Accepted Manuscript

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PII: S0166-4328(18)30060-3
DOI: https://doi.org/10.1016/j.bbr.2018.07.002
Reference: BBR 11495

To appear in: *Behavioural Brain Research*

Received date: 12-1-2018
Revised date: 20-6-2018
Accepted date: 2-7-2018

Please cite this article as: Niechwiej-Szwedo E, Tapper A, Gonzalez D, Bradley RM, Duncan R, Saccade latency delays in young apolipoprotein E (APOE) epsilon 4 carriers, *Behavioural Brain Research* (2018), https://doi.org/10.1016/j.bbr.2018.07.002

Saccade latency delays in young apolipoprotein E (APOE) epsilon 4 carriers

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The apolipoprotein E (APOE) epsilon 4 isoform has been associated with a significantly greater risk of developing late onset Alzheimer's disease (AD). However, the negative effects of APOE-ε4 allele on cognitive function vary across the lifespan: reduced memory and executive function have been found in older individuals but, paradoxically, young APOE-ε4 carriers perform better on cognitive tests and show higher neural efficiency. This study aimed to assess the association between APOE genotype and saccade latency using a prosaccade and antisaccade task in young individuals (N=97, age: 17-35 years). Results showed that prosaccade latency was significantly delayed in a group of ε4 carriers in comparison to non-carriers, which was due to a lower rate of signal accumulation rather than a change in the criterion threshold. In contrast, there was no significant genotype difference for antisaccade latency in this young cohort. These results indicate that prosaccade latency may be useful in establishing the APOE behavioural phenotype, which could ultimately assist with distinguishing between normal and pathological aging.

1. Introduction

The apolipoprotein E (APOE) gene is a significant genetic risk factor for Alzheimer's disease (AD) [1]. However, the physiological mechanisms involving the APOE genotype in normal and pathological aging have not been fully established. APOE is involved in the regulation of lipid transport, storage, and metabolism in the peripheral and central nervous system [2-4]. There are 3 common alleles of the APOE gene: ε2, ε3, and ε4, resulting in 6 genotypes: 3 homozygotes, and 3 heterozygotes (ε2/ε3, ε2/ε4, ε3/ε4). The three isoforms vary in their effectiveness to bind lipids and transport them across the cell membrane, such that the ε4 allele is the least effective. Consequently, presence of the ε4 allele has been associated with increased amyloid-β (Aβ) peptide aggregation and tangle formation in the brain, reduced neurogenesis, increased neuronal toxicity, and impaired cholinergic function [4]. Epidemiological studies have shown that presence of the ε4 allele is associated with a dose-dependent increased risk of AD [5]. For example, the odds of AD are 2.7- to 3.2-fold higher in Caucasian individuals with ε3/ε4 relative to the ε3/ε3 genotype, whereas the odds increase 12.5-14.9 times for ε4/ε4 heterozygotes. Because the pathogenesis of AD is complex and involves multiple physiological mechanisms [6], it is important to have a better understanding of how the APOE genotype affects different cognitive functions. Moreover, establishing an association between the APOE genotype and the corresponding behavioural phenotype across the lifespan can assist with distinguishing between normal and pathological aging. Eye movements provide a sensitive assay to assess a variety of cognitive functions, including decision making and attention [7-10], and could be used to establish the cognitive phenotype. Therefore, the aim of the current study was to assess the association between APOE genotype and saccade latency in healthy young adults. Such knowledge can assist with identifying individuals who are more likely to experience reduced cognitive function with age, and developing targeted interventions that could be delivered early to slow disease progression, improve quality of life, and reduce the financial burden on healthcare.

Establishing the cognitive phenotype associated with the ε4 allele in younger individuals is an important step towards identifying those who might be at a greater risk of age-related cognitive decline later in life. The APOE-ε4 genotype has multiple negative effects on normal brain function, independent of the Aβ aggregation, including reduced myelination, mitochondrial dysfunction, and impaired cholinergic function [6]. A recent systematic review that included 36 studies which investigated the behavioural effects of APOE-ε4 in mid-adulthood highlighted the complex interaction between APOE genotype and age on cognitive functions [11]. In general, studies that used paper and pencil clinical test batteries showed no significant differences between apparently healthy ε4 carriers and non-carriers across different cognitive domains, such as executive function, memory, visuospatial, and language abilities. In contrast, studies that used computerized testing showed that ε4 carriers tend to perform worse on tests of attention, episodic memory, and working memory, specifically under conditions of increased cognitive load. The detrimental effects of the ε4 allele were detected in the second half of the 5^{th} decade, with an accelerated rate of decline in the 6^{th} decade revealed by longitudinal studies. Even more intriguing are findings from studies that focused on young individuals (<30 years old) where the ε4 carriers performed significantly better on tests of executive function, attention, and memory, which supports the antagonistic pleiotropy hypothesis [12-17]. According to this hypothesis, certain genes affect several traits which may have a differential effect on health across the lifespan, hence, the ε4 allele could have a positive effect on cognitive function in young carriers but the opposite effect in older carriers [12, 18]. To summarize, the effects of APOE genotype on cognitive functions across the lifespan, and its interaction with conditions that can influence brain health remains to be established – especially in the younger individuals.

One behavioural approach that has proven useful in providing insight into cognitive functions is eye-tracking [7-10]. Eye movements have been used extensively over the past 30 years to investigate

executive functions, such as attention [19-21], working memory [22-24], and inhibition [25] in normal and pathological cohorts. An important advantage of studying eye movements is that the neural networks involved in their generation have been largely mapped out [26-30], and computational/statistical models that explain behavior have been developed and tested [31, 32]. For example, Carpenter's LATER (Linear Approach to Threshold with Ergodic Rate) model has been used successfully over the past 37 years to explain neural decision making based on the latency distribution of saccadic eye movements [33]. According to the LATER model, eye movements are initiated when the afferent signal reaches a threshold criterion, and a delayed response can be caused by two factors: change in the criterion level or a decreased rate of signal accumulation. Examining the reciprocal of saccade latency distribution provides insight into which of the two factors underlies the delay in saccade latency. Specifically, the reciprocal distribution can be described by two parameters: mean (μ) and variance (σ^2), and changes in these parameters have specific interpretations: changes in μ are associated with the rate of signal accumulation, and changes in σ are associated with the baseline activation and/or threshold criterion. Using the LATER model to analyse saccade latency distributions thus provides insight into the decision-making process.

Despite the potential advantage of using eye movements to study the effects of APOE-ε4 on cognitive function, surprisingly few studies have taken this approach. A notable exception is the study by Velichkovsky et al [34], which examined antisaccade latency – a measure of inhibition control dependent on the integrity of the prefrontal cortex – but found no genotype differences in a cohort of middle age (50 years old) participants. Our investigation extends this work by assessing the effect of APOE-ε4 genotype on saccade latency using both, a prosaccade and antisaccade task in large cohort of young adults. The rationale for choosing a prosaccade task is rooted in neurobiology, in particular, the association between saccade generation and the cholinergic system. The pedunculopontine tegmental

nucleus (PPTN) in the brainstem is a major source of cholinergic neurons with reciprocal connections with the intermediate/deep layers of the superior colliculus and the basal ganglia nuclei involved in saccade generation [29]. Elegant studies by Kobayashi et al using in vitro and in vivo approaches showed that signal transmission in the direct visuomotor pathway involving the superior colliculus is gated by cholinergic inputs from the PPTN [35]. Specifically, administration of nicotine or acetylcholine (Ach) reduced saccade latency [36]. In contrast to the work by Kobayashi et al, pharmacological studies with humans provide mixed evidence for the effect of nicotine on saccade latency. Specifically, no effects of nicotine were found for prosaccade latency among healthy control participants in studies with patients with schizophrenia [37, 38]. On the other hand, studies that examined the effect of nicotine on antisaccade performance reported reduced errors [38], decreased latency [39], or both [40]. Interestingly, the study by Rycroft and colleagues found a comparable reduction in antisaccade latency following administration of nicotine and modafinil, which is a noradrenergic agonist [39]. The authors considered several mechanisms that could explain the common effect of nicotine and modafinil, and concluded that the action of nicotine on antisaccade performance might not be necessarily modulated solely by the cholinergic system.

To summarize, planning and execution of different types of eye movements is mediated by the cholinergic system. Evidence from non-human primates indicates that prosaccade latency is influenced by direct stimulation of the cholinergic brainstem neurons. In contrast, human pharmacological studies found no effect of nicotine on prosaccades, but antisaccade performance was improved. On the other hand, reduced cholinergic function has been found in both normal, and AD diagnosed post-mortem brains of individuals with APOE-ε4 genotype [41, 42]. Given the contribution of the cholinergic system to oculomotor control, it is important to develop a better understanding of the effects of APOE genotype on saccade latency. Therefore, the goal of this study was to assess the effect of APOE-ε4 genotype on

prosaccade and antisaccade latency. We hypothesized that saccade latency will be delayed in our cohort of young ε4 allele carriers.

Methods

2.1 Participants

A sample of convenience was recruited from the undergraduate and graduate student population at the University of Waterloo (52 males/58 females; mean age = 22.7 years; SD 3.2 years; range 18-35 years). Ninety seven participants were recruited using posters placed across the university campus. All participants completed the University of Waterloo healthy history questionnaire, which contained questions regarding general health, concussion history, and symptoms experienced in the past 6 months. There were 27 participants with a history of sports-related concussion who at the time of testing were asymptomatic and cleared to participate in sport activities by a medical doctor. Therefore, these participants were included in the study. The final sample included in the analysis consisted of 86 Caucasian and 11 Asian participants. Participation in the study was voluntary and participants did not receive remuneration. The study's protocol was approved by the University of Waterloo Research Ethics Board Committee (ORE#19965), and consent was obtained prior to beginning the experiment.

2.2 Genotyping

Ninety-seven out of the one hundred and ten recruited participants were eligible- and agreed to provide a saliva DNA sample for analysis of two single nucleotide polymorphisms (rs429358 and rs 7412) on the apolipoprotein E (APOE) gene. Samples were analyzed using Taqman® SNP genotyping assays (Applied Biosystems, Mississauga, Ontario, Canada). Briefly, ~10 ng of genomic DNA was mixed into a 10 μl final volume containing 5 μl of Accustart Genotyping Toughmix (QuantaBiosciences, Beverly, MA, USA) and 0.25 μl of 40× Taqman® SNP genotyping assay mix with the balance of the volume

comprised of 18 Mega-ohm water. Samples were amplified in a CFX96 Real-time PCR detection machine (Bio-Rad Laboratories, Mississauga, Ontario, Canada) using the following conditions: 1 cycle at 95°C for 5 min followed by 40 cycles alternating between 95°C for 5 s and 60°C for 30 s. APOE genotype runs included negative controls lacking DNA template. Duplicate analyses were run on approximately 20% of samples for quality control purposes and this generated 100% concordance of calls. Genotyping was performed by researchers blinded to behavioural results. Participants were not informed about their genotype by the researchers. Instead, they had an option of having their results sent to their primary physician; however, none of the participants requested this option.

2.3 Experimental procedure

Participants completed two oculomotor tests: prosaccades and antisaccades, which were performed in separate blocks, counterbalanced across participants. Oculomotor testing was conducted in a quiet, well-lit room. Participants were seated 80 cm from a 19'' CRT monitor (ViewSonic CRT monitor, resolution 1024x768, refresh rate 85 Hz) with their chin placed in a chinrest. Eye position data were recorded at a sampling frequency of 250 Hz using the EyeLink II eye-tracker (SR Research, Ontario, Canada). A 5-point calibration method was used to calibrate the eye-tracker using a pupil-only collection mode. Validation of eye tracking was performed after the calibration, where the validation acceptance criterion was set at $<1^{o}$ error to ensure reliability.

The experimental procedure was created using the Experiment Builder software (ver. 1.8; SR Research, Ontario, Canada). The visual stimuli were black and they were presented on a white background. At the beginning of each trial participants were instructed to fixate on a central fixation cross (stimulus size 0.25^{o}) which was presented at eye level. Fixation stimulus duration was randomized between 1500 and 2250 ms. As fixation disappeared, a peripheral target (stimulus size 0.25^{o}) was displayed (step paradigm) for 2 seconds at $\pm 10^{o}$ to the left or right of fixation. For the prosaccade task,

participants were instructed to move their eyes to the peripheral target as quickly possible. In contrast, they were instructed to move their eyes in the opposite direction from where the target was displayed in the antisaccade task. Participants completed 50 trials in the prosaccade task. Directional errors were expected in the antisaccade task, therefore, 60 trials were completed.

2.4 Data Analysis

Chi-squared analysis was conducted first to determine whether a history of concussion was associated with the APOE genotype. Prior to testing our main hypothesis, a two sample t-test was conduced to test for potential differences in saccade latency among participants with and without a concussion history.

All eye movement data were analysed offline using the eyetracker's Data Viewer software (ver 1.8; SR Research, Ontario, Canada). Eye position data from each trial were plotted and visually inspected. Trials were excluded if a blink or loss of eye tracking occurred 250 ms prior to or 500 ms following target presentation (8% trials: <1% of trials were anticipatory [i.e., latency <80 ms], the majority of trials were rejected due to blinks and loss of tracking; range across participants 0-16%). Eye movements were detected using a saccade detection algorithm implemented in Data Viewer: 30º/s velocity threshold, and 8000º/s^2 acceleration threshold. The analysis focused on primary saccade latency, which was defined as the interval following target presentation to saccade initiation. Only saccades made in the correct direction in the antisaccade task (i.e., away from the target) were included in the latency analysis.

The main hypothesis was tested using two approaches. First, latency data were averaged across the trials for each participant and task. Next, data were submitted to analysis of variance (ANOVA) with genotype as the between subject factors. Because of unequal number of participants in each genotype group, Wilcoxon rank-sum test for independent groups was also conducted. The second analysis

approach was based on the LATER model developed by Carpenter [43]. SPIC software (available at http://www.cudos.ac.uk/later.html) was used to analyse the saccade latency distributions obtained from each participant and task. Kolmogorv-Smirnov test was used to confirm model's goodness of fit. Two parameters were extracted to characterize each participant's latency distribution: mean μ and variance σ^2. These two outcome measures were subsequently submitted to an ANOVA with genotype as the between subject factor.

Statistical analyses were conducted using the Statistical Analysis System (SAS) Studio, ver. 3.5 Enterprise Edition (SAS Institute Inc., Cary, NC, USA). Descriptive statistics are reported as the mean and standard deviation, as well as the median and interquartile range (IQR).

3 Results

3.1 Genotyping

The distribution of APOE genotypes for the cohort tested in this study is shown in Table 1. The allelic frequency was ε2 (6.2%), ε3 (74.2%) and ε4 (19.6%). The distribution of APOE alleles was consistent with Hardy-Weinberg equilibrium, Chi^2 (df=3) = 4.96, p>0.05 [44][44][44][44][44][44] [calculator can be found at the following website: http://www.husdyr.kvl.dk/htm/kc/popgen/genetik/applets/kitest.htm]. Due to a small number of participants with less common alleles, our main analysis focused on comparing APOE-ε4 carriers and non-carriers. There were 62 participants who did not carry the ε4 allele, and 35 participants who were ε4 carriers.

There was no significant difference in APOE-ε4 carrier frequency among the groups with and without a history of concussion: 37% of participants in the non-concussed group were APOE-ε4 carrier as compared to 33% in the group with a history of concussion (Chi^2 (df=1) = 0.123, p=0.726).

3.2 Oculomotor tasks

Results from a two-sample t-test comparing the groups with and without a history of concussion are shown in Table 2. There were no significant differences between the groups, therefore, this variable was not analysed further.

Prosaccades. Figure 1 shows the distributions of prosaccade promptness (1/latency) and prosaccade latency using a reciprobit plot in APOE-ε4 carriers and non-carriers. Statistical analysis confirmed a significant main effect of APOE genotype ($F_{(1,95)} = 7.38$, $p=0.008$; $\eta_p^2 = 0.13$). Mean saccade latency was 190±28 ms for the APOE-ε4 carriers compared to 174±24 ms for the non-carrier group (Figure 2). Results from the Wilcoxon two-sample test were also significant ($Z=2.56$, $p=0.010$). The median saccade latency and corresponding interquartile range were 175 ms (158-188 ms) for non-carriers, and 184 ms (166-205 ms) for ε4 carriers. Table 3 shows the results of analysis on subpopulations of saccades in the express range (80-134ms), fast regular range (135-180 ms), and slow regular range (181-400 ms). There were no participants in the ε4 carrier group whose saccade latencies were in the express range, and only 3% of non-carrier participants exhibited express saccades. The majority (65%) of non-carrier participants had saccade latency in the fast regular range. In contrast, 43% of carriers had saccade latency in the fast regular range, and 57% had a saccade latency in the slow range. Therefore, the subanalysis indicates a shift towards longer prosaccade latency in the APOE-ε4 carriers.

Analysis using the LATER model confirmed a significant effect of genotype on parameter μ ($F_{(1,95)} = 7.66$, $p=0.007$; $\eta_p^2 = 0.14$), and no significant effect on parameter σ ($F_{(1,95)} = 1.60$, $p=0.209$; $\eta_p^2 = 0.03$). These results suggest that the delay in saccade latency in the APOE-ε4 group is due to a lower rate of signal accumulation, rather than a change in the baseline activity or the criterion threshold. No

significant differences were found between APOE genotypes for prosaccade amplitude or peak velocity (Table 4).

Antisaccades. There was no significant main effect of APOE genotype for antisaccade latency ($F_{(1,95)}$ = 0.31, p=0.581; η_p^2 =0.01), or mean percentage of errors (i.e., saccades in the wrong direction; $F_{(1,95)}$ = 0.37, p=0.545; η_p^2 =0.01). Non-significant results were also obtained with the Wilcoxon test (Z=-0.51, p=0.610). Antisaccade latency was 255±27 ms for the APOE-ε4 carriers compared to 251±35 ms for the non-carrier group (Figure 2). The percentage of errors was also comparable in both groups, APOE-ε4: 17.8±13% vs. non-carrier: 19.5±14%. Results from the Wilcoxon two-sample test were also non-significant (Z=0.94, p=0.346). The median saccade latency and corresponding interquartile range were 244 ms (228-269 ms) for non-carriers, and 256 ms (234-279 ms) for ε4 carriers Analysis using the LATER model showed no significant effect of genotype on parameter μ ($F_{(1,95)}$ = 0.61, p=0.438; η_p^2 =0.01), and no significant effect on parameter σ ($F_{(1,95)}$ = 3.40, p=0.068 η_p^2 =0.07). There were no significant differences between APOE genotypes for antisaccade amplitude or peak velocity (Table 4).

4.0 Discussion

The main aim of this study was to assess the influence of APOE genotype on saccade latency in young adults. Our results show that latency was significantly longer only for the prosaccade task in a group of APOE-ε4 carriers when compared to a non-carrier group. In contrast, there was no difference among the genotypes for antisaccade latency or error rate. We will discuss these findings in the context of the neural circuitry involved in the generation of saccades, and in particular, the influence of the cholinergic system on the generation of eye movements.

Our study provides the first evidence that APOE genotype has a significant influence on prosaccade latency in a young cohort. Saccadic eye movements have been studied extensively in humans and animal models using a variety of experimental paradigms [45], which show that saccade latency is highly task dependent [46-48]. The current study used a step paradigm, and saccade latency interquartile range was as expected, between 158 and 195 ms, with <5% of all eye movements in the express saccade range (i.e., <135 ms). Our results showed that prosaccade latency is ~16 ms longer in APOE-ε4 carriers, which corresponds to a medium effect size. Similar magnitude effects have been previously reported for saccade latency across different experimental manipulations. For example, saccade latency is reliably longer by ~15-20 ms during monocular compared to binocular viewing [49-51]. In the current study, all participants were tested under identical experimental conditions; therefore, our results provide an important new contribution to the literature by identifying that a single individual/genetic factor, APOE polymorphism, has a significant influence on prosaccade initiation. Importantly, the increase in saccade latency is within the normal range of prosaccade latencies; therefore, it does not represent a deficit in the group of young individuals tested in the current study. Future studies should assess saccade latency in older individuals because this outcome measure may be useful in establishing the APOE behavioural phenotype.

A key neural structure involved in the generation of saccadic eye movements is the superior colliculus (SC). Experimental and computational work indicates that a saccade is initiated when the activity of a saccade neuron in the SC reaches a threshold firing rate [52, 53], which has led researchers to propose a stochastic accumulator model to explain initiation of saccadic eye movements [31]. In accordance with this model, increased latency could be associated with a reduced rate of signal accumulation and/or lower level of baseline activity in the saccade neurons. In support of the model, when the stimulus appearance is less predictable, the SC saccade neurons exhibit less activity, and longer saccade latency [54]. In the context of the LATER model, altering the probability of target

appearance would affect parameter σ, while change in the accumulation rate would be reflected by parameter μ. Our analysis confirmed that APOE-ε4 genotype is associated with a lower signal accumulation rate, rather than a change in baseline firing rate.

The SC receives direct input from brainstem cholinergic regions, specifically the parabigeminal nucleus which projects to the superficial (visual) layers, and the pedunculopontine tegmental nucleus (PPTN) which projects to the deeper (motor) layers. These cholinergic inputs terminate in zones that receive multiple afferent inputs, for example, from the frontal eye fields, the parietal cortex, and the basal ganglia; therefore, it is possible that cholinergic inputs are involved in modulation of signal transmission. Support for this hypothesis was provided by Kobayashi et al in an experiment which showed that application of Ach agonist lowered the threshold firing rate of saccade SC neurons resulting in generation of express saccades [36]. Our behavioural results do not support a shift in saccade latency distribution towards the express saccades, and results from the LATER model showed no difference in parameter σ, which suggests that the APOE genotype does not significantly affect the baseline activity of collicular neurons during a prosaccade task. To summarize, our results indicate that APOE genotype does not have a drastic effect on the cholinergic projection to the deeper SC layers, at least in the cohort of young individuals. Instead, the shift in distribution towards longer latencies was subtle, and latency remained within the range of regular saccades.

In addition to direct projections to the superior colliculus, cholinergic neurons have widespread projection to multiple cortical areas involved in sensorimotor and cognitive processing, including attentional enhancement of sensory processing [55-57]. Specifically, PPTN cholinergic neurons projecting to SC bifurcate and send collateral to the lateral geniculate nucleus [58]; therefore, they can influence visual processing at a very early stage, perhaps by increasing the perceptual salience of visual inputs (i.e., increasing the signal to noise ratio of sensory inputs [36]). These findings are in line with the

hypothesis that optimization of task-related sensory processing is mediated by the cholinergic system (for review see: [56, 57, 59]). In light of the fact that APOE polymorphism affects the activity of choline acetyltransferase, it is possible that the baseline cholinergic function is lower in individuals with the ε4 allele. Consequently, the relatively hypocholinergic state would reduce the efficiency of feedforward afferent transmission leading to longer saccade latency in ε4 carriers [58]. This interpretation is consistent with the results from the LATER model, which showed that the delay in prosaccade latency is due to a lower signal accumulation rate. Moreover, our results are consistent with another study that examined the effects of the APOE genotype on attention shifting using a Posner-type paradigm and a manual button press response in a young cohort similar in age to our sample [16]. Although the effects did not reach statistical significance, response latency was ~30 ms slower on validly cued trials in a group of ε4 carriers compared to non-carriers. Lack of significance could have risen because the sample size was relatively small (i.e., n=41), and the inherent variability in manual reaction time is significantly higher in comparison to the variability found for saccade latency distributions. To summarize, the cholinergic system is integral for generating orienting/reflexive responses, and a reduced cholinergic function seems to be associated with a longer latency of responding. Our study clearly shows that only reflexive responses tend to be prolonged in individuals with the ε4 genotype, which maybe due to reduced cholinergic activity. Importantly, our results do not support a general deficit in speed of information processing because the increased latency was only found in the prosaccade task.

Interestingly, the ε4 genotype was not associated with antisaccade latency or error rate. The lack of association between antisaccade latency and APOE genotype is consistent with the findings from Velichkovsky et al [34]. These results can be explained by considering that initiation of saccades depends on an extensive neural network including parietal and frontal areas, which influence the activity of saccade neurons in the SC, either directly or indirectly via the basal ganglia [29]. Ultimately, the

initiation of eye movements depends on the balance of excitatory and inhibitory inputs to the SC. Because the initiation of prosaccades and antisaccades involves different neural circuits [60], these circuits may be differentially dependent on/influenced by the cholinergic system. For example, prefrontal areas show increased activation during antisaccades as compared to prosaccades. Although the frontal cortex receives cholinergic input, studies have also shown that the signalling in the prefrontal cortex is gated by the dopaminergic system [61, 62]. It is possible that the cholinergic system has a greater influence on the neural circuit involved in prosaccade initiation, whereas generation of antisaccades relies more on dopaminergic system function [63]. There is no indication in the literature that the APOE genotype affects the dopamine system, which may be the reason why antisaccade performance is not affected by this polymorphism.

The effect of APOE genotype on cognitive performance and brain function has been studied extensively with the goal of establishing the cognitive/behavioural phenotype that could lead to early detection of AD [11]. Because the presence of APOE-ε4 allele is significantly associated with a dose dependent occurrence of late onset AD, most studies to date used highly cognitive tasks involving episodic memory, working memory, or attention, which are significantly impaired early in the disease process [64]. Increased saccade latency has been also reported in patients with autopsy-confirmed AD [65], however, no studies to date examined the influence of APOE polymorphism on prosaccade latency in ε4 carriers. As highlighted in the review by Lancaster et al [11], cognitive performance and neural function in apparently healthy APOE-ε4 carriers seems to be highly age-dependent. Specifically, studies with participants older than 60 years showed that the ε4 allele is associated with lower memory scores and higher neural activation. In contrast, studies with younger participants showed the reverse effect: APOE-ε4 carriers scored better on memory and attention tests. Moreover, when ε4 carriers and non-carriers were matched on memory test performance, ε4 carriers exhibited decreased neural activation

indicative of more efficient/economic use of neural resources [13]. These findings are consistent with the antagonistic pleiotropy hypothesis whereby the ε4 allele is associated with improved cognitive function at a younger age and reduced cognition at an older age. The cognitive phenotype associated with APOE genotype therefore is expected to vary across the lifespan, although more studies are required to definitively establish this effect. Eye movements can be easily assessed, and therefore, can provide further insight into the APOE behavioural phonotype and the associated mechanisms that may be disrupted by AD.

There are several limitations in our study that need to be acknowledged. First, the sample of participants tested in this study consisted only of university students, and the results might not generalize to randomly recruited participants from a community sample where the level of education might vary considerably. Although the Hardy-Weinberg test was not significant, the percent of ε4 carriers was higher than might be expected based on random sampling. This could be explained in light of an epidemiological study, which reported that ε4 carriers tend to achieve a higher level of education [17]. Another limitation of this work regards the use of the LATER model It has been recommended that 200-300 trials should be used with this analysis; however, our dataset consists of 50-60 trials per participant. We used the Kolmogorv-Smirnov test to confirm the model's goodness of fit to ensure that the parameters obtained from the LATER analysis were valid; however, some caution should be exercised when interpreting the results from the LATER analysis. Finally, and most importantly, saccade latency could be influenced by many polymorphisms in addition to the APOE genotype. Future studies should examine the interaction between APOE genotype and other genetic polymorphisms that influence function of the cholinergic system.

In conclusion, this is the first study to show that APOE genotype has a significant influence on prosaccade latency in a cohort of young participants. Although latency was longer in a group of ε4

carriers, this increase does not represent a general deficit in information processing. In fact, in light of the antagonistic pleiotropy hypothesis, we propose that the increased saccade latency in young individuals could have a positive effect on information processing. Specifically, individuals with the ε4 allele may be less likely to make erroneous eye movements to salient, but behaviourally irrelevant stimuli. These individuals might also have longer fixation durations, and fixation duration has been associated with deeper encoding and better recall accuracy [66]. Our recent work also indicates that eye movements disrupt the encoding of a spatial sequence, and reduce the span of working memory [24]. Therefore, increased saccade latency, as well as fewer eye movements, may provide a benefit for cognitive processing in young individuals. However, the basal forebrain cholinergic system is highly susceptible to age-related decline [67]. Although this decline will occur regardless of APOE genotype, the rate of decline may be accelerated in individuals with the ε4 allele due to greater effects on the dysregulation of cortical cholinergic transmission. Taken together, results from our study provide a strong indication that saccade latency could be used to establish the APOE behavioural phenotype across the lifespan, which may have important implications for identifying individuals at a greater risk of developing AD.

Acknowledgements

The study was funded by a grant from the Propel Centre for Population Health Impact, University of Waterloo.

The authors would like thank Drs. Esther Gonzalez, Luminita Tarita-Nistor, Elizabeth Irving, and Jim Frank for constructive feedback on the previous version of the manuscript.

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Figures

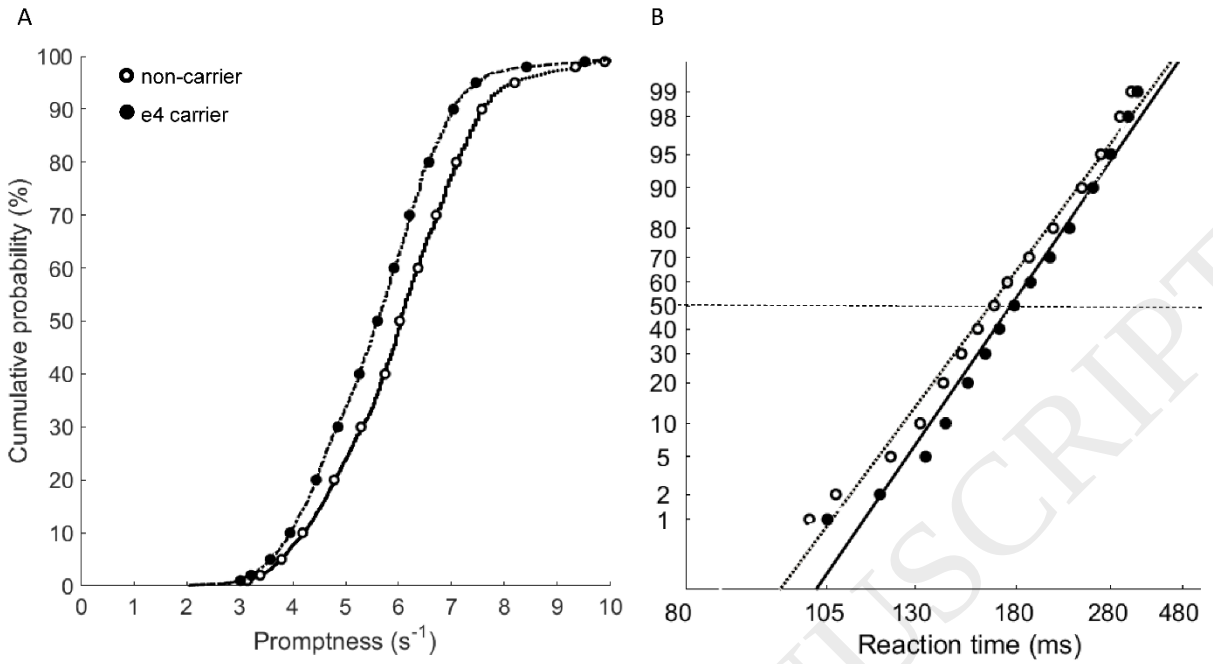

Figure 1: Distributions of prosaccade promptness (1/s) (A), and prosaccade latency using a reciprobit plot (B) in APOE-e4 carriers and non-carriers.

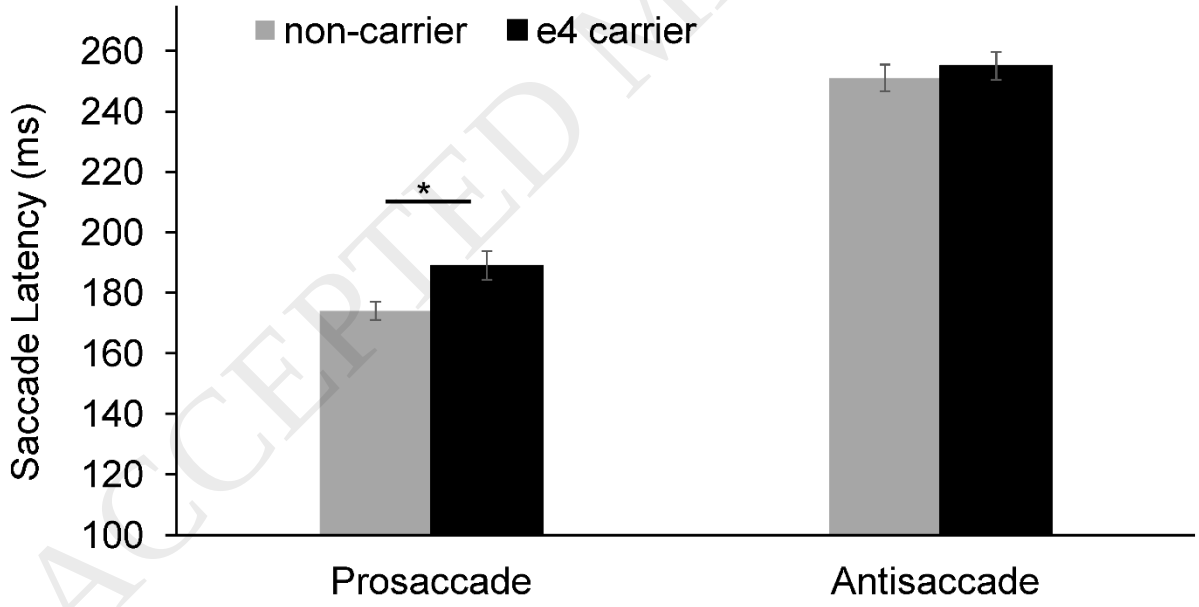

Figure 2: Mean saccade latency for the prosaccade and antisaccade tasks in the e4 carrier and non-carrier groups. Prosaccade latency was significantly longer in a group of e4 carriers whereas antisaccade latency was not significantly affected by genotype. Error bars show standard error of the mean.

Table 1: Demographic information

Genotype	Sex	Age (years)	Concussion history
APOE – ε2ε3	M: 6; F: 5	21.7±1.8	No: 10; Yes: 1
APOE – ε2ε4	M: 0; F: 1	22.0	No: 1; Yes: 0
APOE – ε3ε3	M: 26; F: 25	22.3±3.0	No: 34; Yes: 17
APOE – ε3ε4	M: 14; F: 17	23.7±3.7	No: 23; Yes: 8
APOE – ε4ε4	M: 2; F: 1	24.0±2.4	No: 2; Yes: 1

Table 2: Descriptive statistics for saccade latency and errors in the groups with and without a history of concussion.

	No concussion history (n=70)		Concussion history (n=27)		t-test	Wilcoxon Rank Sum test
	mean±std dev	median (IQR)	mean±std dev	median (IQR)		
Prosaccade latency (ms)	177±25	174 (159-191)	187±29	182 (172-195)	$t_{(95)} = -1.72$ p=0.089	Z=1.85 p=0.065
Antisaccade latency (ms)	249±31	248 (228-269)	261±34	257 (241-287)	$t_{(95)} = -1.63$ p=0.107	Z=1.52 p=0.129
Errors in antisaccade direction (%)	17.9±13.3	13.3 (6.9-28.3)	21.5±13.7	20.3 (6.7-30)	$t_{(95)} = -1.1$; p=0.243	Z=1.15 p=0.250

Table 3: Percent of participants and the corresponding descriptive statistics for latency of express saccades, fast regular, and slow regular saccades.

	APOE genotype (% of participants)	**Mean ± Std dev**	**Median (IQR)**
Express saccade latency range (80-134 ms)	Non-carrier (3%)	131±4	131 (129 - 134)
	ε4 carrier (0%)	NA	NA
Fast regular saccade latency range (135-180 ms)	Non-carrier (65%)	163±12	164 (154 -174)
	ε4 carrier (43%)	165±9	163 (158-171)
Slow regular saccade latency range (181-400 ms)	Non-carrier (32%)	201±19	194 (188 -211)
	ε4 carrier (57%)	208±25	203 (191-219)

Table 4: Mean amplitude and peak velocity for prosaccades and antisaccades in the non-carrier and ε4 carrier groups.

	Saccade amplitude (deg)		Saccade peak velocity (deg/sec)	
	Non-carrier	ε4 carrier	Non-carrier	ε4 carrier
Prosaccade	9.23±1.08	9.25±0.44	329±47	332±43
Antisaccade	9.92±2.64	9.67±3.34	303±54	297±56