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Müller cell and neuronal remodeling in retinal detachment and reattachment and their potential consequences for visual recovery: a review and reconsideration of recent data

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Abstract

Recent evidence suggests that the adult mammalian retina is far more plastic than was previously thought. Retinal detachment induces changes beyond the degeneration of outer segments (OS). Changes in photoreceptor synapses, second- and even third-order neurons may all contribute to imperfect visual recovery that can occur after successful reattachment. Changes that occur in Müller cells have obvious effects through subretinal fibrosis and proliferative vitreoretinopathy, but other unidentified effects seem likely as well. Reattachment of the retina induces its own set of responses aside from OS re-growth. Reattachment halts the growth of Müller cell processes into the subretinal space, but induces their growth on the vitreal surface. It also induces the outgrowth of rod axons into the inner retina.

Keywords: Retinal detachment; Retinal reattachment; Neuronal remodeling; Synaptic plasticity; Photoreceptors; Müller's glia

1. Introduction

The most obvious structural change after retinal detachment (separation of the neural retina from the retinal pigmented epithelium, RPE) is outer segment (OS) degeneration (Anderson, Stern, Fisher, Erickson, & Borgula, 1983; Kroll & Machemer, 1968). It also has been known since the early studies on retinal reattachment that OS have the ability to re-grow (Kroll & Machemer, 1969). If OS have the ability to recover, then why do visual deficits often prevail after successful reattachment surgery? Surgical repair of detachment has a success rate of over 90% in producing reattachment (Williams & Aaberg, 2001). However, if the macula is detached, only 20%–40% of successful reattachments achieve visual acuity of 20/50 or better (Burton, 1982; Tani, Robertson, & Langworthy, 1981, 1980), and other visual deficits may persist as well. Photoreceptor cell death could certainly account for some of these deficiencies, but in the feline retina, less than 20% of the photoreceptor population dies within 3 days of detachment (Erickson, Fisher, Anderson, Stern, & Borgula, 1983), and most of these may be rods. Most macular detachments are probably treated within that time, and since reattachment stops photoreceptor cell death, the actual loss of photoreceptors in a detachment of only a day or two may be quite small (Lewis et al., 2002). Thus, it seems likely that retinal changes other than OS degeneration or actual photoreceptor cell death may account for the changes in vision that occur after reattachment. This hypothesis tends to be supported by recent evidence that the recovery process may be very slow, continuing for months or even years (Liem, Keunen, Meel, & Norrern, 1994). Indeed, comparing results from experiments with animal models to those obtained from human pathology samples provides mounting evidence that detachment induces many changes beyond those in outer segments that may affect visual recovery. Many of these changes involve significant remodeling of retinal architecture by changes in both neurons and Müller cells.

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Detachment of the neural retina may occur in many circumstances as part of disease or trauma, but in recent years it has become part of a surgical procedure for treating age-related macular degeneration by macular translocation (de Juan, Loewenstein, Bressler, & Alexander, 1998; Eckardt, Eckardt, & Conrad, 1999). It also occurs necessarily as part of proposed therapies for retinal degeneration involving RPE transplantation, retinal transplantation (Bok, 1993; Del Cerro, Lazar, & Diloreto, 1997), or the injection of substances into the subretinal space (SRS) (Lewin et al., 1998). In these cases the retina may remain detached for a day or more, adequate time to initiate the events described here since detachment induces responses in important cellular signaling pathways within minutes (Geller, Lewis, & Fisher, 2001). While reattachment has the ability to quickly stop many of these events, and in some cases reverse them, it also induces its own set of cellular changes that may impact the return of vision. Here we provide a review and reconsideration of recently published data (mostly from our own laboratory) relevant to the issues of plasticity and remodeling of the mammalian retina and consider how these events, as described in animal models, may be clinically relevant. The material included here was originally presented at the Sixth Annual *Vision Research* Conference (*Retinal Cell Rescue*), Fort Lauderdale, FL, May, 2002.

2. Experimental detachment and reattachment

Over the past few decades, various procedures have been used to produce detachments and reattachments in animal models. Because the original data presented here was all from our laboratory, we provide a brief description of the methods used in our experimental procedures. The details of these methods have been published previously as noted below.

Our procedure for producing retinal detachment involves removing the vitreous and then slowly infusing fluid into the extracellular space that exists between the neural retina and RPE apical surface through a glass micropipette with a tip diameter of about 100 μm (Lewis, Linberg, Geller, Guérin, & Fisher, 1999). This produces a relatively small hole through the retina and it may not be obvious that this type of detachment models a "rhegmatogenous" detachment (one in which there is a tear through all retinal layers; Aaberg, 1999). However, we have observed the same cellular results in tissue from human rhegmatogenous detachments and from animals in which we have produced a large retinal tear with the detachment. Reattachments are produced by pneumatic retinopexy in which a fluid-gas exchange is performed (with filtered room air), being careful to drain fluid from under the retina and then the air in the eye is replaced with 20% sulfur hexaflouride (also mixed with filtered room air). The details of the immunocytochemistry and microscopy procedures used to produce the original data also have been published elsewhere (e.g. Lewis & Fisher, 2000) and because they are based on standard procedures will not be repeated here. The human tissue samples were obtained with permission of the local Human Subjects Committee and used in accordance with the Declaration of Helsinki.

3. Cellular remodeling produced by detachment and reattachment

3.1. Müller cell remodeling

Müller cells are the radial glia of the retina (Fig. 1A). Their nuclei lie within the inner nuclear layer (INL). Their main stalk has many fine branches, expands at the vitreal border of the retina into an "endfoot," and terminates at the outer limiting membrane where it forms adhearing junctions with photoreceptors and other Müller cells. Microvilli, intensely immunoreactive for the cell adhesion molecule CD 44 (see example in Fig. 3B) project from that border of the cell into the interphotoreceptor space. The example in Fig. 1A is from ground squirrel retina stained with an antibody to the intermediate filament protein vimentin. In this species vimentin extends throughout the cytoplasm of the Müller cells. Vimentin often co-localizes in Müller cells with another intermediate filament protein, glial fibrillary acidic protein (GFAP) but there are prominent species differences in their distribution in normal retina and their reaction to injury. Indeed, antibodies to GFAP do not stain Müller cells in the ground squirrel retina before injury and only rarely afterwards (Linberg, Lewis, Sakai, Leitner, & Fisher, 2000) while they stain the endfoot region heavily in normal feline retina (Fig. 1B). In the common laboratory rat and mouse, anti-GFAP may give little to no positive staining in normal Müller cells but produce a strong reaction throughout the cells shortly after retinal injury (Bignami & Dahl, 1979; Bjorklund, Bignami, & Dahl, 1985). In the cat retina both GFAP and vimentin immunoreactivity begin extending from the endfoot cytoplasm toward the outer retina within a day or two of detachment, so that eventually the entire cytoplasm of the cell is labeled (Fig. 1C). When examined by electron microscopy, this increased immunoreactivity correlates with an unmistakable increase in the presence of intermediate filaments which eventually fill the entire cell (Erickson, Fisher, Guérin, Anderson, & Kaska, 1987; Lewis, Matsumoto, & Fisher, 1995). Thus, the dramatic structural remodeling of the feline Müller cells after detachment can be followed by labeling them with antibodies to either of these intermediate filament proteins. Müller cells grow within the retina so that their main trunks create large columns of intermediate filament-

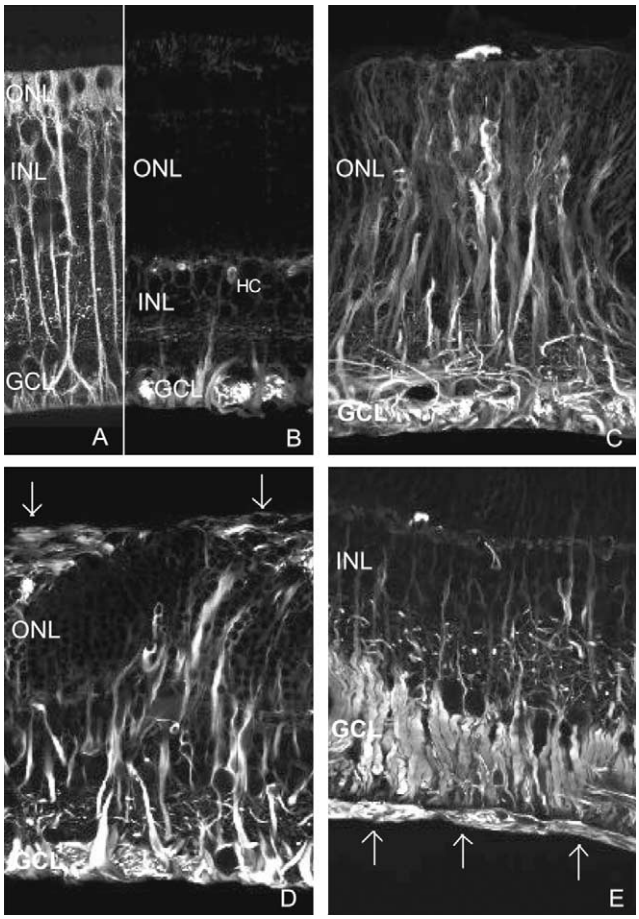


Fig. 1. Laser scanning confocal micrographs of Müller cells labeled with antibodies to vimentin (A) and GFAP (GFAP; B-E). (A) In the ground squirrel retina antibodies to the intermediate filament protein, vimentin label the entire Müller cell, thus illustrating their radial morphology as well as their fine lateral processes. These cells terminate on the borders of the retina where they form the outer and inner limiting membranes. (B) Normal cat retina showing that only the cytoplasm in the endfeet of the Müller cells contain GFAP in this species. Astrocytes in the GCL are brightly labeled while horizontal cell somata (HC) and their processes label faintly. (C) In a cat retina detached for 7 days there is a huge increase of anti-GFAP labeling both in the radial and lateral processes of the Müller cells. (D) In a cat retina detached for 28 days anti-GFAP labeled Müller cell processes have grown through the outer limiting membrane to form a subretinal scar on the photoreceptor surface (arrows). (E) Cat retina *reattached* for 28 days following a 3 day detachment. The GFAP response in the Müller cells is slowed by reattachment, with labeling now extending only into the INL. Reattachment, however, induces Müller cells to grow onto the vitreal surface of the retina forming an epiretinal membrane (arrows). ONL is outer nuclear layer, GCL is ganglion cell layer, and HC is horizontal cell.

filled cytoplasm while their lateral branches enlarge and become chaotically tangled (Fig. 1C and D), especially in the plexiform layers. In the outer nuclear layer (ONL) the expanded Müller cell processes fill in for dying photoreceptors. This can be observed to the extreme in species like the ground squirrel where nearly all of the photoreceptors die, but the Müller cells maintain a perfectly defined "ONL" and outer limiting membrane (Linberg et al., 2000).

As Müller cell processes undergo hypertrophy within the retina, they also begin growing into the expanded SRS where they can form extensive "scars" (Fig. 1D) that inhibit the regrowth of outer segments (Anderson, Guérin, Erickson, Stern, & Fisher, 1986). In humans this condition is called subretinal fibrosis. Indeed, a single Müller cell process lying between the reattached neural retina and the RPE is sufficient to completely inhibit OS regeneration in that region. There appears to be a special relationship between the formation of these subretinal membranes by Müller cells and cone photoreceptors. If a Müller cell process is captured just as it grows beyond the outer limiting membrane (Fig. 3A), that process inevitably occurs adjacent to a cone (Lewis & Fisher, 2000). Labeling with the antibody to CD-44 provides a similar, but slightly different perspective of these events because within a day of detachment, CD-44 labeling is markedly decreased in the Müller cell microvilli, except in those just surrounding the cone photoreceptors where its expression remains almost normal (Fig. 3B and C). At 28 days of detachment, anti-CD-44 labeling associated with Müller cells in the neural retina is lost, except for the apparent "leading edge" of Müller cell processes in the SRS (Fig. 3D) which remains intensely labeled, as if the CD-44 associated with the microvilli in normal retina is now associated with the growing edge of the Müller cell processes as they form a glial scar. If the retina is reattached, CD-44 labeling recovers but is no longer specific to the microvilli, and now occurs uniformly throughout the entire Müller cell (Fig. 3E and F). These data suggest a special molecular relationship between cones and the Müller cell microvilli; a relationship that may be crucial for the growth of Müller cell processes into the SRS. In retina detached for more than 3 days, Müller cell growth into the SRS is common, while we have never observed a similar growth through the inner limiting membrane and onto the vitreal surface of the retina. Thus, vitreal membranes typical of those encountered in human proliferative vitreoretinopathy after reattachment are not observed in the detached feline retina. However, once the retina is reattached, Müller cell processes cease growing into the SRS and can now be found extending through the inner limiting membrane and growing along the vitreal face of the retina (Fig. 1E). These cellular membranes appear to assume contractile properties and produce large folds and eventually re-detachment of the retina.

Müller cells do not only increase in size in the detached retina, but apparently in number as well. When the retina is labeled with a marker for cellular proliferation (e.g. 3H-thymidine or the antibody, MIB-1), labeled nuclei occur in cells with the location and morphology of Müller cells (Fig. 2A and B; Fisher, Erickson, Lewis, & Anderson, 1991; Geller, Lewis,

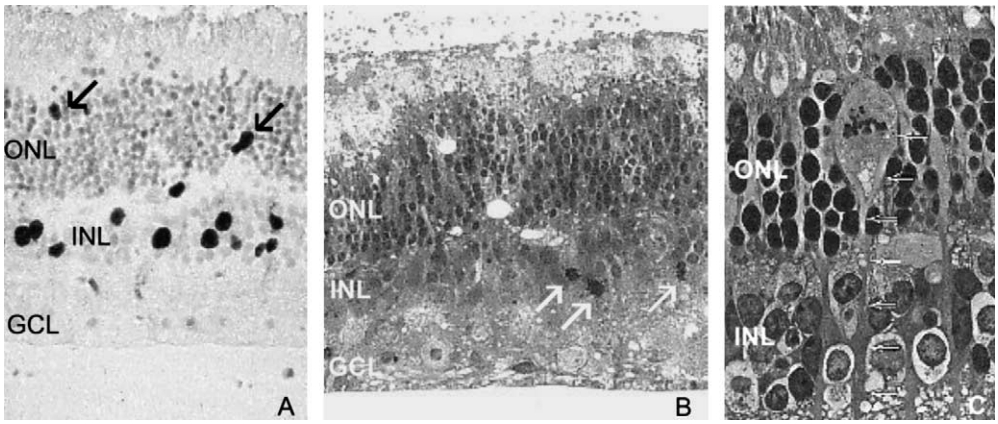
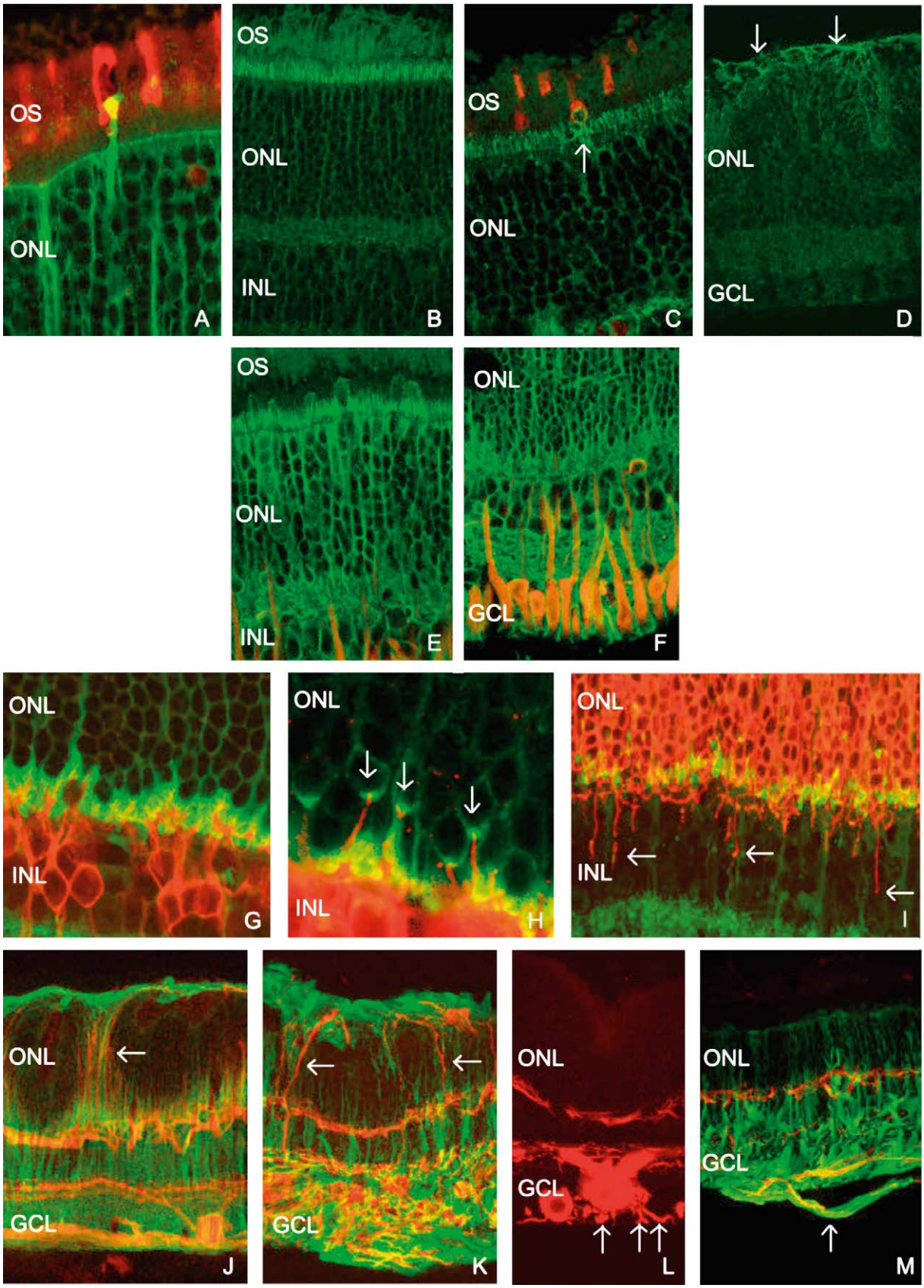


Fig. 2. The proliferative response of non-neuronal cells following retinal detachment in the cat. (A) Light micrograph of a section of retina detached for 3 days and labeled with the MIB-1 antibody to detect proliferating cells. The labeled cells in the INL are probably Müller cells. Cells labeled in the ONL (arrows) may be Müller cells that have migrated into that region. (B) Autoradiograph of a 3 day detached retina labeled with tritiated thymidine to label cells actively synthesizing DNA. Labeled Müller cells in the INL are indicated by the arrows. (C) An electron micrograph showing a mitotic figure in the ONL. The long tail of cytoplasm (arrows) and the large size of the cell body suggests that this is a dividing Müller cell. ONL is outer nuclear layer, INL is inner nuclear layer, and GCL is ganglion cell layer.

Anderson, & Fisher, 1995). This response begins within a day, reaches a maximum at about 3 or 4 days, then declines and continues at a low level as long as the retina is detached. Finding mitotic figures is difficult in detached retina, and we have not observed them in the layer associated with Müller cell nuclei. Most seem to be associated with astrocytes among the ganglion cells (GC) or in large cells along the outer limiting membrane (Fig. 2C; also see Fig. 10 in Erickson et al., 1983). The mitotic figure in Fig. 2C has a long cytoplasmic extension reaching into the inner retina (arrows). Since Müller cells are the only retinal cell type with this general morphology, the image suggest a sequence of events in which the nucleus of a Müller cell migrates from the INL to the outer limiting membrane, the cell ''rounds up'' at that location, and then undergoes mitosis. This cycle occurs among proliferating neuroblasts in the embryonic CNS (Sauer, 1935), including retina (Fujita

Fig. 3. Laser scanning confocal micrographs illustrating some of the neuronal responses to detachment in the cat retina. (A) Retina detached for 3 days and labeled with anti-glial fibrillary acidic protein (GFAP; green), a marker for reactive Müller cells in this species, and peanut agglutin lectin (PNA; red), a label for the extracellular matrix around cone OS. An anti-GFAP labeled Müller cell processes is shown extending past the ONL and into the SRS in association with a cone photoreceptor. (Overlapping GFAP and PNA labeling appears yellow.) (B)–(F) Anti-CD-44 labeling (Green). (B) In normal retina only the Müller cell microvilli extending between the photoreceptor inner segments label with the CD-44 antibody. (C) A retina detached for 3 days and labeled with both anti-CD-44 (green) and peanut agglutinin lectin (red). After detachment there is a general decrease in CD-44 labeling of the microvilli except for those adjacent to a cone photoreceptor (arrow) which retain labeling about equivalent to that found in the microvilli of control retinas. (D) In a retina detached for 28 days anti-CD-44 labeling occurs only at the edge of Müller cell processes that have grown into the SRS (arrows). (E) and (F) Retina detached for 3 days and reattached for 28 days (E) Outer retina. The anti-CD-44 (green) labeling returns to the Müller cell microvilli extending between the OS but it also now extends throughout the Müller cell cytoplasm. (F) Inner retina. Double-labeling with anti-CD-44 (green) and anti-vimentin (red) labeling to show that CD-44 extends to the Müller cell endfeet and that the anti-CD-44 and anti-vimentin labeling do not co-localize because CD-44 occurs in the plasma membrane while vimentin occurs in the cytoplasm. (G) and (H) Anti-synaptophysin labeling = green, anti-PKC labeling = red. (G) In the normal retina anti-synaptophysin labels the synaptic terminals of the photoreceptors which form a compact layer on the border of the ONL, and anti-PK C labels RB cells. Note that the dendrites of the RB cells terminate within the synaptic terminals and do not extend into the ONL. (H) In a retina detached for 3 days, anti-synaptophysin labeled rod synaptic terminals have retracted into the ONL (arrows), and RB dendrites have grown into that layer where they often terminate against the rod terminals. (I) Anti-synaptophysin lableling = green, anti-rod opsin labeling = red. Retina reattached for 28 days after a 3 day detachment. Anti-rhodopsin (red) ''de-localizes'' from its normal location in the OS to label the plasma membrane around rod photoreceptor cells in the ONL. Rod axons that have extended into the INL (arrows) are also labeled with the antibody to rod opsin. Some rod synaptic terminals are still found in the ONL (green = anti-synaptophysin labeling) while many occur in their normal location. Anti-synaptophysin also lightly labels the terminals of the extended rod axons. (J)–(M) Anti-GFAP labeling = green, anti-neurofilament labeling = red. In retina detached for 28 days HC processes labeled with the anti-neurofilament antibody extend through the ONL and into an anti-GFAP labeled Müller cell scar in the SRS. There is often an association between the labeled processes of HCs and those that grow from reactive Müller cells after detachment. **L**. Examples of GC in a retina detached for 28 days labeled with the antibody to neurofilament protein. The fine processes appear on the base of the cell (arrows) are unusual. Cell bodies in the GCL of normal retina do not label with the antibody to neurofilament protein. **M**. A retina reattached for 28 days after a 3 day detachment. An anti-neurofilament labeled GC process (red/yellow) has grown into an anti-GFAP labeled epiretinal membrane (arrow). OS is outer segment layer, ONL is outer nuclear layer, GCL is Ganglion cell layer.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& Horii, 1963), where neuroblasts round-up on the ventricular surface prior to undergoing mitosis. The location of the mitotic figure in Fig. 2C is equivalent in adult retina to the border of the ventricle in embryonic retina. Do all dividing Müller cells undergo this type of cycle in the adult retina? Do both daughter cells re-extend cytoplasmic processes and re-differentiate into Müller cells? These are seemingly fundamental questions if we are to understand the reactivity of Müller cells to retinal injury.

3.2. Neural remodeling

OS degeneration has been well-documented and will not be described further here (Anderson et al., 1983; Kroll & Machemer, 1969; Mervin et al., 1999). Although it was first described in ultrastructural studies nearly two decades ago (Erickson et al., 1983), photoreceptor synaptic terminal remodeling has received less attention until recently. Rods, with their nuclei at all levels of the ONL appear to mount a vigorous response by withdrawing their terminals from the OPL. This remodeling is obvious when the tissue is stained with an antibody to a synapse-specific protein such as synaptophysin. The tightly organized layer of terminals found in normal retina (Fig. 3G) is disrupted and decreased in thickness following detachment, and stained terminals now occur deep in the ONL (Fig. 3H). By electron microscopy, these contain the ribbons and vesicles associated with photoreceptor synapses (Erickson et al., 1983; Lewis, Linberg, & Fisher, 1998). After detachment the dendrites of rod bipolar (RB) cells grow into the ONL where most appear to end against the withdrawn rod terminals even though most of these have lost their deep synaptic invaginations. This process would seem to completely re-shape the morphology of the RB's typical dendritic tree (Lewis et al., 1998), but our attempts to visualize this by staining the detached retina by the Golgi technique has so far been unsuccessful. Photoreceptors are also contacted by horizontal cell (HC) processes. The morphology of HCs changes dramatically in the detached retina, but unlike the RBs, their growth seems much less directed (Fig. 3J and K) even reaching into the SRS where they follow processes that form Müller scars. Indeed, these new HC processes seem to terminate randomly within the ONL and can be frequently observed extending into the inner retina. The HCs undergo a striking change in protein expression as well as structural remodeling. They stain lightly in the normal retina with antibodies to neurofilament protein but stain intensely after detachment (Fig. 3J and K), presumably representing an upregulation of cytoplasmic intermediate filaments, although this has not been demonstrated at the ultrastructural level. Structural remodeling also has been reported to occur in horizontal and amacrine cells in human retinas with the disease retinitis pigmentosa (Fariss, Li, & Milam, 2000). Cone synaptic terminals do not withdraw into the ONL as do those of rods (Erickson et al., 1983; Lewis et al., 1998). Cone pedicles are not unaffected however because their generally uniform shape becomes irregular with some of them flattening out along the border of the OPL, while others assume a more rounded, club-like appearance. By electron microscopy, both rod spherules and cone pedicles appear to lose their deep synaptic invaginations so that postsynaptic processes sit opposite the presynaptic membrane much as the "flat" contacts do in normal retina (Erickson et al., 1983; Lewis et al., 1998). Rod photoreceptors also react to reattachment. In this case rod axons re-extend into the OPL, with many appearing to "overgrow" their target to terminate in the inner retina (Fig. 3I). These endings label with the antibody to synaptophysin, thus presumably containing synaptic machinery (data not shown). Whether they form actual synapses is not known, nor is it known if they eventually withdraw back into the OPL.

The effects of detachment must reach the inner margin of the retina because astrocytes located among the GC proliferate vigorously (Fisher et al., 1991; Geller et al., 1995), and participate in the formation of epiretinal membranes (Van Horn, Aaberg, & Machemer, 1977). Their proliferation can be halted by rapid reattachment (Lewis et al., 2002). Some GCs also react to detachment by increasing dramatically the amount of growth associate protein (GAP) 43 and neurofilament protein they express in their cytoplasm (Coblentz, Radeke, Lewis, & Fisher, in press; Coblentz, Lewis, Radeke, & Fisher, 2001). These same cells appear to sprout neurites, often from their basal surface or axon, giving the cells an unusual morphology (Fig. 3L). When epiretinal membranes form after reattachment, the GC neurites sometimes grow into them, in much the same way that HC processes grow into subretinal Müller cell membranes (Fig. 3M).

4. Discussion and reconsideration of experimental results

4.1. The human condition

We include here three examples of immunolabeled human tissue obtained at the time of surgery to repair retina that had detached after previous reattachment surgery (Fig. 4). The purpose is to show that identical responses to those described in the detached and reattached feline retinas can be identified in human tissue. Fig. 4A demonstrates several events, including the shortening of rod OS, the redistribution of opsin into the inner portions of rod photoreceptor plasma membrane, and axon outgrowth from these cells. It also shows an intense upregulation of GFAP in Müller cells (signals from both anti-rod opsin and anti-GFAP la-

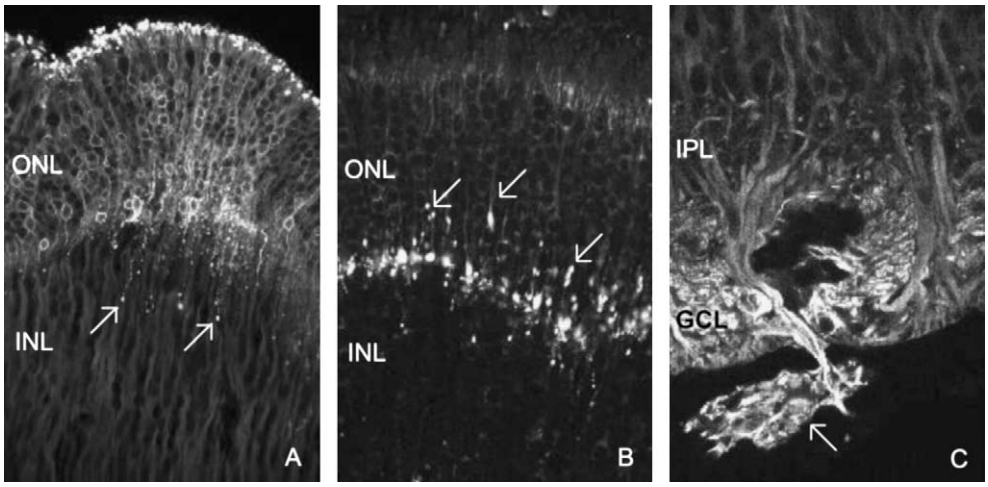

Fig. 4. Laser scanning confocal micrographs illustrating theresponse of the human retina to complex retinal detachment (i.e. re-detachment after reattachment surgery). (A) Labeling with anti-rhodopsin illustrates labeling of the plasma membrane throughout the rod cell bodies in the ONL, a characteristic of detached feline retina; and the labeling of rod axon extensions into the INL, characteristic of reattached feline retina. This section was also labeled with an antibody to GFAP which appears as fainter labeling of Müller cell fibers running radially the INL. (B) Labeling with anti-synaptophysin illustrates the retraction of rod synaptic terminals into the ONL (arrows) characteristic of detached feline retina. The labeling that occurs within the INL represents labeling of the terminals of rod axons that have extended into the INL. (C) An epiretinal membrane (arrow) formed by an anti-GFAP labeled Müller cell process extending through the GCL onto the vitreal surface of the retina. IPL, inner plexiform layer. ONL is outer nuclear layer and INL is inner nuclear layer.

beling are collapsed into a black and white image in this figure since labeling from the two is non-overlapping). Fig. 4B shows anti-synaptophysin labeling in both the ONL and INL, demonstrating the presence of rod synapses that have been retracted into the former and extended into the latter. Fig. 4C shows an example of an epiretinal membrane formed by anti-GFAP-labeled Müller cell processes extending beyond the inner limiting membrane into the vitreous cavity. These data validate the use of the feline model as a system in which to study events relevant to the human retina. However, it should be kept in mind that these observations are made in the periphery, where the human retina, like the feline retina, is rod-dominated. Whether or not similar events occur in the cone-dominated fovea is unknown.

4.2. Cellular remodeling after detachment

The structural remodeling that has been described here after retinal detachment and reattachment is summarized in Fig. 5. As stated earlier, the remarkable regenerative capacity of OS is well established. Retinal detachment and reattachment provides a system in which OS degeneration and regeneration can be manipulated and their effects on the retina studied isolated from other defects. This in turn presents opportunities for identifying and studying molecular factors that underlie these events. Finding the differences between genes expressed in normal, detached and reattached retina can lead to the discovery of molecules that control these processes and that eventually may be used to decrease damage or promote the regeneration of OS.

Besides loss of OS, photoreceptors also undergo some internal remodeling by the loss of mitochondria and the disorganization of organelles involved in protein synthesis and trafficking (Anderson et al., 1983; Mervin et al., 1999). They also change their expression profile for a variety of proteins with significant differences in the reaction of rods and cones (Rex et al., 2002). A similar difference has been shown in human photoreceptors in the disease retinitis pigmentosa (John, Smith, Aguirre, & Milam, 2000). Further remodeling occurs at the synaptic pole of these sensory neurons. Rods withdraw their synaptic terminals into the ONL, while cones do not. The synaptic terminals of both rods and cones change their morphology to appear more like synapses in developing retina (Erickson et al., 1983; Lewis et al., 1998; Linberg & Fisher, 1990). After reattachment, rod axons appear to re-grow into the OPL with some apparently ''over-shooting'' that layer and growing into the inner retina. This phenomenon has been identified in developing retina (Johnson, Williams, Cusato, & Reese, 1999) and in retinas afflicted with retinitis pigmentosa (Li, Kljavin, & Milam, 1995). RB cells grow dendrites into the ONL after detachment, probably as a response to the loss of rod terminals with which they connect. Interestingly, most of these dendrites appear to remain connected to rod photoreceptors and thus their growth appears to be target-directed. Whether these dendrites remain physically connected to the rod terminals and ''follow along'' as the terminals are withdrawn or whether they become disconnected and then sprout anew and grow towards their presynaptic target is unknown. Some rod bipolar processes grow deep in the ONL and

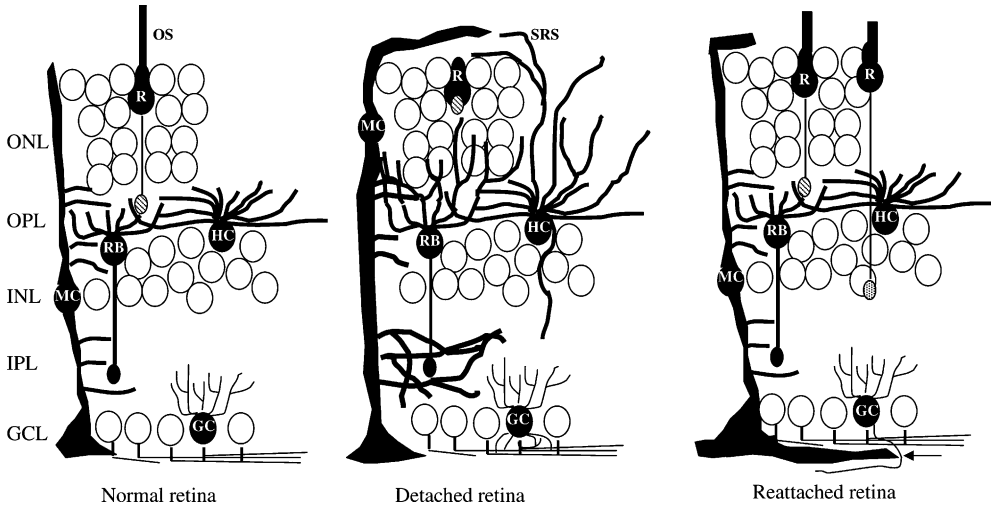

Fig. 5. A schematic representation of cellular remodeling in detached and reattached retina. The light sensitive OS of rod photoreceptors (R) degenerates and their synaptic terminal retracts from the outer plexiform layer (OPL) so that rod synapses now occur deep in the ONL (ONL). RB and HCs, grow neurites into the ONL in response to detachment. The new RB dendrites often end adjacent to withdrawn rod synaptic terminals, while the new HC processes appear to grow randomly into both the outer and inner retina. Müller glial cells (MC), with their main stalk of cytoplasm extending across the width of the retina, undergo several changes in morphology. Their nucleus often migrates into the ONL, their main process and fine lateral branches increase in size and fill with intermediate filaments. In detached retina, the main stalk of the Müller cell often grows onto the surface of the ONL, that is, into the SRS where it can form a "glial scar." The fine lateral processes appear to expand and grow within the retina with unknown effect. A subpopulation of GC shows many small neuritic sprouts along the basal side of the cell, not a location from which dendrites grow in normal retina. Reattachment allows the re-growth of OS and rod axons, although some of these now grow past the OPL, their normal target layer, and grow into the inner retina. Reattachment inhibits the hypertrophy of MC within the retina and in the SRS, but appears to allow the growth of these cells onto the vitreal surface of the ganglion cell layer (GCL) where they form epiretinal membranes. The neuritic sprouts from GC often intermingle with the MC processes that form epiretinal membranes (arrow). Early reattachment probably inhibits the plastic changes in RB and HC, and later reattachment may stimulate the withdrawal of many of the neurites that grew from these cells during the time of detachment.

do not terminate near rod terminals suggesting that at least some are newly developed processes. Whether or not these synapses, which now often appear on the base of the rod synaptic terminal rather than in deep invaginations, are functional isn't known, nor have molecular changes in the organization of the synapses (e.g. postsynaptic receptor distribution) been examined. Understanding events of this type is probably important to understanding the retina's overall responses to injury and diseases that affect the photoreceptors. HCs, by comparison to RB cells, extend processes that can grow wildly throughout the retina in a response more reminiscent of glial reactivity than specific neuronal remodeling. They also exhibit increased immunoreactivity to antibodies against the neurofilament proteins, molecules that form intermediate filaments, another similarity to the glial response where intermediate filaments formed by GFAP and vimentin show huge upregulation. There is now evidence that both gene expression and dendritic tree structure are affected in the GC, presumably a "downstream" consequence of initial changes in photoreceptors. The expression of both neurofilament protein and GAP 43, a molecule generally associated with axon growth and targeted synaptogenesis during development, are upregulated in GC after detachment (Coblentz et al., in press, 2001). Thus, the effects of detachment extend across the entire neural network of the retina. How this effects the processing of visual information and ultimately visual recovery, is unknown.

It has been known for many years that detachment has an effect on Müller cells. Their hypertrophy within the retina and growth into SRS has been well-documented; the effects of their proliferation and molecular factors that control it less so. There is evidence that the growth factor, bFGF (FGF2) plays a role in both proliferation and intermediate filament synthesis (Geller et al., 2001; Lewis, Erickson, Guérin, Anderson, & Fisher, 1992) of Müller cells. Like the photoreceptors, these cells react by a significant amount of molecular remodeling, including up-regulating their expression of GFAP and vimentin, and down-regulating carbonic anhydrase, glutamine synthetase, and cellular-retinaldehyde binding protein expression (Lewis, Guérin, Anderson, Matsumoto, & Fisher, 1994). It is not known if these are protective mechanisms or have an adverse effect on retinal cells, arguments can probably be made for both, but little real data exist (Marc, Murry, Fisher, Linberg, & Lewis, 1998). Certainly the hypertrophy of Müller cells and their growth onto the retinal surfaces is of practical significance because growth into the SRS inhibits almost entirely the regeneration of OS (Anderson et al., 1986) and growth into the vitreous produces cellular membranes that contract causing re-detach-

ment. A fibrotic scar composed of a single Müller cell process may extend for a long distance within the SRS but cannot be detected by routine ophthalmologic observation. Thus, unexplained changes or incomplete recovery of vision could occur with a retina that is anatomically reattached and has a normal appearance. Improvements in in vivo imaging technology may lead to a better understanding of the prevalence of this condition in human reattachments. Inhibiting both the subretinal growth and vitreal growth of Müller cells is desirable but so far has not been reliably achieved. Indeed, Van Horn, Aaberg and Machemer wrote in 1977, "The major remaining obstacle to the reattachment of the detached human retina is epiretinal membrane formation. . .," and there is little to change in this statement a quarter of a century later.

A significant response of the Müller cells involves their proliferation (Fisher et al., 1991; Geller et al., 1995). The proliferation of cells in the adult mammalian retina has received little attention, probably because of the assumption that proliferating cells produce cells of the same type (Fisher et al., 1991). Recent evidence from the chick retina provides some evidence that this may not be the case (Fischer & Reh, 2001) and suggests an important area for further investigation in mammalian retina.

As an experimental system, the induction of retinal remodeling by detachment and reattachment presents an opportunity to study many aspects of the retina's response to injury, including some that have received little attention to date. For example, the effect of neuronal changes described here on the retinal output by GC is a completely unexplored area. Presently there is little known about responses of interneurons in the cone neural pathways because of a lack of markers for cone bipolar cells that provide the reliability and degree of detail obtained by anti-PKC labeling of RB cells. Even the basic issue of cone survival after detachment has proven difficult to study because the cones rapidly lose immunoreactivity to all of the antibodies that serve as cone-specific markers in normal retina (Linberg, Lewis, Shaaw, Rex, & Fisher, 2001). Currently the development of techniques for photoreceptor transplantation, electronic retinal implants, and macular translocation as therapies for retinal degenerative diseases are well underway. The fact that the inner retina has the ability to remodel is, in fact, critical if photoreceptor transplantation will ever succeed because the second-order neurons must be plastic enough to respond and re-establish contact with the transplanted photoreceptors. On the other hand, a prolonged period of photoreceptor degeneration in the host retina may lead to undesirable inner retinal remodeling that renders a successful photoreceptor transplant non-functional. Similar issues will occur for any implanted electronic device that relies on intact and functional retinal circuitry. Translocating the fovea to an area devoid of invading choroidal vessels may successfully achieve the rescue of surviving photoreceptors, but if the detachment required to produce the translocation induces changes in retinal circuitry or a gliotic response by Müller cells, then the overall success may be in jeopardy.

4.3. Reattachment does more than return the retina to "normal"

It may seem reasonable to assume that retinal reattachment represents returning the retina to its "normal" state (i.e. attached to the RPE/choroid), but data from the animal model suggests otherwise. We have not observed axon outgrowth from rod photoreceptors in detached retinas, even after a month of detachment, yet we observe this process routinely in reattached retina. Growth of Müller cell processes into the SRS occurs routinely in the detached feline retina, and reattachment has the ability to stop this process (Lewis et al., 2002). However, reattachment appears to stimulate the formation of epiretinal membranes in the vitreous and the development of proliferative vitreoretinopathy. Understanding the molecular mechanisms underlying the differences between detached and reattached retina may prove to be critical to controlling conditions such as PVR and may also lead to an understanding of the phenomenon of rod axon outgrowth which has now been identified in a number of retinal degenerations.

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