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RESEARCH ARTICLE

Oculomotor Abnormalities during Reading in the Offspring of Late-onset Alzheimer's Disease

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Abstract: ***Introduction*:** Eye movement patterns during reading are well defined and documented. Each eye movement ends up in a fixation point, which allows the brain to process the incoming information and program the following saccade. In this work, we investigated whether eye movement alterations during a reading task might be already present in middle-aged, cognitively normal offspring of late-onset Alzheimer's disease (O-LOAD).

***Methods*:** 18 O-LOAD and 18 age-matched healthy individuals with no family history of LOAD participated in the study. Participants were seated in front of a 20-inch LCD monitor, and single sentences were presented on it. Eye movements were recorded with an eye tracker with a sampling rate of 1000 Hz.

***Results*:** Analysis of eye movements during reading revealed that O-LOAD displayed more fixations, shorter saccades, and shorter fixation durations than controls.

***Conclusion*:** The present study shows that O-LOAD experienced alterations in their eye movements during reading. O-LOAD eye movement behavior could be considered an initial sign of oculomotor impairment. Hence, the evaluation of eye movement during reading might be a useful tool for monitoring well-defined cognitive resources.

Keywords: Offspring of late-onset Alzheimer's disease, reading performance, oculomotor behavior, cognitive impairments

1. INTRODUCTION

There is strong evidence of a close relationship between eye movement patterns and fluent reading, in particular when analyzing word processing [1]. There are some well-documented eye movement measures that inform about cognitive processes, i.e., fixation duration, number of fixations, and saccade amplitude [1, 2, 3, 4, 5, 6, 7, 8]. Research on the perceptual span established that parafoveal visual information extending to about 10 characters in reading direction can influence the word processing in progress. In healthy readers, this information is used for selecting the next saccade target and for determining the saccade's length [3, 4, 5]. Several authors propose that once fixation is made, information processing is critical for programming the next saccadic movement. Fixation duration usually lasts between 150 and 250 ms, with values stretching from 50 ms to over 700 ms. Generally, when processing a target word, the shorter the gaze duration, the more efficient the cognitive processing of the observed individual [6, 9]. In previous works, Fernandez *et al.* [7, 10] showed that the number of fixations (i.e., how many times a subject needs to fixate on a word) increased when readers were processing less frequent words or when readers needed more resources for extracting word information. When reading sentences, it was shown that the distance between two corresponding ocular fixations, in part, depends on the difficulty of the reading context. In general, the easier the reading process, the longer the outgoing saccade [9, 11, 12, 13]. In addition, words exert a well-differentiated impact on fixation duration when reading the initial, middle, and last words of the sentence suggesting that word position is a relevant indicator for understanding the meaning of a sentence [8]. When reading a sentence, each word is part of a construct [14, 15] and the amount of cognitive effort required to process it reflects the interplay of word

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processing and expectancy-driven processes [1, 5]. Further, healthy readers perform strategies to compensate for their perceptual limitations in text processing by making more use of world knowledge and other top-down processes [16]. This strategic change could be reflected in increased neural connectivity across brain regions [17]. Although most functional neuroimaging studies of aging have focused on age effects on regional activity, there is evidence that functional connectivity is also modulated by initial aging processes, including increases in PFC connectivity [18]. If age-related increases in PFC activity can be attributed to compensation processes, then it is possible that age-related increases in PFC connectivity could also be compensatory. In accordance, healthy readers would use some strategies when processing words, producing a decreased number of fixations and longer outgoing saccades when compared with subjects who cannot apply reading strategies efficiently.

In Alzheimer's disease (AD), progressive neuropathological changes within the neocortex make patients prone to visual and attentional disturbances [19]. Mendez *et al.* [20] reported visual field deficits, prolonged visual evoked potentials, abnormal eye movement recordings, and even visual hallucinations, among other disturbances in AD patients. In addition, disturbances and abnormal eye movements during reading were observed in these patients [21]. During the progression of AD, affected individuals evolve from an initial mild cognitive impairment to a severe loss of mental function. Patients with early to moderate AD usually exhibit impairment in learning and deficits in episodic and working memory, which lead to the diagnosis of the pathology. On the other hand, subtle alterations in movement coordination and planning that may also be present while performing fine motor tasks, such as writing or reading, are harder to detect and frequently unnoticed [22, 23]. Networks and structures involved in a range of eye movement behaviors are well defined, including those that measure working memory, pupil dilation, and saccadic execution [24]. The existing knowledge on eye movement control could be extrapolated to improve our understanding of more complex behaviors such as attention, inhibitory control, working memory, and decision-making processes [25, 26, 27]. All the above processes are affected in AD, which reflect early modifications in neurological connectivity that disrupts the processing of incoming information [28, 29]. Recent research strongly suggests that these non-amnestic abnormalities are already present among middle-aged, not cognitively altered offspring of LOAD patients [30]. Family history increases the risk of developing LOAD [31]. Persons who are at risk of developing LOAD because of their family history show altered functional and anatomical connectivities [32, 33], changes in cognitive variables [34, 35], and abnormal brain structure [36]. In a previous study, functional connectivity was related to subtle cognitive alterations in a sample of offspring of LOAD (O-LOAD) patients' capacity to recover from semantic interference effects during learning when compared to healthy control participants [32].

Rösler *et al.* [37] reported that AD patients evidenced a delayed target detection, where patients made more fixations and exhibited longer fixations times when searching arrays of letters. These delays could be interpreted as inefficient planning of a search strategy [38]. Furthermore, a recent work proposed that overt shifts of attention through eye movements are associated with higher accuracy in a relational visuospatial memory task [39]. Given that the broad spectrum of events involved in reading requires information processing, coordination, and planning, we hypothesize that subtle changes in such processing might lead to eye movement alterations in O-LOAD. Our results provide evidence that even in this clinically asymptomatic stage, O-LOAD showed shorter fixation durations and an increased number of fixations compared to control individuals. This suggests that O-LOAD was less efficient when extracting word information with just one fixation, among other things. An analysis of eye movements during reading might also provide new insights into the pathogenesis of AD.

2. METHODS

2.1. Ethics Statement

The investigation adhered to the principles of the declaration of Helsinki and was approved by the Bioethics Committee of FLENI Foundation, Argentina. All participants signed informed consent prior to their participation in the study.

2.2. Participants

As LOAD pathophysiological process begins decades before the first clinical symptoms appear [40], individuals were recruited to participate in a larger, exploratory, cross-sectional study with the aim of assessing early neuroimaging, cognitive and circadian markers in asymptomatic, middle-aged O-LOAD, which are believed to be at greater genetic risk of developing LOAD [41, 32, 42, 36, 43, 44, 45, 46]. Accompanying family members of LOAD patients were informed of the study and invited to participate by the patients' physicians."

18 O-LOAD were recruited at the Service of Psychiatry, Fleni Foundation, Buenos Aires, Argentina; Table 1 describes the demographic and clinical characteristics of the sample, including neuropsychological performance. All participants were asked to fill in the names, dates of birth, age of AD onset, and clinical information of all affected family members. The information was confirmed with other family members and by an interview with the examining physician, discussing the parents' symptomatology and progression of the disease. Only individuals whose parents had lived to age ≥65 were included. For individuals who had received no treatment at Fleni Foundation, the parents' diagnosis of O-LOAD was clinician certified.

The inclusion criteria for O-LOAD were as follows: (1) having at least one parent diagnosed with probable LOAD according to the DSM-5 criteria, (2) being 40–65 years old at the time of recruitment, (3) having seven or more years of formal education, (4) having a Mini-Mental State Examination (MMSE) score >26 [47], (5) having no evidence of current progressive neurological disease or medical conditions likely to impair cognitive function, (6) having no history of substance abuse (alcohol, marijuana, stimulants, benzodiazepines, cocaine, or other illicit drugs), and (7) having a Hachinski score < 4 to screen out subjects with vascular-derived cognitive impairment [48].

The control group consisted of 18 middle-aged adults (Table 1) with no known neurological or psychiatric disease according to their self-report and no evidence of cognitive decline or impairment in daily activities. An ophthalmologist performed an exam of visual acuity. Participants presenting ophthalmological diseases, such as glaucoma, visually significant cataract or macular degeneration, as well as visual acuity less than 20/20, were excluded from the study.

2.3. Cognitive Assessment

A thorough neuropsychological battery standardly used in clinical practice was administered to all participants to assess attention, memory, executive functions, language, and visuospatial abilities.

Although no participants reported subjective cognitive complaints, a thorough neuropsychological assessment was performed by a trained neuropsychologist blind to participants' status (O-LOAD, CS) as part of the recruitment process. The neuropsychological battery is comprised of the following tests, all of which are widely and standardly used in clinical practice and do not require detailed explanation; Trail Making Test A (TMT A, sustained attention) and B (TMT B, cognitive flexibility) [49], Rey's Auditory Verbal Learning Test (RAVLT, verbal episodic memory) [50], semantic fluency, "animals" category (verbal productivity, semantic memory) and phonemic fluency, letter "P" (verbal productivity) [51], D-KEFS design fluency (non-verbal productivity) [52], WAIS-III Vocabulary subtest (semantic memory) [53], WAIS-III Digits Forward (attention span) and Digits Backward (working memory) [53], WAIS-III Similarities subtest (abstract thinking) [53], Drexel Tower of London (TOL, planning, and problem solving) [54], Stroop Color and Word Test (inhibition) [55].

Additionally, the Word Accentuation Test - Buenos Aires version (WAT-BA) [56] was implemented to estimate participants' intellectual quotient (IQ) as a measure of premorbid intelligence level. The Beck's Depression Inventory (BDI II) [57] was administered to screen for depressive symptoms, which might impact cognitive performance.

All subjects exhibited cognitive and intellectual performance within normal limits on all administered tests according to local norms. Therefore, none of the individuals met the clinical criteria for mild cognitive impairment or dementia. Regarding the depression screening, no subjects met the criteria for major depression; therefore, no impacts on cognitive function were observed. Table 1 shows neuropsychological test results.

2.4. Sentence Corpus

The sentence corpus was composed of 40 regular sentences in Spanish, which is the native language of all participants (*e.g.,* "Yesterday I talked to Laura about her daughter") [58]. The sentences comprised a well-balanced number of content and function words and had similar grammatical structures.

Word and Sentence Length: Sentences ranged from a minimum of 5 words to a maximum of 14 words. The mean sentence length was 8.1 (SD=1.4) words. Words ranged from 1 to 14 letters. The mean word length was 4.6 (SD=2.5).

2.5. Apparatus and Eye Movement Data

Single sentences were presented at the centerline of a 20-inch LCD monitor (1024 x 768 pixels resolution; font: regular New Courier, 12 point, the vertical size of one character: 0.5º of visual angle). Participants were seated in front of the monitor at a distance of 60 cm. Head movements were minimized using a chin rest. Eye movements were recorded with an EyeLink 1000 Desktop Mount eye tracker (SR Research, Ontario, Canada), with a sampling rate of 1000 Hz and an eye position resolution of 20-s arc. Eye movement data were screened for the loss of measurement and blinks. Data of sentences without problems were reduced to a fixation format after detecting saccades as rapid binocular eye movements by using a binocular velocity-based detection algorithm that was originally developed for the analyses of saccades in attention-shifting experiments [59]. Fixations shorter than 51 ms and longer than 750 ms, as well as fixations on the first and last word of each sentence, were removed from the analysis [5].

2.6. Procedures

Participants' gaze was calibrated with a standard 13-point grid for both eyes. After validation of calibration, a trial began with the presentation of a fixation point on the position where the first letter of the sentence was to be presented. As soon as both eyes were detected within a 1º radius relative to the fixation spot, the sentence was presented. After reading the sentence, participants had to direct their eyes to a dot in the lower right corner of the screen to end the trial. Occasionally, external factors such as minor movements and slips from the headgear could cause small drifts. To avoid them, we performed a drift correction before each spot presentation.

2.7. Statistical Analysis

Our first approach was aimed at corroborating the discriminative power of eye movements analysis during reading to distinguish between O-LOAD and controls. To fulfil that aim, we defined three linear models comprising a between-subjects factor group (controls versus O-LOAD). The first model has log fixation duration (in milliseconds) as the dependent variable, the second model has the number of fixations as the dependent variable, and the third model has saccade amplitude (in degrees) as the dependent variable. Additionally, we added word number into each model as an independent variable because it is a significant predictor when readers process words embedded in a sentence [60, 61]. Statistical analyses were performed in R version 3.1.1 (RDevelopment Core Team). Finally, group differences (i.e., controls versus O-LOAD) when considering neurocognitive tests were analyzed with one-way ANOVA and t-test.

3. RESULTS

3.1. Neuropsychological Tests

As shown in Table **1**, while all participants scored within the normal range for all tests (not shown), O-LOAD exhibited a lower performance than controls on the Rey Auditory Visual Learning Task (RAVLT). Significant differences were only observed in recognition when comparing controls

Table 1. Demographic and Clinical Data.

-	Group				t-Statistic	P-value
	CS (n=18)		O-LOAD (n=18)			
	Mean or Frequency	SD or %	Mean or Frequency	SD or %		
Age	51.3	7.4	54.3	7.0	T=-1.230	.228
Female	15	75	7	43.8	X^2=3.653	.087
Education	17.6	2.4	18.1	3.5	T=-.517	.609
BDI II	7.7	7.8	7.7	5.9	T=.016	.987
Estimated IQ	108.3	6.1	107.4	5.7	T=.436	.666
TMT A	31.8	9.8	31.9	8.3	T=-.011	.991
TMT B	62.1	19.8	64.7	18.1	T=-.412	.684
RAVLT						
Learning Curve	46.4	9.4	44.3	7.3	T=.731	.470
Max. Learning	11.9	1.6	10.9	2.0	T=1.726	.095
Delayed Recall	10.1	2.0	8.8	2.9	T=1.529	.138
Recognition	13.9	1.3	13.1	1.2	T=2.04	.047
Semantic Fluency	22.7	4.6	22.0	3.1	T=.526	.602
Phonemic Fluency	18.1	3.5	17.7	4.6	T=.216	.831
Design Fluency						
Trial 1	11.4	2.8	11.7	3.3	T=-.230	.820
Trial 2	12.5	3.4	12.1	2.9	T=.347	.731
Trial 3	8.1	2.0	7.8	1.8	T=.369	.714
Vocabulary	50.3	5.9	48.9	8.5	T=.574	.571
Digits Forward	9.7	2.2	9.4	1.8	T=.365	.718
Digits Backwards	6.8	2.7	6.8	1.8	T=.039	.969
Similarities	26.2	4.1	26.7	4.4	T=-.320	.751
Tower of London						
Correct Exercises	3.7	2.0	4.8	2.3	T=-1.488	.147
Total Moves	32.6	14.6	30.6	14.4	T=.408	.686
Start Time (s)	54.2	27.0	77.9	43.8	T=-1.890	.071
Execution Time (s)	213.8	96.5	232.3	92.4	T=-.577	.568
Total Time (s)	267.9	111.1	302.1	119.3	T=-.871	.390
Stroop	3.9	9.5	5.0	6.5	T=-.403	.690
MMSE	29.5	0.7	28.9	1.0	T=1.7	.08

BDI II: Beck's Depression Inventory, second edition; TMT: Trail Making Test, RAVLT: Rey Auditory Verbal Learning Test. Our criterion for referring to an effect as significant was t+- 2.0 and p-value <0.05.

vs. O-LOAD (t=2.04, p<0.01). There were no significant differences between controls vs. O-LOAD when considering other neuropsychological tests (Table **1**).

3.2. Eye Movement Behavior

We hypothesized that O-LOAD would show minor difficulties in processing and interpreting acquired data. As shown in (Table **2** and Fig. **1**), the mean fixation duration significantly decreased in O-LOAD compared to controls when reading sentences (t=-2.40, p<0.01). In addition, word number significantly increased fixation duration (t=10.07, p<0.001).

When considering the mean number of fixations, we noted that it increased in O-LOAD when compared to controls (t=2.34, p<0.01). When we evaluated whether word number affected the number of fixations, we noted that the number increased as readers moved forward along the sentence (t=5.76, p<0.001) (Table **3** and Fig. **2**).

Finally, as shown in (Table **4** and Fig. **3**), the mean saccade amplitude decreased significantly when comparing O-LOAD and Controls' saccades (t=-6.06, p<0.001). Saccade amplitude decreased significantly when increasing ordinal word number (t=-42.69, p<0.001).

4. DISCUSSION

There is consensus about the usefulness of eye-tracking metrics for the study of high-level activities [62]. Previous works showed that patients with moderate AD exhibited abnormalities in eye movements while reading a text [7, 15, 21]. Given that the broad spectrum of events involved in reading requires information processing, coordination, and planning, we investigated whether subtle changes in word processing might lead to eye movement alterations detectable by the eye tracker. Three oculomotor metrics have been proved particularly informative: fixation duration, the number of fixations, and saccade amplitude [1]. Fixation duration provides an index of the time spent on a word for extracting relevant information. In our work, O-LOAD presented shorter fixation periods when compared to controls. Only controls displayed longer fixations when moving forward along the sentence suggesting that they were processing and integrating the whole meaning of a sentence. As suggested, this effect seems to be impaired in O-LOAD, given that their fixations were consistently shorter and similar throughout the sentence (Table **2** and Fig. **1**). O-LOAD's shorter fixations probably reflect difficulties in the extraction of enough word information when reading fluently, which suggests they may need to fixate more time on each word, producing an increase in their number of fixations. Our results suggest that this is the case when considering their fixation patterns (Fig. **2**). The number of fixations provides an index for the number of times a person looks at a particular place, which reflects the number of attentional resources needed to process a particular stimulus [63]. As a moment-to-moment measure of the focus of attention, the number of fixation path analysis can inform the strategies and approaches people use to solve problems [62, 64]. The increased number of fixations in O-LOAD, when compared to controls, is consistent with a previous work where asymptomatic familial AD subjects (E280A-PSEN-1) showed deficits in visual memory recognition, alterations in their processing speed, and their short-term memory (Fernandez *et al.*, 2020, manuscript in preparation). Further, in previous works, mild AD showed shorter durations, particularly while encoding targets [8, 65]. This suggests that poor word extraction mechanisms during fluent reading could be the source of impairment found in O-LOAD. Furthermore, a reduction in fixation duration and an increase in the number of fixations during reading may be the output of inefficient cortical integration mechanisms responsible for holding fluent word processing. Future studies will need to investigate the extent to which impaired eye movements and shorter fixations in O-LOAD may share neurocognitive mechanisms.

In previous works [9, 11, 12, 13], the amplitude of saccades was proposed to depend on the complexity of the visual stimulus. Generally, the easier the processing of the fixated region, the longer the saccade amplitude. Fernandez *et al.* [15] showed that the amplitude of the saccades during reading was consistently shorter in mild LOAD when compared to controls. In our current work, O-LOAD showed shorter saccades when compared to controls. Previous works related altered saccadic movements in AD as a sign of neurodegeneration in basal ganglia, substantia nigra, the caudate nuclei, the reticular formation, and the superior colliculus, among others [66, 67, 68, 69, 70]. As Hikosaka [71] described, the basal ganglia control saccades through connections with the superior colliculus, which receives convergent input from cortical areas. The basal ganglia play a crucial role when performing a saccade to a particular target. In our work, O-LOAD performance during reading suggests that some impairment may be already present at basal ganglia and superior colliculus, reducing saccade amplitudes. The sensorimotor signals carried by the basal ganglia neurons are strongly modulated depending on the behavioral context, which reflects working memory, expectation, and attention, all cognitive processes.

Recent works [72, 73] suggested that in healthy readers, word's expectations and syntactic and grammatical congruency act as an attentional filter to facilitate the extraction of information, resulting in performance benefits across multiple domains, including saccades and reduced number of fixations. In our study, an impairment in O-LOAD's recollection function may produce less efficient predictions and word's online integration. The current O-LOAD eye movement patterns are consistent with their memory performance measured by the RAVLT (i.e., recognition), suggesting that they showed a diminished capacity for recognizing words when compared to controls. Moreover, we have recently detected subtle but significant executive functioning deficits in O-LOAD [30]. Presumably, eye movements during reading and executive function tasks performance share at least some processing circuits involving particular frontal cortical regions. In this regard, this group of individuals displays amyloid deposit and volumetric abnormalities in frontal regions, presumably subserving both executive functioning and eye movements during reading [36]. If this relationship is confirmed in further studies, the present method could represent a candidate noninvasive biomarker for subtle, early Alzheimer´s disease-related neuropathology. We are currently exploring this possibility by establishing the relationship between *in vivo* amyloid deposit, executive functioning, and the eye movement abnormalities described herein.

CONCLUSION

The main conclusion of the present work is that O-LOAD evidenced altered eye movements during word reading, as shown by shorter fixation durations and saccade amplitudes,

Table 2. The log fixation duration (milliseconds) of O-LOAD and age-matched individuals (controls) while reading 40 sentences were compared. Linear models were computed. Our criterion for referring to an effect as significant was t+- 2.0 and p-value <0.05. In addition, controls had longer fixation durations when compared with O-LOAD (Mean=5.28, SD=0.50; Mean=5.20 SD=0.51; controls and O-LOAD, respectively).

-	-	Log Fixation Duration (milliseconds)	-	-
	M	**SE**	**t-value**	**P-value**
Fixed effects				
Mean log Fixation Duration	5.21	0.02	**41.43**	**p<0.001**
Word Number	0.01	0.00	**10.07**	**p<0.001**
Controls vs. O-LOAD	-0.01	0.00	**-2.40**	**p<0.01**

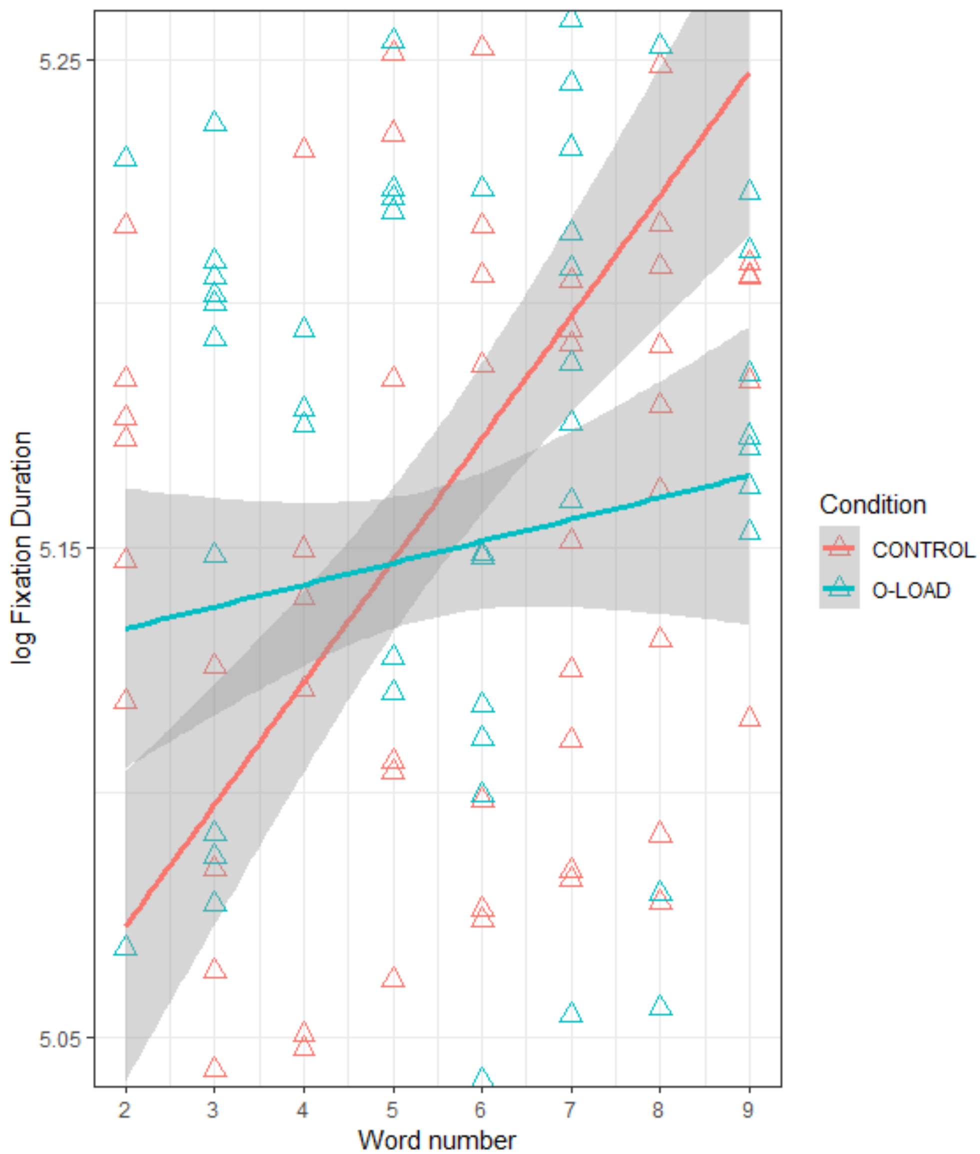

Fig. (1). Effect of the number of words in sentences on normalized log fixation duration. The panel reflects regression of log fixation duration on words on the respective number of words when considering controls vs. O-LOAD (t=-2.40, p<0.01). Gray areas represent 95% confidence intervals.

Table 3. The number of fixations of O-LOAD and age-matched individuals (controls) while reading 40 sentences were compared. Linear models were computed. Our criterion for referring to an effect as significant was t+- 2.0 and p-value <0.05. In addition, controls had longer fixation durations when compared with O-LOAD (Mean=1.22, SD=0.50; Mean=1.29 SD=0.56; controls and O-LOAD, respectively).

-	-	Number of Fixations	-	-
	M	**SE**	**t-value**	**P-value**
Fixed effects				
Mean Number of Fixations	1.48	0.05	**27.62**	**p<0.001**
Word Number	0.01	0.00	**5.76**	**p<0.001**
Controls vs. O-LOAD	0.02	0.00	**2.34**	**p<0.01**

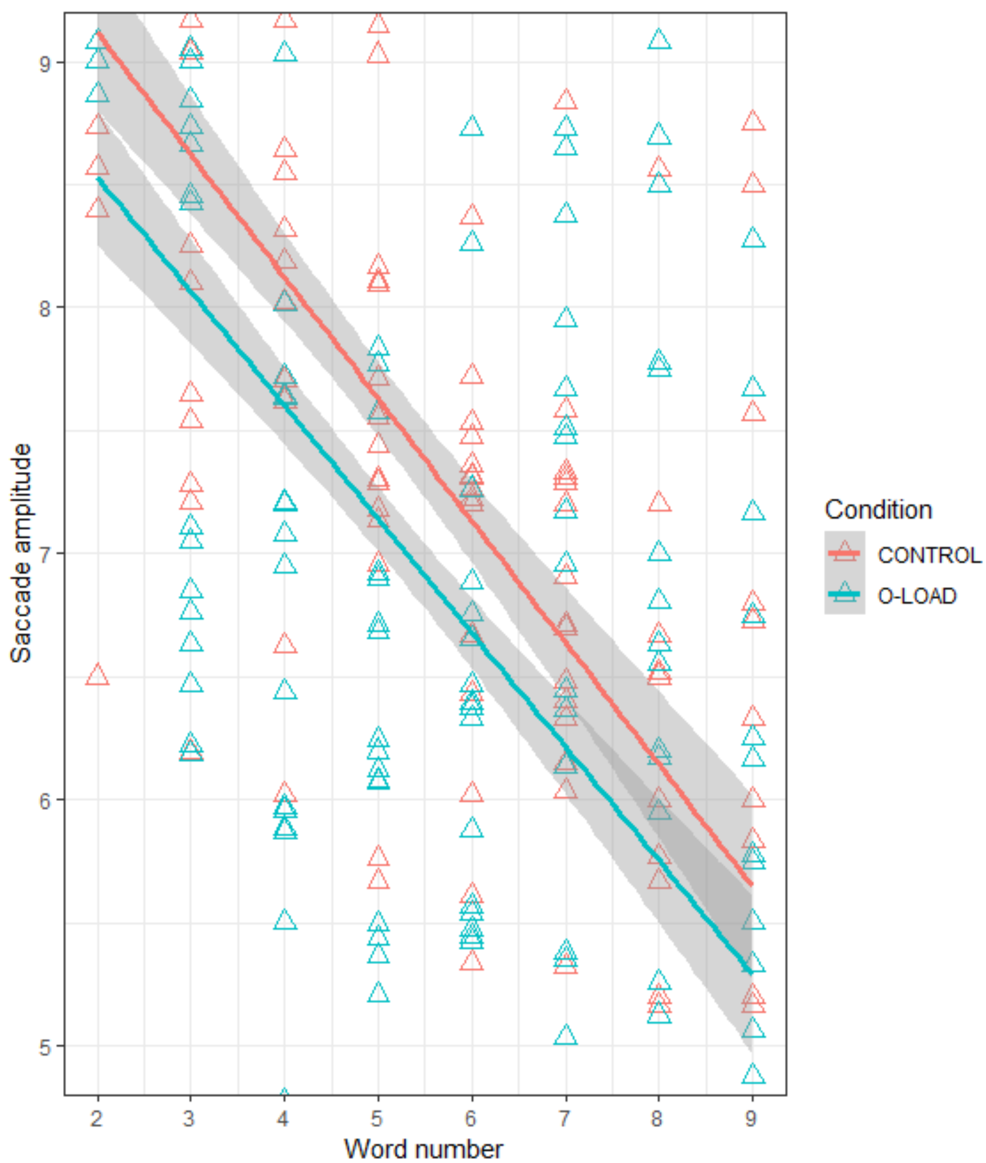

Fig. (2). Effect of the number of words in sentences on normalized saccade amplitude. The panel reflects regression of saccade amplitude on words on the respective number of words when considering controls vs. O-LOAD (t=-6.06, p<0.001). Gray areas represent 95% confidence intervals.

Table 4. The saccade amplitude (degrees) of O-LOAD and age-matched individuals (controls) while reading 40 sentences were compared. Linear models were computed. Our criterion for referring to an effect as significant was t+- 2.0and p-value <0.05. In addition, controls had longer fixation durations when compared with O-LOAD (Mean=8.94, SD=5.60; Mean=8.01 SD=3.93; controls and O-LOAD, respectively).

-	-	Saccade Amplitude (Degrees)	-	-
	M	SE	t-value	P-value
Fixed effects				
Mean Saccade Amplitude	9.12	0.49	**18.35**	**p<0.001**
Word Number	-0.89	0.02	**-42.69**	**p<0.001**
Controls vs. O-LOAD	-0.54	0.08	**-6.06**	**p<0.001**

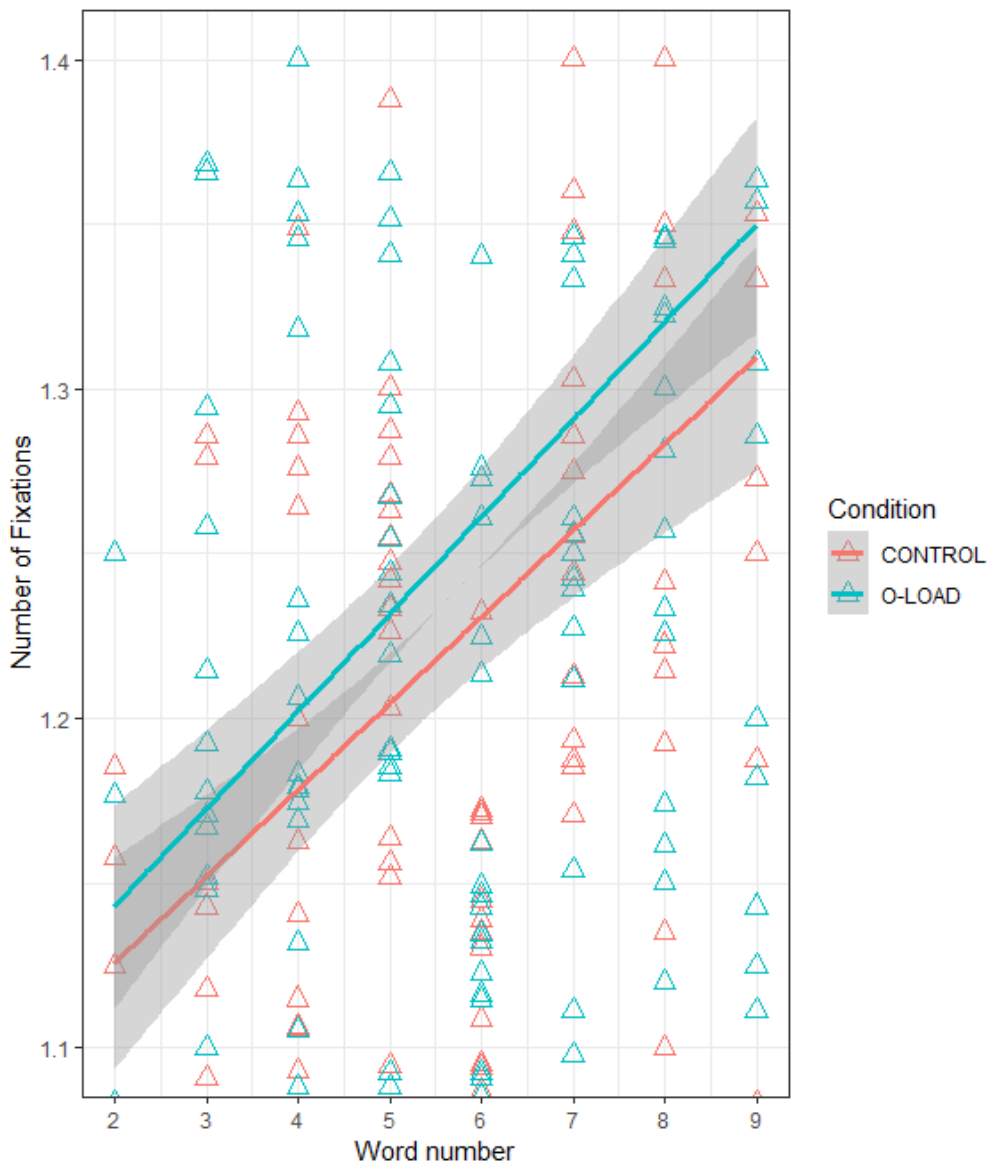

Fig. (3). Effect of the number of words in sentences on the normalized number of fixations. The panel reflects regression of the number of fixations on words on the respective number of words when considering controls vs. O-LOAD (t=2.34, p<0.01). Gray areas represent 95% confidence intervals.

and increased number of fixations. This eye movement pattern could be considered an early marker of oculomotor impairment. Further research is needed to determine if this reflects the early compromise of Alzheimer´s disease-related brain areas and its relationship with actual clinical and functional deterioration.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The investigation was approved by the Bioethics Committee of FLENI Foundation, Argentina.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

All participants signed informed consent prior to their participation in the study.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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