Retinal Imaging

Change in retinal structural anatomy during the preclinical stage of Alzheimer’s disease

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Abstract

Introduction: We conducted a 27-month longitudinal study of mid-life adults with preclinical Alzheimer’s disease (AD), using spectral domain optical coherence tomography to compare changes in volume and thickness in all retinal neuronal layers to those of age-matched healthy control subjects.

Methods: Fifty-six older adults (mean age $= 65.36$ years) with multiple risk factors for AD completed spectral domain optical coherence tomography retinal imaging and cognitive testing at baseline. Twenty-seven months later, they completed the same examinations and an $^{18}$F-florbetapir positron emission tomography imaging study.

Results: Compared to healthy control subjects, those in the preclinical stage of AD showed a significant decrease in macular retinal nerve fiber layer (mRNFL) volume, over a 27-month follow-up interval period, as well as a decrease in outer nuclear layer and inner plexiform layer volumes and thickness in the inferior quadrant. However, only the mRNFL volume was linearly related to neocortical positron emission tomography amyloid standardized uptake value ratio after controlling for any main effects of age ($R^2 = 0.103; \rho = 0.017$). Furthermore, the magnitude of mRNFL volume reduction was significantly correlated with performance on a task of participants’ abilities to efficiently integrate visual and auditory speech information (McGurk effect).

Discussion: We observed a decrease in mRNFL, outer nuclear layer, and inner plexiform layer volumes, in preclinical AD relative to controls. Moreover, the largely myelinated axonal loss in the RNFL is related to increased neocortical amyloid-\textbeta accumulation after controlling for age. Volume loss in the RNFL, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving. However, this retinal change does appear to be modestly related to relative decrements in performance on a measure of audiovisual integration efficiency that has been recently advanced as a possible early cognitive marker of mild cognitive impairment.

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1. Background

The formation of the eye begins in the third week of human embryologic development, with the retina being a crucial component of the ocular globe and the central nervous system. The retina is derived from pluripotent neuroectodermal
cells that migrate from the diencephalic invagination of the neural tube [1], and so it is structurally, physiologically, and functionally brain tissue—with the retina sometimes described as a “protrusion” from the brain. As is the case throughout the neocortex, the retina consists of discrete neuronal cell layers, with multiple types of neurons and neurotransmitter systems, glial cells, and microvasculature. Unlike the rest of the central nervous system, however, the retinal neuronal cell layers can be noninvasively visualized through high-resolution optical methods such as spectral domain optical coherence tomography (SD-OCT) [2].

A recent review [3] cites the wide variety of retinal biomarkers that have been explored in patients with Alzheimer’s disease (AD), ranging from retinal anatomical and vascular markers to curcumin binding studies [4] and retinal oximetry and electroretinogram studies. Clinically, it is reasonable to suspect the presence of early ocular involvement in AD because visual system changes such as decreased vision, abnormal pupillary reaction, visual field changes, motion detection abnormalities, impaired color vision, and decreased contrast sensitivity have been identified in mild-to-moderate AD [3,5].

SD-OCT allows for the precise segmentation and measurement of the retinal cell layers, thereby promoting exploration of neuronal changes that might be directly related to specific neurodegenerative diseases. Over the past 2 decades, there have been over 70 peer-reviewed publications exploring retinal optical coherence tomographic (OCT) correlates of AD, in humans, nonhuman primates, and other animal models of AD disease. With respect to humans, most prior reports have consisted of cross-sectional studies comparing groups of AD patients to groups of seemingly “healthy controls”. When compared with healthy age-matched controls, patients with AD have reduced numbers of ganglion cell axons and are three times more likely to have an increased optic nerve cup-to-disc ratio, a potential consequence of ganglion cell and nerve fiber loss [6]. Such reports have confirmed an earlier report of substantial loss of ganglion cells in AD patients, based on histopathology of autopsy materials [7]. Furthermore, peripapillary retinal nerve fiber layer (pRNFL) thickness has been found to be significantly thinner, suggesting the presence of optic atrophy in patients with mild cognitive impairment (MCI) and mild-to-moderate AD when compared with age-matched controls [8]. Reduction of macular RNFL (mRNFL) volume also has been identified in AD [9]. The loss of RNFL tissue in the retina may constitute an early biomarker of AD. If so, a reduction in RNFL thickness or volume may be observed before widespread damage to the mesiotemporal central nervous system network that is characteristic of AD [10–12].

With respect to the RNFL, we have surveyed all available literature, including those studies relying on other imaging approaches, such as 2D fundus photography and histological analyses, and we have found variable reports of decreased RNFL and/or ganglion cell layer (GCL) thicknesses in AD and MCI. A search on PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) was performed (25 August, 2017) to find all published articles, using the search terms “Alzheimer’s” and “retinal layer”. This search led to 134 articles identified and, of these, only 34 articles consisted of human studies that compare retinal layer morphology changes in Alzheimer’s patients to healthy individuals (see Table 1).

Nearly all of these 34 publications resulted from cross-sectional studies based on comparisons of cases to putatively “healthy controls”. Most often the healthy controls had no biomarker confirmation to indicate that they did not fall within the preclinical stage of AD. One study did search for thickness differences in all 10 retinal layers [20], with the remaining 33 studies concentrating on measurement differences for the mRNFL, pRNFL, and the GCL. Of note, the GCL was only once reported as a single layer [20], being most often considered in conjunction with an adjacent cell layer, either as the ganglion cell–inner plexiform layer complex (GC-IPL) or as the retinal nerve fiber layer–GCL complex (RGCL or GCC).

From the 33 articles seeking to compare group differences for the RNFL, 29 found RNFL thinning in AD compared with age-matched controls (see Table 1). Only seven of these 29 published reports appear to have accounted for participants’ age as a statistical covariate in their analyses. This is important because there is normal age-related thinning of the RNFL and other retinal layers [44]. Of these seven publications that did account for effects of aging, one determined that the observed thinning was due primarily to the main effect of aging rather than disease burden [26]; one study found RNFL thinning in MCI but not in AD [30]; two studies reported RNFL thinning solely in one or two specific quadrants [13,32]; and three studies reported robust disease burden after accounting for age [15,17,18]. Only two of the 33 reviewed studies reported within-subjects longitudinal results (both in symptomatic AD patients vs. controls), and they both reported increased RNFL thinning compared with controls [18,27].

AD-related amyloid-β (Aβ) plaques can start to accumulate abnormally in the brain up to 20 years before symptom onset, and this stage is classified as preclinical AD [45,46]. Only two of the studies reviewed previously, both relying solely on cross-sectional data, recruited individuals in the preclinical stage of the disease. One from the same larger study that we draw from in the current report [16] found a thicker inner plexiform layer (IPL) in preclinical AD compared with healthy controls. Another study [14] found no difference in the RNFL thickness between AD, preclinical AD, and healthy controls. Golzan et al. reported a significant difference in the RGCL thickness across the three groups, and yet they found no association between retinal structural measurements and positron emission tomography (PET) Aβ binding in the neocortex. Aside from these two cross-sectional studies of preclinical AD [14,16], none of the other published reports compared PET imaging evidence of neocortical amyloidosis with the retinal OCT measurements for this earliest stage of the disease. In this
In our present study, we compared morphological changes in retina with cognitive performance. Previous studies examining this relationship have relied on the Mini–Mental State Examination (MMSE) as a screening measure of general cognitive function, and these studies...
have led to conflicting results. Some studies have found no relationship between RNFL thinning and MMSE scores in AD [41,47] and MCI [39], whereas one study found a correlation between RNFL thickness and MMSE scores in MCI [29]. Another study reported a correlation between macular volume thinning (whole retina) and MMSE scores in patients with AD [9]. For individuals in the preclinical stage of disease severity, the MMSE would be a poor choice as a cognitive marker, due to several psychometric limitations for this test [48]. With respect to the MCI stage of disease severity, two prior investigators have reported surprisingly inverse relationships between performance on measures of verbal episodic memory and RNFL thickness [49,50]. Importantly, both of these studies reported cross-sectional data, and it is unclear as to whether this inverse relationship (i.e., relatively enhanced performance on verbal episodic memory tests associated with relatively diminished RNFL thicknesses) would persist if participants were followed up longitudinally and as the disease progresses.

Our intent was to explore this very question, that of the relationship between cognitive functioning and evidence of morphologic change in the retina, over the course of several years and within the very early, preclinical stage of the disease. Based on prior literature in this specific patient population, we chose the Groton Maze Learning Test (GMLT) as a measure of learning efficiency, problem solving, and working memory [51,52], and the International Shopping List Test (ISLT) to measure verbal episodic memory [53,54]. Finally, we administered an audiovisual McGurk task, a speech processing paradigm that relies on the working memory [51,52], and the International Shopping List Test (ISLT) as a measure of learning efficiency, problem solving, and working memory [51,52]. Based on prior work has shown disruption of functional integration for posterior sensory regions in AD [55,56]. Because alterations in white matter integrity may occur in early stages of the disease [57–59], similar early changes in the RNFL, which comprised mostly myelinated axons from the cell bodies in the GCL, might be directly related to the loss of white matter tracts in the cortex and hence correlate with performance on this cognitive task that specifically assesses corticocortical connectivity and has recently been advanced as a potential biomarker of early-stage AD pathology [60].

2. Methods

2.1. Participants

A total of 56 adults aged between age 55 and 75 years (mean age = 65.36 years) with two well-established risk factors for AD, namely, a self-reported first-degree family history of the disease and self-identification of subjective memory concerns, were recruited using a selection process described previously [61]. All participants underwent a detailed medical screening interview. Exclusion criteria included a diagnosis of MCI or AD following National Institute on Aging - Alzheimer’s Association diagnostic criteria [45,62], history of neurological or psychiatric disorder, any significant systemic illness or unstable medical condition (e.g., active cardiovascular disease), and current use of any medications known to affect cognition (e.g., use of sedative narcotics). Subjects with histories of cataract surgery, corneal Laser-Assisted In-Situ Keratomileusis (LASIK) surgery, age-related macular degeneration, or subjects with known ophthalmic pathology were excluded. Inclusion criteria included having an MMSE score of ≥27 and performance within normal limits on a battery of cognitive tests described previously (listed in Table 2) [63,64]. Because RNFL thinning is a characteristic of glaucoma, subjects with glaucoma were excluded. Participants were also excluded if they had a history of optic neuritis, intraocular surgery apart from cataract extraction, or a history of visual loss

Table 2

Demographic characteristics

| Main outcome                  | Full sample (n = 56) | Aβ+ (n = 15) | Aβ− (n = 41) | P     | Cohen’s d |
|------------------------------|----------------------|--------------|--------------|-------|-----------|
| Sex                          | Number of females    | 35 (62.5)    | 11 (73.3)    | 24 (58.5) | .311      |
| APOE                         | Number of ε4 carriers| 27 (4.2)     | 8 (53.3)     | 19 (46.3) | .643      |
| Age                          | Number of years      | 65.36 (5.55) | 68.25 (5.81) | 64.56 (5.26) | .06       | 0.68      |
| Education                    | Number of years      | 17.31 (2.77) | 17.75 (3.91) | 17.19 (2.42) | .54       | 0.19      |
| Florbetapir PET SUVr         | SUVr                 | 1.02 (0.2)   | 1.32 (0.18)  | 0.94 (0.09) | .000      | 3.17      |
| GDS                          | Total score          | 1.4 (1.87)   | 0.91 (0.94)  | 1.52 (2.02) | .34       | 0.34      |
| Body Mass Index              | Body mass index      | 26.69        | 26.58 (4.36) | 26.86 (6.05) | .879      | 0.35      |
| MAC-Q                        | Total score          | 21.90 (3.08) | 21.97 (2.81) | 21.90 (3.08) | .527      | 0.23      |
| MMSE                         | Total score          | 29.25 (1.29) | 28.72 (1.85) | 29.38 (1.10) | .132      | 0.49      |
| ISLT–Total Recall            | Total words recalled | 26.07 (3.79) | 26.00 (3.52) | 26.09 (3.90) | .944      | 0.02      |
| GMLT–Total Errors            | Total number of errors | 8.40 (5.31)  | 8.27 (5.33)  | 8.44 (5.71)  | .92       | 0.03      |

Abbreviations: APOE, apolipoprotein E; GDS, Geriatric Depression Scale; GMLT, Groton Maze Learning Test; ISLT, International Shopping List Test; MAC-Q, Memory Complaint Questionnaire; MMSE, Mini–Mental State Examination; PET, positron emission tomography; SUVr, standardized uptake value ratio. NOTE. Bold values denote significant group differences.
apart from refractive error as these conditions may affect the RNFL thickness [65–67]. In addition, individuals were excluded if the epiretinal membrane was observed using OCT imaging [68]. All participants live independently, most were engaged in full-time or part-time employment, and many were caretakers for a parent with AD. The amount of neocortical Aβ protein aggregation and apolipoprotein E (APOE) genotype were unknown at the time of assessment and were not used to determine enrollment. Although Aβ status and APOE genotyping were conducted as a part of the study protocol, researchers remained blinded to these results throughout testing.

From this larger sample, we identified 15 participants who were categorized as presenting with preclinical-stage disease based on evidence of both (1) elevated neocortical Aβ burden as determined by PET amyloid imaging [45,46] and (2) relative cognitive impairments in response to a challenge with a very low–dose muscarinic anticholinergics [59,63].

The study was approved by and complied with the regulations of Rhode Island Hospital’s Institutional Review Board, and all participants provided written informed consent in accordance with the Declaration of Helsinki. The study complied with Health Insurance Portability and Accountability Act regulations.

2.2. Aβ PET imaging

To assess neocortical amyloid burden, all participants had an Aβ PET scan at baseline and another at 27-month visit. A 370 MBq (10 mCi 1/2 10%) bolus injection of 18F-florbetapir was administered intravenously. Approximately 50 minutes after injection, a 20-minute PET scan was performed with a head computed tomography scan for attenuation correction purposes. Images were obtained using a 128 × 128 matrix and reconstructed using iterative or row action maximization likelihood algorithms. PET standardized uptake value data were summed and normalized to the whole-cerebellum standardized uptake value, resulting in a region-to-cerebellum ratio termed standardized uptake value ratio (SUVR).

An SUVR threshold of 1.1 or greater was used to discriminate between Aβ+ and Aβ−. These SUVR calculations were performed using the MIMneuro software, with a normative database of 74 healthy normal individuals (48 males and 26 females), aged between the ages of 18–50 years, who all had negative amyloid scans on visual assessment [69]. For all cases, Aβ positivity was confirmed by consensus overread by two board-certified radiologists who were also board certified in nuclear medicine.

2.3. SD-OCT imaging

Participants were administered two drops of tropicamide (Mydriacyl 1%) per eye for pupil dilation before OCT imaging. The Heidelberg SPECTRALIS SD-OCT was used to acquire retinal OCT scans of the optic nerve head and the macula for the right and left eyes of all participants at baseline and 27 months. Heidelberg SPECTRALIS has automatic segmentation and quantification of retinal layers and uses the eye-tracking image alignment for repeated measures (TruTrack; Heidelberg Engineering, Inc). Outcome measures for the SD-OCT imaging, following the Anatomic Positioning System protocol sequences, included pRNFL, mRNFL, GCL, IPL, inner nuclear layer, outer plexiform layer, and outer nuclear layer (ONL). For each individual participant, the volume and thickness of all retinal neuronal layers were measured and averaged for both eyes.

The mean macular thickness of each retinal layer was measured within four sectors (superior, inferior, nasal, and temporal) leading to a macular volume measurement for each retinal layer (see Fig. 1). For the pRNFL, the mean thickness also was calculated in four sectors (superior, inferior, nasal, and temporal) centered in the optic nerve, and the thicknesses of all areas were averaged to result in the mean pRNFL thickness.

Fig. 1. Representative cross-sectional OCT image through macular region, with the fovea in the center (green line). Labels are shown for the RNFL, GCL, and IPL. The mean macular thickness of each retinal layer was measured within four sectors (superior, inferior, nasal, and temporal), extending 3.45 mm from the center of the fovea, leading to a macular volume measurement for each retinal layer. Mean volumes (mm³) for each layer and thickness (µm) for each quadrant (right and left eyes averaged) were computed for both the baseline and the 27-month time points. Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; OCT, optical coherence tomography; RNFL, retinal nerve fiber layer.
The volumes for all layers were obtained separately, across the entire macular region extending 3.45 mm from the center of the fovea. Mean volumes (mm$^3$) for each layer and thickness (µm) for each quadrant (right and left eyes averaged) were computed for both the baseline and the 27-month time points. The difference between these two examination time points was obtained for each subject and for each layer. Multicolor imaging, which uses individual laser wavelengths [70] to characterize anatomic and pathologic detail at different retinal depths, served to identify and exclude individuals with background diabetic retinopathy and retinal microbleeds within the region of interest.

2.4. Cognitive assessment

The GMLT (http://www.cogstate.com) is a computer-administered hidden maze learning test, designed by one of the authors (P.J.S.) to measure spatial working memory and problem-solving functions [71]. The GMLT has been described previously both in terms of performance within the context of studies with healthy elderly adults [51,72,73] and in MCI [74].

In addition, all subjects completed the ISLT (www.cogstate.com) as a measure of verbal episodic memory [75], the MMSE as a measure of general cognitive function, and the Memory Complaint Questionnaire [76] as a measure of subjective memory complaints.

As noted previously, we also administered an audiovisual McGurk task that has recently been described as a potential new marker of early-stage AD [60]. The McGurk effect is a compelling misperception in which discrepant visual and auditory speech information presented simultaneously results in the listener hearing a fused audiovisual speech sound, rather than the veridical auditory sound (e.g.,/ba/auditory + /ga/visual is heard as/da/rather than/ba/) [77]. The integrity and efficiency of the audiovisual integration process can be measured by comparing accuracy and response times to identify the auditory information under congruent (matching audio and visual components) and incongruent (conflicting audio and visual components) conditions. For the purpose of this study, we focused on a measure of integration efficiency, defined as the proportional increase in response time from congruent to incongruent conditions for the weaker McGurk stimulus in this task. This stimulus condition was particularly sensitive at discriminating patients diagnosed with amnestic MCI from healthy elderly [60]. Higher efficiency scores reflect greater sensitivity to binding strength.

All cognitive measures were performed by trained staff supervised by a licensed clinical neuropsychologist.

2.5. Statistical methods

For the demographic variables, t-tests were performed for each variable between the preclinical AD and healthy control groups. For the layers RNFL (macular and peripapillary regions), GCL, ONL, outer plexiform layer, inner nuclear layer, and IPL, paired 1-sided significance level of 0.05 t-tests was performed, and the effect sizes for each comparison were computed with the Cohen’s $d$ statistic. For each layer, the differences between baseline and 27-month measurements and between Aβ+ and Aβ− subjects for the total volume and the thickness of the average and the quadrants (N3.45, T3.45, S3.45, and I3.45) were computed. Linear regression models were performed with neocortical PET imaging SUVr entered as a response variable, and retinal layer measures as the explanatory variables, and covarying for effects of normal aging. Although age was not significantly different between groups, it is a key predictor of volumetric loss over time in the macula. To assess whether a correlation exists between retinal layer thickness and any of the cognitive tests, linear regression analysis (Pearson’s test) was adopted, a $P$ value less than .05 was considered significant, and the magnitude of differences was quantified using Cohen’s $d$.

3. Results

3.1. Sample demographics

Of the 56 participants enrolled, 35 were female and 11 of them were amyloid positive on PET imaging. There were no significant differences between Aβ+ and Aβ− groups with respect to sex ($P = .311$). Likewise, there were no group differences in the proportion of individuals with the APOE ε4 genetic risk marker for AD. The mean age for the total sample was 65.36 years, and this sample had an average of 17.31 years of education. All relevant demographic information, for both groups, is provided in Table 2. There were no group differences with respect to body mass index, subjective memory complaints, and cognitive performance at baseline examination (on any of the tests described previously). By definition, both groups significantly differed with respect to neocortical amyloid aggregation as measured via PET imaging ($P = .000$).

With respect to genetic risk for AD, eight of 15 individuals (53%) in the preclinical AD group (florbetapir PET SUVr scores ≥1.10) had at least one copy of the APOE ε4 allele, whereas 20 of 45 individuals (45%) in the control group (florbetapir PET SUVr scores <1.10) had at least one copy of the APOE ε4 allele. Hence, roughly half of the subjects in each group presented with this additional risk factor for AD, but due to small sample sizes, we were not able to further evaluate the specific effect of APOE genetic risk on retinal layer measurements. Subject demographic information, for both groups, is provided in Table 2.

3.2. Retinal layer measurements

At baseline examination, no group differences were found with respect to the thicknesses of any macular quadrant for any neuronal layer, nor were there any group differences observed with respect to total volumes for each layer in the same region (Table 3).
from the same measurements obtained at the 27-month visit.

Retinal measures Preclinical (n = 15) Control (n = 41) P-value

| Layer | Preclinical | Control | P-value |
|-------|-------------|---------|---------|
| mRNFL |             |         |         |
| Total volume (mm³) | 0.227 ± 0.021 | 0.231 ± 0.025 | .283 |
| Average thickness | 28.58 ± 3.08 | 28.92 ± 3.57 | .383 |
| Inferior | 33.04 ± 3.53 | 32.69 ± 4.39 | .599 |
| Nasal | 28.87 ± 3.87 | 29.12 ± 4.70 | .433 |
| Superior | 32.12 ± 4.42 | 32.74 ± 5.10 | .353 |
| Temporal | 20.29 ± 1.20 | 21.12 ± 2.76 | .157 |
| pRNFL |             |         |         |
| Average thickness | 103.36 ± 5.36 | 101.48 ± 9.76 | .726 |
| Inferior | 134.25 ± 8.15 | 129.05 ± 13.49 | .882 |
| Nasal | 83.04 ± 17.36 | 85.3 ± 19.88 | .368 |
| Superior | 127.45 ± 10.32 | 124.17 ± 13.72 | .765 |
| Temporal | 67.86 ± 11.07 | 67.71 ± 11.68 | .612 |
| GCL |             |         |         |
| Total volume (mm³) | 0.444 ± 0.018 | 0.428 ± 0.029 | .942 |
| Average thickness | 51.15 ± 2.20 | 48.94 ± 3.61 | .964 |
| Nasal | 53.60 ± 2.03 | 51.23 ± 3.70 | .970 |
| Temporal | 50.90 ± 3.77 | 49.01 ± 4.41 | .889 |
| Superior | 48.75 ± 4.01 | 48.08 ± 3.88 | .683 |
| Inferior | 51.35 ± 1.94 | 47.42 ± 4.18 | .997 |
| IPL |             |         |         |
| Total volume (mm³) | 0.353 ± 0.030 | 0.357 ± 0.027 | .339 |
| Average thickness | 39.40 ± 3.67 | 39.18 ± 3.27 | .578 |
| Inferior | 39.00 ± 4.92 | 37.54 ± 3.78 | .862 |
| Nasal | 40.37 ± 3.37 | 40.66 ± 3.66 | .403 |
| Superior | 37.50 ± 3.63 | 37.39 ± 3.68 | .534 |
| Temporal | 40.71 ± 4.73 | 41.10 ± 3.28 | .369 |
| OPL |             |         |         |
| Total volume (mm³) | 0.294 ± 0.373 | 0.301 ± 0.037 | .031 |
| Average thickness | 31.32 ± 3.40 | 32.10 ± 3.89 | .267 |
| Inferior | 30.91 ± 3.38 | 31.51 ± 4.68 | .410 |
| Nasal | 33.87 ± 6.19 | 35.33 ± 7.42 | .620 |
| Superior | 30.16 ± 3.36 | 30.65 ± 3.42 | .442 |
| Temporal | 30.33 ± 3.54 | 30.88 ± 3.50 | .483 |
| INL |             |         |         |
| Total volume (mm³) | 0.338 ± 0.020 | 0.348 ± 0.028 | .124 |
| Average thickness | 38.08 ± 1.39 | 38.54 ± 3.13 | .312 |
| Inferior | 37.54 ± 1.23 | 38.45 ± 3.04 | .158 |
| Nasal | 39.70 ± 1.55 | 39.67 ± 4.66 | .310 |
| Superior | 38.08 ± 1.81 | 38.70 ± 3.18 | .645 |
| Temporal | 37.00 ± 2.83 | 37.34 ± 3.37 | .372 |
| ONL |             |         |         |
| Total volume (mm³) | 0.679 ± 0.066 | 0.671 ± 0.067 | .644 |
| Average thickness | 67.09 ± 7.02 | 66.48 ± 7.04 | .604 |
| Inferior | 64.16 ± 7.38 | 64.00 ± 7.94 | .529 |
| Nasal | 67.92 ± 8.66 | 64.51 ± 8.97 | .877 |
| Superior | 68.08 ± 7.14 | 68.34 ± 6.63 | .453 |
| Temporal | 68.20 ± 7.17 | 69.09 ± 7.68 | .361 |

Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer.

NOTE. All measurements, for each layer, are acquired from all radial quadrants from the Early Treatment Diabetic Retinopathy Study circular grid, with a 3.45-mm diameter centered on the fovea. Data from both eyes are averaged. Values are mean ± SD. All values are in micrometer (µm), unless otherwise indicated.

Change over the 27-month follow-up period, for each of these measures, was calculated by subtracting the volumes (mm³) and thickness (µm) obtained at the baseline visit from the same measurements obtained at the 27-month visit.

As shown in Table 4, a significant group difference was found for the mRNFL (P = .050), ONL (P = .026), and IPL volumes (P = .020). In all cases, the preclinical AD group showed a larger reduction in volume over this time period, compared to the healthy control group, and in all cases these group differences were of a moderate effect size. The only significant changes observed in thickness after controlling for effects of age were in the inferior quadrant of the ONL (0.026) and IPL (P = .028), with a larger reduction in the preclinical group, and in the temporal quadrant of the outer plexiform layer (0.040), with a larger increase in the preclinical group compared to the control.

Considering each subject group separately, the change from baseline to 27 months in the total volume (mm³) of the mRNFL was significantly decreased for the preclinical AD group (−0.032 ± 0.003, P = .002) and for the healthy control group (−0.0190 ± 0.003, P = .03).

With respect to the pRNFL, although we found an overall nonsignificant difference in the magnitude of the thinning between the two groups, over the 27-month period, we observed substantially greater ranges of variance of measurements for both groups, as reflected by markedly larger standard deviations of measurement (Table 3). This region of the pRNFL is thought to have greater within-subject and between-subject variability because it contains a multitude of larger diameter blood vessels, particularly, with respect to vascular innervation within the regions of the superior and inferior arcuate bundles, individual differences in optic canal sizes, the presence or absence of space-occupying nerve head drusen, and other individual differences in this region [78].

3.3. Retinal layer change in relation to neocortical amyloid aggregation

A multivariate linear regression model, controlling for participants’ age, with total neocortical PET amyloid ligand binding (SUVr) entered as the dependent measure was conducted. Macular RNFL volume change, over the 27-month study interval, accounted for 10% of the variance in PET amyloid neocortical binding at the end of the study (adjusted R² = 0.106, ρ = 0.017; Fig. 2). By comparison, change in the GCL over the same time was related to participants’ age (ρ = 0.05), but not significantly related to PET amyloid SUVr (ρ = 0.78). For all neuronal cell layers, other than the mRNFL, the observed change over time was not related to neocortical PET amyloid SUVr.

3.4. Retinal layer change in relation to cognitive performance

There were no significant relationships found between volume reductions for any of the retinal layers over 27 months and performance on measures of spatial working memory and learning efficiency (GMLT) or on a measure of word-list learning and episodic verbal memory (ISLT) (P > .005).
Table 4
Change over 27 months by groups

| Location | Preclinical AD (N = 15) | Healthy controls (N = 41) | P-value | Effect size (Cohen’s d) |
|----------|-------------------------|---------------------------|---------|------------------------|
| mRNFL    |                         |                           |         |                        |
| Volume (mm³) | −0.032 ± 0.003 | −0.019 ± 0.003 | .050    | 0.550                 |
| Average  | −4.45 ± 3.68           | −3.26 ± 3.94              | .448    | 0.3072                |
| Inferior | −4.45 ± 3.314          | −3.09 ± 4.323             | .656    | 0.3352                |
| Superior | −5.10 ± 3.671          | −4.50 ± 4.845             | .348    | 0.1318                |
| Nasal    | −4.50 ± 3.801          | −2.41 ± 4.416             | .196    | 0.4917                |
| Temporal | −3.75 ± 3.936          | −3.04 ± 2.212             | .77     | 0.2528                |
| pRNFL    |                         |                           |         |                        |
| Average  | −3.389 ± 2.058         | −1.181 ± 4.057            | .060    | 0.605                 |
| Inferior | −1.39 ± 6.22           | 1.63 ± 9.10               | .386    | 0.357                 |
| Superior | −3.33 ± 11.92          | −1.41 ± 14.10             | .631    | 0.141                 |
| Nasal    | 1.67 ± 5.97            | 3.81 ± 7.79               | .381    | 0.290                 |
| Temporal | −1.04 ± 4.13           | 0.18 ± 4.51               | .400    | 0.276                 |
| GCL      |                         |                           |         |                        |
| Volume (mm³) | −0.016 ± 0.016 | −0.011 ± 0.019            | .206    | 0.29                   |
| Average  | −1.96 ± 2.59           | −1.34 ± 3.03              | .590    | 0.209                 |
| Inferior | −2.4 ± 2.97            | −0.86 ± 3.33              | .091    | 0.464                 |
| Superior | −1.0 ± 3.51            | −1.43 ± 4.18              | .614    | 0.107                 |
| Nasal    | −0.65 ± 3.54           | −0.05 ± 3.54              | .321    | 0.166                 |
| Temporal | −3.8 ± 2.87            | −3.0 ± 3.83               | .590    | 0.210                 |
| OPL      |                         |                           |         |                        |
| Volume (mm³) | 0.017 ± 0.04  | −0.003 ± 0.032            | .965    | 0.603                 |
| Average  | 0.843 ± −3.05          | 0.605 ± 2.89              | .932    | 0.495                 |
| Inferior | 0.75 ± 3.51            | −0.84 ± 2.59              | .956    | 0.566                 |
| Superior | 0.5 ± 3.37             | −0.23 ± 3.57              | .262    | 0.208                 |
| Nasal    | −0.83 ± 4.88           | −1.77 ± 6.65              | .329    | 0.144                 |
| Temporal | 2.95 ± 4.31            | 0.43 ± 4.53               | .040    | 0.562                 |
| ONL      |                         |                           |         |                        |
| Volume (mm³) | −0.029 ± 0.030 | −0.007 ± 0.035            | .026    | 0.646                 |
| Average  | −1.61 ± 2.86           | −0.068 ± 3.39             | .077    | 0.469                 |
| Inferior | −2.25 ± 5.66           | 0.84 ± 4.54               | .026    | 0.644                 |
| Superior | −1.70 ± 2.60           | −1.43 ± 3.73              | .405    | 0.078                 |
| Nasal    | 0.87 ± 3.58            | 2.92 ± 7.27               | .176    | 0.305                 |
| Temporal | −3.37 ± 7.62           | −2.60 ± 5.52              | .347    | 0.128                 |
| IPL      |                         |                           |         |                        |
| Volume (mm³) | −0.014 ± 0.015 | −0.006 ± 0.011            | .020    | 0.686                 |
| Average  | −1.80 ± 1.91           | −1.06 ± 1.66              | .099    | 0.427                 |
| Inferior | −2.41 ± 3.32           | −0.84 ± 2.17              | .028    | 0.638                 |
| Superior | −1.91 ± 1.50           | −1.06 ± 2.49              | .133    | 0.368                 |
| Nasal    | −0.95 ± 2.23           | −1.07 ± 2.011             | .567    | 0.055                 |
| Temporal | −1.91 ± 2.11           | −1.29 ± 2.57              | .22     | 0.250                 |
| INL      |                         |                           |         |                        |
| Volume (mm³) | 0.004 ± 0.019 | 0.001 ± 0.022             | .64     | 0.12                   |
| Average  | 2.04 ± 2.14           | 2.30 ± 5.50               | .43     | 0.276                  |
| Inferior | 2.04 ± 2.32           | 0.19 ± 2.67               | .98     | 0.708                  |
| Superior | 0.33 ± 1.91           | 0.011 ± 2.38              | .665    | 0.140                  |
| Nasal    | 0 ± 1.97              | 0.47 ± 3.94               | .34     | 0.131                  |
| Temporal | 0.70 ± 2.75           | 0.39 ± 2.25               | .655    | 0.131                  |

Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer.

NOTE: All measurements, for each layer, are acquired from all radial quadrants from the Early Treatment Diabetic Retinopathy Study circular grid, with a 3.45-mm diameter centered on the fovea. Data from both eyes are averaged. Values are mean ± SD, unless otherwise indicated. All values are in micrometer (μm), unless otherwise indicated. Bold values denote significant group differences.

However, an interesting correlation was found between the magnitude of mRNFL volume reductions and increasing difficulties on the McGurk task of efficiency for audiovisual integration. That is, individuals with greater volume reduction in mRNFL showed reduced sensitivity to the binding strength of the audiovisual stimulus (ρ = 0.037, 1-tailed; Fig. 3).

4. Discussion

The average RNFL thicknesses for both groups in our study are similar to the control groups reported by others [5,39] and to the measurements obtained by Golzan et al. in their preclinical AD [14]. Likewise, our OCT measurements
of the GCL and IPL layers are quite similar to those reported by others [22]. Of the prior studies we reviewed and described previously, Garcia-Martin et al. reported retinal layer thickness values that differed substantially from both our measurements and from most other published reports [20].

Most published research on this topic has relied on cross-sectional study designs, with measurements of the thickness and volume of various retinal layers at a single point in time. Such studies are likely to be limited in their ability to identify retinal markers of the preclinical stage of AD, as such individuals are still relatively young and such between-groups comparisons have not generally accounted for a variety of confounding variables including, but not limited to, effects of sex differences, ethnicity, axial length, optic disc area, and refractive status on OCT measurements [44,79–81]. Hence, we performed this within-subjects longitudinal study over 27 months to measure individuals' structural parameters that can be reliably remeasured at different time points using the Eye Tracker software for SPECTRALIS SD-OCT [82]; we then calculated the differences for each of the measures collected (Table 4) for both the preclinical AD and the healthy control groups. To our knowledge, this is the first attempt both to explore within-subject changes in retinal neuronal layer structures, in the preclinical stage of AD, and to relate any such observed changes to performance on a cognitive assay that has been shown to be sensitive to detection of early-stage disease burden. Our findings suggest that a decrease in mRNFL volume is the earliest detectable structural retinal change associated with AD. Moreover, this change in mRNFL volume is related with neocortical Aβ accumulation in very early AD (Fig. 1), even after correcting for expected age-related decline [83] (Table 5). By comparison, the decrease in GCL volume observed over the same time period was principally related to the effects of aging (p = 0.05) rather than due to cortical Aβ aggregation (p = 0.78), at least during this earliest detectable stage of disease progression.

The macular region of the retina is physiologically very active in healthy normal eyes [7], and this “hyperexcitation” might be diminishing in the preclinical stage of AD. In support of this hypothesis, postmortem histological studies have found prominent pathological alteration of retinal ganglion cells in the macular region in AD patients [26,84] and a preferential loss of larger axons, suggesting early involvement of the magnocellular retinal ganglion cells in AD as they contribute large caliber fibers to the optic nerve [85]. The RNFL is adjacent to the GCL, and it is composed largely of ganglion cell axons that are organized in superior and inferior arcuate bundles, and the papillomacular bundle, that lead to the optic nerve. Early in multiple sclerosis, there is demyelination without axonal loss. Likewise, the
mRNFL may be thinned due to demyelination, and possibly only when there is axonal loss, will we see loss of ganglion cells (GCL). Our results suggest early demyelination in the RNFL in the preclinical stage of AD, and our data confirm at least one other report of mRNFL changes occurring before pRNFL changes in AD [86]. Moreover, there is some suggestion that this might be most readily observed in the superior quadrant of the mRNFL [86], and this would make sense in light of at least one clinical report of preferential inferior visual field loss in AD [87]. However, in a meta-analysis of 17 studies comparing AD patients with healthy controls and five studies comparing individuals with MCI with controls, there were significant decreases in all four quadrants compared to controls, thus suggesting that the degenerative process affects the entire macular region [8].

There have been several prior reports of GCL thinning in the symptomatic stages of AD [5,15,22]. Moreover, Martin et al. [20] studied 150 patients with AD and 75 age-matched controls to model how changes in RNFL’s and other neuronal cell layers’ thicknesses are associated with disease duration and severity. This work suggests that there is axonal degeneration in the RNFL early in the disease followed by degenerative changes to the cell bodies in the GCL and then progression to deeper neuronal layers [20]. This progressive pattern of mRNFL loss before GCL loss is further confirmed by our results reported previously. In addition, we found that only loss of mRNFL tissue, and not loss in any other neuronal layer, was correlated with Aβ protein aggregation in the cerebral cortex. These results fit nicely with data from a large population-based epidemiological study (Rotterdam Study, 2007–2012) showing that having a thinner RNFL at a baseline examination was significantly associated with increased risk of later developing dementia [88].

4.1. Relationship of RNFL thinning to cognitive function in preclinical AD

Contrary to a few prior reports [49,50,89], we did not observe any correlation between any retinal layer changes and change in either episodic verbal memory (ISLT) or for performance on a working memory and reasoning task for visuospatial information (GMLT). However, because white matter degeneration is readily observable in the early stages of AD [90] and the cerebral white matter is principally composed of myelinated axons and glial cells—as is the retinal RNFL—we chose to administer a cognitive task that is putatively sensitive to functional disruption of corticocortical connections caused by disruption of white matter integrity [60]. Both prodromal and preclinical AD patients with high Aβ burden have been previously found to display changes in audiovisual integration efficiency that are likely due to AD-related disruptions in functional connectivity between posterior sensory regions. Using McGurk-like audiovisual speech stimuli (i.e., video clips of an individual mouthing a speech sound while the audio channel provides either consistent or inconsistent auditory speech information), we found that greater volume reduction in the mRNFL was significantly associated with reduced sensitivity to the binding strength of the audiovisual stimulus (Fig. 2). While healthy individuals typically show faster response times to consistent stimuli than inconsistent stimuli (i.e., response times are faster when the auditory and visual components provide the same compared to conflicting speech information), we found that this response time difference decreased with greater RNFL volume reduction. In fact, greater volume reduction in the mRNFL was significantly associated with reduced sensitivity to the binding strength of the audiovisual stimulus, suggesting that mRNFL volume reduction is related to white matter loss. Consistent with previous findings [60], this reduced sensitivity may reflect subtle disruptions of corticocortical projections within unimodal and heteromodal sensory cortices that indicate individual risk of AD.

4.2. Change in retinal neuronal layers, other than the RNFL, in preclinical AD

As noted previously, we observed significant changes in ONL (P = .026) and IPL volumes (P = .002), and inferior quadrant thicknesses for these same layers, respectively (ρ = 0.026; ρ = 0.028), in the preclinical AD group compared to the controls, over 27 months. However, neither of these changes was related to PET imaging severity of neocortical amyloidosis at the end of the 27-month interval (Table 5). We have previously reported, with the same cohort of subjects at their baseline examinations, an increase in the IPL volume in preclinical AD that may be related to either Aβ deposition or an inflammatory process [16]. Twenty-seven months later, we have now found evidence of tissue loss in the IPL, within our preclinical group relative to controls, suggesting that although there may be an initial early stage of IPL volume increase due to an inflammatory process, there is nonetheless some volume loss in this structure over continued disease progression.

Finally, the ONL consists of photoreceptor cell bodies, and thinning of this layer has previously been shown in patients with age-related macular degeneration in association with tears in the retinal pigment epithelium [91]. In the context of early AD, we believe that our observation of tissue loss in the ONL could suggest retrograde transsynaptic degeneration, but future exploration would be necessary to support this hypothesis.

5. Conclusion and future directions

To our knowledge, this is a first report of a within-subjects, prospective, longitudinal study of retinal anatomic changes in the preclinical stage of AD. We have found that thinning of the mRNFL may be the earliest
anatomic marker of retinal neuronal loss in the preclinical stage of AD, and such loss appears to account for 10% of the variance in observed PET imaging measurement of neocortical amyloidosis. Whereas most reports have relied on clinical examinations for determining disease burden, our study used the PET imaging biomarker of disease burden. Our study is limited by a small sample size, and so these findings require replication in a larger patient population. Second, we chose to recruit participants who were at high risk for the very early stages of the disease, that is, during a prodromal stage that is defined by a relative absence of readily identifiable cognitive and/or functional impairments, as well as by biomarker evidence of disease burden that is subtle and for which clear diagnostic criteria have not yet been established [46]. Third, retinal imaging is not only being studied to identify novel biomarkers for this neurodegenerative disease, but changes in GCL and RNFL thicknesses have also been reported in Parkinson’s disease and multiple sclerosis [92–95].

Future work on this topic will require the recruitment of larger populations of participants, across the entire disease severity spectrum (from healthy controls to mild AD), and ideally such a large cohort should be followed up for an even longer time interval to better model the natural history of retinal anatomic changes over the entire course of disease progression. Additional imaging modalities should be included in such a study (e.g., OCT angiography), as retinal blood flow appears to be reduced in MCI and AD [19,40], and the disease has been associated with a retinal venular stenosis, reduced complexity of the branching pattern and geometry, and reduced and tortuous venules [96]. We also would recommend inclusion of scanning laser polarimetry methods to measure RNFL retardance in preclinical AD because, in glaucoma, RNFL retardance seems to occur before actual RNFL thinning [97]. The mechanisms of glaucomatous injury appear to be related to damage to the integrity of axonal cytoskeletal ultrastructure before the point of RNFL thinning detectable by OCT [97–99]. The axonal cytoskeleton disruption that causes RNFL retardance [97] may possibly be similar to the effects of Aβ plaques and tau tangles in the AD brain.

These results point to the first retinal anatomic changes occurring in the early stages of AD. Characterizing retinal changes at this stage with a noninvasive method will facilitate the search for treatments that can delay or stop the progression of AD. This study also demonstrates that, although many different approaches have already been taken in this field, there is still much more to explore and much that we do not understand with respect to the effects of AD on the retina.

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RESEARCH IN CONTEXT

1. Systematic review: We reviewed 15+ years of literature on the relationship between the morphology of retinal neuronal layers and Alzheimer’s disease (AD), and the evidence of disease-related retinal change over the progression of AD. This literature is mixed, with inconsistent findings, and it consists mostly of cross-sectional studies in patients with symptomatic disease. We were unable to identify prior publications that have sought to explore within-subjects longitudinal change in retinal morphology during the preclinical stage of AD for all retinal neuronal layers.

2. Interpretation: After controlling for expected effects of normal aging, we observed a decrease in the macular retinal nerve fiber layer that is moderately correlated with positron emission tomography imaging evidence of amyloid aggregation. Volume loss in the macular retinal nerve fiber layer, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving but is modestly related to relative decrements in performance on a measure of visual-auditory integration of new information. This may be a first report of neuronal retinal layer volumetric changes in a within-subjects, prospective, longitudinal study of preclinical AD.

3. Future directions: Larger populations of participants, across the entire disease severity spectrum (from healthy controls to mild AD), should be followed up for an even longer time interval to better model the natural history of retinal anatomic changes over the entire course of disease progression. Additional imaging methods could improve our understanding of the mechanisms of disease-related retinal change, such as optical coherence tomography angiography and scanning polarimetry.

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