Morphological and functional retinal impairment in Alzheimer’s disease patients

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Abstract

**Objective:** Our study aims to assess the optic nerve fiber layer thickness in vivo, the function of the innermost retinal layer and whether a correlation exists between morphological and functional parameters in patients affected by Alzheimer’s Disease (AD).

**Methods:** Seventeen AD patients (mean age 70.37 ± 6.1 years, best corrected visual acuity >8/10 with refractive error between ±3 sf, intra-ocular pressure (IOP) < 18 mmHg) were enrolled. They were compared to 14 age-matched controls. Nerve fiber layer (NFL) thickness was measured by optical coherence tomography (OCT). Three different measurements in each quadrant (superior, inferior, nasal, and temporal) were taken and averaged. The data in all quadrants (12 values averaged) were identified as NFL Overall. Retinal function was assessed by pattern electroretinogram (PERG) recordings using high-contrast (80%) checkerboard stimuli subtending 15 min of the visual arc and reversed at the rate of two reversals/s.

**Results:** In AD eyes, there was a significant (P < 0.01) reduction in NFL thickness in each quadrant and in the NFL Overall evaluation compared with the values observed in control eyes. PERGs showed a significant (P < 0.01) delay in N35, P50 and N95 implicit times, and reduction in N35-P50 and P50-N95 amplitudes. NFL Overall values were significantly correlated (P < 0.01) to the PERG P50 and N95 implicit times and P50-N95 amplitude. No correlations (P > 0.01) between NFL values and other PERG parameters (N35 implicit time, N35-P50 amplitude) were found.

**Conclusions:** Our results suggest that in AD patients, there is a reduction of NFL thickness evaluated in vivo by OCT and this morphological abnormality is related to a retinal dysfunction as revealed by abnormal PERG responses. © 2001 Published by Elsevier Science Ireland Ltd.

**Keywords:** Alzheimer’s disease; Pattern electroretinogram; Retinal ganglion cells; Optical coherence tomography

1. Introduction

A depletion of optic nerve ganglion cells and their axons has been histologically observed in Alzheimer’s Disease (AD) patients (Hinton et al., 1986). Further morphometric analyses performed by the paraphenylene–diamine technique confirmed this finding and showed a predominant involvement of the largest retinal ganglion cells (M-cells) that contribute with large caliber fibers to the optic nerve (Sadun and Bassi, 1990). However, more recent studies (Rizzo et al., 1992; Curcio and Drucker, 1993) failed to confirm the above-mentioned findings. This discrepancy has been, at least partially, attributed to methodological bias such as a different postmortem delay in axon count and/or difficulties in obtaining well-preserved myelinated axons.

An objective method of quantifying in vivo the optic nerve axons and retinal thickness has been proposed. This method consists of a new non-invasive technology allowing cross-sectional imaging of the eye by optical coherence tomography (OCT) (Huang et al., 1991; Hee et al., 1999).
1995a,b; Puliafito et al., 1995; Schuman, 1997), and so far has been widely employed in assessing the nerve fiber layer (NFL) thickness in ocular hypertension or glaucoma (Parisi et al., 1999a, 2001; Manni et al., 1999; Schuman et al., 1995) and multiple sclerosis (Parisi et al., 1999b). Recently, Chauhan and Marshall (1999) raised some criticism regarding the accuracy of the OCT in the NFL thickness measurement. However, in the same report, they showed a good correlation between excimer-laser induced ablation of the inner retina and the signal recorded by OCT, stating that ‘… the thickness of the inner band was reduced by the same amount as the ablation step height’. Therefore, although the accuracy of OCT in quantifying NFL thickness is still a matter of debate, we can assume that progressive changes in the OCT signal coming from the inner retina (including NFL, inner plexiform layer and ganglion cell layer) are paralleled by similar changes occurring in the tissue.

The bioelectrical activity of ganglion cells and their fibers can be objectively assessed by recording electroretinographic signals in response to patterned stimuli (PERG) (Maffei and Fiorentini, 1981, 1990). Several studies have shown the presence of an abnormal PERG in AD patients and this finding was considered as further evidence of retinal ganglion cell dysfunction (Katz et al., 1989; Trick et al., 1989).

A group of AD patients was evaluated by means of combined morphometric (OCT) and electrophysiological (PERG) analyses in order to assess: (a) the optic nerve fiber layer thickness in vivo; (b) the function of the innermost retinal layer; (c) whether a correlation exists between morphological and functional parameters. Preliminary results have been previously published as an abstract (Parisi et al., 2000).

2. Subjects and methods

2.1. Subjects

According to the inclusion criteria (see above), 17 subjects (mean age 70.37 ± 6.1 years) were selected from a group of 40 patients with diagnosis of AD. The diagnosis was performed using the established criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). For this study, we included patients with a mild severity of cognitive impairment, according to criteria described in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) (American Psychiatric Association, 1987).

The general inclusion criteria for AD patients and their age-matched controls (N = 14) were: no evidence of vascular dementia, no history of alcohol abuse, no psychiatric disorders, no dismetabolic diseases, no arterial hypertension, no other neurological diseases, no psychotropic therapy. Ocular inclusion criteria were: best corrected visual acuity >8/10 with refractive error between ±3 sf, IOP <18 mmHg, absence or previous history of optic media opacity, cataract or early lens opacity, glaucoma, retinal detachment, early age-related macular degeneration, or other macular degeneration, optic neuropathy, retinal vascular diseases.

Each AD patient underwent psychometric testing including the Mini Mental State Examination (MMSE) (Folstein et al., 1975), the Alzheimer’s Disease Assessment Scale (ADAS) (Rosen et al., 1986; Mohs and Cohen, 1988) and the Immediate Visual–Spatial Memory (IVM) (Gainotti et al., 1978).

Furthermore, magnetic resonance imaging (MRI) was performed in each AD patient, showing a variable degree of cerebral and cortical atrophy.

Informed consent was received from all subjects involved in the study. The research followed the tenets of the Declaration of Helsinki and the protocol was approved by the local Ethical Committee.

2.2. Methods

2.2.1. Optical coherence tomography examination

OCT (Humphrey, Dublin, CA, USA), including the fiber optic delivery system coupled with slit-biimicroscope, was used. This system provides the operator with a video-camera view of the scanning probe beam on the fundus and OCT imaging acquired in real time on a computer monitor. After dilatation with 1% tropicamide, each eye was scanned 3 times using a circle size of 3.4 mm (1.7 mm radius). Near-infrared light (840 nm wavelength) was used. Throughout scanning, the patient kept each eye constantly fixed on an internal target provided by the equipment. The measurements were obtained from 3 non-consecutive scans (i.e. the patient was allowed to rest for a few seconds before being re-positioned to proceed to the following scan). As previously reported, the OCT software provides an automated computer algorithm that identifies the anterior and posterior borders of the retina (Schuman, 1997). This has been claimed as offering the possibility of calculating both NFL and total retinal thickness (Schuman, 1997). The software allows the mapping of the thickness data according to both quadrant-by-quadrant and a clock hour analyses. Retinal thickness was determined by computer as the distance between the first reflection at the vitreoretinal interface and the anterior boundary of the second reflective layer, corresponding to the retinal pigment epithelium and the choriocapillaris. As discussed elsewhere, NFL thickness was automatically assessed by computer assuming the correlation with the red highly reflective layer at the vitreoretinal interface (Schuman, 1997). The posterior margin of the NFL is automatically located by the software, by starting within the photoreceptor layer (posteriorly) and searching forward in the image (Schuman, 1997).

We considered the average values of 3 different measurements per quadrant (superior, inferior, nasal and temporal):
the overall data obtained in all quadrants (12 values averaged) were identified as NFL Overall.

2.2.2. Pattern electroretinogram recordings

According to previously published studies (Parisi, 1997, 2001; Parisi et al., 1997, 1998a,b, 1999a,b,c, 2001), PERG recordings were performed using the following method. The subjects under examination were seated in a semi-dark, acoustically isolated room in front of the display surrounded by a uniform field of luminance of 5 cd/m². Prior to the experiment, each subject was adapted to the ambient room light for 10 min and the pupil diameter was about 5 mm. Mydriatic or miotic drugs were never used. Stimulation was monocular after occlusion of the other eye. Visual stimuli were checkerboard patterns (contrast 80%, mean luminance 110 cd/m²) generated on a TV monitor and reversed in contrast at the rate of two reversals/s. On the basis of previous studies (Parisi et al., 1999b), the check edges subtended 15 min of visual angle. The screen of the monitor subtended 18°.

The bioelectrical signal was recorded by a small Ag/AgCl skin electrode placed over the lower eyelid. PERGs were derived bipolarly between the stimulated (active electrode) and the patched (reference electrode) eye using the method previously described (Fiorentini et al., 1981). As the recording protocol was extensive, the use of skin electrodes with an inter-ocular recording represented a good compromise between signal-to-noise ratio and signal stability. A discussion on PERG using skin electrodes and its relationship to the responses obtained by corneal electrodes can be found elsewhere (Hawlina and Konec, 1992; Porciatti and Falsini, 1993). The ground electrode was in Fpz. The inter-electrode resistance was lower than 3 kΩ. The signal was amplified (gain 50 000), filtered (band pass 1–30 Hz) and averaged with automatic rejection of artifacts (200 events free from artifacts were averaged for every trial) by BM 6000 (Biomedica Mangoni, Pisa, Italy). The analysis time was 250 ms. The transient PERG response is characterized by a number of waves with 3 subsequent peaks, of negative, positive, negative polarity, respectively. In normal subjects, these peaks have the following implicit times: 35, 50 and 95 ms (N35, P50, N95).

Since an intra-individual PERG variability, related to inattention or optical defocus, has been described in AD patients (Prager et al., 1993), the recording session was continuously monitored (with an infrared video camera) to check whether the patient tested maintained a stable fixation over a small red target (0.5°) placed in the center of the stimulus field. The refraction of all subjects was corrected for viewing distance.

We accepted PERG signals with signal-to-noise ratio >2. The noise was measured by recording the bioelectrical signals (200 averaged events), while the monitor was screened by a cardboard and a retinal noise (peak-to-peak measure) <0.1 mV (mean 0.086 mV) was observed in all subjects tested. For all PERG recordings, the implicit time and the peak-to-peak amplitude of each of the averaged waves were measured directly on the displayed records by means of a pair of cursors.

2.3. Statistical analyses

The data are reported as mean values ±1 standard deviation. The differences between control and AD eyes were statistically evaluated with Mann–Whitney U-test. In order to assess whether a correlation exists between NFL thickness and electrophysiological parameters, linear regression analysis (Pearson’s test) was adopted. In both statistical analyses, a P value less than 0.01 was considered significant.

OCT scans were performed by one observer. Test–retest variability was maintained within the 5% limit. In case of a variability >5%, a new set of 3 independent scans was repeated until proper values were recorded.

In the recording session, PERGs were recorded at least twice and the resulting waveforms were superimposed to check the repeatability of the results. The test–retest variability of PERG parameters proved to be <5%.

3. Results

The clinical characteristics of AD patients are shown in Table 1.

3.1. Optical coherence tomography examination

The examples of NFL assessment in two AD eyes (AD1, AD13) and in one control eye are shown in Fig. 1. The mean data and statistical results are shown in Table 2. NFL Overall thickness was within 86.7 and 111.7 μm (mean: 99.9 ± 8.95 μm) in control subjects and within 29.5 and 105.4 μm (mean: 59.5 ± 16.70 μm) in AD patients. NFL thickness evaluated in the separate quadrant (inferior, superior, nasal and temporal) or in the Overall evaluation was significantly (P < 0.01) reduced when compared with that of control subjects.

3.2. Pattern electroretinogram

The examples of PERG recordings from one control and two AD eyes are shown in Fig. 1. The mean data and statistical results are shown in Table 2. AD eyes showed the N35, P50 and N95 implicit times significantly (P < 0.01) delayed compared with those of control eyes; N35-P50 and P50-N95 amplitudes were significantly (P < 0.01) reduced compared with those of control eyes.

3.3. Optical coherence tomography vs. pattern electroretinogram

The correlations between NFL thickness and PERG parameters are shown in Table 3. In AD eyes, the NFL Overall
Table 1
Clinical, morphological (OCT), psychometric (MMSE, ADAS, IVM) and electrophysiological (PERG) data in patients affected by Alzheimer’s Disease (AD)*

<table>
<thead>
<tr>
<th>AD</th>
<th>Eye</th>
<th>S</th>
<th>Age (ys)</th>
<th>VA</th>
<th>IOP</th>
<th>NFL Overall</th>
<th>NFL Inferior</th>
<th>NFL Superior</th>
<th>NFL Nasal</th>
<th>NFL Temporal</th>
<th>MMSE</th>
<th>ADAS</th>
<th>IVM</th>
<th>PERG N35</th>
<th>PERG P50</th>
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<tr>
<td>AD1</td>
<td>LE</td>
<td>F</td>
<td>69</td>
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<td>14</td>
<td>29.5</td>
<td>19.3</td>
<td>48.0</td>
<td>9.3</td>
<td>27.0</td>
<td>18.05</td>
<td>33</td>
<td>16</td>
<td>45</td>
<td>76</td>
<td>111</td>
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<td>0.5</td>
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<td>47.0</td>
<td>33.0</td>
<td>57.0</td>
<td>31.4</td>
<td>18.75</td>
<td>46</td>
<td>11</td>
<td>44</td>
<td>73</td>
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<td>0.7</td>
</tr>
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<td>M</td>
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<td>14</td>
<td>48.5</td>
<td>82.3</td>
<td>56.3</td>
<td>28.6</td>
<td>27.0</td>
<td>18.25</td>
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<td>47.8</td>
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<td>68.5</td>
<td>28.5</td>
<td>28.5</td>
<td>14.27</td>
<td>50</td>
<td>9</td>
<td>41</td>
<td>73</td>
<td>108</td>
<td>0.6</td>
<td>0.6</td>
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<tr>
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<td>RE</td>
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<td>15</td>
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<td>74.0</td>
<td>51.0</td>
<td>45.0</td>
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<td>11.99</td>
<td>46</td>
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<td>61.5</td>
<td>66.5</td>
<td>49.5</td>
<td>39.6</td>
<td>18.09</td>
<td>19</td>
<td>26</td>
<td>48</td>
<td>69</td>
<td>106</td>
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<td>0.7</td>
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<td>RE</td>
<td>F</td>
<td>74</td>
<td>0.9</td>
<td>14</td>
<td>51.6</td>
<td>78.0</td>
<td>42.0</td>
<td>31.0</td>
<td>55.5</td>
<td>17.86</td>
<td>19</td>
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<td>43</td>
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<td>0.8</td>
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<tr>
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<td>LE</td>
<td>M</td>
<td>62</td>
<td>1.0</td>
<td>14</td>
<td>58.1</td>
<td>82.0</td>
<td>79.5</td>
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<td>26.0</td>
<td>11.27</td>
<td>52</td>
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<td>42</td>
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<td>28.0</td>
<td>19.05</td>
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<td>0.8</td>
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<td>M</td>
<td>68</td>
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<td>16</td>
<td>59.3</td>
<td>53.0</td>
<td>94.6</td>
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<td>36.6</td>
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<td>57</td>
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<td>63</td>
<td>106</td>
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<td>0.7</td>
</tr>
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<td>M</td>
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<td>17</td>
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<td>93.6</td>
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<td>57.2</td>
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<td>57</td>
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<td>0.5</td>
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<td>70.5</td>
<td>97.2</td>
<td>30.5</td>
<td>41.5</td>
<td>16.86</td>
<td>57</td>
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<td>80.0</td>
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<td>56</td>
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<td>42</td>
<td>60</td>
<td>101</td>
<td>0.7</td>
<td>1.3</td>
</tr>
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</table>

95%CL of controls – – – 0.3* 20* 82.0# 96.46# 84.4# 66.2# 62.9# 23# 18* 17# 42.7* 66.4* 105.3* 0.85# 1.22#

* Abbreviations. S, sex; Ys, years; VA, best corrected visual acuity; IOP, intra-ocular pressure; NFL, nerve fiber layer (micron); PERG N35, P50, N95, PERG N35, P50, N95 implicit times (ms); PERG N35-P50 and P50-N95, PERG N35-P50 and P50-N95 amplitude (μV). *Upper 95% confidence limit (CL); # lower 95% confidence limit.
values were significantly correlated ($P < 0.01$) to the PERG P50 and N95 implicit times and PERG P50-N95 amplitude. No significant correlations ($P > 0.01$) between other PERG parameters (N35 implicit time and N35-P50 amplitude) and NFL values were found. In control eyes, no significant correlation between PERG parameters and NFL thickness was observed.

3.4. **Optical coherence tomography vs. patient’s age and psychometric parameters**

In AD patients, no significant correlations ($P < 0.01$) between the NFL values and age or psychometric parameters (MMSE, ADAS, IVM) were observed.

4. **Discussion**

AD patients showed a reduction in NFL thickness significantly correlated to abnormal PERG responses.

Our OCT results show an NFL reduced thickness in each quadrant examined indicating that an involvement of the neuroretinal tissue occurs in AD patients. An age-related reduction of NFL thickness in normal subjects has been reported (Gramer and Dirmeyer, 1998); however, the reduc-

<table>
<thead>
<tr>
<th>Group</th>
<th>NFL ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inferior</td>
</tr>
<tr>
<td>Controls ($N = 14$)</td>
<td>116.2 ± 9.87</td>
</tr>
<tr>
<td>AD ($N = 17$)</td>
<td>77.9 ± 26.4*</td>
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<table>
<thead>
<tr>
<th>Group</th>
<th>PERG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N35 implicit time (ms)</td>
</tr>
<tr>
<td>Controls</td>
<td>36.3 ± 3.23</td>
</tr>
<tr>
<td>AD</td>
<td>43.1 ± 2.28*</td>
</tr>
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</table>

* Statistics: Mann–Whitney U-test with respect to controls, *P < 0.01.
tion in NFL thickness observed in our AD patients was significantly greater than that observed in the age-matched controls and therefore it cannot be exclusively ascribed to aging. These data confirm previous observations obtained by histological studies (Hinton et al., 1986) or by other methods of evaluating the NFL in vivo. Blanks and coworkers histologically observed a total decrease of 25% of neurons in the ganglion cell layer at the level of the central retina (foveal/parfoveal retina) in AD patients (Blanks et al., 1996a); the greatest decrease was observed in the foveal region (43% decrease) with the most prominent loss over the temporal region. Similar results were obtained in AD patients with the evaluation of the peripheral retina: the neuronal loss was more pronounced in the superior and inferior quadrants (50–59%) and a significantly increased ratio of astrocytes to neurons was detected when compared with control eyes (Blanks et al., 1996b). Studies performed in vivo by various methods have confirmed that AD patients have optic nerve fiber damage. By using an optic nerve head analyzer, Tsai et al. (1991) have observed an increased cup-to-disc ratio and cup volume and decreased disc rim area in AD patients compared with controls. A retinal nerve fiber layer photograph study was recently performed in 26 AD patients (Hedges et al., 1996): a higher proportion of AD patients showed retinal NFL abnormalities when compared with controls; however, since the inter-observer agreement was 76.7% only, the applicability of this method seems to be low.

The loss of ganglion cells and their fibers observed in AD patients could be ascribed to a neurodegenerative process involving the neuroretinal structures. The method used in our study (morphometric evaluation in vivo of the NFL thickness) does not allow us to establish the mechanisms involved in producing ganglion cell fiber loss in AD. An appropriate evaluation of this parameter may be accomplished by means of histochemical studies. In this sense, it is worth noting that beta-amyloid and amyloid-associated proteins tau and amyloid precursor protein (APP) that have been related to the pathogenesis of the AD are expressed in the human retina at the level of ganglion cells and fibers (Loeffler et al., 1995). Furthermore, experimental studies performed in the rabbit have shown that beta-APP is synthesized in retinal ganglion cell periaxis and then transferred to the axonal plasma membrane and presynaptic nerve terminals (Morin et al., 1993) and that amyloidogenic C-terminal fragments are generated during axonal transport of APP in the optic nerve (Amaratunga and Fine, 1995). Despite these findings, histopathological studies have revealed that the characteristic features found in the brain of AD patients (neurofibrillary tangles, neuritic plaques and granulovacuolar degeneration) are not present in the retina (Hinton et al., 1986), while amyloid deposits in the lateral geniculate nucleus, neuritics plaques and neurofibrillary tangles in the superior colliculus have been observed (Leuba and Saini, 1995).

Therefore, other studies are needed in order to give further explanations about the relationship between the pathological mechanisms underlying AD and the reduction in NFL we have observed.

In our AD patients, PERG responses with delayed N35, P50 and N95 implicit times and reduced N35-P50 and P50-N95 amplitudes were observed. The delayed P50 and N95 implicit times and the reduced P50-N95 amplitudes were significantly correlated with the NFL reduced thickness while no significant correlations between delayed N35 implicit time, reduced N35-P50 amplitudes and reduced NFL thickness were observed.

The delay in implicit time and the reduction in amplitude of the PERG response observed in our AD patients is similar to that observed in other diseases that are known to affect the inner retina, such as diabetes (Parisi et al., 1997; Prager et al., 1990; Parisi et al., 1998a; Parisi and Uccioi, 2001), glaucoma (Parisi, 1997, 2001; Pfeiffer et al., 1993; Salgarello et al., 1999; Parisi et al., 1999c, 2001) or multiple sclerosis (Porciatti et al., 1996; Parisi et al., 1998b, 1999b). Since our AD patients were screened to exclude any disease that potentially induces an impairment of the inner retina (see inclusion criteria), the abnormality of the PERG response could be ascribed to changes, induced by Alzheimer’s disease, on retinal function. Our electrophysiological findings are in agreement with previous studies, in which similar PERG abnormalities were found in AD patients (Katz et al., 1989; Trick et al., 1989).

In our AD patients, as in other pathologies in which there is a retinal impairment (Parisi, 1997, 2001; Parisi et al., 1997, 1998ab, 1999ab, 2001), we found an increase in P50 implicit time. On the other hand, a shortening of P50 implicit time has been observed in pathologies in which there is a primary retinal ganglion cell dysfunction such as dominant optic atrophy (Holder at al. 1999), optic nerve compression (Holder, 1997) and surgical optic nerve section (Harrison et al., 1993). These human data are supported by a
recent paper by Viswanathan et al. (2000), in which a shortening of the P50 implicit time has been observed after inducing experimental glaucoma or TTX treatment in monkey eyes. Therefore, all these mentioned studies suggest that in the presence of ‘pure’ retinal ganglion cell dysfunction, there is a shortening and not a delay of the P50 component. On the basis of these studies by Holder et al. (1999), Holder (1997), Harrison et al. (1993) and Viswanathan et al. (2000), we believe that the delay in the P50 component observed in our AD patients cannot be exclusively ascribed to ‘pure’ ganglion cell dysfunction.

In fact, since full-field electoretinogram (ERG) or oscillatory potentials have not been recorded in our patients, we cannot exclude that a dysfunction of preganglionic elements may also occur, although data of Katz et al. (1989) suggested that no anatomical and functional evidence of damage in the outer retina is present in AD patients. A possible dysfunction of preganglionic elements could explain the increase in P50 implicit time observed in our AD patients and this is supported by data obtained in glaucoma or in multiple sclerosis in which the delay of the P50 implicit time (Parisi et al., 1999a,b,c, 2001) could be ascribed to a dysfunction of both ganglionic and preganglionic elements (Velten et al., 2001; Lightman et al., 1987).

Nevertheless, the significant correlation between the delay in P50 implicit time and the reduced NFL thickness observed in our AD patients, lead us to believe that a contribution of the inner retinal neurons to the P50 component cannot be entirely excluded. On the contrary, the correlation between the delay in N95 implicit time or the reduction in P50-N95 amplitude and the reduced NFL thickness can be explained, in agreement with Viswanathan et al. (2000), by the hypothesis that the N95 component depends exclusively on ganglion cell function. The lack of correlation between NFL thickness and PERG N35 implicit times and N35-P50 amplitudes could be explained by the hypothesis that these transient PERG components could reflect both the function of the innermost retinal layers and a large contribution of those outer retinal elements that are sensitive to uniform luminance changes (preganglionic cells located in more distal retinal layers) (Tobimatsu et al., 1989; Parisi et al., 1999b).

In conclusion, our results suggest that in AD patients there is a reduction of NFL thickness evaluated in vivo by OCT, and this morphological involvement is related to a retinal dysfunction as revealed by abnormal PERG responses.

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