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NEURONAL AND GLIAL ALTERATIONS IN COMPLEX LONG-TERM RHEGMATOGENOUS RETINAL DETACHMENT

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ABSTRACT

Purpose: To explore neuronal and glial alterations in eyes with complex long-term rhegmatogenous retinal detachment (RRD).

Methods: Morphological analysis was performed on eight retinal specimens derived from patients treated with peripheral retinectomy for RRD complicated by retinal shortening or retinal thinning. All eyes had undergone previous surgeries including silicone oil tamponade, and had a median total detachment time of 2.5 months (range 2-12). Specimens were examined with hematoxylin and eosin staining and immunohistochemistry directed against activated Müller cells, ganglion cells, rod bipolar cells, and photoreceptors.

Results: Retinal specimens displayed severe loss of photoreceptor and rod bipolar cells. Remaining neuronal cells exhibited disorganized perikarya and neurites with disruption of the normal retinal lamination. Müller cell activation was evident in all specimens with subretinal and epiretinal hypertrophy present in tissue derived from shortened retinal detachments.

Conclusion: Long-term RRD leads to retinal remodeling characterized by loss of first and second order retinal neurons, disruption of the entire retinal lamination and gliosis. The severity of histopathological changes indicates that anatomical as well as functional recovery of the involved retina is precarious. The findings may be important when devising surgical strategies to avoid permanent retinal detachment.

KEY WORDS

Degeneration; PVR; Retinectomy; Vitrectomy; Gliosis

INTRODUCTION

Rhegmatogenous Retinal detachment (RRD) continues to constitute the most common condition within the field of vitreoretinal surgery. An extraordinary development of tools and techniques has led to high final anatomical reattachment rates, but a small percentage of retinas fail to heal completely despite repeated surgical intervention.¹⁻³ The mechanism behind reattachment failure is most often attributed to development of the clinically defined and classified proliferative vitreoretinopathy (PVR) process.^{4,5} Several risk factors for PVR have been identified, including surgical intervention and detachment of long duration.⁶⁻⁸ To increase understanding of processes involved in complex RRD beyond clinical observations, a more detailed study of retinal pathology is warranted. For the present work, we wanted to explore cellular alterations in retinas at high risk of permanent detachment. We therefore choose to examine histopathological findings in eyes with long-term RRD in which surgery had failed to achieve reattachment despite silicone oil tamponade. These eyes were all subjected to new surgical intervention including peripheral retinectomy with retinal biopsy, and we here report the glial and neuronal histopathological changes in the obtained specimens.

METHODS

Patients

Eight retinal specimens derived from a consecutive series of eight patients (eight eyes) were used for the study. The tissue was obtained at surgery including peripheral retinectomy as a treatment for complicated RRD (Fig. 1). Indications for the procedure were severe intraretinal shortening as a part of a PVR process (n=6), or extensive thinning of the detached retina (n=2). All surgeries were conducted by one consultant (FG) at Lund University Hospital. Clinical data was obtained from patient records as well as from the surgical database kept at the Department of Ophthalmology (FileMaker Pro®, FileMaker Inc., Santa Clara, CA, USA). Five patients were male, three female, and their median age was 57.5 years (range 22-63). Subjects had gone through a median of 2.5 (range 1-5) previous surgeries for RRD in the studied eye. These procedures included scleral buckling (n=4) and/or vitrectomy and tamponade (all eyes). All procedures had been combined with laser retinopexy, and a limited retinectomy had been performed in three eyes. All eyes had previously received silicone oil. The estimated median total detachment time was 2.5 months (range 2-12). The median extent of detachment was 2.5 quadrants (range 1-4), and all included the inferior retina. Four detachments included the macula and the remaining four had done so at least once previously. Individual patient data is given in Table 1.

All patients provided informed consent for microscopical analysis of retinal tissue excised during surgery and the study was approved by the Regional Research Ethics Committee in Lund.

Tissue preparation

The specimen extracted from the peripheral detached retina was immediately fixed in 4% paraformaldehyde in 0.1 M Sørensen's phosphate buffer pH 7.4 for 1 h at 4°C. It was then washed, first in Sørensen's 0.1 M phosphate buffer at pH 7.4 and thereafter with the same buffer containing sucrose at increasing concentrations (10% and 25%). The specimens were embedded in Yazulla (a custom made gelatin (3%) and egg albumin (30%) mixture), serially sectioned on a cryostat at 12 µm and mounted on glass slides. Every 10th slide was stained with hematoxylin and eosin for light microscopy. For immunohistochemical labeling, retinal sections were incubated with phosphate buffered saline (PBS) containing 0.25% Triton X and 1% bovine serum albumin for 30 minutes at room temperature. The slides were then incubated overnight with antibodies raised against the following antigens: Recoverin for labeling of rod and cone photoreceptors (polyclonal, 1:10000, kind gift from Dr A.M.Dizhoor, Wayne State University, Detroit, USA); Phospho-PKCpan (protein kinase C) (rod bipolar cells, polyclonal, 1:200, Cell Signaling, Beverly, MA, USA); NeuN (Ganglion cells, monoclonal, 1:100, Chemicon International Temecula, CA, USA) and Glial Fibrillary Acidic Protein (GFAP) (activated Müller cells, monoclonal, 1:200; Chemicon International).

After incubation, the slides were rinsed 3 x 5 min and incubated at with Texas Red-conjugated antibodies ((Jackson ImmunoResearch, West Grove, PA) or fluorescein isothiocyanate (FITC; Sigma, St. Louis, MO) for 50 minutes at 1:200, rinsed again and mounted in Vectashield mounting medium (Vector Laboratories Inc., CA, USA). Negative controls were obtained by performing the complete labeling procedure without primary antibody. After examination in the fluorescence microscope, each slide was

stained with hematoxylin and eosin for light-microscopic evaluation of the corresponding immunoreactive areas.

Normal adult porcine eyes (n=4 eyes from 2 animals) were used as positive controls. Animals were euthanized by means of captive bolt and incision of the carotid arteries. Both eyes were enucleated and fixed for 30 min in formaldehyde (4% generated from paraformaldehyde) at pH 7.4 in Sørensen phosphate buffer (0.1 M). The anterior segment was then removed, and the posterior eyecup was postfixed in the same solution for 4 h. Tissue specimens were obtained as 4- to 5-mm wide pieces, including the superior peripheral area, the area of the optic nerve and the inferior peripheral area. After fixation, the specimens were washed with Sørensen phosphate buffer (0.1 M; pH 7.4) and then washed again using the same solution with added sucrose at rising concentrations (10 and 25%) before serial sectioning in the vertical plane at 12 μ m on a cryostat. Slides were stained with hematoxylin and eosin and labeled with immunohistochemical markers as above. All proceedings and animal treatment were in accordance with the guidelines and requirements of the government committee on animal experimentation at Lund University and with the ARVO statement on the use of animals in ophthalmic and vision research.

RESULTS

Light Microscopy

In hematoxylin and eosin stained sections, specimens from eyes with shortened retina displayed fewer cells than normal within the nuclear layers (Fig. 2A-C). Outer and inner nuclear layers (ONL and INL) were discernible in the central part of four specimens (Fig. 2A), and in the major part of one specimen (Fig. 2B). Occasional outer segments were seen in one specimen (Fig. 2A), but in all other areas, the retinas were devoid of normal retinal lamination and no outer or inner segments were seen. Gliotic reactions were seen both epi- and subretinally (Fig. 2B and C). In specimens derived from eyes with thinned retina, no retinal lamination could be distinguished and very few remaining cells were seen (Fig 2D).

Immunohistochemistry

In adult porcine control eyes, photoreceptors in the ONL displayed strong recoverin labeling throughout the length of the cells (Fig. 3A). Rod bipolar cells were well labeled with the PKCpan antibody (Fig. 3B) while ganglion cells of various sizes were seen in NeuN labeled sections as previously described (Fig. 3C).⁹ GFAP labeled horizontal astrocyte processes were seen in the innermost part of the retina, whereas vertical Müller cell fibers displayed very weak labeling intensity throughout the retinal layers (Fig. 3D).

In the human retinal specimens, remaining photoreceptors displayed strong recoverin labeling but were significantly fewer than normal, especially in thinned retinal specimens where only one-two rows of cells were found (Fig. 4A and C). In shortened specimens, labeled cells extended randomly organized neurites on various levels in the inner retina and an outer plexiform layer could only be seen occasionally.

Strongly labeled PKCpan positive rod bipolar cells were found in all shortened retinal specimens (Fig. 4E). Labeled cells were not organized in the normal manner, but displayed a seemingly random arrangement with perikarya located at various levels of the INL extending axons towards the inner retina in a disorganized array. In thinned retinal specimens, only a few scattered rod bipolar cells were found (Fig. 4G).

Well labeled ganglion cells were found in both shortened and thinned retinal specimen using the antibody raised against NeuN (Fig. 5A and C). Labeled cells were not organized in a distinct GCL, but rather found at various levels of the inner retina.

Müller cells in all specimens displayed GFAP upregulation in their entire inner-to-outer aspects indicating activation. In shortened retinas, Müller cells had often retained their normal vertical arrangement within the retina but displayed epiretinal and subretinal hypertrophy (Fig. 5E). In thinned retinas, the normal vertical arrangement of Müller cell fibers was absent and GFAP labeled fibers appeared randomly organized (Fig 5G).

DISCUSSION

The literature on human rhegmatogenous retinal detachment (RRD) histopathology is not abundant, whereas experimental detachment has been studied in great detail in several animal models. The laboratory work has shown that neuroretinal separation from the retinal pigment epithelium (RPE) elicits a cascade of acute, as well as chronic events in both entities.¹⁰⁻¹⁶ Early reactions, such as outer segment retraction and loss of RPE microvilli, are reversible if the retina is reattached within days.^{10,17,18} Long-term detachment, however, leads to permanent damage in the form of remodeling of the neuronal circuitry, subretinal gliosis, and photoreceptor loss.

Clinical RRD differs from the experimental induced condition in several respects. First, the rhegmatogenous origin of detachment is difficult to reproduce in the laboratory, and most models of long-term detachment depend on retina-RPE separation by viscoelastic agents. Second, animal models of PVR do not replicate dynamic events occurring in clinical detachment; and third, existing models do not take into account the impact of surgical intervention in the development of complex detachment. Thus, prudence is warranted when translating laboratory results to the clinic.

The main goal of the present paper was to explore cellular level pathology in eyes affected by complex RRD to increase our understanding of the condition beyond clinical examination and gross description. Pastor and colleagues described the role of activated Müller cells in retinal shortening in 2006.¹⁹ In an extensive immunohistochemical analysis of retinal specimens derived from various forms of RD, Sethi and colleagues described neuronal cell death, gliosis and heterotopic neuronal sprouting.²⁰ They point out however, that in their specimens, most components necessary for recovery are retained. Charteris and colleagues found similar changes in their anterior PVR specimens,

but also concluded that the degree of pathological change was associated with a potential for functional recovery.²¹ We found that the long-term detached retina displays remodeling of a severity which may be inconsistent with functional or anatomical recovery. Specifically, these findings includes: 1) degeneration of photoreceptors and rod bipolar cells; 2) disruption of the neuronal circuitry by remaining retinal cells; and 3) subretinal gliosis. These detrimental alterations in retinal architecture are best explained by ischemic insult caused by physical separation of the neuroretina from its choroidal supply. In accordance, ganglion cells, which are less dependent on choroidal nutritive support, appear to be less affected. Further, the potential impact of previous surgical intervention on remodeling cannot be ignored. Laser retinopexy, silicone oil tamponade and vitrectomy have all been linked to PVR development and may thus facilitate gliosis.^{2,6,8,22} Surprisingly, we did not find any apparent correlation between detachment duration and severity of histopathological alterations which may be due to our relatively small sample size. Another explanation is that retinal remodeling is initiated and stabilized within the first months of detachment.

The dynamics of retinal remodeling and the impact of surgical intervention in RRD may have important practical implications consistent with the long-established adage of "getting it right the first time". When primary surgery fails, the detailed nature of pathological events become even more important since proper measures need to be taken without aggravating the condition. In a complex case of redetached shortened or thinned retina, peroperative anatomical reattachment can be achieved by adapting the eye wall to the affected retina with the use of a buckle, or by tamponade with normal or heavy silicone oil.²³⁻²⁴ However, if the normal outer segment-RPE complex is disrupted by the absence of photoreceptors and the presence of subretinal gliosis, permanent attachment is un-

likely, in our opinion.²⁵ In accordance, the profound alterations in outer retina morphology in our eyes well explain why the retina failed to attach previously in spite of extensive surgical management including silicone oil tamponade and laser retinopexy. If stable reattachment of the remodeled retina cannot be obtained, retinectomy with removal of the affected tissue constitutes a radical but acceptable option to rescue the remaining retina from permanent detachment. Clinical studies of retinectomy for advanced RRD indicate a highly variable final anatomical success rate, ranging from 50 to 93%, but also show that visual acuity can be stabilized (albeit at a low level) or even slightly improved in most cases.²⁶⁻³⁰

To summarize, long-term rhegmatogenous retinal detachment in human eyes subjected to previous surgical intervention leads to profound changes in the retinal cytoarchitecture. An increased understanding of complex RRD on the cellular level may help us devise suitable surgical strategies to avoid permanent detachment.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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TITLES AND LEGENDS TO FIGURES

Figure 1. Peroperative image (surgeons view) of the retinectomy procedure. After peeling epiretinal membranes, a 180° retinotomy has been made with automated scissors. A 4x5 mm full-thickness neuroretinal biopsy is being cut from the peripheral inferior part of the detachment.

Figure 2. Hematoxylin and Eosin staining (cryostat sections) derived from four different retinectomy specimens. All four specimens display a reduced number of retinal cells. **A** (patient 5) displays a transition area between the shortened and the more normal retina. On the right side, the outer and inner nuclear layer (ONL and INL) as well as some outer segments (OS) can be seen whereas on the left side, the layers cannot be identified, and the tissue appears disorganized with subretinal gliosis (arrowheads). In **B** (patient 1, moderate pathology), the ONL, INL and ganglion cell layer (GCL) are relatively well preserved, but gliosis is evident stretching from the inner retina and forming an epiretinal mass (EPI). In **C** (patient 4), deconstruction of the retinal layers is evident and there is a gliotic mass present in the subretinal space (SR). **D** (patient 8) displays a retina devoid of any normal laminated organization, and with very few remaining cells. Scale bar = 50 μ m.

Figure 3. Immunohistochemistry of adult porcine control retina. **A:** Photoreceptors in the outer nuclear layer (ONL) display strong labeling throughout the length of the cells. **B:** Rod bipolar cells are well labeled with the PKCpan antibody and display vertical axons terminating in the inner part of the inner plexiform layer (IPL). **C:** Ganglion cells in the innermost part of the retina are seen in an NeuN labeled section. **D:** Horizontal astrocyte processes in the innermost retina display strong GFAP labeling. Weak labeling of vertical Müller cell fibers is seen in the remaining retina. Scale bar = 50 μ m.

Figure 4. Immunohistochemistry of retinectomy specimens. Left column: Immunolabeled sections; Right column: Hematoxylin and Eosin staining of the corresponding area. **A** and **C**: Remaining photoreceptors in the outer nuclear layer (ONL) display strong recoverin labeling. The lack of organization is evident and labeled cells **in the shortened specimen (A)** display random sprouting towards the inner retina (**arrowheads**). The thinned retina (**C**) retains only one-two rows of photoreceptors. PKCpan labeled rod bipolar cells (**arrowheads; E**) terminate in a disorganized manner in the inner plexiform layer (IPL) where no sub lamination can be seen. In the thinned specimen (**G**), very few PKCpan labeled cells are seen (**arrowhead**). Scale bar = 50 μm . GCL = Ganglion Cell Layer; INL = Inner Nuclear Layer; OPL = Outer Plexiform Layer

Figure 5. Immunohistochemistry of retinectomy specimens. Left column: Immunolabeled sections; Right column: Hematoxylin and Eosin staining of the corresponding area. Ganglion cells are well labeled with the antibody against NeuN in both the shortened (**A**) and thinned (**C**) retinal specimen. The cells are not normally organized at the innermost part of the retina, but are found at various levels of the inner retina. Yellow clumps of RPE derived pigment can be seen within the retina. **E** and **G**: Müller cells display strong GFAP labeling throughout the retina indicating activation. In **E**, the Müller cells have retained their normal vertical arrangement in their major part whereas in **G**, the normal organization is lost. Epiretinal (EPI) and subretinal (SR) hypertrophy is evident. Scale bar = 50 μm .

TABLE

No	Previous RRD Surgeries Number	Retinectomy indication	PVR	Extent of detachment (quadrants)	Total Detachment Duration (Months)	Preop BCVA
1	5	retinal shortening	C1	2	2	0.02
2	2	retinal shortening	C1	4	3	HM
3	2	retinal shortening	C1	2	2	0.2
4	3	retinal shortening	C2	3	12	HM
5	4	retinal shortening	C3	4	3	0.02
6	1	retinal thinning	A	1	6	0.02
7	1	retinal shortening	C1	2	2	0.2
8	5	retinal thinning	A	3	2	0.04

Table 1. Clinical patient data. The grade of PVR (Proliferative VitreoRetinopathy) was determined according to the Retina Society classification.⁴ Detachment duration was calculated from patient records adding the current episode. BCVA=Best Corrected Visual Acuity (Monoyer). HM=Hand Motion

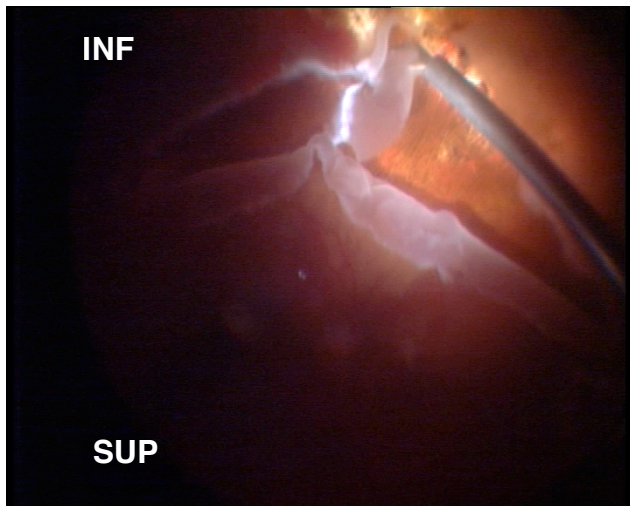


Figure 1. Ghosh and Johansson

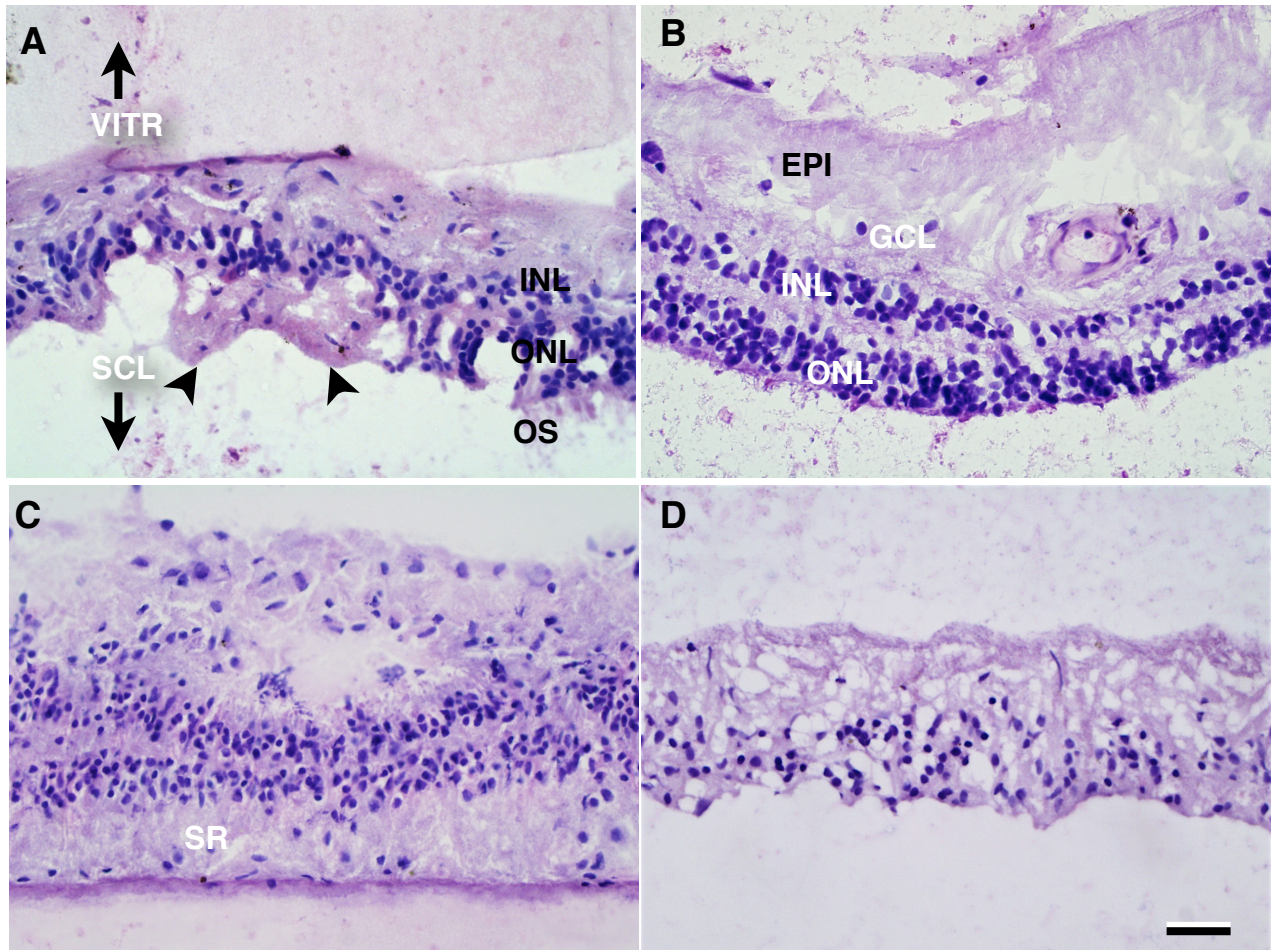


Figure 2. Ghosh and Johansson

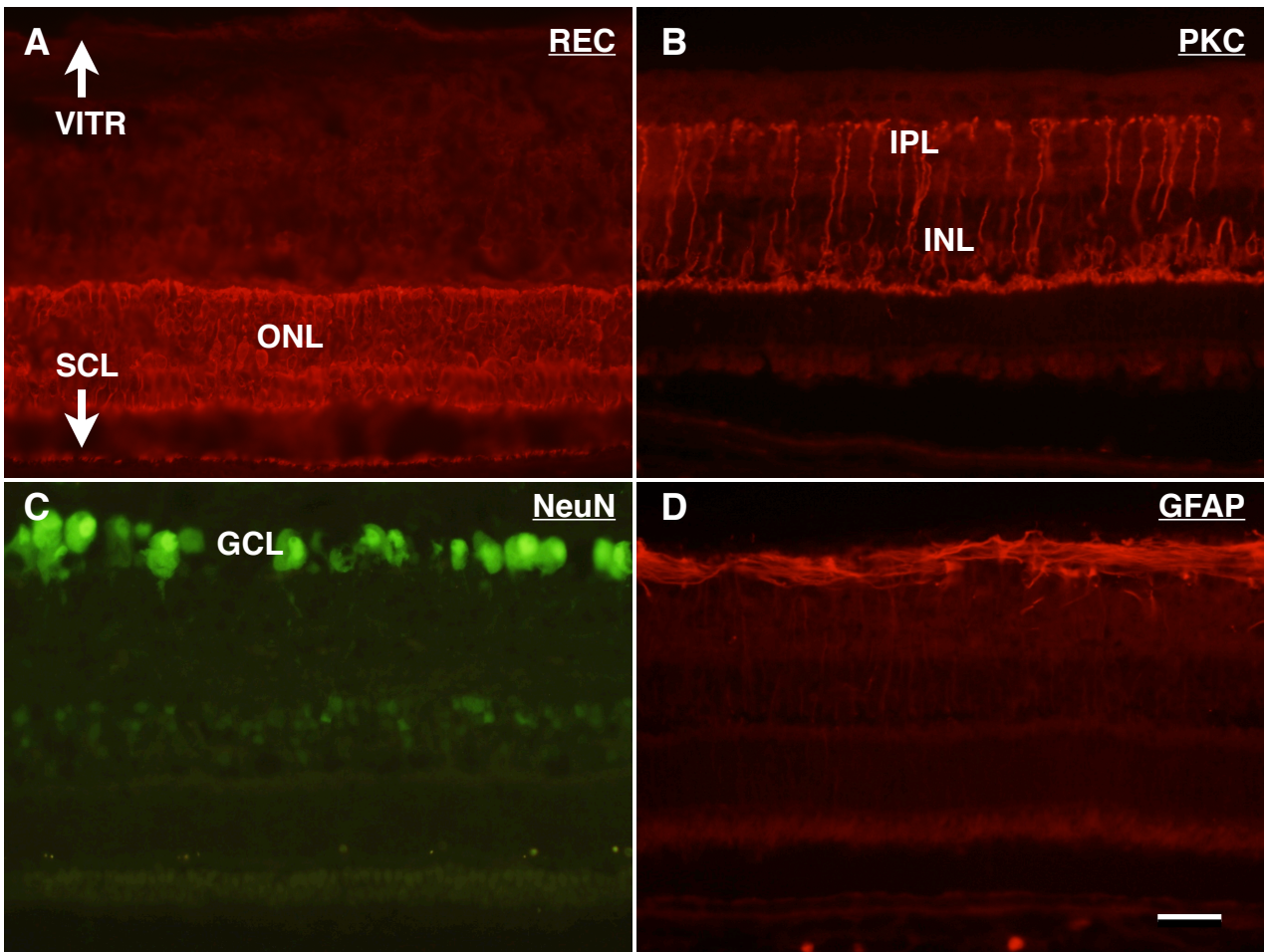


Figure 3. Ghosh and Johansson

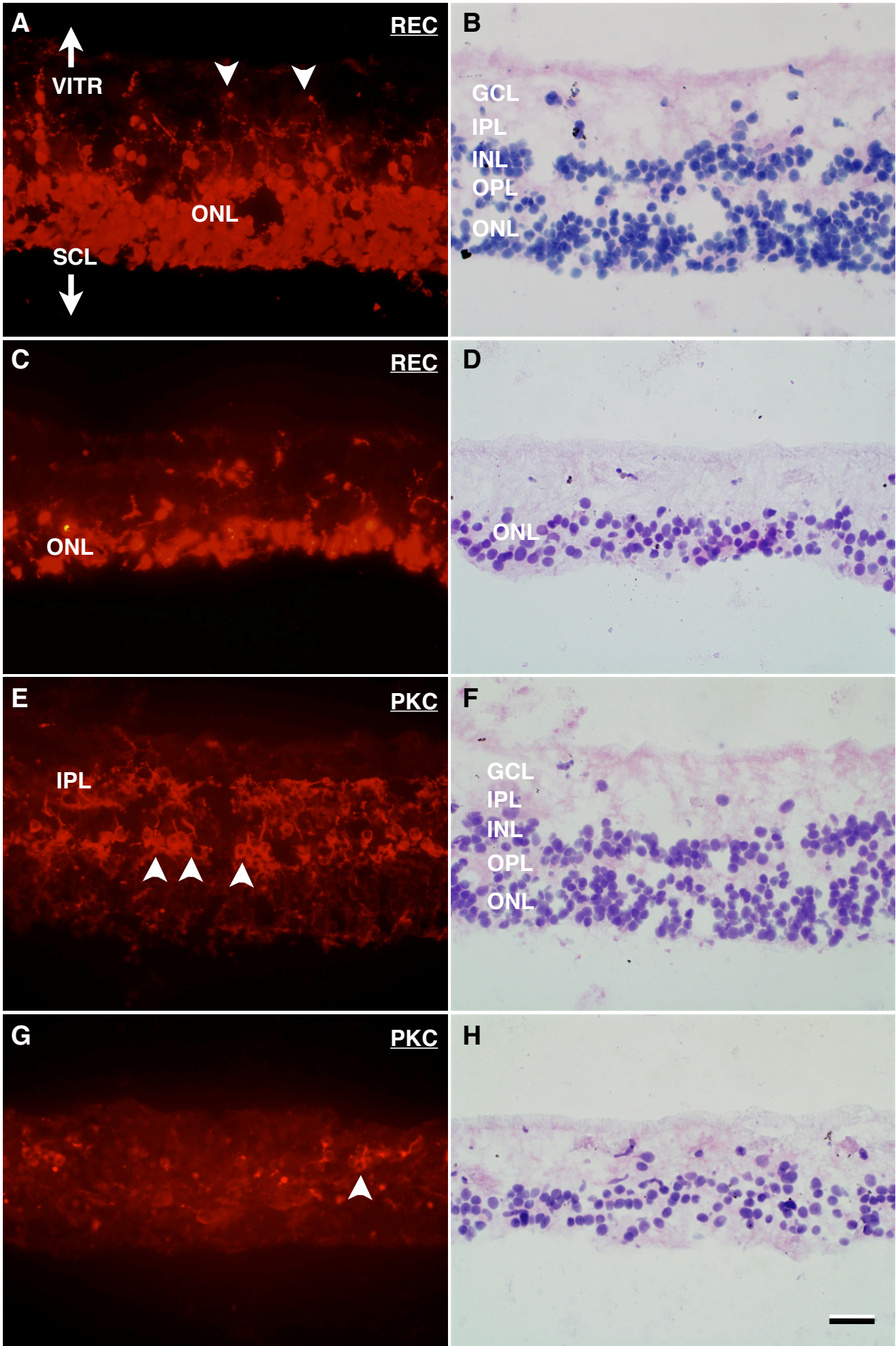


Figure 4. Ghosh and Johansson

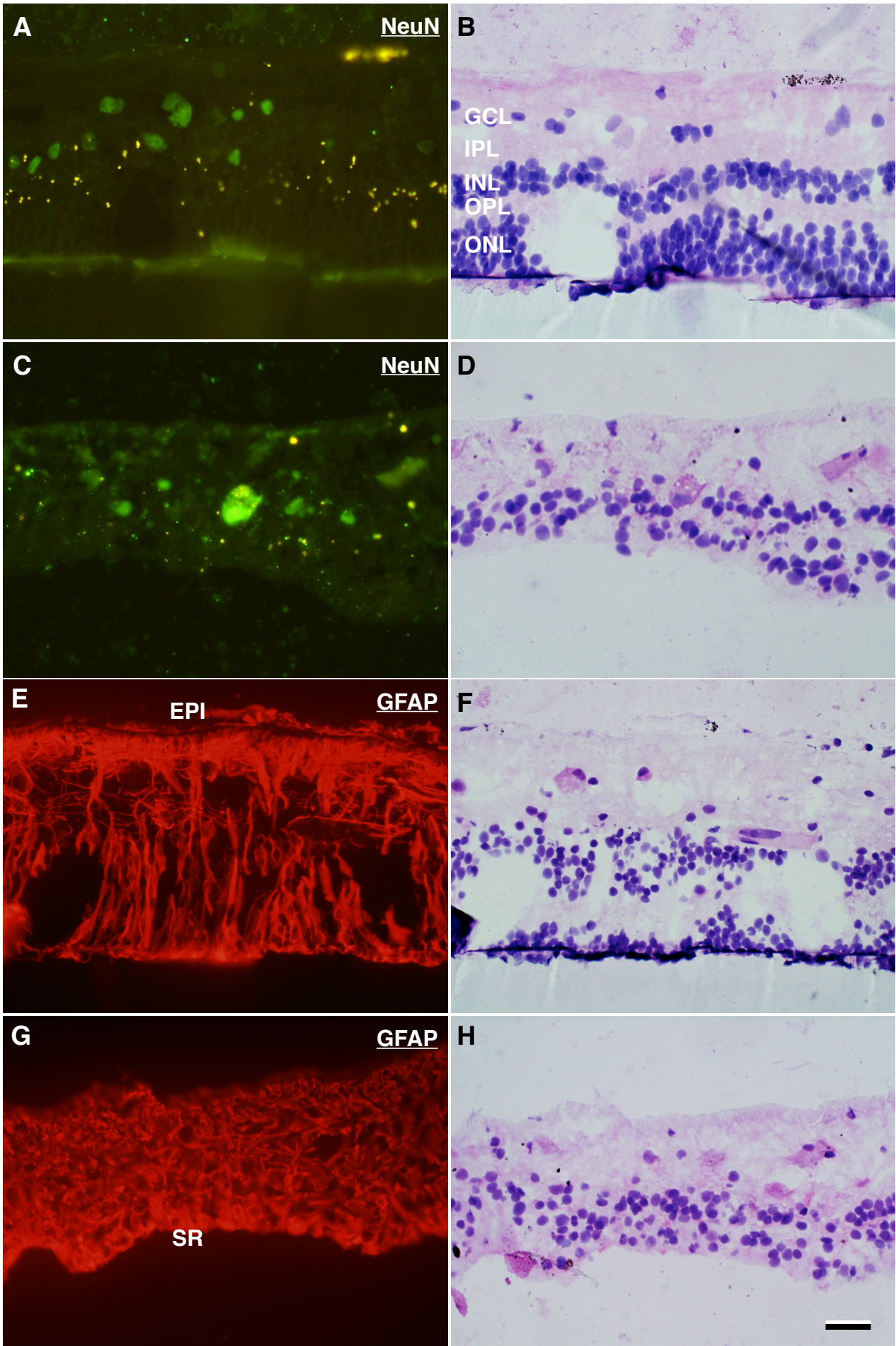


Figure 5. Ghosh and Johansson