Amino Acid Signatures in the Detached Cat Retina

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Purpose. Expressions of certain macromolecules are altered by experimental retinal detachment in the cat. Related alterations in micromolecular signatures of neurons, Müller cells, and the retinal pigment epithelium (RPE) were investigated.

Methods. High-performance immunochemical mapping, image registration, and quantitative pattern recognition were combined to analyze the amino acid contents of virtually all retinal cell types after 3 to 84 days of detachment.

Results. Retinal micromolecular signatures showed a spectrum of alterations. The glutamate contents of Müller cells increased and remained elevated for weeks after detachment. Multispectral signatures of Müller cells showed massive metabolic instability in early detachment stages that ultimately resolved as a homogeneous profile significantly depleted in glutamine. Retinal pigment epithelial cell signals also changed dramatically, displaying an initial glutamate spike and then a prolonged decline, even as taurine levels followed an opposite pattern of initial loss and slow restoration. Neurotransmitter signatures of surviving neurons showed extensive precursor-level variation, and, in one case, GABAergic horizontal cells displayed anomalous sprouting.

Conclusions. Dramatic changes in Müller cell amino acid signatures triggered by retinal detachment are partially consistent with losses in glutamine synthetase activity. Taurine signal variations suggest that orthotopic RPE cells attempt to regulate abnormal taurine concentrations in the enlarged subretinal space. Surviving neurons possess characteristic neurotransmitter signals, but their metabolite regulation seems abnormal. On balance, microchemical and structural anomalies develop in the detached cat retina that represent serious barriers to recovery of normal visual function. (*Invest Ophthalmol Vis Sci.* 1998;39:1694-1702)

The cytologic and biochemical sequelae of experimental retinal detachment are diverse but stereotypical within cell groups. These effects partially involve glial migration and proliferation,[1] with concomitant alterations in overall morphology and expression patterns of macromolecules in Müller cells.[2,3] Among the many roles of Müller cells, their abilities to clear perisynaptic overflow of glutamate and γ-aminobutyric acid (GABA)[4] and to recycle those carbon skeletons are especially noteworthy, because glutamate-GABA clearance is presumed essential for maintenance of channel isolation in visual processing, glutamate clearance prevents excitotoxic glutamatergic activation of neurons causing cell death,[5] and glial carbon skeleton recycling is a key process in retinal metabolism.[6,7] Previously, we did not know whether Müller cells in detached retinas performed any of these normal acute processes. Losses in the expression of glutamine synthetase (GS) activity after detachment suggest that glutamate cycling capacity must be lost,[2] but alternative pathways are well known[8] and may suffice to sustain glutamate conversion.

The retinal pigment epithelium (RPE) is particularly vulnerable in detachment and may proliferate, migrate into mounds, or form complex strands of undetermined polarity that extend into the subretinal space,[9] all of which presumably compromises the topology and efficacy of RPE-photoreceptor interactions, even if proper apposition is restored. However, no critical assessment has yet been made of the status of the remaining orthotopic RPE cells. Is that transport layer functional? How might it respond to a large volume of anomalous diluent in the subretinal space?

Finally, although neurons do not proliferate in response to detachment, apparent losses in outer and inner nuclear layer elements occur,[10] and new data show substantial sprouting of horizontal cell and rod bipolar cell neurites into ectopic sites.[11] These observations suggest that more detailed examination of neurochemical status is appropriate, especially because it is unknown whether surviving neurons retain normal expression of metabolic signatures and form.

Our previous work[12] has characterized the normal amino acid signatures of most cell types in the cat retina, including those of Müller cells, RPE cells, and 12 classes of retinal neurons. By examining the aspartate, glutamate, glutamine, glycine, GABA, and taurine signals in identified cell types between 3 and 84 days after detachment, we have now documented a clear disruption in Müller cell glutamate metabolism, anomalous amino acid signals in the RPE that probably reflect responses to a changing micromolecular environment, and aberrant metabolism and growth of retinal interneurons.

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METHODS

Tissue Preparation and Immunocytochemistry

All animal care was in accordance with institutional animal care and use guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Analyses of detached retinas were performed on samples previously processed for ultrastructural studies and were verified to have sustained large detachments. Details of the surgical and detachment procedures have been published.[1,2,9] Cat retinas with large detachments of 3, 6, 13, 30, and 84 days' duration were processed for electron microscopic grade embedding in resin (Araldite 6005; Polysciences, Warrington, PA). Serial 200-nm sections were probed with IgG targeting glutaraldehyde-conjugate haptens at the following serum dilutions: γ-aminobutyric acid (γ), 1:32000; aspartate (D), 1:2000; glutamate (E), 1:64000; glycine (G), 1:2000; glutamine (Q), 1:16000; and taurine (τ), 1:64000. All IgG was produced by the laboratory of REM or obtained from Signature Immunologics (Salt Lake City, UT).

Primary IgG signals were detected with goat anti-rabbit IgG coated with 1-nm gold particles and visualized by silver intensification. These methods are detailed in Marc et al.[13] and Kalloniatis et al.[14]

Image Calibration, Signal Interpretation, and Image Registration

All image capture, digital montages, registrations, and classifications were performed exactly as detailed in our previous publications.[12-14] In particular, gray levels acquired for individual cell classes were converted to estimated intracellular concentrations as described by Marc et al.[13]

Image Visualization, Pattern Recognition, and Statistical Analysis

Image data were explored as aligned serial monochrome images, red-green-blue (rgb)-mapped images, and *N*-dimensional data sets resolved into statistically separable theme classes by pattern recognition.[13] Theme classes were used as masks to extract the patterns of amino acid content for individual cell populations over time. Only one retina was analyzed at each detachment time, because comprehensive signature analysis is extremely time-intensive. Nevertheless, any individual detached retina displays aberrations in glial, RPE, and other cellular signatures that may vary more than 3 standard deviations from those of a large cohort of normal cat and other mammalian retinas. Single samples cannot represent the temporal profile of any single detachment, but presumably reflect the range of possible responses over time. Individual measurements of theme-class amino acid content were calibrated as previously described[12] and represent samples of 10 to 100 individual cells, with a coefficient of variation less than 0.2.

RESULTS

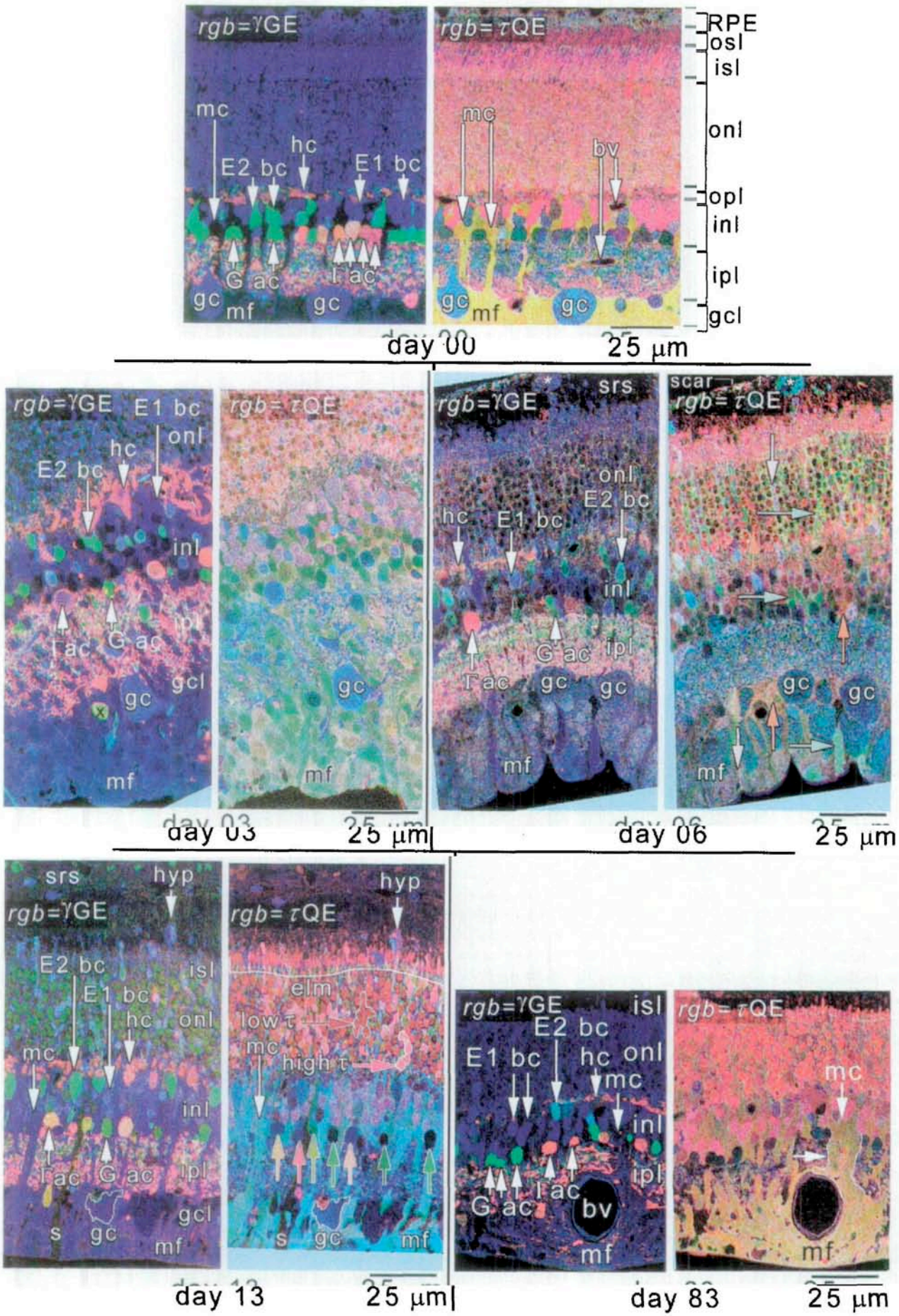
There were two obvious biochemical sequelae of retinal detachment: glutamate levels began to rise in Müller cells by day 3 of detachment and remained at concentrations far above those seen in normal glia for as long as 12 weeks (Fig. 1), and the amino acid signatures of the orthotopic RPE layer were altered by day 3 of detachment but slowly reverted to the normal RPE signature after approximately 4 weeks (Fig. 2). Detailed changes in amino acid contents were complex in Müller cells, and subtle anomalies emerged in the secondary signatures of the neuronal cohort.

Müller Cells

Normal Müller cells in all species display extremely low levels (<100 μM) of intracellular glutamate, GABA, and glycine, but high levels of glutamine (1-3 mM) and taurine (5-10 mM). This gave them a characteristic black signal in γGE → rgb mappings and a bright orange-yellow signal in τQE mappings (Fig. 1; day 0). A history of Müller cell biochemistry can thus be read from these mappings in detachments of varied durations. Quantitative glutamate, aspartate, glutamine, and taurine profiles provided a concerted view of biochemical changes (Fig. 3), derived by extracting *N*-dimensional theme classes and parsing a pure signal for each amino acid in each class.[13] At all detachment durations, the normally black γGE mapping of Müller cells was transformed into blue hues of varied intensities, providing direct evidence that Müller cells could no longer convert their endogenous glutamate load efficiently (Fig. 1; days 3-83). Glutamate content in Müller cells was quickly elevated and remained at supramillimolar levels never achieved in normal retina, with an apparent decline at prolonged detachment durations (Fig. 3). These primary effects were accompanied by secondary alterations in τQE signals and biochemical instability among individual Müller cells. Glutamine levels showed an apparent mild rise at short detachment durations but begin to decline by day 6, never to recover (Fig. 3). After a delay, taurine levels declined radically, and Müller cells took on a cyan-τQE mapping that was the biochemical inverse of the yellow-orange τQE signal in normal retinas. By 2 weeks of detachment, Müller cells had accumulated an inordinate amount of glutamate and had lost glutamine and much of their taurine. Taurine signals eventually recovered to normal or supranormal levels in detachments of long duration (Fig. 3), resulting in a Müller cell compartment characterized by modest levels of glutamate and glutamine and high taurine levels, with red-orange τQE mapping (Fig. 1; day 83). In the day 6 sample, τQE mapping uncovered a range of signal mixtures in neighboring foot pieces, somas, and distal processes of Müller cells, with some tinted lavender (red τ and blue E), others orange (red τ and weak green Q), and some green-cyan (green Q and blue E), none of which were attainable in normal Müller cells (Fig. 1). Structural responses to detachment such as hyperplasia of distal processes that can coalesce into glial scars appeared quickly (Fig. 1; days 6 and 13). Finally, even although Müller cell somas are often immediate neighbors in normal mammalian retinas, they never form confluent cartridges such as those observed in the detachments on days 13, 30, and 83 (indicated in Fig. 1, day 83), suggesting that proliferation of Müller cells triggered by detachment resolved into stands of sibling Müller cells.

Retinal Pigment Epithelium

Most RPE cells overlying detachments remained orthotopic and showed few cytologic alterations other than occasional pigment clumping. Because the RPE is the transport portal for gases, ions, and all organic species carried by the choroidal circulation, the acute insertion of a diluent into the subretinal space through trauma to the retina (tears) or experimental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injection of sodium hyaluronate represented a significant change in the composition of the medium bathing the apical RPE surface and reactive alterations in the amino acid signatures were expected. The normal RPE contained moderate glutamate, aspartate, glutamine, and taurine signals (Fig. 2). Although aspartate and glutamine signals are strongly correlated with glutamate content and metabolism in primate retinal neurons,[14] no evidence of key converting enzymes such as GS, aspartate aminotransferase, glutamate dehydrogenase, or phosphate-activated glutaminase has been reported in the RPE. Alternatively, taurine transport has long been known to be a hallmark of the RPE,[15] and a stable taurine signal representing the normal balance between taurine import and export may be a reasonable index of RPE status. At the earliest stages examined (Fig. 2), glutamate content spiked to supramillimolar levels, whereas aspartate content declined. At longer times, glutamate and glutamine signals seemed simply to decline. Taurine signals displayed a different profile, declining radically after a delay and then returning to normal or even supranormal levels. A particularly exotic taurine signal emerged at intermediate times (Fig. 2; days 6 and 13), when intracellular contents were extremely low but the adjacent extracellular matrix was enriched in taurine apparently trapped by fixation. Because elevated taurine and protein concentrations are required to generate a strong signal in postembedding immunocytochemistry, this pattern implied that taurine was fluxing into the subretinal space from some unknown source.

Neurons

The neuronal cohort of the cat retina was composed of photoreceptors with **E0** signatures; bipolar cells dominated by **E1** (rod bipolar and cone OFF center cells) and **E2** signatures (cone ON center cells); ganglion cells dominated by **E4** signatures; horizontal cells with a **Γ1** signature; two kinds each of γ^+ (**Γ2**, **Γ3**) and G^+ (**G1**, **G2**) amacrine cells and a dual **GΓ** amacrine cell. Regardless of the duration of the detachment and despite cell losses in outer and inner nuclear layers,[10] no selective loss of a class could be documented. **E1** and **E2** classes contain OFF and ON center cone bipolar cells, respectively,[14] and both can be found at every duration of detachment (Fig. 1), demonstrating that the prime mechanism of neuronal loss does not discriminate the type of glutamate receptor borne by each class.[16] Likewise, inhibitory neurons such as GABAergic horizontal cells, GABAergic amacrine cells, and glycinergic amacrine cells have persisted at every duration so far examined.

Metabolic instabilities that are likely to be associated with the death of photoreceptors were especially evident in τQE mappings. Normal photoreceptors contained supramillimolar levels of taurine, and τQE → rgb mapping in the outer nuclear layer yielded a homogeneous magenta field with dark red outer segments. As early as day 3 after detachment (Fig. 1), the outer nuclear layer was mottled in τQE mapping with patches of nuclei becoming extremely dark. Later (day 6), detached cells displayed even more dramatic aberrations and most of the photoreceptors displayed altered cytosolic signals and extremely dark nuclei. As the photoreceptor layer thinned, the remaining cells displayed chaotic signatures (day 13) with some patches showing τQE mapping similar to normal photoreceptors, some with detectable but depressed taurine signals and others with a polychrome array of hues. Detachments of long duration showed a restoration of the τQE signature of surviving photoreceptors and a fairly homogeneous outer nuclear layer (days 30 and 83). Even as taurine signals fluctuated dramatically in rods, glutamate signals declined and did not return to predetachment levels.

It was not possible to document the survival of all of the subtypes of γ^+ and G^+ amacrine cells because distinguishing secondary metabolic profiles were vastly altered by detachment and showed persistent declines in glutamate and glutamine as early as day 6. Some specific amacrine cell types may have died, but we could not track them with the methods used in the present study. The most obvious alteration in ganglion cells was a steady decline in glutamate content that, in parallel

Figure 1. Variations in γ-aminobutyric acid-glycine-glutamate (γGE) and taurine-glutamine-glutamate (τQE) signatures of cat retina after detachment. Day 0, γGE mapping: Normal cohorts of glutamate-rich photoreceptors (class **E0**), bipolar (class **E1**, **E2**) and ganglion cells, glycine-rich amacrine cells (class **G**), and γ-aminobutyric acid-rich amacrine cells (class Γ). Day 0, τQE mapping: Müller cells showed the normal orange-yellow signature characteristic of their glutamine-taurine-rich contents and negligible glutamate content. Day 3, γGE mapping: The basic signatures of all major neuronal classes were present, but the glutamate content of Müller cells was clearly elevated, giving them a blue hue. A misplaced glycine-rich cell is labeled ×. Day 3, τQE mapping: In addition to the elevation of glutamate content, the taurine signal of the Müller cells had declined radically, shifting their signature to a green-blue, glutamine-dominated state. Day 6, γGE mapping: All retinal signals were weakened, although the basic neuronal types were still evident. Photoreceptors in particular show massive loss of signals, and ganglion cells displayed no more glutamate than Müller cells. A large, glutamate-rich process, possibly derived from Müller cells is labeled *. Day 6, τQE mapping: Müller cells were shown to be extremely heterogeneous in amino acid content with neighboring cells appearing taurine-dominated (*orange upward arrows*), glutamine-dominated (*green horizontal arrows*), or glutamate-taurine-rich (*lavender downward arrows*). A layer of tangled Müller cell processes distal to the remaining photoreceptor inner segments is labeled scar. Day 13, γGE mapping: All major neuronal classes persisted, but ganglion cell signals were completely buried in the glial signature, indicated by the outlined ganglion cell gc. Müller cell GABA content was slightly elevated, giving them a purplish cast. Day 13, τQE mapping: Photoreceptor heterogeneity was revealed as patches of cells with low and high taurine signals. Amacrine cell somas virtually devoid of normal metabolite signals are indicated by *arrows* colored to match their corresponding γGE signals. Day 83, γGE mapping: The retina was substantially thinner, although the basal signals were similar to those in normal retina. Day 83, τQE mapping: Taurine signals were nearly normal, but the Müller cell compartment had clearly expanded, creating large stands of confluent cells. ac, amacrine cell; bc, bipolar cell; bv, blood vessel; elm, external limiting membrane; hc, horizontal cell; hyp, hyperplastic Müller cell process; inl, inner nuclear layer; ipl, inner plexiform layer; isl, photoreceptor inner segment layer; mc, Müller cell; mf, Müller cell end feet; onl, outer nuclear layer; opl, outer plexiform layer; osl, photoreceptor outer segment layer; rpe, retinal pigment epithelium; s, extracellular space; srs, subretinal space. All images were captured as density-scaled signals, registered, converted to intensity scaling, and viewed as red-green-blue triplets. Scale bar, 25 μm.

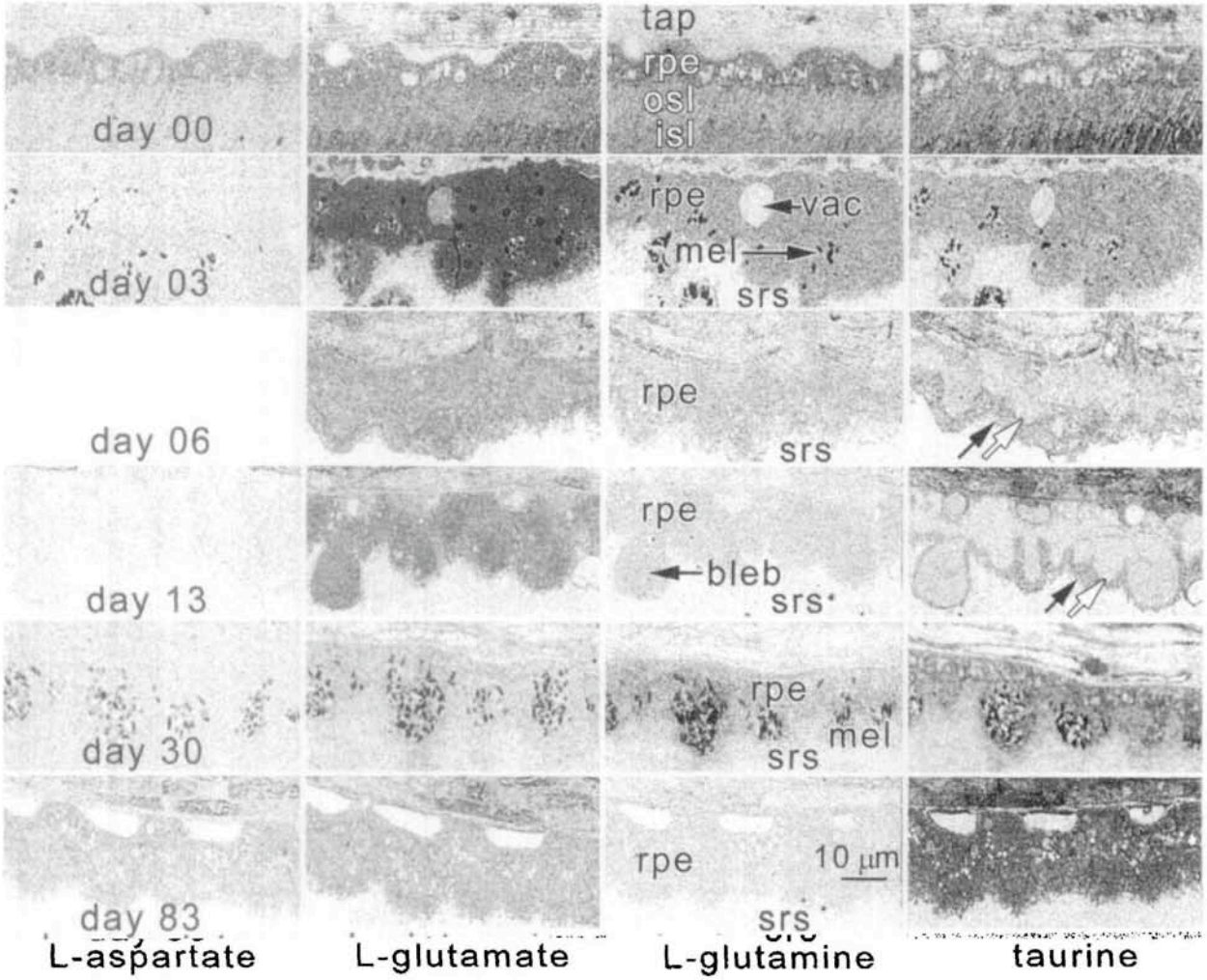

FIGURE 2. Alterations in amino acid signals of the retinal pigment epithelium after detachment, viewed as density-scaled images. Day 0: The retinal pigment epithelium possessed a distinctive signature with aspartate and glutamine contents higher than photoreceptor outer and inner segments, glutamate levels almost matching photoreceptor inner segments, and taurine signals slightly lower than photoreceptor inner segments. Day 3: Aspartate signals dropped below the detection limit, whereas glutamate signals spiked massively. Glutamine signals began to decline, whereas the taurine content held steady. Day 6: Glutamate, glutamine, and taurine levels continued to decline. Note the differential intracellular (*open arrow*) and extracellular (*black arrow*) taurine signals. The serial aspartate section was lost in this series. Day 13: A moderate glutamate signal persists, although intracellular aspartate, glutamine, and taurine levels remain low. Day 30: All amino acid levels were low except for taurine, which displayed substantial recovery. Day 83: Aspartate levels showed some recovery, glutamate and glutamine levels remained low, but taurine content recovered to supranormal levels. isl, photoreceptor inner segment layer; mel, melanin granules; osl, photoreceptor outer segment layer; rpe, retinal pigment epithelium; s, extracellular space; srs, subretinal space; tap, tapetum; vac, large vacuole (normal in the retinal pigment epithelium).

with the rise in glutamate levels in Müller cells, caused the submersion of the γGE signature of ganglion cells in the glial background by day 3 and persisted thereafter.

In one specimen (84 days of detachment), horizontal cells showed dramatic changes in form, with large-caliber neurites or stalks emerging from their normally smooth proximal faces and extending toward the inner plexiform layer (Fig. 4). Such aberrations imply that the normal retina suppressed neurite growth or that this particular detached retina possessed factors that promoted anomalous growth. Descending processes were not observed in horizontal cells from shorter duration detachments. There is little evidence of abnormal amacrine cell or ganglion cell neurite growth and, on balance, the density of γ^+ and G^+ structures in the inner plexiform layer was much lower than in normal retina, consistent with overall losses in neuronal numbers. In summary, neurons of all fundamental types persisted in all detachment durations, even though fluctuations in metabolite signatures were clear.

DISCUSSION

Müller Cells

All vertebrate classes so far examined display a common Müller cell signature.[14] Experimental retinal detachment evokes changes in the signatures of Müller cells, the RPE, and neurons that correspondingly imply alterations in macromolecule expressions and the composition of the extracellular environment. Lewis et al.[2,3] have detailed the apparent activation of genes coding for cytoskeletal elements and suppression of GS

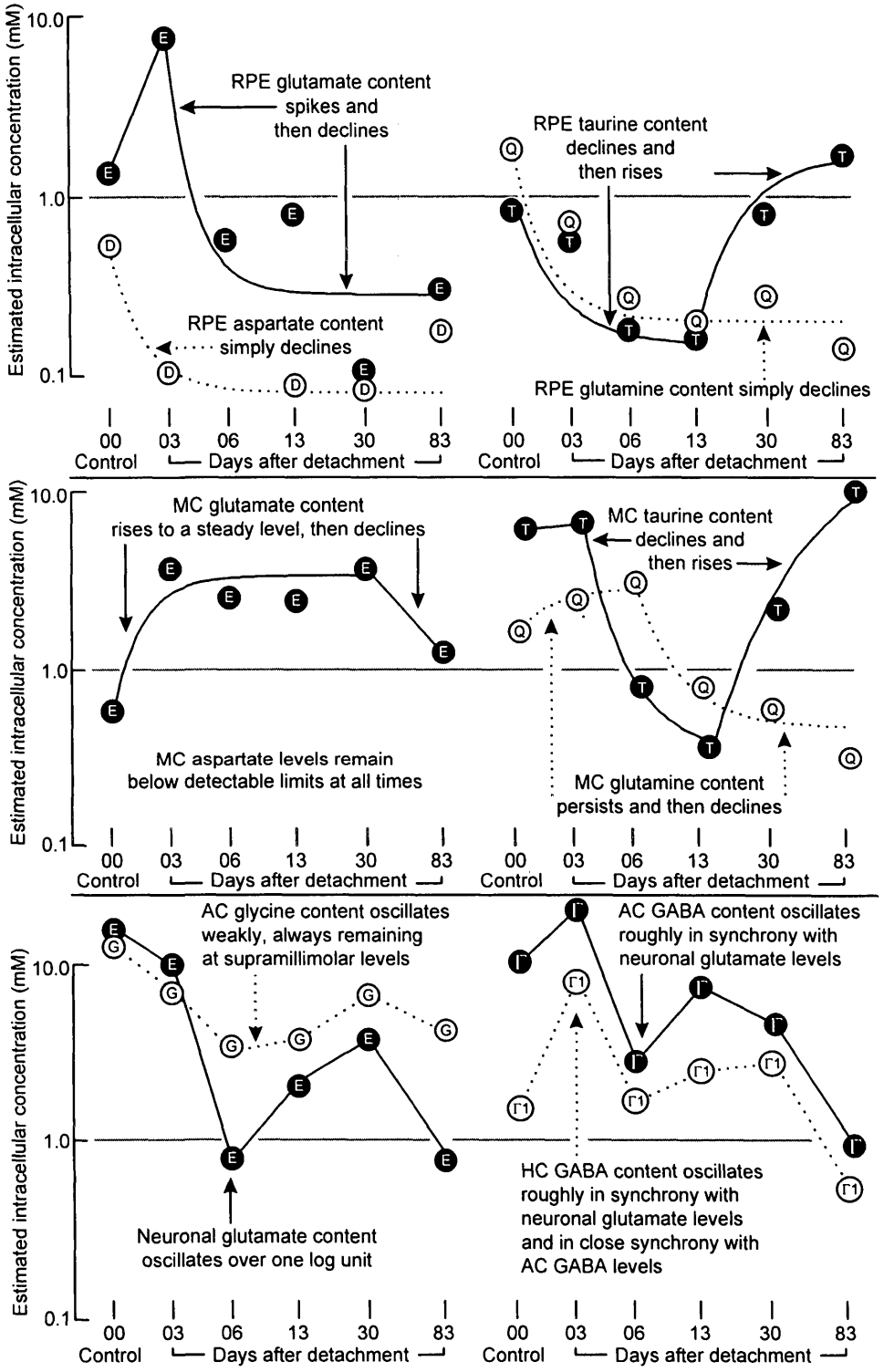

Figure 3. Temporal profiles of amino acid contents in the retinal pigment epithelium (RPE; *top*), Müller cells (*middle*), and neurons (*bottom*). Each symbol represents the mean gray levels of 10 to 100 cells of each class in each retina, converted to estimated intracellular concentrations.[13] The coefficient of variation for each mean was less than 0.2. The *y*-axis is logarithmic. Symbols are labeled according to the amino acid of interest: aspartate (D), glutamate (E), glycine (G), glutamine (Q), and taurine τ, or cell class (Γ1 for horizontal cells and Γ for amacrine cells). The smooth curves associated with each plot are either straight line segments or scaled single exponentials. Plots are split into left and right panels for visual ease, and 1.0 mM is marked as a continuous *horizontal line* parallel to the time axis. AC, amacrine cell; MC, Müller cell; GABA, γ-aminobutyric acid; HC, horizontal cell.

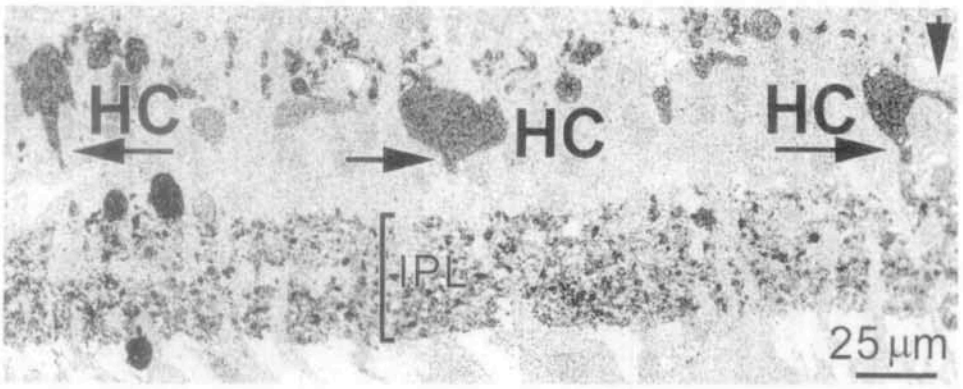

FIGURE 4. Anomalous horizontal cell morphology after 83 days of detachment visualized by GABA immunoreactivity. Three large horizontal cell somas show abnormal processes arising from their proximal surfaces (*horizontal arrows*). An additional process extending from the lateral surface of a horizontal cell shows a potential arc toward the inner retina (*downward arrow*). HC, horizontal cell; IPL, inner plexiform layer. Scale bar, 25 µm.

expression in Müller cells after retinal detachment. Because Müller cells are the sole compartment in which neuronally derived glutamic acid released by synaptic activity is converted to glutamine, loss of GS activity implies a reactive decline in neuronal glutamate synthesis. The amino acid signatures in the present study were largely consistent with this notion: the glutamate contents of Müller cells became elevated by day 3 of detachment, at which time retinal GS content assayed by immunocytochemistry and immunoblots had declined substantially.

Immediately after an acute detachment, extracellular glutamate levels were likely to rise because of photoreceptor rupture and en masse mechanical stimulation of photoreceptor and bipolar cells. Retinal pigment epithelial cells are known to possess some form of glutamate transport[17] and the strong glutamate signal therein at day 3 is evidence that glutamate levels in the subretinal lumen were elevated. The apical processes of Müller cells form the proximal border of the detachment lumen, and concordant increases in Müller cell glutamate similarly argue that glial glutamate transport is nominally intact. The enhanced glial glutamate signal could then be because of an acute overload of GS capacity or rapidly emerging deficiencies in GS expression. However, the decline in GS content is rapid,[3] and Müller cell glutamate levels remained elevated after the glutamate signals in the RPE declined (Fig. 3). This suggests that the extracellular glutamate load is not sustained and that a selective defect in glial capacity to metabolize glutamate underlies the persistent Müller cell glutamate signal. The normal mammalian retina is unable to release sufficient glutamate to overload GS capacity, even with excitotoxic depolarization,[18] and the rise in glial glutamate content after detachment was most simply attributable to declines in GS expression. Müller cell glutamate levels remained elevated even though glutamine levels slowly declined. However, if GS is the sole mechanism for the conversion of glutamate skeletons,[19] why did glial glutamate levels not continue to rise and why did neuronal glutamate signals not disappear altogether? One possibility is that a subthreshold level of GS persisted, but that would not explain why glutamine levels in Müller cells continued to decline in the presence of a steady glutamate load. Although neuronal glutamate levels declined gradually, they were not negligible, and photoreceptors and bipolar cells continued to display some glutamate content. Glial cells probably express several other possible glutamate conversion paths, and the functional "uncovering" of these pathways and possibly others is probable for an additional reason. Glial conversion of GABA to succinate semialdehyde by GABA-transaminase (GABA-T) depends on amino group transfer from GABA to α-ketoglutaric acid, producing even more glutamate. Because glial GABA levels do not rise much if at all, sufficient α-ketoglutaric acid must be present, and any additional glutamate must be rapidly shuttled back into the α-ketoglutaric acid pool. This is strong evidence of an alternative path, because glial GABA-transaminase capacity is extremely susceptible to overloading.[18] Provided glial GABA transport remains intact, significant conversion paths for glutamate other than GS must exist in cat. This is inconsistent with the view that glutamate synthesis in certain retinal neurons is obligatorily derived from glial glutamine pools, as inferred from disruption of rabbit retinal glutamate metabolism after methionine sulfoximine treatment to inhibit GS activity.[19] Methionine sulfoximine is not GS-selective, however. It powerfully inhibits γ-glutamylcysteine synthetase, thus blocking essential glutathione metabolism in all cells.[20] Thus, the inability of neurons to recover substrates from Müller cells is not simply attributable to GS suppression. The presence of multiple glutamate conversion paths must be considered in any model of altered Müller cell metabolism.[21] Whether the reduced provisioning of neurons by the altered Müller cells of detached retinas has consequences for recovery of normal neurotransmission cannot be resolved presently.

The data from 6 days after detachment show that single Müller cells may also become extremely unstable in their expressions of amino acid signals, with immediate neighbors displaying vast differences in their πQE mappings (Fig. 1). Such variations could not plausibly arise from microenvironmental variations. More probably, detachment ushers Müller cells into a chaotic regimen of gene expression that resolves as an anomalous homogeneous state, which in turn implies that gene expression patterns of normal Müller cells are actively coordinated. Normal Müller cells may form homologous gap junctions but seem to be poorly coupled, when judged by dye-coupling studies.[22] Thus the mechanism of normal glial coordination remains unknown.

Retinal Pigment Epithelium

After detachment, amino acid signals in the orthotopic cat RPE varied in patterns that suggest the RPE cells sense and perhaps attempt to regulate the composition of the subretinal space. The glutamate spike in the RPE cells occurred at the same time that the aspartate signal declined and cannot be attributed to a general increase in RPE amino acid synthesis. Although the origin and fate of aspartate in the RPE is currently inexplicable, glutamate transport by RPE cells has been reported by Miyamoto and Del Monte[17] and a major increase in RPE cytosolic glutamate levels is a plausible direct response to elevated glutamate levels in the subretinal space. Glutamate signals declined to low levels thereafter, suggesting that the subretinal space was eventually depleted of excess glutamate, perhaps by the dual action of Müller cell and RPE glutamate transport.

Taurine is an essential dietary amine in cats, and taurine deficiency in cats causes retinal degeneration,[23] perhaps because taurine is a major osmolyte for many retinal cells, as it is in other tissues.[24,25] Taurine is imported by the RPE at its apical face,[15,26] but must also efflux through some path, perhaps through taurine-permeant basolateral anion channels. Similar to glutamate, there was a rise in taurine in the RPE just after detachment, followed by a long decline and then a surprising recovery to apparently normal levels. Detailed examination of light microscopic images at days 6 and 13 after detachment shows substantial taurine immunoreactivity surrounding the apical surfaces of the RPE, apparently in the extracellular space. Although this observation requires electron microscopic verification, it suggests that some intraretinal source of taurine slowly mediated the flux of taurine into the subretinal space and that an amine-rich matrix to which the taurine can be fixed was closely associated with the RPE. The recovery of RPE taurine levels indicates that taurine levels became equilibrated in the subretinal space. The amino acid contents of the orthotopic RPE after detachment may not exclusively reflect responses to the composition of the subretinal fluid. As in Müller cells, alterations in the expressions of enzymes and transporters could also affect signature changes: For example, Campochiaro et al.[28] have described the emergence of apparently anomalous platelet-derived growth factor receptor immunoreactivity in the orthotopic, detached rabbit RPE. Significantly, the signatures of immediate neighbors in the orthotopic RPE have identical signals, even when Müller cells show extensive intercellular variability in the same tissue. Mammalian RPE cells are well coupled by gap junctions,[29,30] which can presumably coordinate RPE metabolism and transport.

Neurons

All the basic neurotransmitter signature types seemed to survive detachment. Neuronal losses occurred, but no class selectivity was detectable. The spike in glutamate levels observed at day 3 in the RPE was probably a remnant of a massive glutamate efflux event arising from detachment, possibly accompanied by significant glutamate excitotoxicity. But are extracellular glutamate levels still elevated at day 3 and beyond? We cannot measure this directly, but such a persistent efflux event should also have another characteristic sign: the elevation of GABA signals in Müller cells caused by swamping of GABA-transaminase by excessive extracellular GABA levels.[18] Because Müller cell GABA levels at day 3 were not significantly elevated, any hypoxic and excitotoxic event must have long since subsided. Thus, subsequent cell losses in the inner nuclear layer must have been caused by mechanisms other than persistent excitotoxicity. At the same time, there is no question that the metabolic signatures of neurons changed radically, especially in their content of glutamine. This makes the preservation of the residual glutamate and GABA signals in various cells all the more mysterious, because it suggests that neuronal glutamate production persisted despite the apparent failure of Müller cells to supply adequate glutamine for glutamate synthesis through phosphate-activated glutaminase. Some neurons are capable of producing glutamate from lactate,[6,8] and lactate production and export by Müller cells may support neuronal needs.[6,7]

All is not well, even for surviving neurons. Lewis et al.[11] have reported hyperplastic horizontal cell and rod bipolar cell dendrites in the outer plexiform layer of the detached cat retina. We found no consistent evidence of anomalous neurite extension from the inner plexiform layer to the optic fiber layer during the period of the present study. However, in one retina (an 84-day detachment) GABAergic horizontal cells possessed large processes arising from their proximal surfaces coursing toward the inner retina, which is abnormal for the mammalian retina. Peichl and Bolz[31] were able to induce aberrant proximal neurite extension from cat horizontal cells after intravitreal injections of low doses of kainic acid, and Chu et al.[32] showed that horizontal cells in the RCS rat possessed anomalous neurite patterns. However, descending horizontal cell processes are not a consistent consequence in detachment. Lewis et al.[11] found no evidence of descending processes from cat horizontal cells after detachments of 28 days' duration. It is possible that the case reported in the present study was atypical or a consequence of longer detachment. Such a response, however rare, bodes ill for the restoration of normal function after prolonged detachment. Normal signal processing could be seriously corrupted to the extent that such processes were capable of functional synaptic connectivity in an ectopic target.

Interpretations, Implications, and Future Directions

There is far more to know about the micromolecular and macromolecular profiles of neurons, glia, and the RPE after pathophysiological insults, but the data presented here are consistent with the view that detachment challenges all retinal cells to some extent. If signaling between the RPE and Müller cells is required to maintain Müller cells, a large fluid void could represent simple dilution of that signaling. The stability of individual Müller cells after detachment can now be explored by detailed examination of the boundaries between detached and normal retina. The evaluation of reattached retinas will also determine the extent to which normal signature restoration correlates with structural status. Finally, the ultimate question is whether neurons in the detached region function normally. The existence of horizontal cell and rod bipolar cell neurite alterations suggests not.

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