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Eye Movement Latency Coefficient of Variation as a Predictor of Cognitive Impairment: An Eye Tracking Study of Cognitive Impairment

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Abstract: Studies have demonstrated impairment in the control of saccadic eye movements in Alzheimer's disease (AD) and people with mild cognitive impairment (MCI) when conducting the pro-saccade and antisaccade tasks. Research has shown that changes in the pro and antisaccade latencies may be particularly sensitive to dementia and general executive functioning. These tasks show potential for diagnostic use as they provide a rich set of potential eye-tracking markers. One such marker, the coefficient of variation (CV), has so far been overlooked. For biological markers to be reliable they must be able to detect abnormalities in preclinical stages. MCI is often viewed as a predecessor to AD with certain classifications of MCI more likely than others to progress to AD. The current study examined the potential of CV scores on pro and antisaccade tasks to distinguish participants with AD, amnestic MCI (aMCI), non-amnesiac MCI (naMCI) and older controls. The analyses revealed no significant differences in CV scores across the groups using the pro or antisaccade task. Antisaccade mean latencies were able to distinguish participants with AD and the MCI subgroups. Future research is needed into CV measures and attentional fluctuations in AD and MCI individuals to fully assess this measures potential to robustly distinguish clinical groups with high sensitivity and specificity.

Keywords: Alzheimer's disease; Saccades; Eye movements; Latency; Coefficient of variation

1. Introduction

Eye movements are a powerful tool for assessing cognitive functioning [1-3]. Alzheimer's disease is a prominent neurodegenerative disease that results in abnormalities in the control of eye movements [4-6]. Due to the current clinical diagnostic tests, AD often goes undiagnosed until later stages making treatments and interventions less effective. Treatments for AD are most effective when administered in the early stages of the disease prior to neurodegeneration in the brain becoming widespread and rendering treatments ineffective [7]. Current diagnostic methods which are capable of detecting AD in the early stages are either invasive (lumbar puncture for cerebrospinal fluid sample) or expensive (neuroimaging). Eye tracking could provide an invaluable indicator for neurodegenerative disorders and impaired cognitive functioning offering a cost effective and non-invasive alternative [8-10]. Multiple eye tracking markers for impairment have not been assessed or compared. The current study aims to assess potential impairment markers on pro and antisaccade tasks and their sensitivity in identifying established dementia and the preclinical stages, mild cognitive impairment.

In clinical populations and healthy adults, the antisaccade task has been widely used to assess inhibitory control [11,12]. The antisaccade task requires a participant to inhibit shifting their gaze towards the displayed target and instead look towards the opposite

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side [13, 14]. Due to a reduction in inhibitory control, disengagement of attention and a decline in working memory and executive functioning [15] people with AD are significantly slower at performing pro and anti-saccadic eye movements resulting in an increase in mean latencies [16-19]. In an addition to cognitive slowing, Crawford et al [15] demonstrated higher error rates and uncorrected errors in AD on the antisaccade task that correlated with dementia severity. Apparently, top-down executive control is required to inhibit the eye gaze from shifting towards the target and this top-down processing requires working memory resources often impaired in people with AD [20].

Deficits in eye tracking performance are evident when assessing antisaccades in people with AD [21], however, this has not been fully investigated in earlier, preclinical stages such as aMCI and naMCI groups. For a biological marker to be beneficial it must be sensitive enough to detect subtle signs of impairment in the preclinical stage. MCI is a clinical syndrome characterised by cognitive impairments which are atypical for a person's age. MCI has traditionally been classed as a distinct stage of dementia due to the deficits not being sufficiently severe to significantly impact on a individuals daily living and capabilities [22, 23]. However, there is a growing case that MCI should be classed as a preclinical stage between normal cognitive health and AD [7]. There are two subgroups of MCI, amnesic MCI (aMCI) and non-amnesic MCI (naMCI) [24]. People with aMCI experience greater memory impairments than naMCI whereas people with naMCI often have preserved memory but display other cognitive impairments such as executive functioning deficits. People with aMCI are deemed at a greater risk of progressing to AD then naMCI [25, 26]. Previous research assessing MCI subtypes in relation to eye movement performance found that eye movement paramotors such as latencies and error rates were able to distinguish between naMCI and aMCI [27]. Interestingly results showed aMCI participants performed more similarity on the antisaccade task to AD participants and naMCI more similarity to healthy controls. This provided further support for the antisaccade task as a useful task to identify and monitor cognitive impairment and even be successful in distinguishing subtle differences between MCI subgroups [28].

Research to date indicates that fluctuations of eye movement latencies could serve as an additional impairment marker [17]. When programming a saccadic eye movement there is a decisional process that takes place prior to the eye movement [29]. This decisional process is often measured as the time taken between target onset and threshold for triggering the goal-directed saccade. The time required to initiate a saccadic eye movement relies on the resources of executive functioning and attentional processing capabilities therefore impairments in these operations can result in reductions in processing speed and increased latency fluctuations. Therefore, latency variability could be an indicator of attentional fluctuations when completing these tasks. Participants with attentional deficits often show a greater fluctuation of task latencies and scores [17]. This indicates less consistency and reductions in sustained attention across the course of the task indicating attentional processing deficiencies [30]. A measure of latency variability on pro and antisaccade tasks may offer markers for further distinctions between healthy adults and people with memory impairments.

The current study investigated attentional fluctuations using a measure of relative variability termed the coefficient of variation (CV). This measure takes the ratio of the standard deviation in relation to the mean. The higher the CV, the greater the level of dispersion around the mean score. The lower the CV percentage the more precise and less variability the measure is. CV could be an additional biological marker for impairment, alongside existing other eye tracking makers such as mean latencies and error rates. Yang et al [17] assessed CV scores on prosaccade eye movements on a gap and overlap version of the task. Results showed higher CV in latencies for AD participants than for healthy adults and aMCI participants. Increased variability of accuracy and speed was also

abnormality higher in AD participants in both vertical and horizontal saccades [18]. This indicates the potential for CV in latencies on the prosaccade task to distinguish between AD and healthy adults. The current study expanded on this research by assessing CV in latencies on a wider range of tasks (prosaccade and antisaccade) and in a wider group of participants with the addition of naMCI participants. The addition of the naMCI will provide information on the potential of latencies CV scores to distinguish between subgroups of MCI participants which is vital in identifying more at-risk groups for AD.

In summary, the current study investigated the potential of mean latencies, latency CV measures and error rates as biological markers for impairment on prosaccade and antisaccade tasks. These measures will be evaluated on their potential to detect cognitive impairment particularly in distinguishing preclinical stages of dementia by comparing AD, aMCI, naMCI in relation to healthy older adults.

2. Materials and Methods

2.1. Participants

The study included 65 participants with diagnosis of dementia due to AD (Mean age =74.15, SD= 7.75), 42 with aMCI (Mean age =73.71, SD=7.42) and 47 naMCI (Mean age = 69.26, SD = 6.89) and 98 older adult controls (Mean age =67.80, SD= 8.10). The AD and MCI participants were recruited from various NHS sites and memory clinics across the UK. The AD participants met the requirements for the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) for AD. All AD and MCI participants had received a full assessment from a qualified NHS dementia specialist. The MCI participants had a formal diagnosis and met the following criteria [31]: (1) subjective reports of memory decline (reported by individual or caregiver/informant); (2) memory and/or cognitive impairment (scores on standard cognitive tests were >1.5 SDs below age norms); (3) Activities of daily living were moderately preserved. To subgroup the MCI participants into aMCI and naMCI, the Free and Cued Selective Reminding test with Immediate Recall (FCSR-IR) task (see below) scores were used for classification [21].

Control participants were recruited via opportunity sampling. Participants with focal cerebral lesions, history or neurological disorders, neurodegenerative disease, cerebrovascular disease or alcoholism were excluded. Control participants who scored less than 26 on the Montreal Cognitive Assessment (MoCA) [32] were excluded from the final analysis. All participants were deemed to have capacity to consent to participation in the study and informed consent was obtained from all subjects involved in the study. Ethical Approval was granted by Lancaster University Ethics committee and NHS Health Research Authority, Greater Manchester West Research Ethics Committee.

2.2. Cognitive assessments

Participants completed four cognitive assessments. The Montreal Cognitive Assessment [32] assessed cognitive impairment with a score lower than 26 an indicator of probable dementia. The digit span assessed verbal working memory taken from the Wechsler Adult Intelligence Scale III [33] both forwards and backwards versions of the task. Spatial memory was assessed using the Spatial Span task via the use of the Corsi block [33] for both forwards and backwards versions. As recommended by the International Working Group on Alzheimer's Disease, the FCSR-IC task was conducted [34] due to its high sensitivity in differentiating between AD and MCI subgroups [35]. Participants were asked to memorise 16 drawings (presented 4 at a time), and these were linked to category cues to be used as memory prompts. Participants were asked to search the 4 imagines, point to

and name the item (for example onion) based on the category clue verbally given (a vegetable). The card was then removed, and participants asked to recall the four items based on the category clue. Participants were reminded of any items and corresponding cue if unable to recall or identify. This procedure was repeated for all 16 items. The test phase consisted of three recall trials each preceded by a 20 second counting distractor task. For each trial, participants were given two minutes to freely recall the items. Following this, category cues were provided for items they were unable to recall. The task provides a measure of free recall and cued recall for correct responses (a total of 48 for both scores). MCI participants who scored equal to or below 27 on the free recall score were classified as aMCI and scores over 28 classified as naMCI as recommended by Lemos et al [31].

2.3. Eye Tracking Tasks

Eye movements were recorded via the EyeLink Desktop 1000 at 500Hz. A chin rest was used to reduce head movements. Participants sat approximately 55cm away from the computer monitor (60Hz). Participant's gazes were calibrated and validated using 9-point calibration prior to each task. The stimulus was created and controlled via the use of Experiment Builder Software Version 1.10.1630. The data were analysed and extracted using Data Viewer Software Version 3.2.

2.3.1. Prosaccade Task

Participants were presented with 36 gap trials followed by 12 overlap trials. A white fixation target was displayed for 1000ms in order to centre the participants gaze, followed by a red target presented randomly to the left or right at 4° for 1200ms. Participants were instructed to first look towards the white fixation point at the centre of the screen and then towards the red target as quickly and accurately as possible. For the gap condition, there was a blank interval screen displayed for 200ms between the extinguishment of the white fixation target and the initial appearance of the red target. This resulted in a temporal gap in stimuli presentation (figure 1a). In the overlap condition, the target was presented while the central fixation point remained on the screen for 200ms. There was an overlap in stimuli presentation resulting in the target and the fixation point being displayed simultaneously for 200ms (figure 1b). After a short period, the central fixation was removed, and the target presented singularly for 1200ms.

Figure 1a. Timings and display presentation screens for the prosaccade task gap condition. Task instructions required participants to look towards the red target.

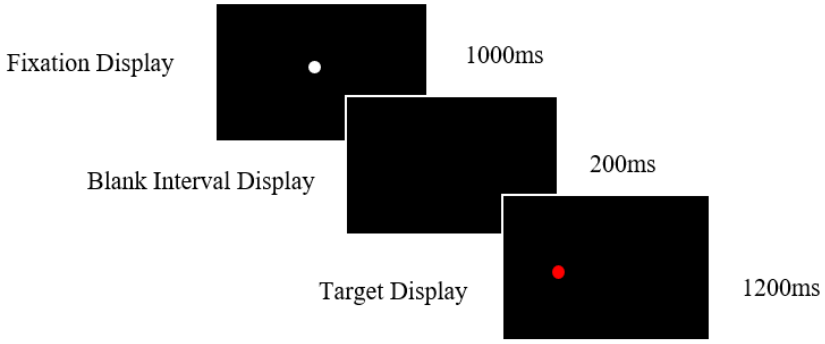
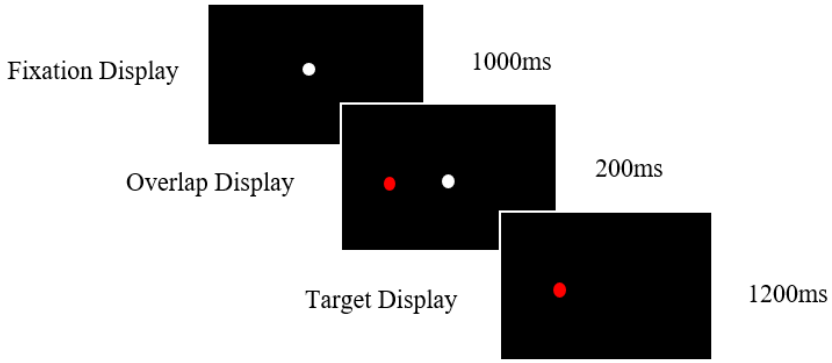


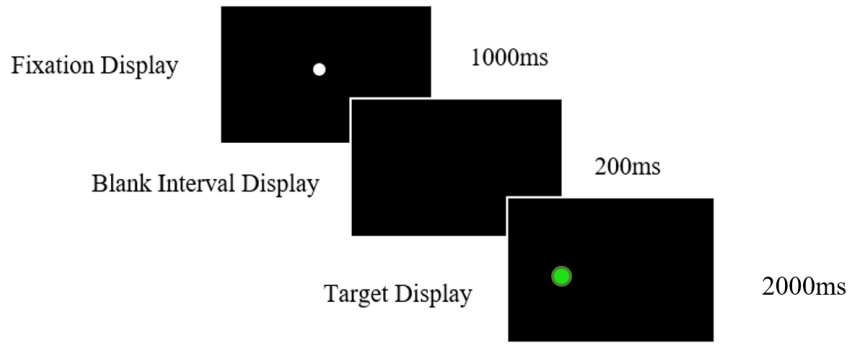
Figure 1b. Timings and display presentation screens for the prosaccade task overlap condition. Task instructions required participants to look towards the red target.



2.3.2. Antisaccade Task

Participants completed 24 gap trials and 4 practice trials. Participants were presented with a central white fixation for 1000ms followed by a green target on the left or right side of the screen presented for 2000ms. Participants were instructed to direct their gaze and attentional focus to the opposite side of the screen to which the target appeared (figure 2). There was a 200ms gap in presentation of the fixation point and the target in which a blank interval screen appeared. Participants needed to generate the saccade to the opposite side of the screen to which the target was displayed to perform a successful anti-saccade.

Figure 2. Timings and display presentation screens for the antisaccade task. Task instructions required participants to ignore the green target and move their gaze to the opposite side of the screen.



2.4. Data Processing

The raw data was extracted and analysed via EyeLink using DataViewer Software Version 3.2. A bespoke software [36] was then used to analyse the data offline. This software removed spikes and noise by filtering out frames with a velocity signal greater than 1,500 deg/s or with an acceleration signal greater than 100,000 deg2/sec. The EyeLink Parser was used to detect the fixations and saccadic events and the saccades were extracted alongside multiple temporal and spatial variables. Trials were removed in cases when the participant did not direct their gaze to the central fixation. The temporal window of 80-700ms used and measured from the onset of the target display. Anticipatory saccades made prior to 80ms and excessively delayed saccades made after 700ms were removed. Latency CV scores were calculated using the following formula: latency standard deviation/mean latency*100.

2.4. Statistical Analysis

The results were analysed using ANOVA models via SPSS version 28. Participant's eye tracking mean latencies and latency standard deviations were compared with performance on the cognitive assessments and group effects were assessed. One MCI participant was excluded from the analysis due insufficient eye tracking data. To examine the effect of group on cognitive performance (MoCA, digit span, spatial span and FCSR-IC) an ANOVA was performed. For the eye tracking tasks (prosaccade gap, prosaccade overlap and antisaccade task) ANOVA's were performed comparing the effects of participant group on eye tracking mean latencies and CV scores. Pearson Correlations assessed the relationship between the eye-tracking markers and cognitive assessment performance.

3. Results

3.1. Cognitive Assessments

An ANOVA was performed to assess the effect of group on cognitive performance on the MoCA, Digit span, spatial span and FCSR task. For the MoCA results revealed a significant effect of participant group, $F(3, 247) = 73.99$, $p < .001$. Post hoc comparisons revealed AD produced significantly lower scores compared to older adults and naMCI participants. There was no significant difference between AD and aMCI participants on MoCA score. There was a significant difference between the MCI subgroups with naMCI producing significantly higher scores then aMCI. Further aMCI and naMCI participants also expectedly scored lower when compared to older controls (see Table 1).

For the digit span task, there was an effect of participant group ($F(3, 228) = 6.98$, $p < .001$) with AD participants scoring lower than older controls on the task. Further aMCI also scored significantly lower than controls on the task, although no significant difference was found between controls and naMCIs. There were no further significant differences between the groups.

There was a significant group effect on spatial task performance, $F(3, 222) = 15.10$, $p < .001$. AD participants scored lower compared to controls and naMCI participants. Both MCI subgroups produced significantly lower scores when compared with controls. There were no further significant differences between the MCI subgroups.

The FCSR task has a significant effect of participant group $F(3, 163) = 20.96$, $p < .001$ when assessing total task score with AD participants scoring lower then controls and both MCI subgroups. There were no significant differences between the MCI subgroups and the controls.

Table 1. Table displaying means, standard deviations and post hoc contrasts for MoCA, Digit Span, Spatial span and FCRS task score for all participant groups.

	Alzheimer's Disease (n=65)		**aMCI (n=42)**		**naMCI (n=46)**		**Healthy Older Controls (n=98)**		**Post Hoc Contracts (P values)**					
											Disease Effects			
	M	SD	M	SD	M	SD	M	SD	AD vs OC	AD vs aMCI	AD vs naMCI	aMCI vs naMCI	aMCI vs OC	naMCI vs OC
MoCA	19.98	5.71	20.93	4.46	25.34	2.17	28.02	1.79	<.001*	.577	<.001*	<.001*	<.001*	<.001*
Digit Span	15.64	4.12	16.35	3.66	16.66	4.79	18.72	4.48	<.001*	.850	.631	.988	.023*	.050
Spatial Span	11.34	3.12	12.58	3.10	13.00	2.55	14.56	2.81	<.001*	.178	.022*	.919	.004*	.021*
FCSR-IC	36.48	14.72	45.10	4.41	47.39	1.29	47.73	0.94	<.001*	<.001*	<.001*	.592	.401	.996

Note. Dependent variable: Task score.

*Significant at p<.05 level

3.2. *Prosaccade Task - Gap Condition*

3.2.1. Mean reaction times and coefficient of variation group effects

Results revealed no significant effects of participant group on prosaccade mean reaction times, F(3, 169) = 1.78, p =.153 (Table 2). When assessing CV measures, there was a significant effect of participant group on CV scores, F(3, 169) = 2.70, p =.047. Post hoc comparisons revealed that the older adult group displayed lower coefficient of variation scores indicating less variation in prosaccade reaction times during the task however this was not statistically significantly. Interestingly there was no significant difference between AD and older controls.

Table 2. Table displaying means and standard deviations for mean latencies and CV scores and post hoc contracts for the prosaccade task gap condition.

	Alzheimer's Disease (n=31)		**aMCI (n=29)**		**naMCI (n=27)**		**Healthy Older Controls (N=71)**		**Post Hoc Contracts (P values)**					
											Disease Effects			
	M	SD	M	SD	M	SD	M	SD	AD vs OC	AD vs aMCI	AD vs naMCI	aMCI vs naMCI	aMCI vs OC	naMCI vs OC
Mean Latencies	215	31.88	201	39.14	226	60.33	203	48.56	.648	.770	.826	.351	.997	.163

Coefficient of Variation	23.14	10.03	26.93	17.09	25.57	15.62	19.77	12.41	.627	.687	.916	.720	.060	.271

Note. Dependent variable: Reaction times.

*Significant at p<.05 level

3.2.2. Correlations between prosaccade markers and cognitive assessments.

Correlations were conducted to compare the eye tracking measures (mean latencies and CV scores) and the cognitive assessment scores. Due to the variations between the participant groups, correlations were assessed for the groups individually. Interestingly there was no single task which consistently correlated with mean latencies or CV across the groups. The aMCI group showed correlations between CV score and the digit span task backwards version (r(17) = -.486, p = .048) and for the spatial span task, forwards (r(17) = -.492, p = 046), backwards (r(17) = -.512, p = . 036) and total scores (r(17) = -.548, p = . 023) and also for MoCA task score (r(17) = -.551, p = .022). Participants with higher task scores produced lower CV indicating less variation in latencies across prosaccade trials. The aMCI group also showed a significant correlation between mean latencies and MoCA task score (r(17) = -.543, p = . 024). However, this was not consistent across the other groups. The controls showed a significant correlation between CV score and backwards digit span score (r(56) = -.299, p = .025) and total score (r(56) = -.268, p = .046), again with higher task score correlating with less fluctuation in latencies. Further the AD and naMCI group did not show any correlations between eye tracking latencies and cognitive assessments indicating a weak link between these markers.

3.3. Prosaccade Task – Overlap Condition

3.3.1 Mean reaction rimes and coefficient of variation group effects

When assessing group effects on mean reaction times table 3 revealed there were no significant differences between the groups, F(3, 167) = 2.55, p =.058. The overlap condition often leads to a delay in disengaging attention from the fixation point which may have resulted in less variation between groups when initiating the saccade. Table 3 revealed no significant differences in CV scores across the participant groups (F(3, 167) = .354, p =.786), indicating limited potential for distinction between participants groups for this task.

Table 3. Table displaying means and standard deviations for mean latencies and CV scores and post hoc contracts for the prosaccade task overlap condition.

	Alzheimer's Disease (n=43)		**aMCI (n=29)**		**naMCI (n=27)**		**Healthy Older Controls (n=69)**		**Post Hoc Contracts (P values)**					
												Disease Effects		
	M	SD	M	SD	M	SD	M	SD	AD vs OC	AD vs aMCI	AD vs naMCI	aMCI vs naMCI	aMCI vs OC	naMCI vs OC
Mean Latencies	274	57.61	234	62.45	273	74.51	254	71.51	.462	.070	.999	.127	.509	.601

Coefficient of Variation	37.94	19.29	38.96	18.20	36.44	19.04	34.93	18.15	.857	.997	.989	.966	.814	.986

Note. Dependent variable: Reaction times.

*Significant at p<.05 level

3.3.2. Correlations between prosaccade markers and cognitive assessments-overlap

Similar to the prosaccade gap condition there was little consistency across groups when assessing correlations. The aMCI group showed a correlation between mean latencies and spatial span total score (r(23) = .454, p = .030) and FCSR free recall score (r(29) = .418, p = .024) but unlike the gap condition here were no correlations between CV scores and cognitive task score. The control group showed a significant correlation between mean latencies and the FCSR total score with participants who score higher on the task displaying lower mean latencies (r(31) = -.442, p = .013). There were no significant correlations found for the AD and naMCI consistent with the gap condition.

3.4. Antisaccade task

3.4.1. Correct trials mean reaction times and coefficient of variation group effects

Results revealed a significant effect of participant group on antisaccade mean reaction times, F(3, 238) = 13.54, p <.001. Post hoc comparisons revealed that the AD group produced significantly slower saccade reaction times compared to healthy older adults (Table 4), indicating reductions in processing speed and inhibitory control deficits. The AD and aMCI group produced comparable saccade reaction times supporting previous research that AD and aMCI show similar impairments and deficits. The AD and naMCI produced significantly different results with the AD group producing slower saccade reaction times then the naMCI group. The naMCI group performed similarly to healthy controls with no significant difference in saccade reaction times. The aMCI group produced significantly slower saccade reaction times than the naMCI group which again supports previous research on distinctions between naMCI and aMCI participants with aMCI performing more similarly to the AD and the naMCI more similarity to the healthy older controls (Table 4). There were no significant differences in measures of CV between the participant groups, F(3, 238) = 2.21, p = .087 . This indicates that the variability of scores and performance on the antisaccade task is not affected by disease. The AD and MCI group do not display differences in CV when compared to healthy adults indicating comparable and typical levels of attentional fluctuation on the task.

Table 4. Table displaying means and standard deviations for mean latencies and CV scores and post hoc contracts.

Alzheimer's Disease (n=65)		**aMCI (n=42)**		**naMCI (n=47)**		**Healthy Older Controls (n=88)**		**Post Hoc Contracts (P values)**					
								Disease Effects					
M	SD	M	SD	M	SD	M	SD	AD vs OC	AD vs aMCI	AD vs naMCI	aMCI VS naMCI	aMCI vs OC	OC naMCI VS
404.34	86.34	418.91	81.70	363.05	61.61	338.12	83.91	<.001*	.804	.041*	.008*	<.001*	.320

Mean Latencies														
Coefficient of Variation	23.57	10.43	20.55	5.80	25.04	6.79	24.74	10.30	.858	.376	.854	.133	.080	.998

Note. Dependent variable: Reaction times.

*Significant at p<.05 level

3.4.3. Correlations between antisaccade markers and cognitive assessments

In contrast to the prosaccade task, the AD group revealed a significant correlation between antisaccade mean latencies and the digit span forwards score (r(60) = -.324, p = . 011). Further CV score correlated with FCSR total scores (r(44) = -.389, p = . 009). Participants who score higher on these cognitive tasks produced lower and less variable mean latencies. The only correlation found for the aMCI group was between CV score and digit span forwards task score with again higher task score indicating lower CV scores and less variable latencies (r(38) = -.357, p = . 028). For the naMCI, the only correlation was between CV score and spatial span forward score (r(43) = -.416, p = .006). The control group showed correlations between saccadic mean latencies and MoCA score (r(88) = -.294, p = . 005). These results indicate that there is not a sole cognitive task that consistently correlate with the eye tracking markers across the groups. However, it is clear from the results that higher cognitive functioning and higher task scores often leads to lower mean latencies and saccadic processing speeds and less variation in latencies indicating less attentional fluctuation.

3.5. Error rates

An error was defined as a saccade in the direction of the presented distractor target. This was determined based on the first saccade in the direction of left or right. An ANOVA was performed to assess the group effects on percentage of error trials. Results revealed a significant effect of participants group on percentage error rate (F (3, 243) = 12.96, p < .001), as previously reported in this cohort [18]. Post hoc comparisons revealed that AD participants displayed a significantly higher number of errors compared to naMCI and controls (Table 5). AD participants produced a similar number of errors on the task to aMCI resulting in no significant difference between AD and aMCI participants. The aMCI group produced significantly higher percentage error rates compared to naMCI and controls, indicating that they performed more similarly to the AD group then the naMCI group. Further there was no significant difference between error rates when comparing the naMCI and the control group. This indicates that naMCI produce error rate more similarly to controls then aMCI and AD participants. Error rates on the antisaccade task may be successful at distinguishing between AD and aMCI participants from naMCI and controls.

Table 5. Table displaying mean and standard deviations and post hoc contracts for percentage error rates for all participant groups.

	Alzheimer's Disease	**aMCI**	**naMCI**	**Healthy Older Controls**	**Post Hoc Contracts (P values)**
					Disease Effects

	M	SD	M	SD	M	SD	M	SD	AD vs OC	AD vs aMCI	AD vs naMCI	aMCI VS naMCI	aMCI vs OC	OC naMCI VS
Percentage error rate	26.13	28.80	30.11	30.02	12.40	10.75	10.36	10.98	<.001*	.773	.004*	.001*	<.001*	.951

Note. Dependent variable: Percentage error rate.

*Significant at p<.05 level

4. Discussion

The current study assessed the effectiveness CV as an additional biological marker alongside well-founded measures such as mean latencies and antisaccade error rates. The study assessed mean latencies and CV on the prosaccade and antisaccade tasks. The CV measure provides a proxi measurement of latency fluctuations throughout the task. Given previous research finding greater attentional fluctuation (determined by higher CV scores) on prosaccade eye tracking tasks in people with MCI and AD [17,18], it was predicted that this finding would be replicated in the current study and may be evident on other similar eye tracking tasks such as the antisaccade task. However, results from the current study showed no significant differences in CV measures across the groups on the pro or antisaccade task. This failure to replicate could be due to a lack of sensitivity and robustness of CV scores particularly in detecting more subtle variations between AD, MCI subgroups

Another key finding revealed that antisaccade mean latencies were able to distinguish participants with AD from older controls and between the MCI subgroups showing high sensitivity. Participants with AD produced significantly slower mean latencies indicating a greater difficulty in generating the saccade and a reduction in processing speed. This finding is supported by previous research showing inhibitory control impairments resulting in difficulties performing correct anti-saccades leading to speed reductions and increased difficulty in triggering saccades [37, 38]. Previous research [39] has demonstrated eye movement latencies greatly rely on attentional processes, often impaired in people with AD [40]. The slowing in saccade latencies is likely the result of these attentional impairments [41]. The current study provides further support for the effectiveness of mean latencies and indicates sufficient sensitivity to distinguish between MCI subgroups and preclinical stages of AD.

It has been previously demonstrated that people with AD show more variable latencies than older controls and people with MCI which suggests that higher latency variability is related to greater attentional fluctuation [30,42]. More variable latencies on the task indicate that people with AD have less sustained attentional focus on the task compared to older controls and MCI participants and this is likely to be due to damage to regions of the brain responsible for executive functioning and attentional processing. Yang et al [17] found a higher latency CV, increased variability of accuracy and abnormally high latencies for people with AD compared to healthy adults and MCI participants. It was stated that the latency and latency variability abnormalities reflect deficits of cerebral areas involved in the execution and triggering of saccades. However, the results from the current study do not support these findings and instead showed that levels of variation and CV scores were comparable across the groups. It is possible that variations in attentional fluctuation may only be evident in more advanced stages of AD, however it is also possible that the experimental tasks and analysis methods employed in the current study are not sensitive enough to detect more subtle CV variations in early to moderate stages of AD.

CV scores on other eye tracking tasks may prove more sensitive to variations in CV scores in early to moderate stages of AD and preclinical stages and this requires further assessment in the literature. However, previous research has shown higher CV scores and increased attentional fluctuation in MCI participants on the tasks used in this study which does not support this conclusion [17]. These inconsistent finding indicate that CV may not be a reliable and robust marker for cognitive impairment as previously thought in the literature. More research is needed to assess CV scores and their robustness for distinguishing clinical and non-clinical groups on eye tracking tasks

A further key finding was the clear distinction seen on antisaccade task between the MCI subgroups. The aMCI group produced significantly higher antisaccade mean latencies compared to naMCI. This indicates that aMCI have greater deficits in generating and executing saccadic eye movements and the decisional process prior to an eye movement. The time required to initiate a saccade relies on executive functioning and attentional processing capabilities and therefore impairments in these areas results in a slowing in processing speed and increased latencies. The current study indicates reduced capabilities in executive functioning and attentional processes in aMCI compared to naMCI. Antisaccade mean latencies were comparable for the AD and aMCI and significantly different from the naMCI and controls, indicating similar processing and executive functioning capabilities between aMCI and AD participants. The naMCI group performed more similarly to controls again further emphasising this MCI distinction. People with aMCI are more likely to progress to develop AD whereas naMCI are less likely to progress to an AD diagnosis and the pattern of results in the current study supports this deviation. The antisaccade task appears to be a useful tool at highlighting the distinction between these MCI subgroups and provide support for the argument of MCI particularly aMCI to be assessed as a preliminary stage prior to AD or full-blown dementia. The clear distinctions between these groups on the antisaccade task is valuable when assessing biological markers between MCI subgroups to provide vital information on the likelihood of an individual to develop AD and an indication on the severity of this progression.

The relationship of eye tracking mean latencies and CV with paper-based cognitive assessments was assessed. The results revealed that cognitive task scores correlated with mean latencies and CV scores, however the specific cognitive assessment correlating with the eye tracking measure varied for each participant group. The overall tread showed that higher scores on the cognitive assessments correlated with faster mean latencies and lower CV scores. This finding adhered with previous research findings that cognitive ability is reflected in pro-saccade and antisaccade eye movement performance [43, 44]. However, these results also indicate that different cognitive tasks are more effective in predicting mean latencies and CV depending on the participant's group. This brings into question the robustness of eye tracking measure in directly predicting cognitive ability as mean latencies and CV score only correlate with certain cognitive assessments which vary depending on participant group and ability. Further it must also be considered that the cognitive assessments are not sensitive enough to correlate with more subtle variations and changes in mean latencies and CV scores across the groups. This should be assessed with a wider battery of cognitive assessments to further assess consistency between groups.

In summary, the current study assessed the disease effect on pro and antisaccade eye movement latencies, CV and error rates. Certain parameters on the antisaccade task are capable of distinguishing between AD participants, MCI subgroups and older control participants but it is clear that research into the effectiveness of CV as a biological marker for impairment is required further as results do not provide clear evidence of increase attentional fluctional in AD and MCI participants. This conflicts with previous findings which have shown promising findings for CV as an additional biological marker however more research is required to fully assess the robustness and full potential of this variable.

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References

1. Wolf, A., & Ueda, K. (2021). Contribution of eye-tracking to study cognitive impairments among clinical populations. Frontiers in Psychology, 12, 590986.
2. Liu, Z., Yang, Z., Gu, Y., Liu, H., & Wang, P. (2021). The effectiveness of eye tracking in the diagnosis of cognitive disorders: A systematic review and meta-analysis. PloS one, 16(7), e0254059.
3. Tokushige, S. I., Matsumoto, H., Matsuda, S. I., Inomata-Terada, S., Kotsuki, N., Hamada, M., & Terao, Y. (2023). Early detection of cognitive decline in Alzheimer's disease using eye tracking. *Frontiers in Aging Neuroscience*, *15*.
4. Readman, M. R., Polden, M., Gibbs, M. C., Wareing, L., & Crawford, T. J. (2021). The potential of naturalistic eye movement tasks in the diagnosis of Alzheimer's disease: a review. Brain Sciences, 11(11), 1503.
5. Molitor, R. J., Ko, P. C., & Ally, B. A. (2015). Eye movements in Alzheimer's disease. *Journal of Alzheimer's disease*, *44*(1), 1-12.
6. MacAskill, M. R., & Anderson, T. J. (2016). Eye movements in neurodegenerative diseases. *Current opinion in neurology*, *29*(1), 61-68.
7. Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., & Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia, 7(3), 280-292.
8. Crawford, T. J. (2013). Alzheimer's disease: is the clue in the eyes?. Neurodegenerative Disease Management, 3(1), 5-7.
9. Molitor, R. J., Ko, P. C., & Ally, B. A. (2015). Eye movements in Alzheimer's disease. Journal of Alzheimer's disease, 44(1), 1-12.
10. Fernández, G., Castro, L. R., Schumacher, M., & Agamennoni, O. E. (2015). Diagnosis of mild Alzheimer disease through the analysis of eye movements during reading. *Journal of integrative neuroscience*, *14*(01), 121-133.
11. Hutton, S. B., & Ettinger, U. (2006). The antisaccade task as a research tool in psychopathology: a critical review. Psychophysiology, 43(3), 302-313.
12. Fernandez-Ruiz, J., Peltsch, A., Alahyane, N., Brien, D. C., Coe, B. C., Garcia, A., & Munoz, D. P. (2018). Age related prefrontal compensatory mechanisms for inhibitory control in the antisaccade task. *Neuroimage*, *165*, 92-101.
13. Munoz, D. P., & Everling, S. (2004). Look away: the anti-saccade task and the voluntary control of eye movement. Nature Reviews Neuroscience, 5(3), 218-228.
14. Crawford, T. J., Higham, S., Mayes, J., Dale, M., Shaunak, S., & Lekwuwa, G. (2013). The role of working memory and attentional disengagement on inhibitory control: effects of aging and Alzheimer's disease. Age, 35(5), 1637-1650.
15. Baddeley, A. D., Baddeley, H. A., Bucks, R. S., & Wilcock, G. K. (2001). Attentional control in Alzheimer's disease. Brain, 124(8), 1492-1508.
16. Peltsch, A., Hemraj, A., Garcia, A., & Munoz, D. P. (2014). Saccade deficits in amnestic mild cognitive impairment resemble mild Alzheimer's disease. *European Journal of Neuroscience*, *39*(11), 2000-2013.
17. Yang, Q., Wang, T., Su, N., Xiao, S., & Kapoula, Z. (2013). Specific saccade deficits in patients with Alzheimer's disease at mild to moderate stage and in patients with amnestic mild cognitive impairment. Age, 35(4), 1287-1298.

18. Yang, Q., Wang, T., Su, N., Liu, Y., Xiao, S., & Kapoula, Z. (2011). Long latency and high variability in accuracy-speed of prosaccades in Alzheimer's disease at mild to moderate stage. Dementia and geriatric cognitive disorders extra, 1(1), 318-329.
19. Polden, M., Wilcockson, T. D., & Crawford, T. J. (2020). The disengagement of visual attention: An eye-tracking study of cognitive impairment, ethnicity and age. Brain Sciences, 10(7), 461.
20. Polden, M., & Crawford, T. J. (2021). Active visual inhibition is preserved in the presence of a distracter: A cross-cultural, ageing and dementia study. Cortex, 142, 169-185.
21. Opwonya, J., Doan, D. N. T., Kim, S. G., Kim, J. I., Ku, B., Kim, S., ... & Kim, J. U. (2022). Saccadic eye movement in mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. *Neuropsychology Review*, *32*(2), 193-227.
22. Crawford, T. J., Parker, E., Solis-Trapala, I., & Mayes, J. (2011). Is the relationship of prosaccade reaction times and antisaccade errors mediated by working memory?. Experimental brain research, 208(3), 385-397.
23. Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. Journal of internal medicine, 256(3), 183-194.
24. Glynn, K., O'Callaghan, M., Hannigan, O., Bruce, I., Gibb, M., Coen, R., & Robinson, D. (2021). Clinical utility of mild cognitive impairment subtypes and number of impaired cognitive domains at predicting progression to dementia: A 20-year retrospective study. *International Journal of Geriatric Psychiatry*, *36*(1), 31-37.
25. Fischer, P., Jungwirth, S., Zehetmayer, S., Weissgram, S., Hoenigschnabl, S., Gelpi, E., & Tragl, K. H. (2007). Conversion from subtypes of mild cognitive impairment to Alzheimer dementia. Neurology, 68(4), 288-291.
26. Ward, A., Tardiff, S., Dye, C., & Arrighi, H. M. (2013). Rate of conversion from prodromal Alzheimer's disease to Alzheimer's dementia: a systematic review of the literature. Dementia and geriatric cognitive disorders extra, 3(1), 320-332.
27. Koçoğlu, K., Hodgson, T. L., Eraslan Boz, H., & Akdal, G. (2021). Deficits in saccadic eye movements differ between subtypes of patients with mild cognitive impairment. *Journal of Clinical and Experimental Neuropsychology*, *43*(2), 187-198.
28. Wilcockson, T. D., Mardanbegi, D., Xia, B., Taylor, S., Sawyer, P., Gellersen, H. W., & Crawford, T. J. (2019). Abnormalities of saccadic eye movements in dementia due to Alzheimer's disease and mild cognitive impairment. Aging (Albany NY), 11(15), 5389.
29. Hutton, S. B. (2008). Cognitive control of saccadic eye movements. Brain and cognition, 68(3), 327-340.
30. Kapoula, Z., Qing, Y., Vernet, M., Orssaud, C., Samson, M., Dieudonne, B., et al. (2010). Preservation of automatic ocular saccades in healthy elderly: alteration in patients with dementia with Lewy body. Psychologie & Neuropsychiatrie du Vieillissement, 8(4), 295-306
31. Lemos, R., Simões, M. R., Santiago, B., & Santana, I. (2015). The free and cued selective reminding test: Validation for mild cognitive impairment and A lzheimer's disease. Journal of Neuropsychology, 9(2), 242-257.
32. Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., & Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. Journal of the American Geriatrics Society, 53(4), 695-699.
33. Wechsler, D. (1997). WAiS-iii. San Antonio, TX: Psychological Corporation.
34. Grober, E., Merling, A., Heimlich, T., & Lipton, R. B. (1997). Free and cued selective reminding and selective reminding in the elderly. Journal of Clinical and Experimental Neuropsychology, 19(5), 643-654.
35. Cummings, J. L., Dubois, B., Molinuevo, J. L., & Scheltens, P. (2013). International Work Group criteria for the diagnosis of Alzheimer disease. Medical Clinics, 97(3), 363-368.
36. Mardanbegi, D., Wilcockson, T., Sawyer, P., Gellersen, H., & Crawford, T. (2019, June). SaccadeMachine: Software for analyzing saccade tests (anti-saccade and pro-saccade). In Proceedings of the 11th ACM Symposium on Eye Tracking Research & Applications (pp. 1-8).
37. Boxer, A. L., Garbutt, S., Seeley, W. W., Jafari, A., Heuer, H. W., Mirsky, J., & Miller, B. L. (2012). Saccade abnormalities in autopsy-confirmed frontotemporal lobar degeneration and Alzheimer disease. Archives of neurology, 69(4), 509-517.
38. Kaufman, L. D., Pratt, J., Levine, B., & Black, S. E. (2012). Executive deficits detected in mild Alzheimer's disease using the antisaccade task. Brain and behavior, 2(1), 15-21.
39. Clark, K., Squire, R. F., Merrikhi, Y., & Noudoost, B. (2015). Visual attention: Linking prefrontal sources to neuronal and behavioral correlates. Progress in Neurobiology, 132, 59-80.
40. Malhotra, P. A. (2019). Impairments of attention in Alzheimer's disease. *Current opinion in psychology*, *29*, 41-48.
41. Levinoff, E. J., Li, K. Z., Murtha, S., & Chertkow, H. (2004). Selective attention impairments in Alzheimer's disease: evidence for dissociable components. Neuropsychology, 18(3), 580-588.
42. Li, T., Liao, Z., Mao, Y., Hu, J., Le, D., Pei, Y., & Yu, E. (2021). Temporal dynamic changes of intrinsic brain activity in Alzheimer's disease and mild cognitive impairment patients: a resting-state functional magnetic resonance imaging study. *Annals of translational medicine*, *9*(1).

43. Boxer, A. L., Garbutt, S., Rankin, K. P., Hellmuth, J., Neuhaus, J., Miller, B. L., & Lisberger, S. G. (2006). Medial versus lateral frontal lobe contributions to voluntary saccade control as revealed by the study of patients with frontal lobe degeneration. Journal of Neuroscience, 26(23), 6354-6363.
44. Garbutt, S., Matlin, A., Hellmuth, J., Schenk, A. K., Johnson, J. K., Rosen, H., et al. (2008). Oculomotor function in fronto-temporal lobar degeneration, related disorders and Alzheimer's disease. Brain, 131(Pt 5), 1268-1281.