

## CHAPTER 40

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# EFFECTS OF RETINAL DETACHMENT ON S AND M CONE FUNCTION IN AN ANIMAL MODEL

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### Introduction

Detachment can physically damage the retina and disrupt the normal metabolic traffic between photoreceptors and the choroidal blood supply. These changes initiate a series of cellular and molecular events in the retina that can lead to profound retinal remodeling (Fisher and Anderson 2001). Among the many functional changes that may ensue are alterations in color vision. Nearly a century ago Köllner reported that blue-yellow defects are characteristic of patients with retinal detachment (Köllner 1907). Although subsequent research has revealed a more complex picture of color vision change than he described, the generalization that retinal detachment often leads to blue-yellow color defects remains intact (Pokorny *et al.* 1979; Fletcher and Voke 1985). Recent investigators have sought indications of detachment-induced changes in the retina that might correlate with the onset of blue-yellow color vision defects. Two such effects have been reported. First, the relative contributions from S and M/L cones of patients who had undergone reattachment surgery were assessed through measurement of the amplitudes of different waveform components of the electroretinogram (ERG). A lowered ratio of signals from S versus M/L cones was inferred suggesting that S cones were relatively more vulnerable to the damaging effects of detachment (Hayashi and Yamamoto 2001). Second, histochemical staining of human retinas that had suffered recent detachment indicated that S cones were almost entirely lost from the detachment zone while M/L cones were still abundantly present (Nork *et al.* 1995). Both of these outcomes imply that blue-yellow color defects following retinal detachment may result from alterations in the photoreceptors themselves and that, specifically, S cones are more susceptible to change under these conditions than M/L cones.

There are obvious limitations imposed on attempts to understand the biological basis of change induced by retinal detachment in clinical patients. Consequently, researchers have often turned to animal models in which the details of detachment can be controlled,

their consequences can be more directly studied, and various therapeutic interventions can be evaluated. We have recently been examining the dynamics of retinal detachment and reattachment in ground squirrels. These rodents have cone-rich retinas and therefore offer an especially favorable context for studies of the influence of retinal detachment on contributions from S and M/L cone populations.

## Methods

### Subjects

Adult California ground squirrels (*Spermophilus beecheyi*) of both sexes were used. An abundance of information about retinal organization and vision in this species has been accumulated. The features that make this animal an attractive target for studies of cone-based vision are summarized elsewhere (Jacobs *et al.* 2002). For present purposes the most important of these is that the retina of the California ground squirrel contains two classes of cone totaling about 7.5 million receptors with M cones ( $\lambda_{\text{max}} = 518 \text{ nm}$ ) outnumbering S cones ( $\lambda_{\text{max}} = 436 \text{ nm}$ ) by an overall ratio of about 14: 1.

### Apparatus and procedures

ERGs were differentially recorded from contact lens electrodes installed on the eyes of ground squirrels anesthetized with a mixture of xylazine (9 mg/kg) and ketamine (70 mg/kg). Through a dilated pupil the animal viewed the screen of a computer-controlled color monitor (Radius, Intellicolor) positioned to subtend a rectangular area of 116 x 101 deg. The stimulus was a spatially uniform field, temporally modulated as a 37.5 Hz square wave (mean luminance = 50 cd/m<sup>2</sup>). The control software was written in Matlab using extensions as described earlier (Brainard *et al.* 1999).

Stimuli modulating the contrast seen by either the S or the M cones were designed based on estimates of the spectral absorption properties of the ground squirrel cones as modified by measurements of absorption by the lens of this species (Jacobs *et al.* 2001). The maximum contrasts were 63.1 per cent for M cone stimulation and 76 per cent for S cone stimulation. The procedures for stimulus calibration and the recording apparatus have been described in detail (Jacobs *et al.* 1996; Brainard *et al.* 1999). In brief, analog hardware was used to window the amplified ERG signal with a sinusoid set to the frequency of the stimulus train (37.5 Hz). For each stimulus sequence the position of the window was shifted to maximize its correlation with the ERG signal. The window positions could then be used to extract information about the relative phases of the cone signals. Responses were averaged over the last 50 of a total of 70 stimulus sequences and these amplitudes were recorded from a computer display. For S and for M cone modulation, the contrast level was varied from the maximum available downward in nine or ten steps to levels as low as 0.7 per cent. Five complete stimulus sequences were recorded at each contrast level and these values were averaged.

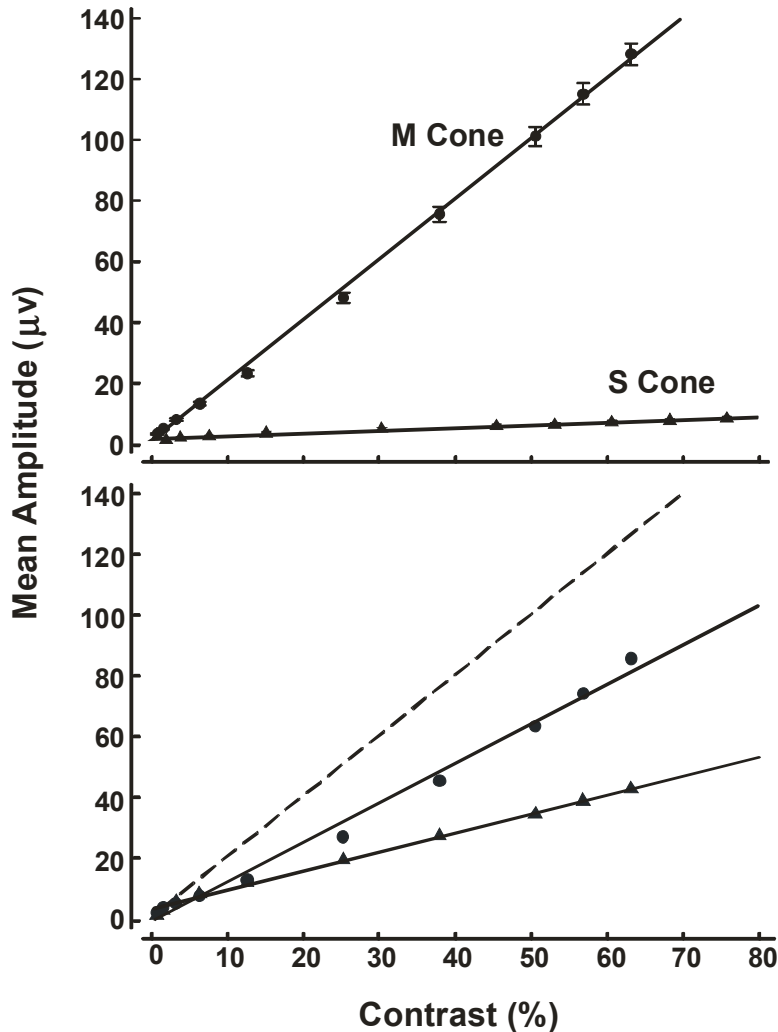
ERG contrast-response functions were obtained from normal animals, from animals 24 hours following experimental retinal detachment, and at various time points following

retinal reattachment. Retinal detachments were created by infusing sodium hyaluronate (0.25 per cent) in a balanced salt solution from a glass micropipette inserted through the sclera at the region of the pars plana to a site between the neural retina and the pigment epithelium (Anderson *et al.* 1986). For a subset of animals, the retinas were subsequently reattached by injecting a 0.2 ml mixture of sulphur hexafluoride (20 per cent) and room air (80 per cent) following paracentesis to control intraocular pressure. At the end of the experiment animals were killed with an overdose of pentobarbitone, the retina was removed and flattened, and the size and position of the detached region was measured. From maps of the density distributions of S and M cones in the ground squirrel retina (Kryger *et al.* 1998) the number of receptors of each type that lay in the zone encompassed by the detachment could be determined.

## Results

As documented elsewhere, ground squirrels give large and reliable ERG responses to fast flicker (Jacobs *et al.* 2002). Over the contrast range examined, ERG amplitude increases linearly with increasing contrast. Figure 40.1 (top) illustrates this relationship for both M and S cone isolation. Note that intersubject variability is relatively small. By convention, the slope of the fitted line defines the contrast gain for each mechanism (Kremers *et al.* 1999). For data from a large sample ( $N = 41$ ) of ground squirrels the mean ratio of M and S contrast gains was 21.4 (SD = 8.0). The retinas were subsequently detached in a number of these animals and then, 24-hours following surgery, a second set of ERG measurements were made. As illustrated by the M-cone contrast/response functions shown for two animals in Fig. 40.1 (bottom), detachment reduces the amplitude of the ERG. These functions are also well captured by linear regressions so that the magnitude of the loss engendered by detachment is reflected in a lowered contrast gain relative to the normal. The results obtained for S-cone isolation were similar in nature. For a subset sample of 22 ground squirrels, the mean M/S contrast gain ratio prior to detachment was 25.79 (SD = 8.62), while following detachment the corresponding value was 18.66 (SD = 12.55). These values are reliably different (paired samples  $t = 2.359$ ; d.f. = 21;  $p < 0.03$ ). Note that for this comparison the detachment appears to have had a proportionally larger impact on M-cone than on S-cone signals.

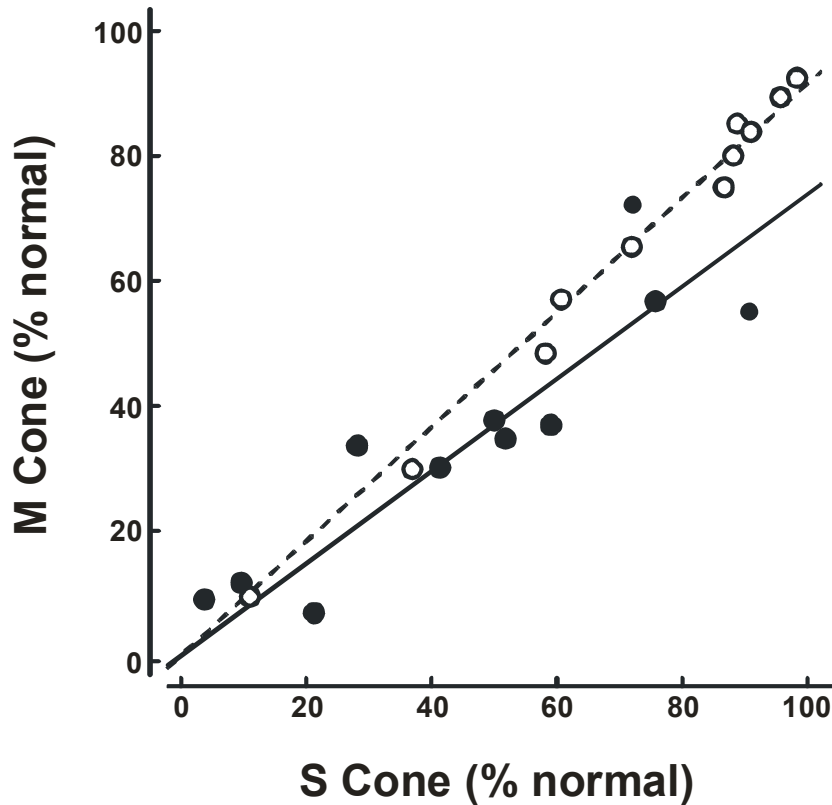
The lower M/S contrast gain ratio detected by this comparison could have a number of interpretations. For one thing, detachments can obviously vary from animal to animal. The retinal distributions of different cone types are heterogeneous so, for example, detachments of equal size but varying locations can potentially have unequal impacts on the different cone types. Consequently, a better evaluation of the differential effects of detachment on cone function requires an accurate mapping of the detached area and a determination of the cone populations in this region. To accomplish this, we examined 11 animals that were tested prior to surgery and then again 24 hours following detachment. In this experiment an attempt was made to systematically vary the size of the detached region. Following the procedures described above, an indication of the



**Figure 40.1** Top: Contrast response functions for ERG signals obtained from modulation of S and M cones in the ground squirrel. The plotted points are mean values obtained for S cone isolation ( $N = 25$ ) and M cone isolation ( $N = 35$ ). Error bars show  $\pm 1$  SEM. Bottom: Contrast response functions obtained for M cone isolating stimuli in normal animals (dashed line is the average taken from the top panel) and two animals whose retinas had been detached (continuous lines).

number of S and M cones that lay in the zone of the detachment was retrieved from anatomical examination of a flat mount of the retina. From this information we could make comparisons between the proportions of receptors of each type in the detachment zone and the ERG results.

Figure 40.2 summarizes the relationships that emerge from this experiment. The open circles indicate for each animal the numbers of both types of cone (expressed as a percentage of the total in the normal retina) located outside of the detachment zone. It can be seen that the detachments covered almost the full span of potential sizes including both very small and very large portions of the retina. The dashed line is a linear regression providing the best fit to these size measurements. The excellent fit of this line ( $r^2 = 0.987$ ) shows that detachments varying greatly in size always include the same proportion of S and M cones, but since the slope of this line is less than 1, the proportion of receptors in the detached region is always somewhat smaller for S than for M cones. The second set of data shown in Fig. 40.2 (solid circles) is the contrast gains for M and S cone stimulation,



**Figure 40.2** Effects of retinal detachment on receptor and contrast gain loss in 11 ground squirrels. The open circles plot the proportion of the total cone population for both S and M cones that fell outside of the detachment zone. The dashed line is the best fitting linear regression (slope = 0.916,  $r^2 = 0.987$ ). The solid circles show the contrast gains measured for both S and M cone stimulation, each plotted as a percentage of its pre-detachment value. The best fitting linear regression (continuous line) has a slope of 0.739 and an  $r^2 = 0.801$ . See text for further discussion.

again specified as a percentage of their pre-detachment values. Across the sample there is a large variation in the size of the reduction in contrast gain produced by detachment. These data too are well fit with a linear regression ( $r^2 = 0.801$ ) indicating that the loss of contrast gain following detachment is directly proportional to the magnitude of the detachment. If the loss in contrast gain engendered by detachment were the same as the relationship between the proportions of S and M cones affected, the slopes for the two functions should be the same; alternatively, a proportionally greater effect on S than on M function would predict a steeper slope. In fact, although the differences are not statistically reliable ( $t = 1.329$ ; d.f. = 18; ns), what is observed is a shift in the opposite direction, that is, the slope for contrast gain is shallower than that predicted based on a greater vulnerability of S cones.

ERGs were also recorded from animals following reattachment of the retina. Reattachment leads to at least a partial recovery of the ERG response in all animals, the details of which are currently under study. Of interest here is whether the recovery triggered by reattachment is different for signals originating from the two classes of cone. To examine this, eight animals were tested prior to detachment and then at 24 hours following detachment. Just after this second recording session the retina was reattached and the recordings were repeated an additional three times at test intervals spanning the range from 7-42 days. As noted above, detachment causes a drop in the M/S contrast/gain ratio. Following reattachment, however, we detected no further change in the contrast/gain

ratio even though the actual contrast gain values for both S and M cone activation improved by a factor of about 1.5-2 over the examination period.

### Discussion

ERGs recorded from the cone-rich retina of the ground squirrel can be used to accurately assess the consequences of retinal detachment while the use of cone-isolating stimuli permits examination of signals originating from independent activation of the S and M cone classes. This study was motivated by the classic result that retinal detachment in human patients often leads to blue-yellow color vision defects and by recent observations suggesting that these color vision changes may be the direct result of a greater susceptibility of S cones to the effects of detachment. Our experiments show quite plainly that retinal detachment in the ground squirrel model does not have a larger impact on S cone than on M cone function as indexed by signals originating in the outer retina. To the contrary, to the extent there is any differential effect of detachment on function subserved by the two cone types the results here suggest the loss may be slightly in the opposite direction, that is, a proportionally greater effect of detachment on M than on S cone function.

The results reported here appear contradictory to the claim that there is a selective loss of S cones in retinal detachment, but there are obvious differences in the nature and duration of detachment in that study (Nork *et al.* 1995) and the present one and, of course, they involve different species and different indicators for inferring cone function. The earlier study involved examination of ten human eyes taken at 2.5-11 days following traumatic detachments. Our choice of a relatively shorter detachment time (24 hours) was predicated on the observation that photoreceptor degeneration in the ground squirrel is quite rapid following detachment far faster, for instance, than that seen in the cat, a standard model for studying retinal detachment (Linberg *et al.* 2000). Nork *et al.* (1995) used histochemical and immunocytochemical staining in conjunction with light microscopic observations to assess photoreceptor changes. Carbonic anhydrase (CA) staining served as a principal marker to separately assess the presence of S and M/L cones. In normal retinas M/L cones stain positively for CA while S cones do not stain. In the detached human retinas, unstained cones showed a virtually total dropout and they were completely absent from two retinas that were detached and subsequently reattached (Nork *et al.* 1995). Although that result seems clear cut, there are several reasons to suggest that it may not represent the final word. First, another study of human retinal detachment that utilized a range of different cellular markers found that at least some S cones in fact survive even long-term detachments (Charteris *et al.* 2000). Second, some M/L cones are observed to become CA negative following injury (Nork *et al.* 1995) which suggests the need for caution in using CA labeling as a sole indicator of cone identity. Finally, although ground squirrels are clearly not humans, our results are apparently not just species specific since a study of the rod-dominated cat retina has also failed to show any differential impact of detachment on the subsequent relative numbers of S and M cones (Rex *et al.*, 2001).

Our results are more directly contradictory to the earlier ERG study done on human patients (Hayashi and Yamamoto 2001). While there is no obvious resolution of the two studies, it can be noted that, unlike the present experiment, the human study provided no compelling way of isolating contributions from the different cone classes to the recorded ERG.

If, as our results might suggest, S cones are not inherently more vulnerable to damage associated with retinal detachment, then what is the basis for the observation that blue-yellow color vision defects are frequently documented in cases of retinal detachment? An earlier research summary shows there are in fact quite a number of potential mechanisms that might explain why S cone related visual losses are common in cases of retinal damage (Mollon 1982). These include factors associated with differences in the relative numbers of S and M/L cones, the distinctive pathways through which these different cone classes transmit information into the central visual system, the possibility that diagnoses of blue/yellow defects from standard color vision tests are prone to artifact, as well as the prospect that S cones are inherently more vulnerable to damage. Our results would argue against this last possibility. Paradoxically, in this experiment outer retinal signals from the M cones actually appear somewhat more susceptible to loss than were corresponding signals from S cones and one should note that their preferential loss would also alter the nature of blue/yellow spectral opponency and this too could lead to the appearance of a blue/yellow color vision defect.

## Acknowledgements

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