



An animal model for studying cone function in retinal detachment

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Abstract. In people, retinal detachment often leads to a significant loss in cone-based vision. Most of the animal models commonly used for studying the consequences of retinal detachment have rod-dominated retinas. The purpose of this investigation was to evaluate the possibility that the ground squirrel, a rodent with a heavily cone-dominated retina, might provide a useful model for studying cone function in retinal detachment. Corneal ERGs were recorded from ground squirrels for large-field temporal modulations presented on a computer-controlled color monitor. Modulations were chosen to selectively stimulate either of the two classes of cone found in the ground squirrel retina. Under these test conditions, large and reliable cone ERGs could be readily recorded. In animals in which the retina had been surgically detached, the loss of cone signal was directly related to the number of cones in the detachment zone relative to the total cone population and that relationship did not differ for short-wavelength sensitive (S) and middle-wavelength sensitive (M) cones. Surgical reattachment produced a progressive recovery of cone-based signals. The ground squirrel seems likely to provide a useful animal model for studying the dynamics of cone function in retinal detachment and subsequent events.

Key words: cone, cone-isolating stimuli, electroretinogram, ground squirrels, retinal detachment

Introduction

Retinal detachment initiates a chain of molecular and cellular events in the retina that culminates in loss of visual capacity, often including decreases in visual acuity, visual threshold elevations and alterations in color vision. It is an unfortunate fact that impaired vision often persists even after successful reattachment surgery. There has been considerable effort dedicated to understanding the details of structural and functional changes following retinal detachment, frequently pursued with a view toward proposing and evaluating a variety of therapeutic interventions that might ameliorate visual loss [1–4]. Laboratory studies of retinal detachment typically involve the use of an animal model. Monkeys have been used on occasion, but most often the models

are derived from studies carried out on rabbits and cats. These species are nocturnal or crepuscular, and in accord with such lifestyles each has a retina that is rich with rods but only relatively sparsely populated with cones. These models provide some interpretative limitations since many of the pressing practical consequences of retinal detachment in humans impact cones and their contributions to photopic vision. It would, thus, seem undeniably useful to have a good animal model for examining various aspects of cone-based vision. We propose that the ground squirrel might provide such a model and here report results of observations that led us to that conclusion.

The California ground squirrel (*Spermophilus beecheyi*), like other sciurids, is a strongly diurnal animal. Over the years much has been learned about the retina, the visual system, and the visual capacities of these rodents [5–11]. In brief, the retina of the ground squirrel is heavily cone dominated (about 85% of the photoreceptors are cones). Each retina contains a total of about 7.5 million cones, roughly one and a half times the cone complement of a human retina. As in many other mammals, the ground squirrel has two classes of cone, one containing a photopigment with maximum sensitivity in the short wavelengths (S cones with $\lambda_{\text{max}} \approx 436$ nm) and the other with maximum sensitivity at about 518 nm (M cones)[8]. The packing density of M cones reaches a value of about 45 000/mm² along a horizontal strip of retina located just ventral to the elongated optic nerve head [11]. S cones achieve a high density of about 4500/mm² at the temporal end of the visual streak and there is a small secondary region of very high density (ca. 20 000/mm²) at the dorsal-nasal retinal margin. M cones outnumber S cones overall by about 14:1. These two cone types provide spectral information that supports well-defined dichromatic color vision [12]. The cone receptors, in conjunction with the retinal wiring afforded by a large population of ganglion cells, provide the basis for spatial acuity that is good for a rodent (at the limit, about 4 cycles/deg) and this arrangement also allows particularly high temporal sensitivity [6]. Of importance for the present concern, robust electrical signals can be recorded from the eye of the ground squirrel and at various locations in the visual system under stringent photopic test conditions. With these features in mind, we undertook an examination of the behavior of a retinal gross potential, the electroretinogram (ERG), in intact ground squirrels and in animals in which the retina had been surgically detached.

Methods

Subjects

Adult California ground squirrels (*Spermophilus beecheyi*) of both sexes were studied. All animal care and experimental procedures were in accordance with institutional animal care and use guidelines and the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

ERG apparatus and procedures

Squirrels were anesthetized with a mixture of xylazine (7 mg/kg) and ketamine (70 mg/kg) with supplements given as necessary during recording sessions that lasted approximately 45 min. The pupil of one eye was dilated by topical application of atropine sulfate (0.04%) and phenylephedine hydrochloride (10%). The animal was positioned for recording with the use of a head restraint. ERGs were differentially recorded from a contact lens electrode.

Stimuli were presented on a computer-controlled color monitor (Apple PowerMac 6100; Radius Paintboard Turbo graphics card, 9-bit DAC; Radius, Intellicolor 20-in monitor, model 0461) positioned perpendicular to the optic axis such that the stimulus display subtended a rectangular area of 116×101 deg of visual angle. The stimulus was a spatially uniform field modulated in time with a mean luminance level of 50 cd/m^2 . The monitor refresh rate was 75 Hz. The control software was written in Matlab (The Mathworks, Natick, MA), using the extensions provided by the high-level Psychophysics Toolbox [13] and the low-level Video toolbox [14].

Retinal detachment and reattachment has been shown to differentially impact S and M/L cone function in humans [15–17]. It seemed important, thus, to be able to assess independently the signals generated by ground squirrel S and M cone classes. To accomplish this we used square-wave stimuli that temporally modulated (at a rate of 37.5 Hz) the contrast seen by either the S or the M cones. The general logic of using cone-isolating stimuli in ERG work has been detailed elsewhere [18, 19]. In addition to being able to confidently record signals from single cone classes, another advantage of using temporally modulated stimuli of this kind is that the eye is maintained in a constant state of light adaptation during the recording session. To design the cone isolating stimuli, we used estimates of ground squirrel cone spectral sensitivities [8] as modified by measurements of absorption in the lens of this species [20]. The maximum cone contrasts that could be achieved were 63.1% for M cone stimulation and 76% for S cone stimulation. We also recorded responses to isochromatic modulation, i.e. where the contrasts seen by the S and M cones were identical (maximum contrast=76%). The procedures for

stimulus calibration have been described [19] and the recording apparatus has also been described in detail [21]. Briefly, analog hardware was used to window the amplified ERG signal with a sinusoid set to the frequency of the stimulus train (37.5 Hz). These signals were averaged over the last 50 of a total of 70 stimulus cycles and the resulting amplitudes were read directly from a computer display.

In the recording experiments, responses were obtained for S-cone modulation, M-cone modulation and isochromatic modulation. In each case, the contrast level was stepped downward from high to low values. At least 9 different contrast levels were examined for each condition, ranging from the maximum available down to contrast levels as low as of 0.0125%. At each contrast level, we recorded the averaged responses to five separate stimulus sequences (each of these in turn involving 50 stimulus cycles) and these five sequence averages were subsequently averaged to obtain a final estimate of the response to each stimulus contrast. Responses to maximum contrast stimuli of the three separate types were recorded at the beginning and end of each recording session to assure that no systematic changes in the quality of the recording had occurred during the test session. As an additional control measure, we also recorded responses in the same fashion when stimulus contrast was set to 0%.

Retinal detachment

Retinal detachments were made under sterile conditions and with the anesthetic regimen noted above. The general procedure for producing surgical detachment has been described [22]. Briefly, a glass micropipette was inserted through a 20-gauge hole in the sclera at the region of the pars plana. A solution of 0.25% sodium hyaluronate (Healon; Pharmacia, Piscataway, NJ) in balanced salt solution was infused between the neural retina and the pigment epithelium. A single detachment was made in the right eye of ground squirrels. Across animals an attempt was made to vary the size of the detachment zone.

In the ground squirrel, significant changes in photoreceptor structure are observed 24 h following retinal detachment [23]. ERGs were recorded at that time point and, immediately after the recording session, animals were sacrificed with an overdose of sodium pentobarbitone. The eyes were removed, fixed in 4% paraformaldehyde in sodium cacodylate buffer (0.1 N; pH 7.4), and stored at 4 deg C. The cornea and lens were removed and the retina was carefully dissected from the eyecup by cutting radially from the retinal edges. The edges of the retina were trimmed away to permit flattening of the eyecup and the flat-mounted retina was subsequently photographed.

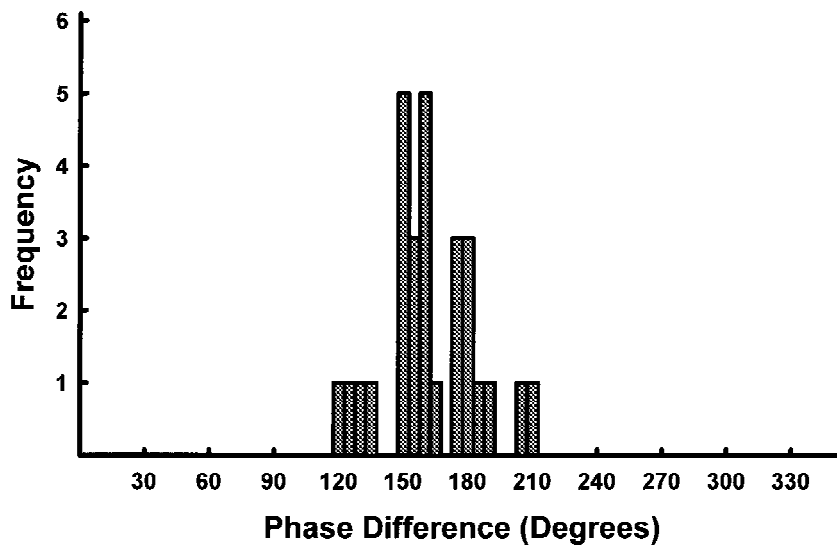


Figure 1. Phase differences in the ERGs recorded for M and S cone-isolating stimuli. Plotted is the distribution of phase differences measured in 28 ground squirrels for 37.5 Hz stimuli presented at maximum contrast. On average, the responses to S-cone isolating modulations lagged that for M cone stimulation by 162.5 deg (SD=21.2 deg).

A defining structural feature of the ground squirrel retina is an elongated optic nerve head that is oriented horizontally above the retinal equator. We used this feature to determine the size and position of the retinal detachment. To accomplish that, the width of the optic nerve head was measured in retinal flat mounts taken from five normal eyes (8.93 ± 0.22 mm). Using that value, the software package Image Tool (University of Texas Health Science Center, San Antonio) was then used to scale the digitized image of the photograph of the retina onto a standard flat mount map of the ground squirrel retina [11]. In this operation, the position of the optic nerve head was aligned with the map and adjusted in size to the standard specified above. The region of detachment was visualized and outlined on the map. Previous immunocytochemical labeling experiments have provided maps of the density distribution of M and S cones in the ground squirrel retina [11]. Accordingly, the total number of receptors of each type that lay in the detached region could be readily calculated. Detachments are here specified as a percentage of the photoreceptors in the detachment zone relative to the total number of photoreceptors of each of the cone classes.

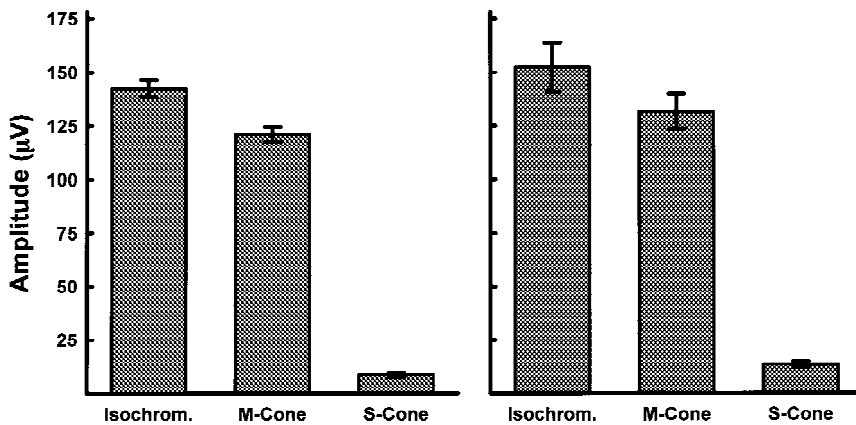


Figure 2. ERG amplitudes recorded for 37.5 Hz maximum-contrast, temporal modulations of three types: isochromatic, M-cone isolating, S-cone isolating. Left panel: Mean values for 23 normal squirrels. Right panel: Mean values from a single animal that was tested on five separate occasions. Error bars= ± 1 SD.

Results

Nature and variability of ground squirrel cone ERGs

Under the conditions of cone isolation specified above, ERG signals could be easily recorded from the eyes of ground squirrels. For each of a large sample of ground squirrels ($N=28$), we measured the relative timing (phase of the fundamental response) to full contrast M and S cone-isolating stimuli. The distribution of these phase differences are shown in Figure 1 from which it can be seen that for this set of test conditions the responses to M and S cone modulation are substantially antiphasic. A similar antiphasic relationship has recently been noted for isolated S cone and isolated rod responses recorded from the eye of a human S-cone monochromat [24]. Figure 2 (left panel) shows the average amplitudes of these signals for maximum contrast stimuli at each of the three test conditions. The average amplitudes obtained for M-cone-isolating and isochromatic stimuli are large (in the range of 120–140 μV) and only modestly variant across a sizable sample of normal animals ($N=23$). Signals could also be readily recorded for all subjects tested for S-cone isolating flicker, although these signals were much smaller (on average about 9 μV) and, relatively, somewhat more variable. We similarly recorded ERGs from a single animal in five separate test sessions, each separated by at least one week, to assess the reliability of the recording. The amplitudes measured for maximum contrast stimuli over the five sessions are summarized in Figure 2 (right panel). The variability within an animal is well within that seen for the large sample.

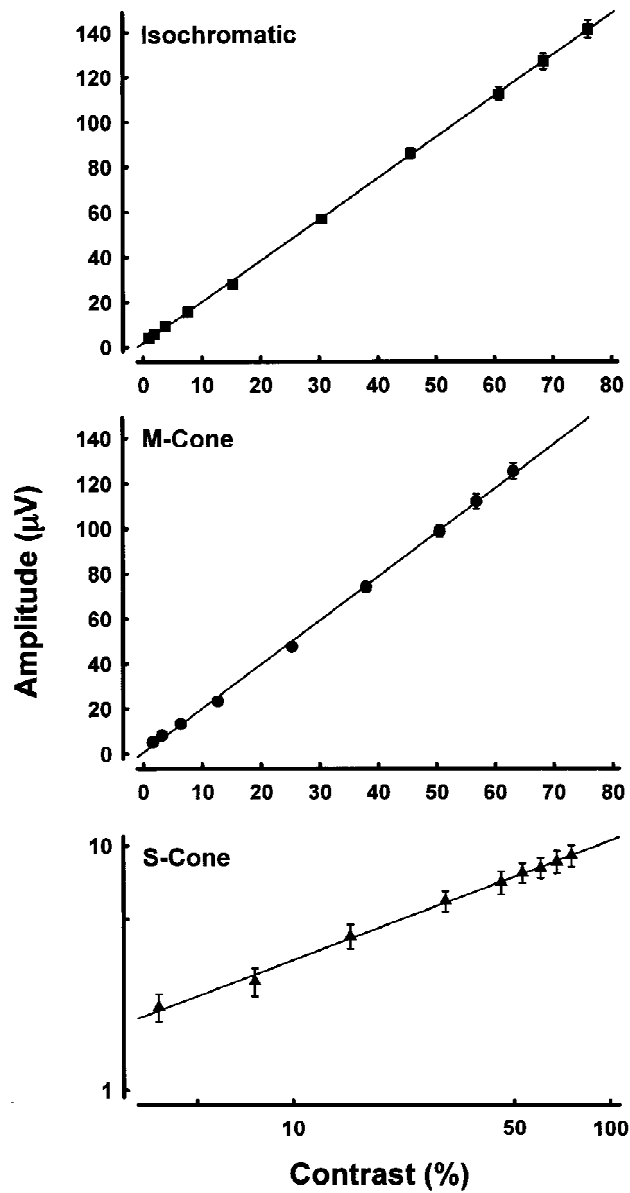


Figure 3. ERG contrast/response functions obtained for temporally modulated stimuli in the ground squirrel. The data points are means and the solid lines are best fitting regressions. Top panel: Isochromatic modulation ($N=24$ animals, $r^2=0.99$); Middle panel: M-cone isolation ($N=23$, $r^2=0.99$); Bottom panel: S-cone isolation ($N=19$, $r^2=0.99$). Error bars enclose ± 1 SEM.

Contrast/Response relationships

For both M cone and isochromatic stimulation, the amplitude of the ERG was linearly related to stimulus contrast over the entire range examined (Figure 3, top two panels). Over most of the contrast range, the response to isochromatic stimuli was slightly, but consistently, smaller than it was for equivalent M cone stimulation. This presumably reflects the fact (above) that under these test conditions the separate responses to M and S cone stimulation are combined antiphasically to yield the summed response given to isochromatic stimuli.

Whereas the contrast/response relationship for both M cone and isochromatic stimulation was linear, that for S cone stimulation was not. For S cone stimulation, ERG amplitude can be well characterized as a compressive response of increasing contrast. Figure 3 (bottom panel) shows in log/log coordinates the average function obtained for S cone stimulation. The best-fit line for that data array has an exponent value of 0.49 ($r^2=0.997$).

Effects of retinal detachment

Retinal detachments were made in 11 ground squirrels. The extent of the detachment zone was determined following the procedures describe above. One such detachment is illustrated in Figure 4. Note that the dissection of the retina causes a flattening of the detached region. To the left is a photograph of the flat mount section with an outline drawn around the area of the detachment. This outlined region was transferred to a computerized version of a standard flat mount map of the ground retina [11]. The latter is illustrated at the right of Figure 4. From that map, the number of cones of each of the two types that were in the detachment zone was calculated. For the 11 squirrels that were studied, the relative losses for the two cone types ranged from 1.9% to 89.0% (S cones) and from 7.6% to 90.6% (M cones).

Figure 5 summarizes the results from ERG measurements made at 24 h following retinal detachment. Plotted there is the change (% of the normal average) in ERG amplitude at a single contrast (63%) level for both M (solid circles) and S (open circles) cone modulation. Four animals with small S cone loss (11% or less) showed no significant change ($p<.01$) in their S cone ERGs relative to those recorded from normal control animals. That fact suggests that the operation *per se* does not induce significant change in the ERG. We reached a similar conclusion by recording ERGs from a control animal that was given a sham operation, i.e. a complete surgical procedure but no retinal detachment. In all other cases, retinal detachment produces a clear decrease in the ERG amplitude. The decrease varied in size as a function of the number of cones in the detachment zone from a low of 20 to 40% loss to a high of near

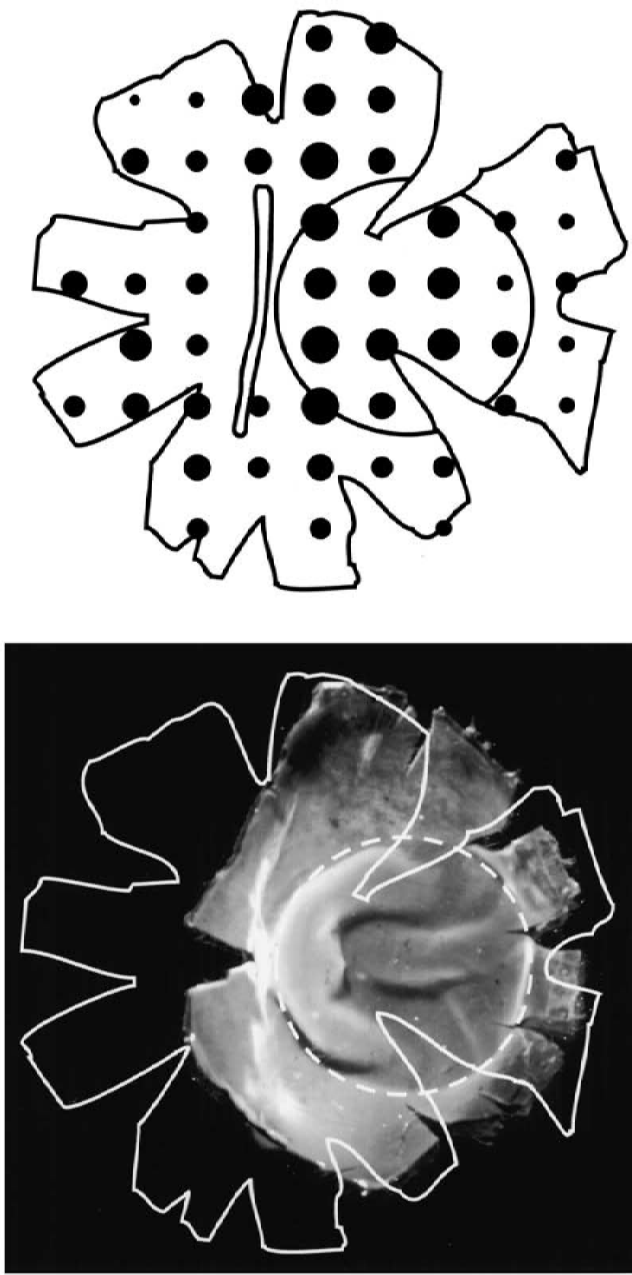


Figure 4. Illustration of the technique used for mapping the extent of experimental detachment of a ground squirrel retina. At the left is a picture of a portion of a retinal flat mount section. The margins of the tissue were trimmed to allow flattening and to gain an accurate viewing of the detached region. The remaining portion of the retina has been aligned over an outline of a standard flat mount map of the ground squirrel retina. The superimposed white circle encloses the boundaries of the detached region that extends ventrally from the elongated optic nerve head shown at the top. At the right, the outline of the detached region has been superimposed onto a standard cone density map of the ground squirrel retina(11). The total cone density (S+M cones) is coded by circle size; it varies in steps of $5000/\text{mm}^2$ from $20\,000\text{--}25\,000/\text{mm}^2$ (smallest circles) to $45\,000\text{--}50\,000/\text{mm}^2$ (largest circles). The total number of cones in the region of the detachment was calculated by summing the regional densities of cones.

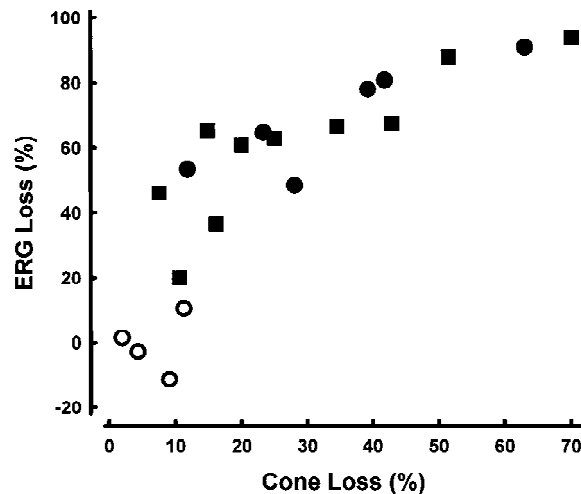


Figure 5. The relationship between the extent of the retinal detachment (specified as % of cones in the detached region relative to those in the whole retina) and the decrease in ERG amplitude relative to the average values obtained from normal animals. Each data point is for an individual animal and results are shown for both M cones (squares) and S cones (circles). The open symbols indicate cases in which the amplitudes recorded from animals with retinal detachments did not differ significantly from normal animals. See text for further details.

complete loss of the ERG signal for the very large detachments. Significantly, within the restrictions of sample size, the relationship between cone and ERG loss was not different for M and S cone populations.

Recovery following retinal reattachment

The above results show that the amplitudes of ground squirrel cone ERGs are reliably related to stimulus contrast and to the number of cones in a region of surgical detachment. Can these same measures serve as a useful indicator of change induced by some potentially therapeutic manipulation? To determine if they could, we recorded ERGs from a ground squirrel 1 d following retinal detachment. The contrast response function for M-cone isolating stimuli recorded at that point is shown in Figure 6. Immediately after the recording, the retina was reattached by injecting 0.2 cc of a mixture of 20% sulphur hexafluoride gas and 80% room air following paracentesis to control intraocular pressure [25]. Recordings were subsequently made at intervals of 12, 26 and 35 days following reattachment. Figure 6 illustrates the progressive improvement in ERG signal amplitudes over this time. It seems clear that the ERG indices employed here are capable of providing an indicator of functional recovery from the effects of detachment and, by

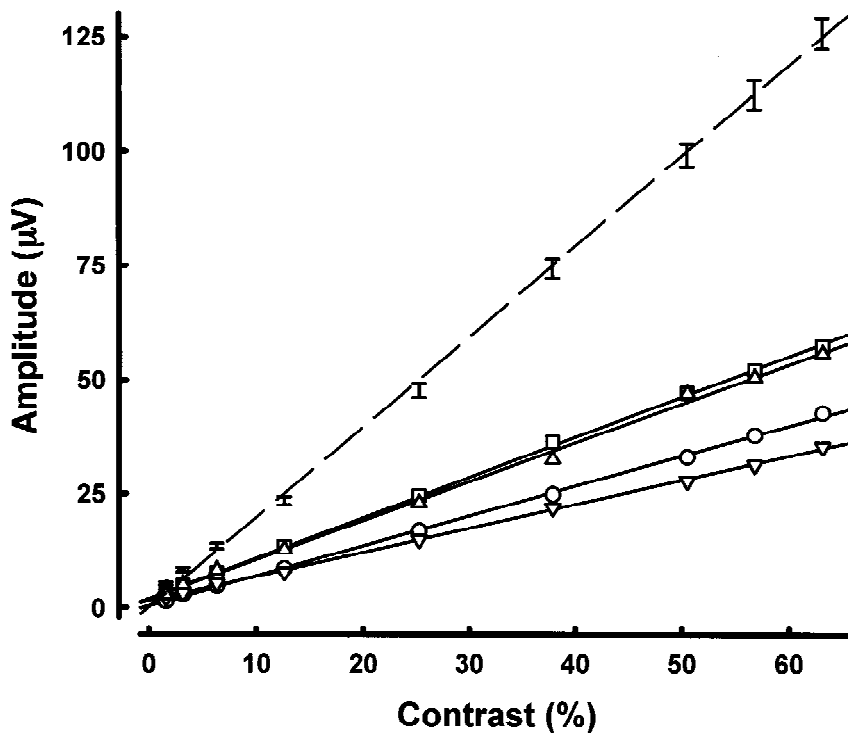


Figure 6. Contrast/response functions obtained from a ground squirrel 1 d following retinal detachment (inverted triangles) and then at three subsequent times after surgical reattachment (circles, 12 d; squares, 26 d; triangles, 35 d). The values are for M-cone isolating stimuli presented at maximum contrast. The dashed line at the top represents averaged values (± 1 SEM) obtained from a large sample of normal animals.

extension, to other manipulations that may impact retinal detachment and reattachment dynamics.

Discussion

The observations reported here suggest that ERGs recorded from ground squirrels using high frequency temporal modulations and cone-isolating stimuli may provide a useful tool for studying and understanding the dynamics of retinal detachment and their impact on cone-based vision. We believe there are several advantages offered by this preparation. First, large amplitude, cone-based signals can be reliably elicited from ground squirrel eyes. For example, ground squirrel ERGs obtained from high contrast M cone and isochromatic stimulation have amplitude values at least twice those recorded

from humans under the standard clinical test conditions for eliciting cone signals [26, 27]. Second, cone responses can be noninvasively measured in successive test sessions. This allows the possibility of following changes over the course of a therapeutic regimen. Third, the use of temporally modulated stimuli keeps the average retinal illuminance constant across the recording session so adaptation state does not fluctuate. This is a potentially important factor in the comparison of results obtained from study of intact and damaged retinas. Fourth, the use of cone-isolating stimuli allows a direct comparison of the relationship between the outputs of different cone classes. This is difficult to convincingly accomplish in any other fashion (for instance, by using accessory chromatic adaptation) and it will, for instance, allow a direct examination of any differential effects of retinal detachment and reattachment on S and M cone types and the visual capacities they support. Finally, an advantage of specifying the size of retinal detachment in terms of the proportion of cones in the detachment zone is that in most retinas, including both ground squirrel and human, cones have heterogeneous distributions that differ for each cone type. This implies that two detachments of similar size could yield quite different cone losses and thus differ significantly in their implication for subsequent visual change.

The stimulus parameters we used were chosen to produce cone-based signals that could be reliably recorded from the ground squirrel. These stimuli, however, are also well within the range of the normal vision capacities for this species. For example, the temporal contrast sensitivity function for the California ground squirrel measured in behavioral discrimination experiments peaks at around 20 Hz with sensitivity down only slightly from this peak at the frequency used for the current ERG recording (37.5 Hz) [6]. At that frequency, animals were able to discriminate stimuli having contrast values of about 10%, a value again well within the range that yields reliable ERGs. Furthermore, in unit recording experiments, it was demonstrated that all of the ganglion cell types of the ground squirrel retina (including those with S cone inputs) easily follow temporal modulation rates as high as 50 Hz [28]. That same investigation involved behavioral measurements of spectral sensitivity functions for flickering lights. The shapes of these functions change significantly for different flicker rates showing a progressive loss of S cone influence as stimulus rate is increased. It is of interest that for highest rates tested (40–50 Hz), model fits made to the spectral sensitivity functions were interpreted as suggesting, ‘the short-wavelength mechanism provides a negative contribution under these test conditions’ [28]. Those test conditions are quite similar to those used for the ERG recording. Perhaps the antiphase relationship between S and M cone contributions (Figure 1) provides an explanation for the shapes of the spectral sensitivity functions.

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References

1. Fisher SK, Anderson DH. Cellular effects of detachment on the neural retina and the retinal pigment epithelium. In: Glaser BM, ed. *Surgical Retina*. 2nd ed. St. Louis: Mosby, 1994;2035–61.
2. Mervin K, Valter K, Maslim J, Lewis G, Fisher S, Stone J. Limiting photoreceptor death and deconstruction during experimental retinal detachment: The value of oxygen supplementation. *Amer J Ophthalmol* 1999;128: 155–64.
3. Lewis G, Mervin K, Valter K, Maslim J, Kappel PJ, Stone J, Fisher, S. Limiting the proliferation and reactivity of retinal Muller cells during experimental retinal detachment: The value of oxygen supplementation. *Amer J Ophthalmol* 1999;128:165–72.
4. Lewis GP, Linberg KA, Geller SF, Guerin CJ, Fisher SK. Effects of neurotrophin brain-derived neurotrophic factor in an experimental model of retinal detachment. *Invest Ophthalmol Vis Sci* 1999;40:1530–44.
5. Jacobs GH. Spectral sensitivity and colour vision in the ground-dwelling sciurids: Results from golden-mantled ground squirrels and comparisons for five species. *Anim Beh* 1978;26:409–21.
6. Jacobs GH, Blakeslee B, McCourt ME, Tootell RBH. Visual sensitivity of ground squirrels to spatial and temporal luminance variations. *J Comp Physiol* 1980;136:291–99.
7. Jacobs GH, Blakeslee B, Tootell RBH. Color discrimination tests on fibers in the ground squirrel optic nerve. *J Neurophysiol* 1981;45:903–14.
8. Jacobs GH, Neitz J, Crognale M. Spectral sensitivity of ground squirrel cones measured with ERG flicker photometry. *J Comp Physiol A* 1985;156:503–9.
9. Jacobs GH. Duplicity theory and ground squirrels: Linkages between photoreceptors and visual function. *Vis Neurosci* 1990;5:311–8.
10. Linberg KA, Suemune S, Fisher SK. Retinal neurons of the California ground squirrel, *Spermophilus beecheyi*: A Golgi study. *J Comp Neurol* 1996;365:173–216.
11. Kryger Z, Galli-Resta L, Jacobs GH, Reese BE. The topography of rod and cone photoreceptors in the retina of the ground squirrel. *Vis Neurosci* 1998;15:685–91.
12. Jacobs GH. The distribution and nature of colour vision among the mammals. *Biol Rev* 1993;68:413–71.
13. Brainard DH. The Psychophysics Toolbox. *Spatial Vis* 1997;10:433–36.
14. Pelli DG. The Video Toolbox software for visual psychophysics: Transforming numbers into movies. *Spatial Vis* 1977;10:437–42.
15. Pokorny J, Smith VC, Verriest G, Pinckers AJLG. *Congenital and Acquired Colour Vision Defects*. New York: Grune & Stratton, 1979.
16. Nork TM, Millecchia LL, Strickland BD, Linberg JV, Chao GM. Selective loss of blue cones and rods in human retinal detachment. *Arch Ophthalmol* 1995;113:1066–73.
17. Yamamoto S, Hayashi M, Takeuchi S. Cone electroretinograms in response to color stimuli after successful retinal detachment surgery. *Jap J Ophthalmol* 1998;42:314–7.

18. Usui T, Kremers J, Sharpe LT, Zrenner E. Flicker cone electroretinogram in dichromats and trichromats. *Vis Res* 1998;38:3391–6.
19. Brainard DH, Calderone JB, Nugent AK, Jacobs GH. Flicker ERG responses to stimuli parametrically modulated in color space. *Invest Ophthalmol Vis Sci* 1999;40:2840–7.
20. Yolton RL, Yolton DP, Renz J, Jacobs GH. Preretinal absorbance in sciurid eyes. *J Mammal* 1974;55:14–20.
21. Jacobs GH, Neitz J, Krogh K. Electroretinogram flicker photometry and its applications. *J Opt Soc Amer A* 1996;13:641–8.
22. Anderson DH, Guerin CJ, Erickson PA, Stern WH, Fisher SK. Morphological recovery in re-attached retina. *Invest Ophthalmol Vis Sci* 1986;27:168–83.
23. Linberg KA, Lewis GP, Chartersis DG, Fisher SK. Experimental detachment in a cone dominant retina. *Invest Ophthalmol Vis Sci* 1999;40:S951.
24. Scholl HPN, Kremers J. Electroretinograms to S-cone and rod isolating stimuli in S-cone monochromacy. *Color Res Applicat* 2000; 26: S136–S139.
25. Guerin CJ, Anderson DH, Fariss RN, Fisher SK. Retinal reattachment of the primate macula. *Invest Ophthalmol Vis Sci* 1989;30:1708–25.
26. Birch DG, Anderson JL. Standardized full-field electroretinography. *Arch Ophthalmol* 1992;110:1571–6.
27. Marmor MF, Zrenner E. Standard for clinical electroretinography (1999 update). *Doc Ophthalmol* 1999;97:143–56.
28. Crognale MA, Jacobs GH. Behavioral and electrophysiological sensitivity to temporally modulated visual stimuli in the ground squirrel. *Vis Neurosci* 1991;6:593–606.

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