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INVITED REVIEW

Retinal remodelling

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Retinal degenerative diseases that progress through loss of photoreceptors initiate a sequence of events that culminates in negative remodelling of the retina. Initially, photoreceptor loss ablates glutamatergic signalling to the neural retina and eliminates coordinate Ca^{++}-coupled homeostatic signalling. Retinal neurons react to this loss of glutamatergic input through retinal rewiring and migration of neurons throughout the axis of the retina. All diseases that kill photoreceptors trigger retinal remodelling as the final common pathway and cell death is a common feature. Retinal remodelling resembles CNS pathologic remodelling and constitutes a major challenge to all rescue strategies.

Key words: amacrine cell, bipolar cell, computational molecular phenotyping, ganglion cell, horizontal cell, photoreceptor, plasticity, remodelling, retina, retinitis pigmentosa.

Retinal degenerations are progressive diseases, the clinical picture of which varies depending on the primary insult. While being the best characterised of the retinal degenerative diseases,[1] retinitis pigmentosa (RP) is only one form of retinal degeneration with numerous aetiologies. More than 160 gene loci have been identified and associated with RP forms of retinal degenerations.[2] In addition to RP, the retinal degenerative diseases encompass myriad forms ranging from cone and cone/rod dystrophies to age-related macular degeneration, to environmental challenges to the retina, all of which can induce photoreceptor stress and cell death. Broadly speaking, retinal degenerations fall into three categories including rod-degenerative, mixed rod/cone degenerative and debris-associated forms (for example, light damage models and *mertk* defects). Even though all these forms of retinal degenerative disease have differing primary mechanisms, they all have the same outcome: Photoreceptor loss followed by continued cell death and neuronal remodelling of surviving cell populations. Ultimately in the most severe forms, the retina deconstructs itself with some surviving neurons even escaping from the retina into the choroid. These findings are in marked contrast to previously held beliefs that the neural retina maintains its anatomy and physiological status after photoreceptors have degenerated, altering our understanding and approach to strategies to rescue vision.

CLINICAL AND HISTOLOGIC FINDINGS

Retinal degenerations present with clinical sequelae that depend on the primary genetic or environmental insult. Initially, rod-cone dystrophies typically present with complaints of night blindness in a patient's early teens. Often, changes in the ERG can be observed prior to ophthalmoscopically detectable signs in the fundus, with the X-linked form demonstrating the most dramatic changes in ERG responsivity from early time points, as rods begin to demonstrate photoreceptor stress followed by cellular death. This is important: photoreceptor signalling through the neural retina is impaired before photoreceptor cell death begins. As photoreceptor cell death progresses, visual loss continues, beginning in the periphery and often leading to ring scotomata by the early 20s. Autosomal dominant forms of RP typically have later onset of clinically observable symptoms and a longer time course of retinal changes. In the cone and cone rod

dystrophies, the clinical presentation is often initially through a loss of visual acuity and/or colour discrimination. These patients frequently present with central scotomata and when evaluated by ERG, show decreases in cone ERG responses that are either attenuated or obliterated, while rod ERG responses remain intact in the pure cone dystrophies. In the cone-rod dystrophies, cone ERG responses are compromised, as are rod ERG responses reflecting the involvement of both major populations of photoreceptors. Ophthalmoscopic visualisation of a typical retina from a patient with the RP form of retinal degeneration presents a pale yellow optic nerve head with possible papilloedema. Pigmented bone spicules are often seen in the periphery and are associated with accumulations of pigment epithelium coalescing into clumps and becoming entombed within the neural retina (Figure 1).

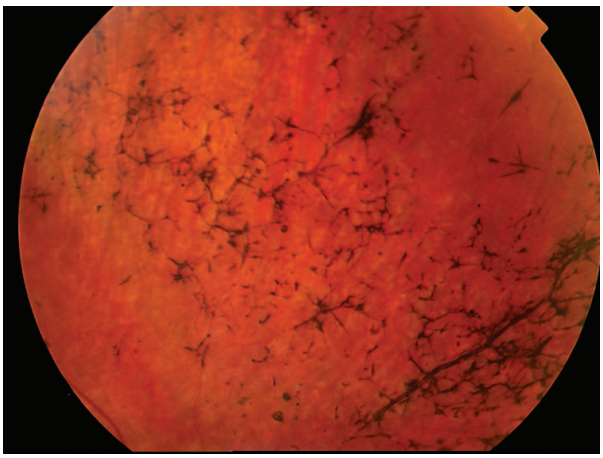

Figure 1. Fundoscopic image from a 48-year-old male patient with diagnosis of retinitis pigmentosa. The presence of stellate pigment hyperplasia or 'pigmented bone spicules' is evident throughout the field. Histologically, bone spicules are accumulations of pigment epithelium that coalesce into clumps, migrating down into the neural retina along glial scaffolds.

The retinal vasculature often presents with thickened walls and may be somewhat attenuated. This is a late-stage attribute. Usually, the macula and fundus are free of pigmentation in all but the most advanced cases of RP, which might also present with lesions or macular holes. Examination of the retina at the cellular level with traditional histologic techniques reveals an invasion of pigment granules from the retinal pigment epithelium (RPE) in regions associated with the pigmented bone spicules and other changes associated with alterations in the borders of the normal lamination of the retina (arrow P in Figure 2D). These standard histologic measures of evaluating the status of the retina have failed to reveal the true extent of pathology to the retinal degenerative community.

Because the initial stages of retinal degeneration were thought to involve only photoreceptor loss, most studies focused on cell counts to define the status of the retina. Some of these studies have attempted to document surviving neuronal cohorts in human subjects by examining retinas post-mortem from individuals with severe forms of RP.[3-5] Santos and colleagues[4] found approximately 20 per cent of inner nuclear layer cells lost in the macula of late stage RP patients, while Humayun and associates[3] documented 20 per cent survival in regions immediately peripheral to the macula with losses far more severe in the peripheral retina. It should be noted that even apparently small losses of 20 per cent in the macula can be dramatic and could largely deplete the ganglion cell layer of functional inputs.[6]

Other approaches have examined more subtle changes to the neural retina and these studies over the course of the past decade have revealed that retinal degenerative diseases possess a more insidious physiological response that more closely mirrors remodelling observed in deafferented CNS systems. The concept of neuronal remodelling, while new to the vision community, has been extensively documented by the epilepsy[7-10] and learning/memory communities,[11-13] though it should be noted that as early as 1974 within the basic science vision research community there were indications of compromised retinal circuitry in human RP.[14]

Remodelling in the retina was not formally recognised until much later with studies that revealed abnormal changes in the inner retina following photoreceptor degeneration that included sprouting of photoreceptors and horizontal cells.[15] Other studies demonstrated specific changes in the bipolar and horizontal cell populations of the *rd1* mouse after photoreceptor loss.[16-18] Milam and her colleagues[19] documented pathological similarities in human RP with those in the RCS rat and subsequently demonstrated neurite sprouting in horizontal cells and rods from human RP tissue.[20] Since then, several other studies have focused specifically on early changes to the neural retina in photoreceptor degenerative diseases. These document alterations in the circuitry of the neural retina and demonstrate progressive events[16-18,21-24] that, taken together with a comprehensive computational molecular phenotyping (CMP) based analysis of retinal remodelling in human and genetic engineered models of degenerative diseases,[6,25] conclusively demonstrated that the retina, like the CNS, exhibits remodelling in response to loss

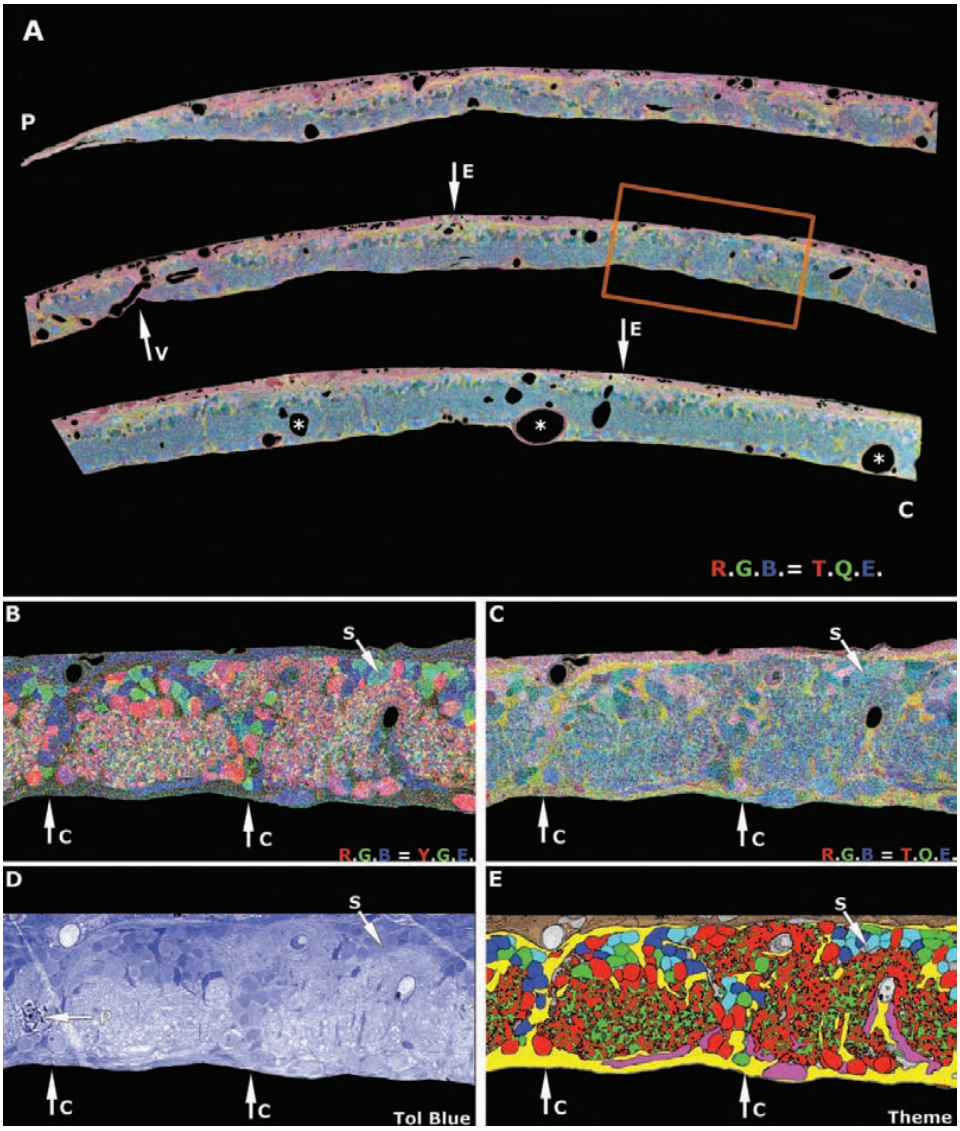

Figure 2. pnd 900 RCS rat retina.

A: a peripheral (P) to central (C) retinal hemisection with τ.Q.E. → rgb mapping assigning taurine, glutamine and glutamate to red, green and blue colour channels respectively allowing simultaneous visualisation. Vascular invasion and thickening (V) are seen in mid-peripheral retina along with fluid channels (asterisk) that develop and are likely to be in response to Müller cell seals, walling off the neural retina from the vascular choroid. Escape zones (E) are also seen where the Müller cell seal is incomplete allowing neuronal emigration out of the neural retina into the choroid. Inset box shows region of retina in B, C, D and E.

B: γ.G.E → rgb assigns GABA, glycine and L-glutamate to red, green and blue channels. Columns (C) of neuronal migration are seen along with an inner plexiform layer stricture (S). Glycinergic amacrine cells (green cells) are seen migrating down into the ganglion cell layer at the bottom of the migration columns; an event never observed in normal retina.

C: As in A, this inset image shows τ.Q.E. → rgb mapping assigning taurine, glutamine and glutamate to red, green and blue colour channels, respectively. Müller cells (gold colour) can be seen sealing off the neural retina from the remnant choroid.

D: Standard toluidine blue histological stain showing structural features of columns (C) and stricture (S). Clumps of pigment (P) are also seen having migrated down along the Müller cell column into the neural retina.

E: Computationally derived theme map from clustering alanine, aspartate, glutamate, glycine, glutathione, glutamine, taurine and GABA. Müller cells can be seen in yellow, glycinergic amacrine cells in green, GABAergic amacrine cells in red, ON cone and OFF bipolar cells in light and dark blue respectively and ganglion cells and axons in pink.

of afferent input. These outcomes impact strategies designed to rescue the retina given current visual rescue paradigms and methodologies.[26-30] Superficially, this remodelling or negative plasticity might appear to preclude the use or implementation of bionic or biological implants but we would encourage more optimism as the plasticity may prove advantageous to retinal rescue. That said, we do not yet know the windows of opportunity for intervention, especially as RP arising from different primary defects progresses at differing rates.[25]

COMPUTATIONAL MOLECULAR PHENOTYPING AND RETINAL DEGENERATIVE MODELS

In terms of evaluating the status of the retina, traditional clinical or histological methods of examination are impoverished techniques and do not reveal the true status of neural populations or connectivity of the neural retina. The development of CMP[31] has allowed us to data-mine tissue samples revealing normal and abnormal circuitry, neuronal phenotype revision and cellular translocation, events that are invisible to standard histological approaches. The development of these technologies in combination with a thorough examination of aged retinas in a spectrum of models allowed us to go beyond simple cell counts as evaluators of the neural retina in retinal degenerations, and have enabled us to capture the dynamic nature of retinal remodelling while documenting specific changes occurring in the surviving cohorts of neurons. The impetus to define timelines for degenerative disease and describe sequelae of degeneration is the movement to create strategies designed to rescue vision through bionic, biological and pharmacological therapies.

CMP is a fusion of molecular and computational technologies to concurrently visualise small molecular species with subcellular resolution and has proven useful in determining cell populations in normal and pathologic retinal tissue.[25,31-36] CMP depends on quantitatively tracking the colocalisations and concentrations of small molecules in tissues to determine cellular identity with IgGs generated against amino acids, which have proven to be good class discriminants for cell populations. The determination of cell classes or populations of cells based on the small molecular contents are not visualised under the microscope *per se*. Rather, the data sets are constructed of individual images of immunocytochemically labelled sections on slides that are then realised as multidimensional data sets. All retinal space can be accounted for by amino acid immunolabelling and, in particular, by the amino acids glutamate, GABA and glycine, which label neuronal populations, and with taurine and glutamine labelling glial populations and neuronal subsets.[32,35]

Glycine and GABA are primarily the neurotransmitters of the horizontal pathway of information flow through the retina, which includes horizontal cells (GABA) and amacrine cells (GABA and glycine), and glutamate is primarily responsible for the vertical flow of information through the retina, including photoreceptors and bipolar cells. Multispectral classification with just these three immunolabels, GABA, glycine and glutamate, allows quick discrimination of bipolar, amacrine and ganglion cells but not with the degree of discrimination obtained with seven or more small molecular labels. In short, CMP allows for multidimensional segmentation of cell classes in retinal tissues with the aim of cataloguing cell classes and elucidating their circuitries. The multidimensional signatures of classes in retina are typically surveyed and visualised as theme maps and selected rgb images, where defined images are encoded into three space images as red, green and blue respectively. For example, γ.G.E → rgb assigns GABA, glycine and L-glutamate to red, green and blue channels.

CMP analyses of retinal degenerations were made with over 200 retinas from naturally occurring and engineered animal models (RCS, *rd1*, *rd2*, *or^{J}*, *pcd*, *nr^{J}* , GHL, rhoΔCTA, TG9N, *rdcl*, P23H, S334ter, *or^{J}* + *P27Kip1$^{-/-}$* , *rho$^{-/-}$*, *elovl4$^{-/-}$*, hrhoG, hrhoG(H), hrhoG + *rd1* and light damage [LD] rat) that were processed along with 18 retinas from human patients with RP diagnoses. The CMP process begins with tissue fixation in 1 per cent paraformaldehyde/2.5 per cent glutaraldehyde in phosphate buffer. Selected samples were osmicated for ultrastructural analysis. After fixation, all samples were dehydrated in graded methanols to acetone and processed in resin matrices as single specimens, stacks or mosaics for thin sectioning at 40 to 250 nm into serial arrays, mounted on 12 spot Teflon coated slides (Cel-Line, Fisher Scientific) and serially probed with IgGs generated against alanine (A), aspartate (D), glutathione (J), glutamate (E), glutamine (Q), glycine (G), GABA (g) and taurine (t), all key retinal metabolites and cell-specific markers. Primary immunohistochemical labelling was followed by silver intensification with a secondary goat anti-rabbit IgG adsorbed to one nanometre gold particles and visualised with silver intensification.[37] All images of immunoreactivity were captured as 8-bit greyscale images and registered to less than 250 nm root-mean-square error. CMP[31] was then employed to classify neurons and non-neuronal populations. EM overlay was used to identify signatures at the ultrastructural level.[38]

The conclusions drawn were made possible through the diversity of models of retinal degeneration used, which include the naturally occurring models (RCS rat, *rd1* mouse, *rd2* mouse, *or^{J}* mouse, *Agtpbp1* mouse, and *nr^{J}* mouse), transgenic models (GHL mouse, rhoΔCTA mouse, TG9N mouse, *rdcl* mouse, P23H rat [3 lines], S334ter rat [4 lines], genetic knockout models (*or^{J}* + *P27Kip1$^{-/-}$* mouse, *rho$^{-/-}$* mouse, *elovl4$^{-/-}$* mouse), genetic knockin models (GFP-human rhodopsin fusion hrhoG, hrhoG(H), hrhoG + *rd1*) and the induced light damage (LD rat) rodent models. The naturally occurring RCS rat contains a *mertk* defect that impairs rod outer segment phagocytosis;[39,40] the natural *rd1* mouse model of autosomal recessive (ar) RP possesses a *pde6* nonsense mutation;[41,42] the natural *rd2* mouse has a partial dominant *prph2* defect exhibiting slow rod degeneration;[43] the natural *or* mouse model of human micro-ophthalmia, possesses a null mutation in the *chx10* homeobox gene[44,45] resulting in reduced numbers of retinal neurons; the natural

Agtpbp1 mouse exhibits both Purkinje cell and slow rod degeneration;[46-49] the natural *nr* mouse, with a chromosome 8 defect causes Purkinje cell and rod degeneration.[50-52] Transgenic models include the GHL mouse model of adRP, with a triple V20G, P23H, P27L rhodopsin mutation;[53] the transgenic rhoCTA mouse possesses a truncated rhodopsin at Ser334;[25] the transgenic TG9N mouse expresses the N-terminal fragment of mouse RGS9;[54] the rodless/coneless *rdcl* mouse[55] is a model lacking both rods and cones; the transgenic P23H rat is a model of autosomal dominant (ad) RP with an N-terminal rhodopsin H mutation at P23;[15,56] the transgenic S334ter rat expresses a truncated rhodopsin at Ser334.[56] The genetic knockout models include the *Chx10/p27^{Kip1}* mouse -*p27^{Kip1}* knockout model, which is a partial rescue of the *or* mouse;[57] the rho-/- knockout mouse model of human RP[58] and the *elovl4$^{-/-}$* mouse model of Stargardt's dystrophy.[59] Genetic knockin models include the GFP-human rhodopsin fusion hrhoG, hrhoG(H), hrhoG + *rd1*[60] and finally, the induced (LD) rat models,[61,62] which are likely to be a model for age-related macular degeneration (AMD) and AMD-like disorders.

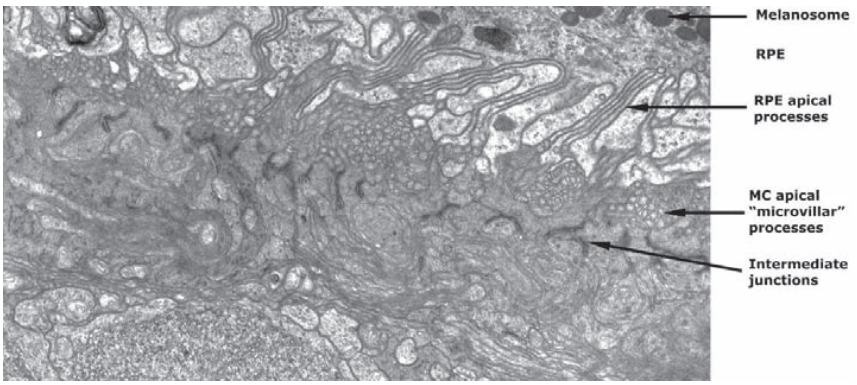

Figure 3: Electron microscopy of the Müller cell seal demonstrating intermediate junctions between Müller cell apical expansions. Normally one would see rod and cone inner segments making intermediate junctions with Müller cells to form the external limiting membrane. However, in retinal degenerations, Müller cells entomb the neural retina, isolating it from the remnant subretinal space.

PHASES OF RETINAL REMODELLING

Typically, retinal degenerations follow three phases over the course of the disease. Retinal remodelling and perhaps occult molecular changes begin early, possibly as soon as photoreceptors become stressed. Some evidence indicates that acquired[61,63] and inherited[64] rodent degeneration models reveal subtle changes in cellular molecular phenotypes in the neural retina, with some changes even preceding rod degeneration.[64]

Phase 1 of retinal remodelling is initiated with cellular stress in RPE cells and photoreceptors as revealed by alterations in small molecular signatures that are likely to be due to uncoupling in RPE cell populations.[25] Early morphological changes observed in phase one include sprouting of photoreceptor processes projecting down into the inner nuclear layer and ganglion cell layers. These processes participate in the misrouting of rhodopsin to the inner segments of photoreceptors[19] and rhodopsin delocalisation throughout processes extends down the fascicles projecting into the inner nuclear and ganglion cell layers.[65,66] In the *rd1* mouse,[16,18] a phototransduction defect induces rod photoreceptor death by postnatal day (pnd) 21 and exhibits subtle remodelling of rod pathways in secondary order neurons.[17] More generally, photoreceptors begin to deconstruct their synaptic terminals as part of the sprouting process and bypass their normal intended targets of bipolar and horizontal cells, which in turn retract their axonal terminals and fine dendrites.

Rod bipolar cell axonal terminals also appear to assume immature synaptic structures of unknown synaptic efficacy. Aberrant processes extend to cone circuits with both cones[67] and cone horizontal cells[18] in the *rd1* mouse sprouting new neurites that extend aberrantly to improper targets. Prior to cellular apoptotic initiation, rods may retract their processes. Rare misplaced rod photoreceptor cells in human RP have been observed in the inner nuclear layer where they appear to survive (BW Jones, CB Watt and RE Marc, unpublished data). Additionally, during human rod degeneration, surviving rods, horizontal and amacrine cells similarly extend anomalous neurites throughout the retina.[20] The sprouting resembles that observed in development where photoreceptor neurites project to the inner plexiform layer and then retract as the outer plexiform layer matures.[68] In developmental and retinal detachment studies,[69] retraction of neurites does not necessarily presage cell death, as it does in retinal degenerative diseases. Rather in retinal degenerative diseases, the failure of synaptic signalling triggers a variety of possible rewiring scenarios, including a retraction of dendrites from bipolar cells, switching of synaptic targets by bipolar cells and extension of horizontal cell processes into the inner plexiform layer.

Phase 2 is characterised by photoreceptor cell death, which eventually deafferents bipolar cell populations, thereby eliminating light mediated signalling to the neural retina. Because of large amounts of extracellular protein resulting from cell death, debris removal becomes important in this stage, perhaps via microglial activation. Formation of the Müller cell (MC) seal is the principal hallmark of stage two and is most likely to occur due to the collapse of the outer nuclear layer, leaving

behind a dense filigree of MC distal elements that entomb the remnant neural retina, sealing it off from the remnant RPE and choriocapillaris.[6,70,71] This seal is made locally complete by the formation of intermediate junctions between MCs (Figures 3 and 4C). RPE invasion and more problematic, neuronal escape from retina can occur in focal regions where the seal is not complete (Figure 2A arrow E).[61,62] These dramatic MC transformations are found exclusively in areas with complete rod and cone loss. Negative neuronal plasticity is most evident in these areas, including bidirectional neuronal migration along hypertrophic MC columns throughout the thickness of the retina (Figures 2 and 4), with some neurons emigrating or escaping from the retina into the remnant choroid.[61,62] Neurons identified by CMP as glycinergic amacrine cells are notable as indices of abnormal migration into the ganglion cell layer, as they are normally restricted to the amacrine cell layer.[25] Statistically, one is more likely to see amacrine cells migrating from the amacrine cell layer to the ganglion cell layer than the reverse, although bidirectional migration is commonly observed with ganglion cells translocating to the inner nuclear layer as other neurons move to the ganglion cell layer. Another feature of phase 2 transformations is a molecular revision in glial signals. It is not uncommon to observe a dramatic more than 10-fold increase in MC glutamine, which occurs only in MCs engaged in seal formation. MCs less than 0.1 mm away from the seal are normal, which suggests that MCs engaged in seal formation have strongly altered their metabolic and perhaps genetic profiles.[36] In LD models of retinal degeneration, irregular two- to four-fold increases in RPE glutamine and rod aspartate levels are observed, perhaps presaging photoreceptor cell death. Before completion of phase 2 remodelling, dendrites of bipolar cells retract and horizontal cells commonly send axonal processes into the inner plexiform layer (IPL).[16-18,72] Finally, some degree of neuronal death may begin in phase 2.[6]

Phase 3, the final and most prolonged stage of remodelling, was originally described in the GHL mouse model.[70] At the

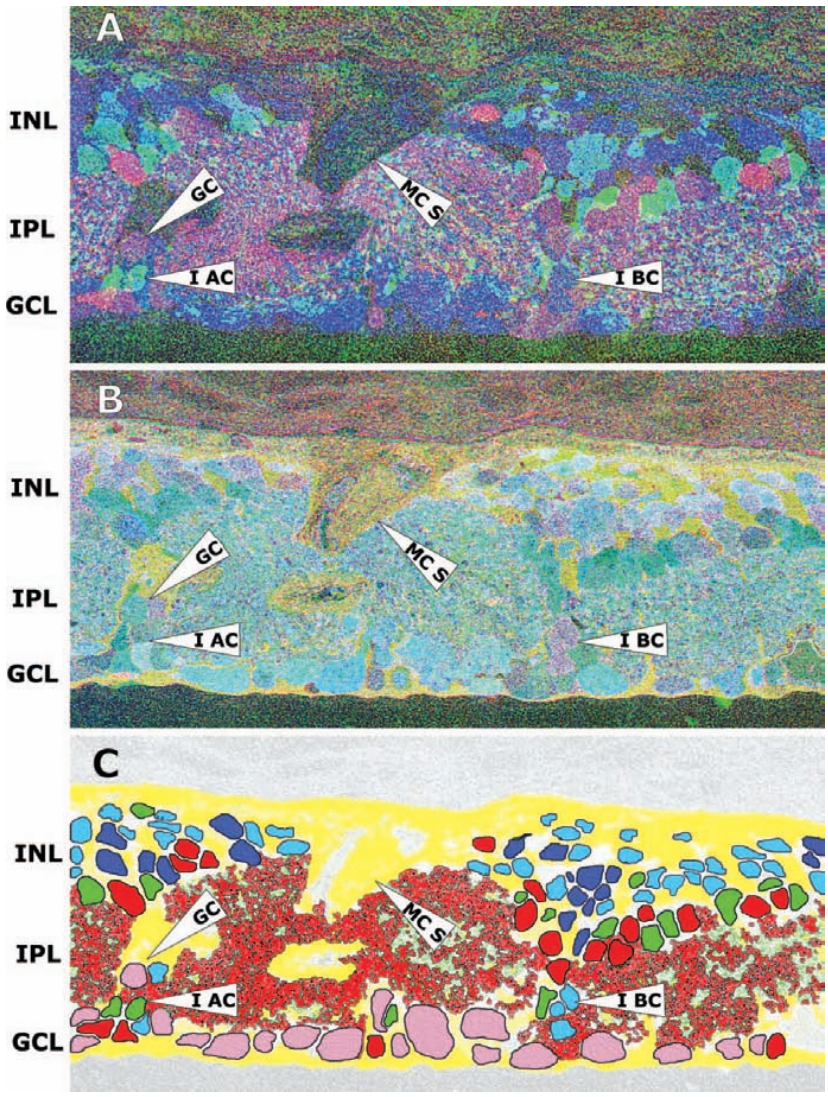

Figure 4.

A: Transgenic GHL mouse pnd 746 with γ.G.E → rgb mapping assigning GABA, glycine and L-glutamate to red, green and blue channels respectively. Alterations in the normal lamination of the retina can be seen along with bidirectional migration of neuronal classes including ganglion cells (GC) migrating upwards, glycinergic amacrine cells (I AC) migrating down into the ganglion cell layer, and bipolar cells migrating into the ganglion cell layer (I BC). Müller cell strictures (MC S) can be observed breaking up the normal lamination and creating isolated regions of retina.

B: τ.Q.E. → rgb mapping assigning taurine, glutamine and glutamate to red, green and blue colour channels respectively. The Müller cell seal can be appreciated in yellow/gold investing the retina and walling it off from the remnant choroid above it.

C: Theme map representing computationally classified neuronal space based on immunolabeling with alanine (A), aspartate (D), glutathione (J), glutamate (E), glutamine (Q), glycine (G), GABA (γ) and taurine (τ).

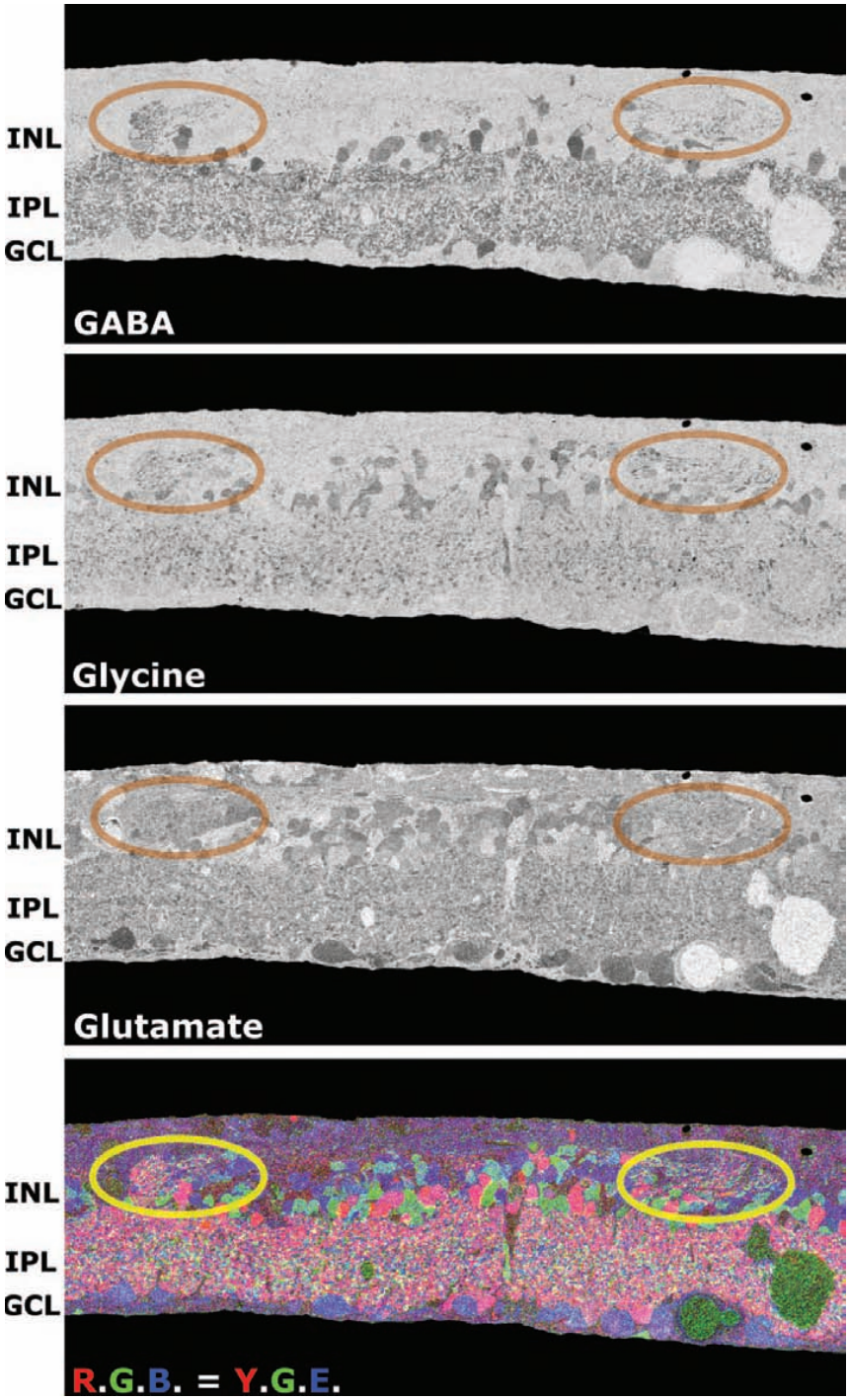

Figure 5. Quantitative GABA, glycine and glutamate immunoreactivity in greyscale with γ.G.E → rgb mapping in the bottom image showing microneuroma formation outside the normal lamination of the inner plexiform layer. A process from a glycinergic amacrine cell migrating into the ganglion cell layer can also be seen just to the right of centre along with large fluid channels forming in the lower right of each image.

time, it was not realised that retinal remodelling was a common theme of all retinal degenerations that result in complete deafferentation of the neural retina. Subsequent work in naturally occurring and genetically engineered models revealed extensive remodelling with alterations in the normal circuitry of the neural retina in response to photoreceptor degeneration.[6,25,61,71,73,74] As amacrine and ganglion cells become isolated from excitatory synaptic inputs due to bipolar cell dendritic retraction in phase 1 and loss of bipolar cell drive, they lose normal Ca^{++} current modulation essential for maintaining normal gene expression. Loss of normal inputs apparently drives neurons to sprout processes to seek lost glutamate drive and thus restore Ca^{++} signalling. Failure to achieve synaptic contact may result in either cell death or cellular somatic migration to other regions of the retina. All surviving cell classes in the retina are vulnerable to cell death, which is followed by MC hypertrophy that partially fills the vacated neuronal spaces (Figures 4B and 4C). Additionally, fluid channels begin to form, most likely arising from hydrostatic pressure induced by the MC seal between the neural retina and the vascular choroid. This seal is likely to prevent normal transretinal water movement,[75] resulting in the formation of fluid channels or cysts within the neural retina (asterisks in Figure 2A). These channels have been observed in a number of models including human RP. As the MCs begin to hypertrophy and migrate throughout the retina, distortions of the normal lamination of the inner and outer plexiform layers occur (Figures 2 and 4). Additionally, surviving retinal neurons are likely to use MCs as pathways for transretinal migration. Amacrine cells begin to migrate along these glial pathways, possibly via association with integrins[76,77] into the ganglion cell layer (Figure 4). Ganglion cells also migrate into the inner nuclear layer as noted above.

Additionally, microneuromas are formed. Microneuromas are tangles of processes from GABAergic amacrine cells, glycinergic amacrine cells, glutamatergic bipolar and ganglion cells (Figure 5), ranging from 20 to 100 μm in diameter and

their number exceeds 30,000/retina in some models (GHL, TG9N, RCS and most notably, hrhoG). Most of the microneuromas form in isolation but some merge with the remnant inner plexiform layer and all contain numerous synapses formed *de novo*. We fused CMP datasets and electron microscopy[38] (EM overlay) to map the participating classes of neurons as well as define the circuitry of microneuromas.[25] Conventional and ribbon synapses are present in microneuromas with conventional synaptic contacts (amacrine → amacrine, amacrine → bipolar, amacrine → ganglion cell, bipolar → amacrine, bipolar → ganglion cell) as well as clear, though infrequent instances of bipolar → bipolar contacts.[6,25] Microneuromas can also form at the terminations of large fascicles of mixed neurite processes coursing for more than 100 microns underneath the glial seal. These fascicles are composed of processes from surviving cohorts of bipolar cells, glycinergic amacrine cells, GABAergic amacrine cells, and ganglion cells that tend to co-segregate within the fascicles.[25] The extensive rewiring that occurs in the retina combined with massive cellular somatic migration within the retina has significant implications for retinal rescues. Specifically, corrupt visual circuitry and self-signalling will impact on the design of retinal implant and transplant approaches to rescue vision. Perhaps most troublesome are findings in phase 3, including gaps in the glial seal permitting RPE invasion of the retina, often in association with choroidal blood vessels. Because cellular movement and migration are bidirectional throughout the axis of the retina, neuronal escape or emigration from the neural retina becomes possible, and is evident at the light and electron microscopic level in AMD-like models.[61,62] These emigrant amacrine and bipolar cells appear to have normal signatures indicating a metabolically stable status. The abandoned neural retina is, for all intents and purposes, dead. Rescue at this point is impossible.

What drives neuronal rewiring? Rewiring in retinal degenerations is evident within microneuroma formation, where extensive new synaptic forms emerge. There is no evidence that suggests microneuromas recapitulate normal circuitry. Rather, all evidence indicates that synaptic connectivity within microneuromas is anomalous and corruptive of visual processing. We theorise that novel circuit formation results from self-signalling that emerges from pre-existing mechanisms in the inner retina, which would be consistent with an accompanying hypothesis that recovery of excitatory inputs is imperative to neuronal survival in retinal degenerative diseases. This self-signalling provides essential excitation for survivor neurons and rewiring, migration and microneuromas are possible mechanisms that remnant neurons exploit to enhance survival. Those neurons that are able to maintain or discover glutamatergic input from other neurons are able to maintain their normal metabolic homeostasis. In the absence of glutamatergic signalling, cells are unable to maintain Ca^{++} mediated metabolic processes and progress through cellular death pathways. It is worth noting that while anatomical surveys of the macula in human RP[3-5] reveal variable ganglion cell loss, from mild to severe, our surveys of human tissue and more than 200 examples of animal models of retinal degeneration reveal that well-preserved regions of the neural retina invariably possess surviving sensory retina harbouring cones, suggesting that cone loss and subsequent neuronal rewiring and loss are inversely related.

CONCLUSIONS AND IMPLICATIONS FOR FUTURE APPROACHES TO RESCUE VISION

While retinal remodelling is common to all retinal degenerations that deafferent the retina by removal of photoreceptors, a general consensus on the status of the neural retina has been missing in the literature. This is likely to be due to a number of factors: lack of availability of aged animals and, most importantly, the lack of appropriate tools with which to evaluate changes in the retina occult to traditional histologic techniques. Typically, most researchers have not allowed retinal degeneration to progress beyond simple photoreceptor degeneration. Remodelling in many rodent models, except the fastest/most aggressive models, does not occur until more than one year old. Although, analysis of animals older than two years have been rare in the literature, it should be noted that the rates of progression in animal models are likely to parallel those found in humans. Specifically, a mouse retina comprises only approximately one per cent of the area of a human retina, and assuming the same rate of progression in many disorders, beginning from a single disease focus, will involve the same percentage of total retinal size in the mouse model, as a human may experience in 20 years.

With respect to visualisation techniques, standard histologic nuclear stains can reveal only coarse information about cell location and number, while comprehensively mapping specific cell populations is much less common. The use of traditional measures of physiologic function, such as ERG, might also miss early subtle changes in wiring of individual populations of cells that might not be detectable within the large population of normal cellular responses. Yet those changes are the harbingers of corrupting cross-channel and reentrant networks that would be incompatible with normal retinal visual processing.[6,25,61,74]

We suggest that all insults resulting in loss of photoreceptors represent fundamental circuit deafferentations of the neural retina from the sensory retina; deafferentations that trigger retinal remodelling in a manner similar to CNS pathologic plasticities. These remodelling events include neuronal loss, growth of new neurites, formation of new synapses, and reorganisation of neuronal and glial somatic positions without dedifferentiation. The new circuitry formed by remodelling is unlikely to support normal retinal processing and we believe represents neurons attempting to find synaptic excitation through glutamatergic mechanisms that activate Ca^{++} permeation and support Ca^{++}-dependent regulatory processes. Even minor rewiring may corrupt signal processing in retinal pathways, rendering many

approaches to bionic and biological retinal rescue untenable. Thus, the sequelae of retinal degenerative disease are far more complex than previously believed and conflict with approaches to rescue vision via bionic implants or stem/engineered cells based on preservation of normal wiring and cell population survival/patterning after photoreceptor death. Retinal neurons die, migrate, create new circuitries and in the most extreme cases, even emigrate from the retina itself into the remnant choroid. While our work has clarified the need for strategies to rescue vision to be refined, all hope is not lost. The retina demonstrates plasticities that may be harnessed to facilitate rescue.

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