Dendritic and Synaptic Plasticity of Neurons in the Human Age-Related Macular Degeneration Retina

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PURPOSE. To determine whether structural plasticity is evident in human retinal tissues in response to age-related macular degeneration (AMD). Remodeling events such as sprouting of neuronal processes and the reconnection of synapses are essential elements in repairing any damage to adult nervous tissues such as might occur in response to insults such as strokes or in AMD.

METHODS. The anatomic architecture of normal and AMD-affected human retinas was examined in the central, midperipheral, and far-peripheral regions. The retina, by virtue of its well-organized laminar structure, allows the identification and analysis of abnormal projections or connections of neuronal elements.

RESULTS. In AMD-affected retinas, but not in normal aged human retinas, a large number of photoreceptor synapses across the entire retina retracted into the outer nuclear layer. This event evokes the subsequent outgrowth of dendrites from the postsynaptic bipolar cells, again across the entire retina, and the subsequent reformation of synaptic contacts between photoreceptor and bipolar cells.

CONCLUSIONS. These findings illustrate that there are anatomic changes in the AMD retina at all eccentricities, not just in the macular region. Aged human retinal neurons have the capacity to form new synapses, and this finding may be important when investigating possible means of repairing the damaged human retina. (Invest Ophthalmol Vis Sci. 2007;48:2782–2791) DOI: 10.1167/iovs.06-1283

Age-related macular degeneration (AMD) is a common blinding disease known to cause the loss of photoreceptors in the central region (macula) of the human retina. There are two clinical forms of macular degeneration, the atrophic (dry) form (characterized by the presence of soft drusen and the loss of retinal pigment epithelium [RPE] and photoreceptors in the absence of neovascular AMD) and the more severe neovascular (wet) form (characterized by features such as abnormal subretinal blood vessels). The prevalence of macular degeneration has been shown to be in the range of 20% to 50% in individuals 75 years of age or older. Most investigations into AMD have focused on the changes in the macula, such as the loss of photoreceptors and RPE. However, little has been done to elucidate the possibility of changes in the peripheral retina. When such studies have been performed on AMD-afflicted or aged retinas, Müller cells and astrocytes have been primarily analyzed, revealing changes in glial cell immunoreactivity for glial fibrillary acidic protein (GFAP) in both normal aged and AMD-afflicted retinas as well as evidence of some dystrophic ganglion cells and photoreceptors.

There is contradictory evidence in the literature as to whether there is a decline in rod-mediated sensitivity associated with aging and early AMD. Some studies have shown evidence of a decline in scotopic responses when measured using electroretinography. Conversely, other studies have found little evidence of functional decline with AMD or age. Previous studies analyzing structural aspects of the inner retina, including those from our own laboratory, have failed to show overt anatomic changes in populations of neurons such as glycergic amacrine cells. Accordingly, there is some ambiguity as to whether any putative changes in rod pathway function are translated through into structural changes, such as dendritic plasticity in second-order neurons such as bipolar cells. The premise for this study was that although neuronal plasticity has historically been characterized as being predominantly a feature of the developing nervous system, there is now significant evidence of adaptive structural changes to neurons in adult tissues after overt injuries. Such changes include altering neurite arborization of cortical neurons after deafferentation in response to ischemic insults and in response to spinal cord injuries and of retinal neurons after insults such as retinal detachment. In light-damaged rat eyes, which exhibit many features that are comparable to those in dry AMD we have shown extensive remodeling of the retina including extensive dendritic remodeling and the extension of neurons along glial cell processes that are also remodeled. Similarly, it has been inferred that in humans, recovery of some function after strokes and closed head injuries may be the result of neuronal plasticity.

A major problem in studying the anatomic aspects of plasticity is in knowing where the neurons or their processes should have been before the insult, to allow inferences to be drawn as to whether they have been anatomically remodeled. The retina is uniquely appropriate to studies of this type, because of its highly predictable, layered structure.

In this study, we have reexamined the human AMD retina, to look for anatomic substrates that could explain the functional changes that are described in some studies. We examined eyes with the dry form of AMD rather than the wet form, since in the wet form the changes in permeability of the blood-retina barrier are likely to cause widespread changes in retinal function due to the influx of molecules such as glutamate from the blood. Because the wet form of AMD is treatable, it is plausible that the pathologic conditions exhibited by eyes with a history of the wet form of AMD are confounded by the prior treatment regimens. These specific confounders were presumed to be of less influence in eyes with the dry form of AMD, which is not treatable. In this study, we did not focus on

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the macular region, as there are significant degenerative changes in this region that obscure any early remodeling events that may have been present in this region of these eyes. We hypothesized that the functional changes observed in some ERG analyses were indicative of anatomic and functional plasticity in the synaptic circuitry, possibly at the level of the photoreceptor–bipolar synapses (because amacrine cells appear to be anatomically normal in such tissues).

METHODS

Human Ocular Tissues

Donated human postmortem retinal tissues were obtained as fixed tissues (4% formaldehyde fixation) from the Lions Eye Banks in Brisbane and Sydney and the Lions Eye Institute for Transplant and Research (Tampa, FL). Eyes were from donors, aged 61 to 85 years of age. The research adhered to the tenets of the Declaration of Helsinki for biomedical research involving human subjects.

Adult human retinas (five normal with no evidence of ocular disease, six with a diagnosis of the dry form of macular degeneration, see Tables 1, 2) were obtained with postmortem delays of less than 8 hours. The eyes were classified as being normal or exhibiting evidence of dry AMD by the eye banks before they were dispatched. Additional clinical information and treatment histories were not made available. However, for each eye we also subsequently confirmed their status by histology using standard criteria. The status of an eye as a normal aged eye was confirmed by the following standard histologic criteria: (1) The macular region had a normal appearance, with intact photoreceptors and no overt evidence of degeneration in any other retinal layer; (2) the RPE was apparently normal, with no breaks in Bruch’s membrane; and (3) drusen deposits were absent. Lack of choroidal neovascularization was also a criterion both in attributing normal status to the eye and in excluding the wet form of AMD. Dry-AMD eyes were confirmed, by histology, the status of an eye as a normal eye or one afflicted by dry AMD.

Antibodies

Immunohistochemistry was performed using rabbit polyclonal antibodies against the vesicular glutamate transporter vGlut-1 (1:1000; Synaptic Systems, Göttingen, Germany), PKCa (C-20; 1:3000; Santa Cruz Biotechnology, Santa Cruz, CA), calbindin (D28K; 1:3000; Swant, Bellinzona, Switzerland), Glyt-1 (ImmunoSolution, Jesmond, NSW, Australia), GLT-1c, and a mouse monoclonal antibody against synaptophasin (1:300; Sigma-Aldrich, St. Louis, MO). Labeling was performed singularly or in conjunction with lucifer yellow (LY)-filled preparations or in combination with other markers.

Immunocytochemistry

Immunohistochemical staining was subsequently performed by using standard immunoperoxidase and immunofluorescence protocols. Immunoperoxidase labeling used biotinylated anti-rabbit secondary antibodies and streptavidin-biotin horseradish peroxidase (HRP) complex (GE Healthcare, Sydney, NSW, Australia) at a dilution of 1:300, and labeling was revealed by using diaminobenzidine (DAB) as the chromogen. Immunofluorescence labeling was performed with species-specific anti-rabbit and anti-mouse antibodies (GE Healthcare or Sigma-Aldrich) coupled to FITC or Texas red at a dilution of 1:200. Immunoperoxidase-labeled sections were viewed with a microscope (BX51; Olympus America, Lake Success, NY), and images were acquired with a digital camera (DP70; Olympus). Fluorescence-labeled sections were also viewed by using a confocal microscope (C1; Nikon, Tokyo, Japan).

Single-Cell Lucifer Yellow Injections

Iontophoretic intracellular injections of fixed tissue slices with Lucifer yellow dye have been described in detail elsewhere.50 In brief, 50-μm-thick sections of retina were prelabeled with a fluorescent dye (4,6-diamidino-2-phenylindole [DAPI]; Sigma-Aldrich), for selective labeling of the nuclei of cells. Sections were mounted on black nitrocellulose filters (0.22-μm pore size; Millipore, Bedford, MA), placed onto a Perspex injection chamber filled with 0.9% saline and positioned onto a fixed stage microscope (Axioskop FS; Carl Zeiss Meditec, Thornwood, NY). Glass microelectrodes (1.5 mm external diameter borosilicate glass; World Precision Instruments, Sarasota, FL) were pulled by using an electrode puller (PC-10; Narishige, Tokyo, Japan) and were filled with 4% LY (Sigma-Aldrich) in 0.1 M LiCl. Cells were selected based on nuclear morphology and fluorescence staining patterns, which allows differential identification of cone and rod photoreceptor somata. Selected cells were impaled, and LY dye was injected by a continuous hyperpolarizing current (up to 50 nA for ~20 seconds) under direct visual guidance. After intracellular injections, the preparations were fixed for 10 minutes in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2). The fluorescence signals in injected cells were detected with a confocal microscope (C1; Nikon).

Table 1. Age and Gender of Human Retinae without Evidence of Retinal Disease

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Table 2. Age and Gender of Human Retinae with Evidence of Dry-AMD

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Fiber Analysis and Density Estimates

To measure the numerical density of rod bipolar cells, photoreceptors, and populations of amacrine cells and the apical dendritic lengths of the rod bipolar cells, we immunolabeled transverse sections of AMD and control retinas with a variety of markers. PKCα was used as a marker of rod bipolar cells. PKCα also labels blue cone bipolar cells and D86 cone bipolar cells.32 Adjacent sections were labeled for GLT-1c, a glutamate transporter splice variant that labels the plasma membranes of both rod and cone photoreceptors and calbindin (which labels cone photoreceptors). Additional sections were immunolabeled for Glyt-1, which labels all the glycinergic amacrine cells.33

Immunoperoxidase-labeled sections were examined using a ×100 objective lens on a microscope (BX51; Olympus) and images were acquired with a digital camera (DP70; Olympus). Total rod bipolar fiber lengths and cell densities were measured in six images (175 μm across) of each specimen, taken at three eccentricities: the central (−5 mm from the optic nerve), midperipheral (−12 mm), and far-peripheral (−22 mm) regions. Data were expressed as aggregate data for AMD and control retinas, respectively. Cell somata were included in counts if they were in the focal plane of the image and were clearly trans- nolabeled for Glyt-1, which labels all the glycnergic amacrine cells.35

Electron Microscopy

Sample sections of formaldehyde-fixed retinas from the normal and AMD-afflicted human eyes were also processed for electron microscopy. Retinal tissues were refixed overnight by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). Tissues were osmicated (1% osmium in 0.1 M sodium cacodylate buffer; [pH 7.2]) for 30 minutes, dehydrated in ethanol and embedded in Araldite resin. Ultrathin sections were cut with an ultramicrotome (LKB, Gaith ersburg, MD) and collected on nickel grids. Sections were then stained in uranyl acetate and lead citrate and viewed with a transmission electron microscope (1200 EXII; JEOL, Tokyo, Japan).

Production of Illustrations

A schematic diagram illustrating the proposed anatomic organization of the retina, and the location of electron micrograph images was created (AppleWorks; Apple Computer, Cupertino, CA).

For illustrative purposes, all digital images were imported into image-analysis software (Photoshop 7; Adobe, San Jose, CA) where minor changes to brightness and contrast adjustments were made. Composite plate images of the digital files were generated (Freehand X; Macromedia, San Francisco, CA).

RESULTS

Histologic Confirmation of the Status of Normal and AMD-Afflicted Eyes

Examination of the macular region of tissues, which we had received from the eye banks as normal aged human eyes confirmed their presumed status, these eyes lacking any histologic evidence of overt damage or disease (Figs. 1A, 1B). Conversely, eyes diagnosed as having dry AMD (Fig. 1C) exhibited classic features of the disease with overt loss of photoreceptors that was restricted to the macular region. In the AMD-affected macula, RPE cells exhibited hypopigmentation. Numerous drusen deposits were present in the macular region of all dry AMD-afflicted eyes. These features validated the prior classifications provided by eye banks. Conversely, none of the eyes exhibited choroidal neovascularization, which is a hallmark of wet AMD.

Synaptic Vesicle Protein Distribution in Normal Retinal Tissues

Examination of retinas from human eyes in which there was no clinical diagnosis of disease or apparent disease (Fig. 2A) revealed that immunoreactivity for the synaptic vesicle proteins

![Figure 1. Microtome sections of normal aged (female, aged 62) macula (A, B) and dry-AMD-affected (female, aged 61) macula (C). In the normal macula (A, B) the RPE layer was continuous, and Bruch’s membrane (BM) was intact. Drusen (D) deposits were not evident. Photoreceptor cells exhibited normal morphologies with intact inner and outer segments and no evidence of cell loss. Conversely, in the AMD-affected macula (C), extensive photoreceptor loss was evident. RPE cells exhibited hypopigmentation, and drusen deposits were evident. Sc, sclera; C, choroid; GCL, ganglion cell layer. Scale bars: (A–C) 50 μm.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933239/ on 02/09/2017)
Synaptic Reconnections in the Human CNS

Synaptic Vesicle Proteins in AMD-Afflicted Retinas

Immunolabeling of sections of midperipheral retinas of human eyes affected by AMD (Fig. 2B) revealed a redistribution of labeling for synaptophysin and vGLUT-1, whereas labeled puncta were still evident in the IPL (though in some cases staining appeared weaker), the OPL was apparently disorganized, with many puncta displaced into the ONL, suggesting the retraction of photoreceptor axonal processes and their synapses back into the ONL. Control (unlabeled) sections (Fig. 2C) of the same retina as in Figure 2B exhibited green autofluorescence in photoreceptor inner and outer segments (IS, OS) in (B) representing autofluorescence, as illustrated in an adjacent unlabeled section (C). Cone photoreceptors filled with Lucifer yellow (pseudocolored red) (D) or immunoperoxidase-labeling for calbindin (E) exhibited normal morphology; synaptic pedicles (circled) were located in the OPL. Horizontal cells (h) also labeled for calbindin and vGLUT-1 (red) were also immunoreactive, with many puncta displaced into the ONL, suggesting the retraction of photoreceptor axonal processes and their synapses back into the ONL. (F, G) Rod photoreceptors filled with Lucifer yellow. Spherules (circled) were located in either the normal position in the OPL (F) or retracted into the ONL (G). (H) Photoreceptor plasma-membranes including retracted rod photoreceptor spherules (circled) immunolabeled for GLT-1c. Scale bars: (A–C) 20 μm; (D–H) 10 μm.

Analysis of Cellular Architecture

To determine whether the photoreceptors had retracted their axonal processes and synaptic terminals, individual photoreceptors were injected with the fluorescent dye Lucifer yellow (LY). All cone photoreceptors examined exhibited normal morphologies, with the characteristic cone pedicles remaining in the OPL (Fig. 2D). Subsequent observations of cone photoreceptors that had been immunoperoxidase-labeled for calbindin (Fig. 2E) confirmed that cone photoreceptors retained an apparently normal morphology in the peripheral retinal regions examined. Horizontal neurons were also immunoreactive for calbindin and exhibited apparently normal morphology; with the cell bodies residing in the INL adjacent to the OPL and their neurites terminating in the OPL (Fig. 2E), without evidence of extension of processes into the ONL. Examination of LY-injected rod photoreceptors in AMD-afflicted retinal tissues revealed a mixture of apparently normal rod photoreceptors (Fig. 2F) and others where the synaptic spherules appeared to have retracted into the ONL (Fig. 2G). This observation that rod photoreceptor synapses and axons had retracted was confirmed by immunostaining for GLT-1c, which labels the plasma membranes of all photoreceptors. Retracted rod spherules were readily identified (Fig. 2H). By contrast, no retracted rod photoreceptor processes were observed in normal retinas (data not shown).

Remodeling of Rod Bipolar Neurons

Immunoperoxidase labeling of normal human retinas for PKCa revealed as expected the predominant labeling of rod bipolar cells (Fig. 3A) which extended finely branched dendrites to the OPL and a single axon that projected to the IPL terminating in several lobular synaptic endings. These architectural characteristics of rod bipolar neurons were in accord with earlier reports. By contrast, in the peripheral and midperipheral regions of AMD-affected retinas, exuberant dendritic sprouting of the rod bipolar neurons was evident (Figs. 3B, 3C). Close analysis of the rod bipolar cells revealed that apical dendrites extended radially or tangentially into the ONL, often with swollen conelike regions that gave rise to multiple fine processes that also have been radially or tangentially oriented (Fig. 3C). Double-immunofluorescence labeling was performed with antibodies to synaptophysin and PKCa, to correlate the relationship between photoreceptor synapses and the rod bi-
polar cell dendrites (Figs. 3D–H). In normal retinas, apical dendrites extended to the OPL where they became invaginated in the synaptic cleft of the rod photoreceptor spherules (Fig. 3D). In the AMD-afflicted retinal tissues, the apical dendrites of rod bipolar cells extended into the ONL (Figs. 3E, 3F). Higher-magnification images (Figs. 3F–I) revealed that most of the rod bipolar dendrites that extended into the ONL were in contact with retracted rod photoreceptor spherules or well-defined puncta at the basal region of rod photoreceptor cell bodies in the ONL. Some of the connections between rod bipolar cell dendrites and photoreceptor synapses were not simply radial in nature, but instead the bipolar cell dendrites extended upward and laterally at an angle (Figs. 3G–I; see also Figs. 4B, 4F).

Quantification of Cellular Changes

Measures of apical dendritic lengths of rod bipolar cells at differing retinal eccentricities in normal and AMD-afflicted retinas (Figs. 4A–I) revealed that there was a marked increase in dendritic fiber length at all eccentricities in the AMD retinas compared with age-matched normal human retinas. Our sum-
mary graph of dendritic length measurements of rod bipolar cells between central and peripheral eccentricities in AMD-afflicted retinas (Fig. 4I) indicate, somewhat surprisingly, that there was apparently more dendritic sprouting in the peripheral regions in comparison to the central region of the AMD-afflicted retinas.

Counts of cell numbers revealed that the number of rod photoreceptors (Fig. 4J) was reduced in the central retina in AMD (as would be expected since AMD is a disease of the central retina characterized by photoreceptor loss), but in the mid- and far-peripheral retina the number of rod photoreceptors was unchanged relative to the control. Analysis of the number of rod bipolar cells (Fig. 4K) and glycinergic amacrine cells (Figs. 5A–C) revealed no evidence of the loss of these cell populations at any retinal eccentricity examined. The glycinergic amacrine cells did not exhibit any overt anatomic changes in the midperipheral or peripheral retina.

Electron Microscopy

Based on the preceding data, schematic diagrams were generated to illustrate the normal (Fig. 6A) anatomy of the photoreceptor complex and the rearrangements of this complex (Fig. 6B) in the AMD retina. To confirm the actual anatomic rea-
Arrangements of the retina, in response to AMD, representative electron micrographs were taken from regions corresponding to the area schematically depicted in Figure 6B.

Electron microscopic examination of AMD-afflicted retinas showed that cone photoreceptor pedicles remained in the OPL, the synaptic complexes contained multiple presynaptic ribbons and a mixture of invaginated or flat postsynaptic connections from horizontal and bipolar cells (Fig. 6C). Analysis of rod photoreceptors confirmed that many of these cells including those that had completely retracted their axonal processes into the ONL, still formed ultrastructurally demonstrable synaptic complexes including presynaptic ribbons and invaginated postsynaptic elements which, on the basis of their ultrastructural characteristics, are most likely to represent rod bipolar dendrites that have extended into the ONL (Fig. 6D).

By contrast, the typical more electron-lucent horizontal cell processes that normally also invaginate into the synaptic complex were not demonstrable, in accordance with light micro-
scopic observations, which indicated that the processes of horizontal cells labeled for calbindin, remained in the OPL.

**Radial and Tangential Connections between Rod Bipolar Cells and Rod Photoreceptors**

The data in Figures 3 and 4 illustrate a reconnection between rod bipolar cells and rod photoreceptors via radially or tangentially directed outgrowth of rod bipolar cell dendrites. The tangential type of rearrangement is schematically illustrated in Figure 7. It necessitates that the connections between the rod photoreceptors and the rod bipolar cells be broken and then reformed. If the contacts between the rod photoreceptors and the rod bipolar cells were not broken, it would require both the concurrent lateral migration of the rod photoreceptor somata by at least three to five somata widths in many cases, as well as the need for the axon–dendrite complex to cut through and around many other neuronal processes and somata. This scenario does not appear to be supported by any of the current data.

**DISCUSSION**

In this study, we demonstrate for the first time that neurons in retinal tissues from human eyes affected by AMD have the capacity to remodel by sprouting processes and to re-form synapses in the OPL. Our study shows that at the light and electron microscopic levels, new synaptic complexes can form and that such complexes contain structural elements such as synaptic ribbons, as well as vesicles that express the appropriate vesicular proteins including vGLUT-1 (needed for the photoreceptor vesicles to package glutamate) and synaptophysin. Moreover, appropriate anatomic interactions between rod photoreceptors and rod bipolar cells are evident.

![Figure 7](image-url)

**Figure 7.** Rearrangements of the human AMD retina. (i) In the normal retina, rod photoreceptor synapses are located in the OPL and connect radially to postsynaptic processes of rod bipolar cells in the OPL. In AMD retinas (ii) many rod photoreceptors retract their synaptic processes into the ONL and lose their synaptic connections with the rod bipolar cells. Conversely (iii), a large number of rod bipolar cells extend their dendrites in a radial fashion to allow radially oriented reconnection with rod photoreceptors. In some cases, the connection between bipolar cells and rod photoreceptors is not radial (iv), but arises from tangentially extended rod bipolar cell dendrites.

This study confirmed our initial hypothesis that significant anatomic changes occur in the midperipheral retinas of human eyes affected by AMD and thus probably represent the anatomic substrate of observed electrophysiological anomalies that are evident in this tissue. Our data show, however, that these changes are not simply widespread degenerative changes, since the cone photoreceptors seem, on the basis of their morphology, to be unaffected at peripheral retinal eccentricities. Moreover, there was no evidence of generalized cell loss at any retinal eccentricity, and only photoreceptor loss in the central retina (as may be expected, since AMD is a disease characterized by the loss of central retinal photoreceptors).

In AMD-afflicted retinas, there was an apparent increase in rod bipolar apical dendrites, particularly at peripheral eccentricities in comparison to the central region. This disparity is at first surprising, since we had assumed that we would find a gradient with greatest remodeling in the central region because of the presumption that any disease process would exhibit a central to peripheral gradient in AMD. We cannot offer an unequivocal reason for this disparity, but the data suggest that rod circuits, in the central retina may be relatively resistant to the disease processes.

The retraction of rod photoreceptor synaptic spherules and the subsequent sprouting of rod bipolar dendrites that selectively reconnect to appropriate target neurons suggests that functionally relevant plasticity is possible, even in the aged human retina. Conversely, we do not believe that the retracting photoreceptor synapses are simply “dragging” rod bipolar cell dendrites (but not horizontal cell dendrites) along with them, since the processes of these bipolar cells appear to undergo extensive anatomic changes including the extension of multiple fine processes from swollen cone-like enlargements of the apical dendrites. These features are suggestive of active reorganization of these bipolar cell processes rather than a “dragging along” of the original apical dendrites. Furthermore, we have shown that in many instances the new rod bipolar cell connections that are established are tangential in nature (that is, the bipolar cell dendrites now often connect to photoreceptors positioned three to five cells lateral to the normal targets). If a simple “dragging” process were to generate this result, it would also require the concomitant lateral migration of the photoreceptors. We are unaware of any evidence of this type of lateral migratory event. Moreover, we do not notice any significant tangential deflection of the photoreceptor axonal processes, which would be expected if the photoreceptor somata were being dragged laterally into new laterally displaced locations. Finally, if the synaptic connection were retained during this type of lateral displacement, the axon–dendrite complex would have to slice through any intervening neuronal or glial elements in a synthelike manner to achieve its final trajectory. Accordingly, our data firmly support the view that the retraction of photoreceptors is associated with the detachment of normal postsynaptic elements and the subsequent outgrowth and reattachment of rod bipolar dendrites (but not horizontal cells) to form new synapses, which may or may not be radially arrayed.

The retraction of rod photoreceptor synapses from the OPL is in accordance with the view that AMD is a very slow and progressive disease, one which we speculate, if the afflicted person was to live long enough, might ultimately encompass the mid and peripheral regions of the retina as well as the macula. Accordingly, we suggest that rod photoreceptor axon and synapse retraction may be an early feature of this disease, which is not normally demonstrable in the macula due to the overt degenerative events normally evident in this area during the disease. This idea that rod remodeling is an early feature of the disease is in accord with prior suggestions that the pathology in the central retina is initially associated with loss or
dysfunction of rod photoreceptors in the perimacular region and that it is the loss of some undefined trophic support from these cells that subsequently leads to the very slow death of cone photoreceptors.\textsuperscript{56,57}

The retraction of rod photoreceptor synapses is a feature that is evident in other overt insults of human retinal tissues such as detached retinas\textsuperscript{25} and in diseases such as retinitis pigmentosa.\textsuperscript{38} Similar ectopic photoreceptor terminals have been noted in the degenerating retinas of Royal College of Surgeons rats.\textsuperscript{39,40} The structural changes evident in each of these studies are associated with overt damage and loss of photoreceptors. Previous studies from our laboratory have also demonstrated that in the ageing rat retina, loss of photoreceptors due to exposure to normal animal house light levels is associated with major anatomic remodeling of glial and neuronal elements.\textsuperscript{11} These changes included the extension of Müller glial processes out of the retina into the overlying choroid and the subsequent extension of neurites and the migration of adult neurons out of the retina into the choroid, where they reform synaptic connections with other neurons.\textsuperscript{14} Recently, it has been demonstrated that in the aged C57BL/6 mouse retina, there was extensive remodeling of the rod bipolar cells in a manner analogous to that shown in the human retina (Eliasieh K, et al. IOVS 2006;47:ARVO E-Abstract 4199).\textsuperscript{41} The mouse data at first appear to indicate that rod bipolar cell remodeling is a feature of normal aging. However, an analysis of the literature revealed that the standard C57BL/6 mouse strain from Jackson Laboratories (http://jaxmice.jax.org/strain/000664.html; Bar Harbor, ME) has a high incidence of microphthalmia and other associated eye abnormalities. The C57BL/6 mouse strain carries a mutation in Cdh23, cadherin 23 gene which is known to be associated with eye abnormalities such as early-onset retinitis pigmentosa and reductions in the a- and b-wave amplitudes, which indicates dysfunction of the ONL.\textsuperscript{42}

This raises the question of what is a normal aged retina and what is a diseased aged retina. Eyes are usually classified as normal if overt abnormalities are absent. In the context of this study, there is clearly room for debate as to whether rod photoreceptor retraction and rod bipolar cell dendrite extension are events associated with normal aging or are symptomatic of disease. Prior studies of normal human rod bipolar cells have typically used the term “normal” aged donor eyes (e.g., Refs. 31,32) and have not shown the effusive sprouting of the apical dendrites. Accordingly, this phenotype has routinely been assumed to be the normal state. We believe that the lack of sprouting in the normal aged human eyes that we report is consistent with the prior literature and is indicative of the normal aged state. However, we suggest that this is not the case. In unpublished studies on an aged macaque monkey retina (17 years old) such retinas do not show effusive sprouting of the rod bipolar cell dendrites (Sullivan and Pow, unpublished observations, 2000). Similar arguments relating to the lack of evidence in previous studies of retracted rod photoreceptor synapses in the human retina again tend to support the view that retraction is a pathophysiological feature rather than a consequence of normal aging.

In this study, extensive neuronal remodeling was evident before any evidence of overt damage or loss of neurons and, most significantly, was accompanied by the reconnection of the presynaptic elements to the postsynaptic bipolar neurons. However, it is probable that this reconnection was imperfect, as the horizontal cell processes (labeled for calbindin) which are also components of the normal photoreceptor synaptic complex, did not appear to extend new neurites into the ONL and thus could not reconnect with the rod photoreceptors. This notion appeared to be supported by our electron microcopy data. However, data indicative of absence cannot exclude the reasonable possibility that some horizontal cells may extend dendrites to form new connections with the retracted photoreceptor synapses.

In conclusion, the data suggest that in the human eye, aged retinal neurons retain an inherent capacity to display adaptive changes in their morphology and connectivity and that this is readily demonstrated in eyes afflicted with dry AMD. An understanding of this adaptive capacity is important, not only for our interpretations of how the retina functions in pathophysiological states but also because it provides insight into how the retina may respond to regimens such as cell or tissue transplant therapies, during therapeutic attempts to restore normal vision.

Our data suggest that the subtle dysfunctions of the midperipheral retinas from eyes affected by AMD may be amenable to further analysis of functional plasticity and thus serve as a good model of human synaptic plasticity. Further investigations into the molecular signals, such as NoGo,\textsuperscript{33} may provide parallel insight into events that occur or may be prompted to occur after insults such as strokes. We conclude that our findings have fundamental implications for understanding adult central nervous system plasticity, which appears to be retained even in the aged human CNS.

References


