets, for a total of 20 possible remembered objects-in-scenes.

2.3.2. Experiment 2: target detection memory task (Fig. 1c)

This task was described previously [36]. 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, lasting 2.3s, whereby the target object appeared to blink, exactly like the reveal in the flicker task. Because the target object was *uncued* during the trial, at encoding, 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. Thus, encoding duration is held constant in this task. Each participant saw a series of 10 images presented 4 times, allowing 3 chances to demonstrate memory for a given image.

2.4. Data analysis

2.4.1. Fixation and search

Fixation times and locations were calculated with iView X iTools IDF Event Detector, using a dispersion based algorithm with a minimum fixation duration of 80 ms and maximum dispersion of 100 pixels [68], corresponding to ~3 dva (3.2, YAs; 2.7 all OAs). This dispersion setting is in accordance with previously-described recommended settings [86] that offer an allowance for reduced fixation stability with aging (see Ref. [89]). Fixation times and locations, and pupil diameter was then analyzed using MATLAB (Natick, MA). Search times were calculated from trial onset to the onset 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 gaze inside 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. These off screen times were infrequent, comprising <10% of viewing time for all participants, and on average (+/−SD): 0.01 s(0.03) for YAs, 0.02 s(0.05) for HOAs, 0.10 s(0.07) for ROAs and 0.98 s(1.14) for the AD group.

To quantify the spatial dispersion of fixations, we calculated entropy from the center of the scan path, obtained using the gaze samples recorded at 60 Hz: $H = \ln(\sigma_1\ \sigma_2)$, where σ_1 and σ_2 represent orthogonal directions of maximal search variance around the search centroid ('H_{path}' in Ref. [52]). 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 old/new judgments (correctly remembered rate – falsely remembered rate) then group differences were statistically evaluated. We compared group responses with the Kruskal-Wallis test (non-parametric ANOVA) due to non-homoscedasticity of data and, where significant, at a two-tailed alpha level of 0.01, planned pairwise post-hoc tests (Wilcoxon rank sum) were run to test for (1) aging effects (comparing young adults to healthy and at-risk older adults) (2) Alzheimer's-related performance differences (comparing AD patients to healthy and at-risk older adults).

2.4.2. Visual angle differences across groups

To determine if object size influences repeated trial search times (rather than memory), we measured in subtended visual angle, to account for the different viewing distances between young and older populations. Because an influence of size on search time would represent a confounding variable, we reduced Type II errors at the cost of Type I errors, pooling the non-AD older adult data and comparing to the pooled younger adult data, with two-tailed tests. For each group, Pearson's correlation coefficient was calculated between a given target's size (DVA of diagonal spanning horizontal and vertical extent of target) and the search time on repeated trials. No multiple comparison correction was applied. In addition, we generated a linear model to measure the prediction of search time by the factors size (DVA), group (Young/Old), and their interaction term.

2.4.3. Pupil velocity

Horizontal pupil diameter was sampled from each trial at 60 Hz. For each trial, values were robust spline fit [32], gaps due to blinks were detected, as were outliers, identified as residual error of the mean response around the time of trial onset, which comprised <10% of trials and was independent of memory condition. This produced a smoothed, time-resolved pupil size that was aligned to stimulus onset, allowed the measurement of the rate of change in pupil size due to stimulus onset (i.e. pupil response velocity). For each individual, velocity profiles from each trial were grouped according to order of presentation (first: 'novel', second: 'repeated'; for Exp. 2: only the first repetition was used). Peak velocities (minima) were compared across participant groups using an ANOVA, followed by planned post-hoc pairwise tests to determine differences between groups. The repetition 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 (i.e. no difference between novel and repeat velocities) were tested and False Discovery Rate (FDR) corrected to account for multiple comparisons [4].

3. Results

3.1. Experiment 1: flicker change detection memory task

3.1.1. Visual scan paths

Fixation durations during search for novel and repeated trials can be found in Table 1. The dispersion of repeated-trial scan paths (Fig. 2A, light blue lines) differed across groups ($H_{(3,44)} = 29.25$, $p = 1.9 \times 10^{-6}$). They were more focused (e.g. less diffuse) in healthy younger adults than in healthy or at-risk older adults (younger – healthy older: $W_{(17,10)} = 190$, $p = 0.013$; younger – at-risk: $W_{(17,8)} = 163$, $p = 6.5 \times 10^{-4}$). Within the aging populations, AD patients displayed the most diffuse repeated-trial search, compared to healthy ($W_{(9,10)} = 133$, $p = 8.7 \times 10^{-5}$) and at-risk ($W_{(9,8)} = 40$, $p = 9.8 \times 10^{-4}$) older adults, and there was a trend for at-risk adults to also show more diffuse search compared to healthy older adults ($W_{(10,8)} = 96$, $p = 0.068$). 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

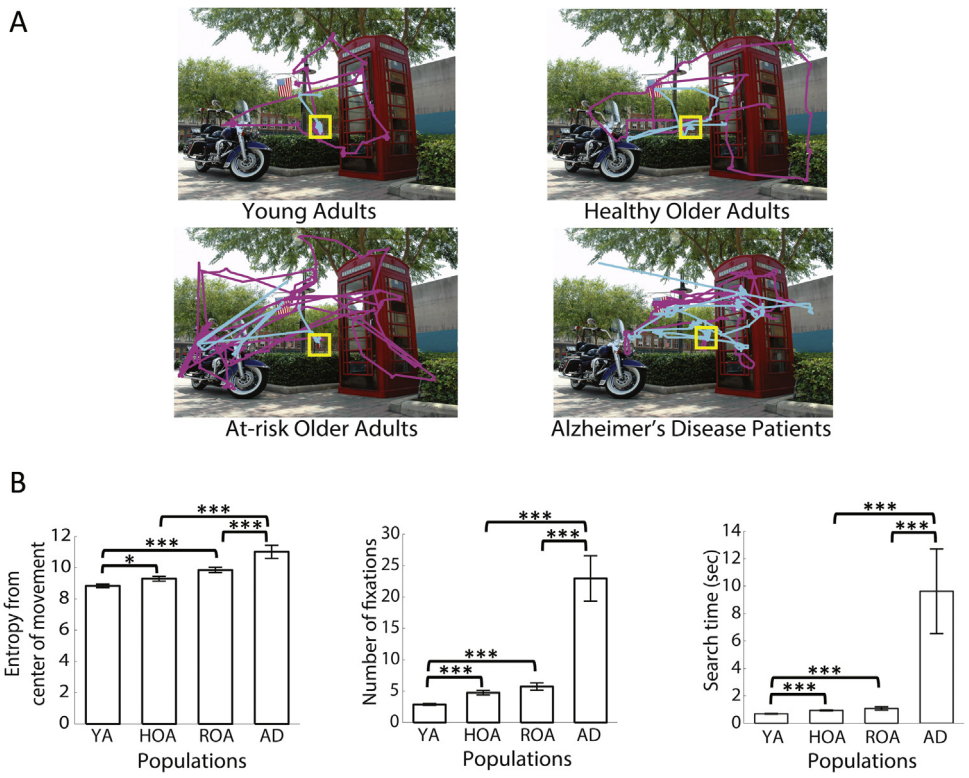

Fig. 2. Memory-guided visual search during flicker change detection. (A) Superimposed scan paths during novel and repeated flicker trials. Scan paths for an example scene were taken from a single participant from each group. Scan paths for novel trials are shown in purple, while scan paths for repeated trials are shown in light blue. (B) Entropy from the 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 $_{n=9}$; * $p = 0.01$; *** $p < 0.001$, here and in C and D). 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.

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 ($H_{(3,44)} = 36.27$, $p = 6.5 \times 10^{-8}$), including an age-related increase in the average number of fixations before target detection (younger adults v. healthy older adults: $W_{(17,10)} = 222$, $p = 4.2 \times 10^{-5}$; younger adults v. at-risk older adults: $W_{(17,8)} = 169$, $p = 1.7 \times 10^{-4}$). AD patients showed a clear impairment, with more fixations than healthy adults ($W_{(9,10)} = 135$, $p = 2.1 \times 10^{-5}$) and at-risk older adults ($W_{(9,8)} = 36$, $p = 8.2 \times 10^{-5}$), shown in Fig. 2.

As expected, more fixations in aged and particularly AD-groups corresponded to longer search times for target detection in repeated trials ($H_{(3,44)} = 34.72$, $p = 1.4 \times 10^{-7}$). Younger adults found the repeated target faster than healthy older adults ($W_{(17,10)} = 219$, $p = 7.9 \times 10^{-5}$) and at-risk older adults ($W_{(17,8)} = 165.5$, $p = 7.3 \times 10^{-4}$), and AD patients took significantly longer to find repeated target objects than their healthy ($W_{(9,10)} = 135$, $p = 2.2 \times 10^{-5}$) and at-risk ($W_{(9,8)} = 36$, $p = 8.2 \times 10^{-5}$) age-matched counterparts, suggesting both age and AD-related memory impairments for the objects in the scenes (Fig. 2).

As a group, young adults had a closer viewing distance to the monitor than the older-population groups, therefore the viewing angle subtended by the targets was larger for the young adult group. If the viewing angle (in degrees visual angle, 'DVA'), affects search time, it could potentially account for the suggested 'memory effects': faster search on repeated trials across the aging comparison. For both younger and older adults, there was no statistical relationship between target size and repeated-trial search time (Pearson's correlation YA: $\rho = -0.08$, $p > 0.1$; OA: $\rho = -0.07$, $p > 0.1$). Furthermore, a linear model constructed with the predictors 'Target size (DVA)' the categorical variable 'Group' (YA/OA) and their interaction 'size*group' on the outcome 'search time' produced only one significant predictor: Group ($t = 3.69$, $p < 0.001$), whereby trials of YAs predicted faster search than those of OAs. This suggests that, after accounting for the differences in object DVA, performance differences remained across populations. In contrast, search time was not predicted by target size ($t = -0.86$, $p > 0.1$) or the interaction of target size and group ($t = -1.53$, $p > 0.1$).

3.1.2. Memory-guided search, grouped by encoding duration

Group differences could be attributable to diminished capacity to detect targets in general, and not memory, per se. Indeed, there were group effects at encoding ($H_{(3,44)} = 33.23$, $p = 2.8 \times 10^{-7}$) with younger adults finding targets faster than the other 3 groups, with no differences among the older groups. Longer encoding durations might improve subsequent memory. In addition, longer encoding durations could artificially inflate the relative speed 'benefit' of repeated trials, even when repeated search is very rapid. To address this, we selected the objects-in-scene images that were found rapidly at encoding ('short' encoding trials < 3.5 s), those found with slightly longer search at encoding ('medium' encoding trials, 3.5–10 s), and those found only after protracted search ('long' encoding trials > 10 s). Under conditions of complete forgetting, one might expect either regression to the mean of search time distributions, or, if saliency leads to highly repeatable scan paths, correlated novel and repeated search times. In contrast, if memory during repeated trial search leads to rapid target detection, irrespective of how concealed or apparent the target was initially, then (memory-

guided) search should be similarly fast across encoding-duration groups.

The group statistics (unpooled) are depicted in Fig. 3A; pooled data are shown in Fig. 3C. Search time improvement (Fig. 3B) is measured as the difference in search duration between first and second presentations of a given image, summed across all images. Thus, longer search times at encoding afford a higher possible improvement score. To visualize the distribution of times across populations, and accounting for encoding duration, we calculated the probability density, kernel-smoothed at a constant bandwidth of 1, which was smaller than the standard deviation of the samples, to prevent over smoothing and track multiple modes in the distribution. Because the integral for each curve is constant, the relative frequency of different search times can be compared across populations with unequal sample sizes, shown in Fig. 3C.

We found a difference in repeated trial performance across groups ($F_{(3,126)} = 51$, $p < 1.0 \times 10^{-8}$), and a trend towards a main effect of encoding trial category ($F_{(2,126)} = 2.78$, $p = 0.06$). Post-hoc testing revealed only the AD group showed different repeated-trial search times as a function of encoding category (Fig. 3A), with times increasing slightly with longer encoding-trial duration ($H_{(3,44)} = 7.56$, $p < 0.05$), though not fully following the encoding durations (Fig. 3B). This difference was due to a rapid-search mode in the distribution of repeated search times (i.e. on a subset of trials, Fig. 3C), that was characteristic of memory in the other groups, and the hallmark of explicit memory for objects in scenes in previous studies [12]). This also reveals a capacity for rapid search, even while longer search times are predominant in the older groups. Indeed, every participant showed an early search mode, these were larger for repeated than for novel trials, though the magnitude of the peak (the probability density at the peak) varied across individuals. In sum, these results suggest that performance differences, i.e. differences in duration of search during encoding, do not account for the age and AD-related deficits with trial repetition.

3.1.3. Scene familiarity assessed with verbal report

Old/new judgments for the scenes were worse for AD patients than all other groups (younger adults: $W_{(9,17)} = 60.5$, $p = 4.8 \times 10^{-4}$, healthy older adults: $W_{(9,10)} = 50$, $p = 2.4 \times 10^{-4}$, at-risk older adults: $W_{(9,8)} = 84$, $p = 1.4 \times 10^{-3}$) indicating that the AD patients were impaired on this measure. No differences were found in old/new judgments for the scene among the young adults and other older adults due to ceiling effects (all group medians = 100%). Even in the AD patients, the median (true positive – false positive) rate was 85% and 3/9 scored > = 90%, suggesting general scene recognition memory may not be the most sensitive measure of memory decline with aging and in AD.

3.1.4. Pupillary responses

Light-induced pupillary responses are known to diminish with aging, but it is unclear whether cognitive factors in search or the recently-described relation between memory and pupillary responses [56] would also show age-related effects. In this study, the onset of both novel and repeated images elicited rapid constriction followed by dilation of the pupil, though the response velocity differed across groups ($F_{(3,39)} = 16.52$, $p = 4.38 \times 10^{-7}$). Younger adults had higher peak velocity than the healthy and at-risk older groups (v. HOA $t_{(25)} = 3.69$, $p < 0.01$; v. ROA $t_{(22)} = 3.76$, $p = 0.01$), and AD patients had lower-velocity responses than did healthy older adults ($t_{(17)} = 3.26$, $p < 0.01$) and trend-level differences with at-risk older adults ($t_{(14)} = 1.83$, $p = 0.09$). Furthermore, repeated trials evoked faster velocities than did novel trials, but only in healthy populations, i.e. in younger ($t_{(16)} = 4.71$, $p = 2.4 \times 10^{-4}$) and older adults ($t_{(9)} = -3.26$, $p = 0.01$), but not in the at-risk or AD groups (Fig. 4b). These results indicate that image onset evokes a general pupillary response that weakens with age and, further, with dementia, and that memory-dependent differences in pupillary responses could segregate healthy from at-risk and AD populations.

3.2. Experiment 2: target detection task

3.2.1. Target detection search times

In the target detection task, a single scene image is displayed continuously for 5 s before the designated target location is revealed as a blinking of the object seen in that location. On repetition, if the target is detected – i.e. fixated – before 5 s, the trial will end with the same giveaway period when the target is revealed (Fig. 1c). Because the target is not changing during the search epoch, there is nothing to 'find' until the end of the 1st trial, therefore, target detection on subsequent scene presentations is attributable solely to memory for the target embedded in that scene context, and encoding duration is held constant. The short trial length relative to the 45-s flicker task, and the absence of a verbal response component at the end of the trials allowed this set to be completed within 6 min, across all participants. All groups detected the targets after several repetitions (Fig. 5), though there were group differences in the rate of learning, as measured by the search times on the second presentation of each image (second presentation search times: $H_{(2,38)} = 22.68$, $p = 4.7 \times 10^{-5}$). Young adults were faster than older adults (v. HOA: $W_{(17,10)} = 187$, $p < 0.05$; v. ROA: $W_{(17,8)} = 155$, $p < 0.01$), and AD patients were impaired compared to their age-matched counterparts (v. HOA: $W_{(9,10)} = 118$, $p < 0.05$; ROA: $W_{(9,8)} = 150$, $p < 0.05$) on the second presentation; however, given another 2 repetitions, the AD group further decreased to the speed the healthy older adults showed on the second presentation, suggesting that all groups understood the task, were capable of rapid target detection, and the tracking performance was adequate to register this improvement (Fig. 5). We also found age-related differences between younger adults and healthy older adults ($W_{(17,10)} = 183$, $p = 0.001$) and with those in the at-risk group ($W_{(17,8)} = 155.5$, $p = 0.003$).

3.2.2. Pupillary responses

In this study, similar to the flicker task, the onset of both novel and repeated images elicited rapid constriction followed by dilation of the pupil, with response velocity diminishing with age and disease status ($F_{(3,39)} = 15.48$, $p = < 0.001$). Younger adults had the highest velocity, faster than their healthy older counterparts ($t_{(47)} = 4.56$, $p < 3.66 \times 10^{-5}$). At the other extreme, AD patients had lower-velocity responses than did their age-matched at-risk counterparts ($t_{(36)} = 4.45$, $p = 8.0310^{-5}$). Furthermore, repeated trials evoked faster velocities than did novel trials in this task, for young and old groups, excluding the AD group (Fig. 6b). Whereas the pupillary response at trial onset was visible across groups, it was clearly diminished with aging and disease status; nevertheless, healthy and at-risk adults still showed memory effects in this shorter, target detection task.

4. Discussion

4.1. Summary of findings

In this study, we measured two nonverbal classes of responses (visual search and pupil dynamics) during performance of an episodic-like memory task in young and old adults, including individuals with memory-impairments and Alzheimer 's disease (AD). The task required explicit, goal-directed search for a concealed target, but for which performance was dramatically improved (search was rendered rapid) if the target was remembered. Memory effects were also observed in the pupillary response, consistent with previous reports in young adults [56]. We will consider first the

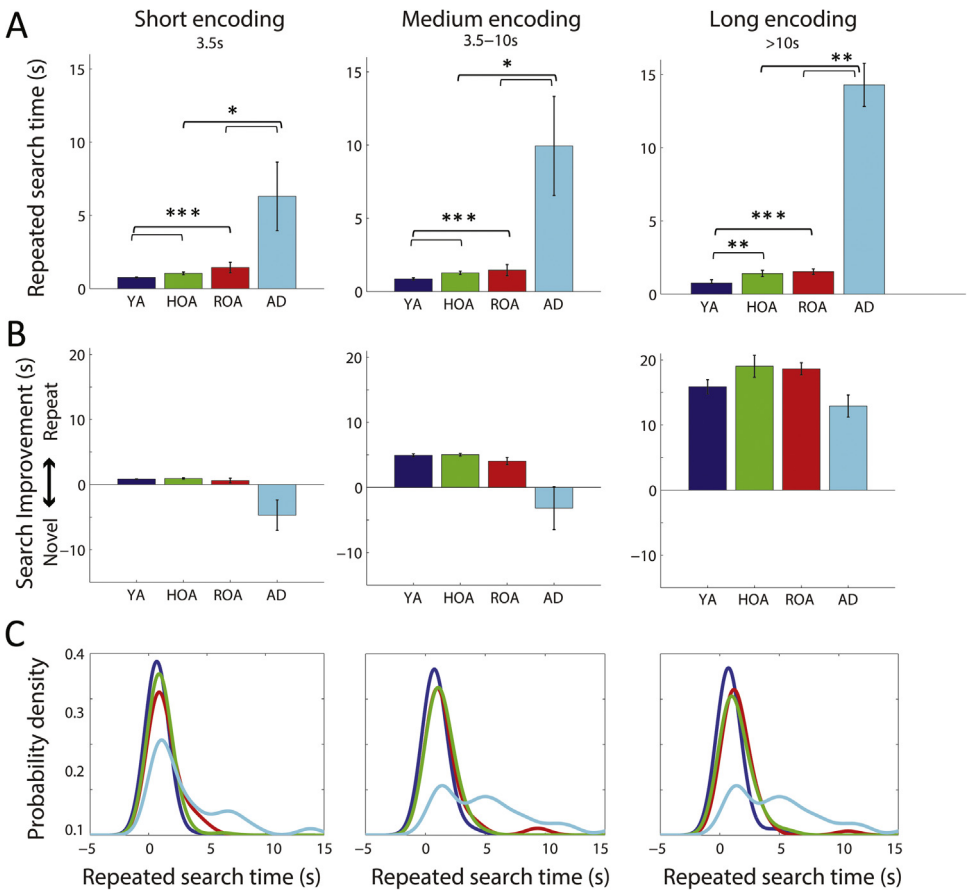

Fig. 3. Search times on repeated trials grouped by encoding-trial duration. (A) Repeated-trial search times that resulted from (left) targets found quickly on initial presentation (short encoding trials, <3.5 s); (middle) from targets initially found between 3.5 and 10 s (medium encoding trials); and (right) targets initially found after 10 s (long encoding trials). Errorbars are SEM around the group means, sample size listed on the respective bars. * = $p < 0.05$; ** = $p < 0.01$. (B) Search time improvement, grouped by encoding-trial duration. Encoding-trial groups and conventions as in A. (C) Distributions of repeated search times, pooling trials across individuals in a group, for a given encoding duration bin (short, medium, long). AD patients show a rapid search mode irrespective of encoding duration, though such trials are less frequent than for the other older adult groups.

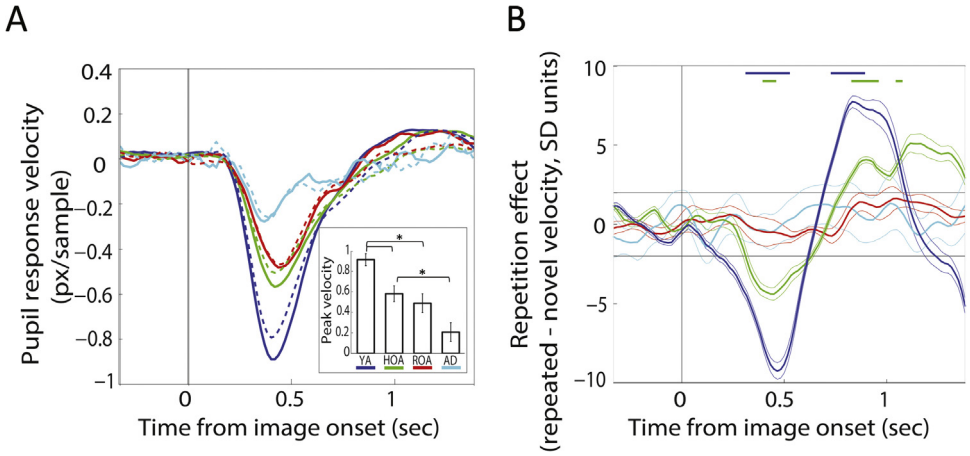

Fig. 4. Pupil-velocity responses following image onset. (A) Average pupil velocity following image onset in novel and repeated trials across groups. Novel-trial velocity indicated with dotted lines; repeated-trial velocity with solid lines. Color conventions described in inset. *Inset*. Average repeated-trial peak velocity for each group, expressed negative-up, for ease of viewing. 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); *$p <= 0.01$. (B) Memory-related differences in pupil velocity responses 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 Fig. 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

implications of the visual search differences, then the pupillary responses, and finally limitations of the study.

4.2. *Differences in visual search with aging and in AD*

When viewing natural scene images, changed objects are detected using contextual cues [8,84,2,37], and this process

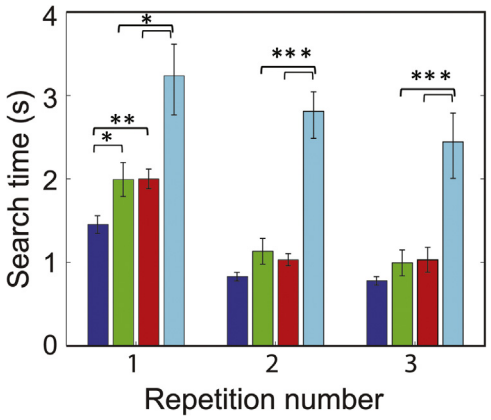

Fig. 5. Search times on repeated scene presentation in Experiment 2 target detection task. The average target detection times per group (YA = Young Adults (N = 17), HOA = Healthy Older Adults (N = 10), ROA = at-Risk Older Adults (N = 8), AD = Alzheimer's Disease patients (N = 4)) on presentations 2–4 (repeats 1–3) of the non-cued target detection scenes. Errors bars represent the standard error of the mean. * = p < 0.05; ** p < 0.01; *** p < 0.001.

depends on the integrity of the medial temporal lobe, including the hippocampus and entorhinal cortex [66,75,12]. In the present study, two variations of object detection in natural scenes revealed impairments with healthy aging and further in individuals with Alzheimer 's disease. Group differences indicated better memory-guided search in younger adults compared to older adults, and in older adults compared to adults with probable early AD.

Traditional cognitive batteries used with the Alzheimer's population included the ADAS-cog and the mini mental state examination (MMSE). These tests are designed for sensitivity to a range of cognitive functions, potentially at the cost of specificity for memory processes that may index MTL degeneration with AD [90,21,25,13,74,80,81,31]. For example, one cognitive subdomain tests memory using word recall, which in some forms is among the best-performing tests for measuring episodic-like deficits characteristic of the disease [90]. Performance on this test may be confounded by language ability, which may have diagnostic value but stem from different underlying neuropathological deficits. As such, evaluating the efficacy of treatments to specific neural pathways or behavioral phenotypes may benefit from non-verbal measures of episodic-like memory.

Virtual environments can be used to measure memory non-verbally, though many VR tasks designed to detect probable dementia use verbal responses [22,17,88]. Other studies using virtual environments for detecting dementia that avoid verbal recall, use joysticks or mice for navigation [3]. Such tasks provide a middle ground that affords testing feasibility and ecological validity/sensitivity to MTL function. Operation of the mouse/joystick devices, however, requires a non-standard mapping of visual and motor commands, known to be compromised in individuals with MCI and AD [67,82,83]. Thus, tests involving the use of joysticks or mice that aim to measure memory impairments have several advantages, but may be confounded by deficits in visuo-motor integration in AD.

Another nonverbal memory test that is used with AD populations is the human analogue of the Morris Water Maze test [44]. Individuals with AD were impaired in navigating to a hidden goal location in a real arena. This task offers strong ecological validity for topographic amnesia, though the feasibility and portability of the task are limited, and it would be important to compare topographic memory performance with tasks designed to test elements of episodic memory, including what-where-which instance memory or associative/relational memory.

Paired associates learning (PAL) – a test that measures the ability to remember object-location associations, is sensitive to detecting impairments in MCI and AD [50,47,29]. Task benefits include ease of testing, standardized designs, and sensitivity to MTL integrity, albeit at the cost of using repeated items that can cause memory interference and using simplistic object and location arrays rather than naturalistic object and scene displays. In contrast to PAL stimuli, scenes from our day-to-day life exhibit 'crowding', that is, a spatially dense collection of features from a multitude of objects that combine to create our perception of the scene environment as a whole. Object recognition in this 'crowded' view is known to be more effortful [60], and therefore benefits from prior knowledge, including memory for the location of a specific item in a given scene context. The present target detection tasks incorporate quasi-naturalistic scene crowding, and provide more realistic simulations of daily life events for which we depend on our memory, such as having to remember where in the house one put one's keys. The

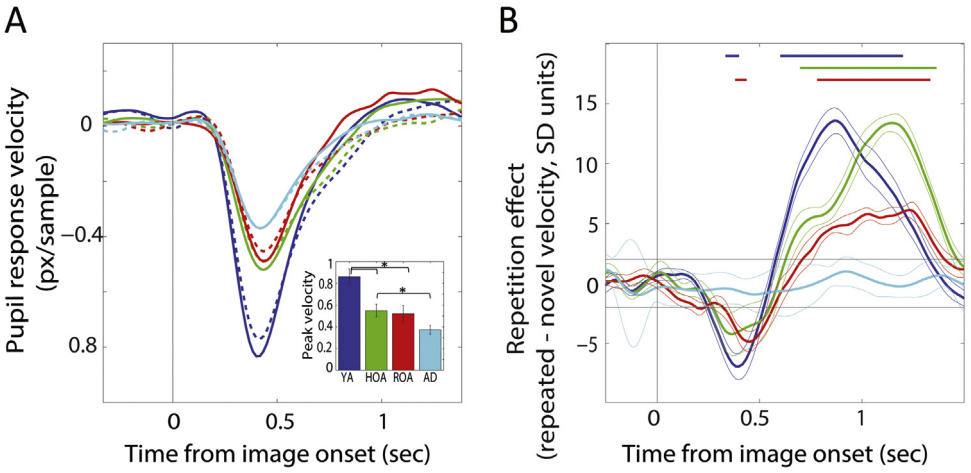

Fig. 6. Pupil-velocity responses following image onset in Experiment 2 target detection task. (A) Average pupil velocity following image onset in novel and repeated trials across groups. Novel-trial velocity indicated with dotted lines; repeated-trial velocity with solid lines. Conventions as in Fig. 4. *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). *p < = 0.01. (B) Memory-related differences in pupil velocity responses 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 Fig. 6A. The times of significant differences between novel and repeated velocities are plotted at the top of the plot, in colors corresponding to the groups: YA in blue, HOA in green (**p < 0.01**) and ROA in red (**p < 0.05**), both FDR corrected for multiple comparisons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

time to detect the target was the intuitive measurement to detect age-related changes in memory in this task, but it was not the only one.

4.3. Pupil dynamics

Pupillary responses to scenes followed the same pattern of changes across groups: they were stronger in younger than in older adults, and were further diminished in the AD group. Furthermore, in the first experiment, we found memory effects in healthy older adults, but not in our memory-impaired individuals (ROA or AD groups). In the second experiment, which was faster and generally easier, we found a similar profile of diminished pupil responses with aging, aged-impairment, and frank AD. In healthy young adults, pupillary responses (constriction followed by dilation, like those described here) have been positively correlated with target detection during search in complex natural scenes [63], with recognition memory of scenes [56], and with paired-associate cued recall [85]. These studies varied in the metrics used to calculate pupillary responses, from absolute levels, to slopes/rates of pupil change. In the present study, we focused on the dynamics of the response, expressed in the pupil size velocity, which is independent of absolute pupil size and slowly shifting baselines.

The pupillary response is known to occur more generally with changes in cognition, as a function of cholinergic (ACh) inputs, as well as from noradrenergic (NE) inputs originating in the locus coeruleus (LC [43,5,69,34]). Change in pupil size and motility also occurs with aging [49], and is further affected in AD [62], and this is attributed not to peripheral neuromuscular atrophy but to underlying changes in neuromodulatory tone [42]. Despite much attention given to the transentorhinal region as one area showing early signs of AD pathology, brainstem nuclei – and specifically the LC – show some of the earliest changes, including abnormal tau protein deposition years before clinical symptoms, followed by frank cell loss near the onset time of cortical histopathology like plaques and tangles [6,7,35,10]. The aging and dementia-related autonomic changes [16,26] have not previously been linked to the ACh/LC-mediated pupillary responses in recognition memory.

We found that AD patients exhibited a decreased pupillary response compared to all other groups, with no difference between the response to novel and repeated trials in either task. Because this measure showed memory-related pupillary responses in healthy populations that were diminished in at-risk individuals and absent in this AD population, it may be a biomarker for early diagnosis. The present experimental design differs from the pupil assay, which uses pharmacological intervention (tropicamide application) to measure changes in baseline pupil size under cholinergic antagonism [70–72]; here, we measure pupillary changes at the onset of goal-directed perception and memory with no pharmacological intervention.

4.4. Limitations and considerations

Limitations of the findings for the Alzheimer's group include the small population sample and the unknown specificity of the effect to AD, as opposed to other neurodegenerative diseases [27,79]. In addition, more research will be needed to determine the relative contribution and interactions between cholinergic and noradrenergic activity with age, with the progression of neurodegenerative disease, and in MTL-dependent memory formation and expression.

The (non-flicker) version of the target detection task may be best-suited for widespread use. The time needed to administer such tests is a major consideration for test viability. 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 thereby rendering less robust results. We found that the shorter target detection task revealed the same impairments in the AD group that they showed in the longer, flickering version of the task, suggesting the flickering image *per se* was not a critical factor in producing impairments. The (non-flicker) target detection task can be rapidly administered with a maximum six-minute time period, making the task a fast, low-cost testing option amenable for use with larger groups across multiple testing sites, such as clinical trials with treatments intended to modify function in the extended hippocampal circuit [46,51,76]. The dynamic range of this task, though, was more limited, with young adults quickly reaching performance ceilings. Adjustment of image size and distractor complexity, and the number of images for retention, may increase difficulty, though potentially at the cost of floor effects in the memory-impaired groups. One potential compromise would be a shortened flicker task, as the full 45-s was not necessary to reveal group differences (Fig. 3C).

Other methodological limitations were differences in viewing distance between young and older adult groups leading to a smaller subtended image size for older adults (~15% smaller), and possible eye detection and perceptual/oculomotor performance differences. The diversity of measures showing differences across groups, and the improvements seen with added repetition in the older groups (Fig. 5), suggest that these methodological limitations did not prevent the detection of valid memory effects. In addition, the visual angle subtended by the target object did not predict performance on repeated trials, though future studies should equate stimuli and viewing distance across groups to balance the possible effects of complex interactions between target size, target and scene semantic and visual content. Furthermore, the aging effects were paralleled among the older groups, who showed similar eye signal and viewing distances. Nevertheless, reduction in eye signal quality and calibration accuracy are common when testing older populations and need to be considered. One testable prediction is that repeated search would be speeded independent of the 'plant' used to respond (e.g. hand versus eye). In this case, touchscreen touches could replace saccade fixations, with the prediction that touch-delivered detection would occur more quickly for remembered trials, and consequently, would reveal the same pattern of responses, namely, memory deficits with aging and in AD.

4.5. Conclusions

The present scene search task measures hippocampal-dependent memory for trial-unique objects-in-context, while avoiding potential confounds with language or visuo-proprioceptive deficits that may also arise in AD populations. The memory impairments seen with age and additionally with AD status were measured using an identical experimental paradigm across age groups and dementia status, demonstrating a wide dynamic range for the task, and general feasibility for populations with varying cognitive abilities. The main dependent variable was target detection times, which could, in principle, be extended to settings without the use of an eye-tracker, though the eye signal also revealed scanning differences across populations. Finally, the degeneration of the LC early in AD, and the role of the LC and ACh in regulating the pupillary response, suggest that pupillometry could indicate early and progressing stages of dementia. We found memory-related pupillary responses in our healthy, but not memory-impaired populations, suggesting an additional potential biomarker warranting further study.

Conflict of interest

None.

Author contributions

Michelle C. Dragan: Designed research, Performed research, analyzed data, wrote the paper.

Timothy K. Leonard: Contributed research/analytical tools, analyzed data.

Andres M. Lozano: Designed research, contributed research/analytical tools.

Mary Pat McAndrews: Contributed research/analytical tools.

Karen Ng: Contributed research/analytical tools.

Jennifer D. Ryan: Designed research, contributed research/analytical tools.

David F. Tang-Wai: Contributed research/analytical tools.

Jordana S. Wynn: Designed research, performed research.

Kari L. Hoffman: Designed research, performed research, analyzed data, wrote the paper.

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