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The contribution of cone responses to rat electroretinograms

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Abstract

The contribution of rods and cones to the scotopic electroretinogram (ERG) of small animals is unclear, with a recent report suggesting that the mouse has no cone a-wave. The present study considered the contribution of cones to the ERG of the rat. Dark-adapted Long Evans rats ($n = 4$) had ERG signals collected following a single flash, which stimulated rods and cones (mixed response), or a twin-flash paradigm (short interstimulus interval, 1 s), which isolated cone responses. Rod signals were derived by digital subtraction of the cone signal from the mixed rod/cone ERG. The rat a-wave was found to be dominated by rod responses but cone responses contributed substantially (45%) to post-receptoral waveforms (b-wave) at higher light levels.

Key words: cone, electroretinogram, rat, rod.

Introduction

It has been suggested that the environment inhabited by various species determines the spectral characteristic and distribution of their photoreceptors.[1] Early data indicated that the rat contained only rod photoreceptors,[2] although more recent work has identified the presence of cones.[3–10]

Although three photoreceptor classes can be recognized using monoclonal antibodies, anatomical studies show that rods dominate the rat retina, with a rod : cone ratio of approximately 100:1.[10] The majority (93%) of cones that are present are sensitive to medium-to-long wavelengths with a small proportion having short wavelength sensitivity.[10]

Behavioural, physiological and electrophysiological experiments have exposed rod and cone-like responses.[3–10] Rat increment thresholds show two spectral responses consistent with rods and cones.[7] Neitz and Jacobs report a photopic system in the rat, with a medium wavelength spectral-sensitivity (λ_{max} = 510 nm).[8] Indeed, the rat electroretinogram (ERG) shows three spectral peaks (450 nm, 520 nm and 560 nm) corresponding to short wavelength, rod, and medium-to-long wavelength sensitive photopigments, respectively.[3]

As the rat is a commonly used laboratory animal in vision research it is important to establish the contribution of cone photoreceptors to the dark-adapted ERG. The present study reports such an evaluation.

Methods

The experiments reported herein were approved by our institutional animal ethics committee and were conducted in accordance with the guidelines set out by the Australian code of practice for the care and use of animals for scientific purposes.[11]

Male Long Evans rats ($n = 4$) were maintained at 21°C in a 50–150 lux environment with a 12-h light/dark cycle (on at 08.00 hours) and fed standard chow. Their average age was 12 weeks postnatal with sexes balanced in test groups.

Rats were dark adapted overnight (> 15 h) prior to experimentation. Animals were anaesthetized with ketamine (35 mg/kg; Ketamil, Troy Laboratories, Smithfield, Australia) and xylazine (5 mg/kg; Xylazil, Troy Laboratories). Mydriasis was induced prior to recording with tropicamide (Mydriacyl 0.5%; Alcon Laboratories, Frenchs Forest, NSW, Australia) and corneal anaesthesia induced with proxymetacaine (Ophthetic 0.5%; Allergan Australia, Gordon, NSW, Australia). Electroretinogram responses were elicited using single flashes of a discharge source (285 V; Vivitar Photographics, Newbury Park, CA, USA) delivered via a Ganzfeld sphere over a five log unit intensity range (–2.5 to +2.5 $\log_{10}$ cd.s/m^2) using calibrated neutral density filters (0–5 ND; Kodak Wratten, Eastman Kodak, Rochester, NY, USA). Signals were collected with custom Ag/AgCl differencing electrodes. A corneal active electrode was referenced to an inactive electrode located in the mouth. A stainless steel needle electrode inserted in the tail served as a common ground. Signals were amplified (× 1000; Grass P55, Astro-Med, West Warwick, RI, USA) and filtered online (0.1 Hz – 3 kHz). In all cases, a 3-min (180 s) interval was allowed between trials in order to allow full recovery of the waveforms.

A twin-flash paradigm was used to isolate cone responses (1 s interstimulus interval). This procedure was based on the method of Birch *et al.*[12] and measured the retinal response to

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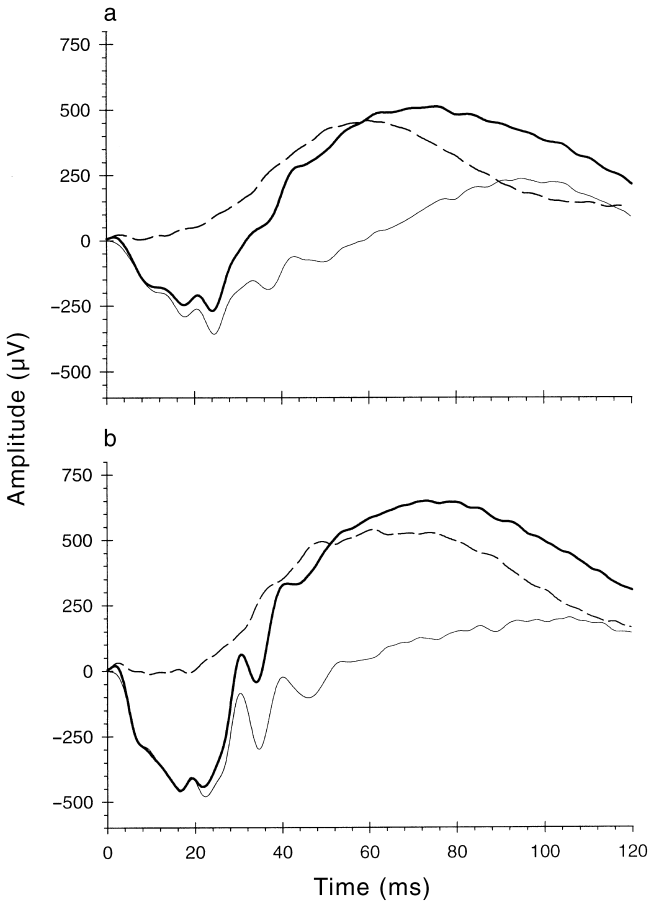

Figure 1. Representative rat waveforms obtained using two interstimulus delays of 180 s (—, mixed rod/cone response) and 1 s (- - -, cone response) and two flash exposures (a) 1.3 $\log_{10}$ cd.s/m^2 and (b) 2.5 $\log_{10}$ cd.s/m^2. Rod responses (—) were achieved by digital subtraction of the 1-s signal from the 180-s signal.

a probe flash after an initial adapting (saturating) flash. Both rods and cones responded to the initial test flash. However, as the rod system is not capable of responding to the test flash at very short interstimulus intervals (1 s), the response to the probe flash was purely cone derived. Two identical flash units were used for testing with their timing controlled electronically (Uniblitz, TS132 shutter-timer; Vincent Associates, Rochester, NY, USA). Isolated rod responses were derived by digital subtraction of the cone response from the mixed rod/cone dark adapted ERG (Fig. 1). The amplitude–intensity relationship was described by either a linear trend or Naka–Rushton relationship as given by Equation 1.

$$A = A_{\max} \frac{E^n}{K^n + E^n} \qquad \text{Equation 1}$$

$A_{\max}$ is the maximal amplitude (μV), E the test exposure ($\log_{10}$ cd.s/m^2), K is the exposure that yields a 50% maximal response and n is a constant that describes the slope. Fitting was achieved by minimizing the root-mean-square error term with a Levenberg–Marquart optimization routine.[13]

Statistical analysis was performed using repeated measures ANOVA with an alpha of 0.05. Post-hoc comparisons were made using Tukey's test. Where error bars are given or errors quoted, these represent the standard error of the mean.

Results

Representative rat waveforms are shown in Fig. 1 for two stimulus intensities (1.3 and 2.5 $\log_{10}$ cd.s/m^2). These waveforms were acquired using a single flash (repeated every 180 s) or a twin-flash paradigm with a 1-s interstimulus delay. Figure 1 shows that short interstimulus intervals abolish the a-wave and expose a fast post-receptoral b-wave (cone). In addition, digital subtraction of the cone-derived response from the mixed response exposes a slower rod b-wave. The characteristics of the putative rod and cone signals are consistent with the known physiology of these processes. In the following, we use the twin flash (1-s delay) to isolate cone responses and digital subtraction from the mixed waveform to yield the rod-isolated response at all intensities in the dark-adapted ERG.

Representative waveforms collected from rats are shown in Fig. 2 for the mixed (rod/cone, Fig. 2a), measured cone (Fig. 2b) and extracted rod (Fig. 2c) responses. From threshold, the rod ERG signal (Fig. 2c) shows a b-wave. As intensity increases, b-wave amplitude increases and an a-wave becomes visible at 0.1 $\log_{10}$ cd.s/m^2. Both a-wave and b-wave amplitudes continue to increase with higher light levels and become faster. A faster cone b-wave (Fig. 2b) is evident at higher stimulus intensities (> 0.1 $\log_{10}$ cd.s/m^2). A small cone a-wave is found at the highest light levels. With higher intensities, the cone b-wave increases in amplitude but decreases in implicit time. The extracted rod response (Fig. 2c) shows less electro-positivity than does the mixed response.

Intensity–response relationships of photoreceptor (a-wave) and post-receptoral (b-wave) amplitudes and implicit times are shown in Fig. 3a and Fig. 3b, respectively. It is evident that the faster cone response is responsible for the speeding up of the ERG waveform at high light levels.

At all intensities > 0.1 $\log_{10}$ cd.s/m^2, the a-wave mixed response amplitude is greater than is the cone amplitude ($P < 0.001$), with a small cone a-wave (–40 μV) being found only at the highest stimulus intensities. The mixed a-wave shows a maximum amplitude of –404 μV compared to the –368 μV of the rod response. The rod a-wave dominates the mixed response at all light levels and only deviates from the mixed signal at the three highest stimulus exposures, indicating that cones have minimal input to rat a-waves.

The b-wave shows a similar trend to the a-wave with exposure except that cones provide a much greater input to the signal (507 μV or 45%). The mixed signal increases in amplitude with light to reach a peak of 1128 μV and rod inputs dominate at low light levels. It is interesting to note a hump in the mixed b-wave intensity–response function, which most likely reflects interactions between the fast cone response and the slower rod response. The cone b-wave is

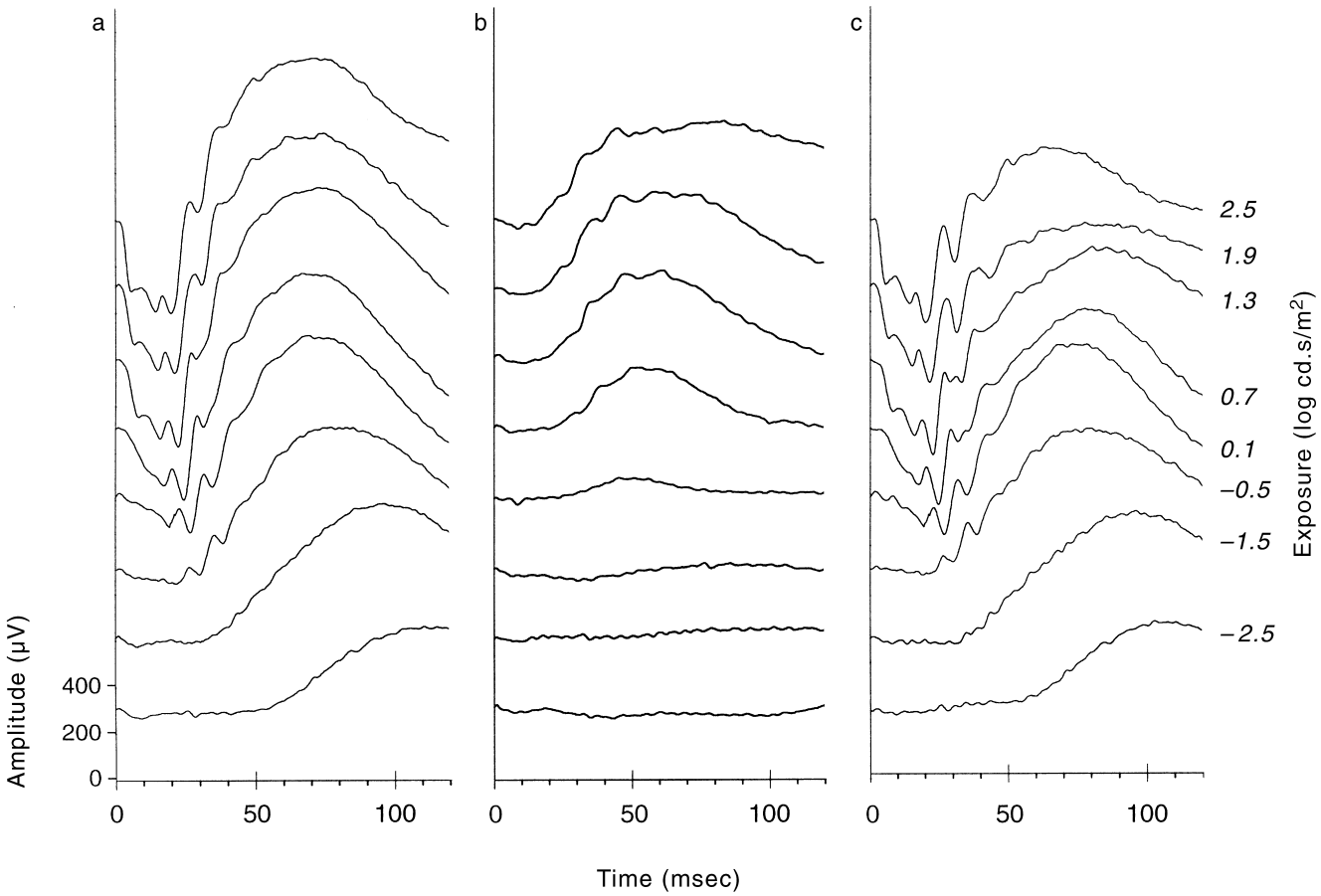

Figure 2. Representative rat waveforms obtained over the range of exposures given in log cd.s/m^2, isolated using the methods outlined in Fig. 1 for (a) mixed rod/cone; (b) cone; and (c) extracted rod responses.

seen to increase in amplitude and saturate at the highest exposures. In contrast, the rod response has a maximum amplitude of 745 μV and shows a slight decline at higher exposures. It is 2.3 log units more sensitive than is the cone response.

Figure 3c and 3d show a-wave and b-wave implicit times, respectively. The mixed a-wave implicit time (22 ± 2 msec) is dominated by the slower rod (21 ± 1 msec) and never reaches the cone implicit time (11 ± 2 msec), consistent with the proposal that cones have minimal impact on this waveform. As expected, the rod a-wave implicit times decrease with intensity ($P < 0.0001$) as shown by the linear regression (Fig. 3c). Rods also dominate the mixed b-wave implicit time which shows a speeding up with increased exposure (107 ± 1 to 71 ± 2 msec, $P < 0.0004$). Of the isolated responses, the rod b-wave implicit time was slower at the highest stimulus intensity (80 ± 6 msec) than was the cone implicit time (70 ± 5 msec) and at this exposure the mixed response is dominated by cone-derived mechanism.

Discussion

It is appreciated that the rat retina contains three receptoral types, with spectral peaks corresponding to rod, short wavelength and long/medium-wavelength pigments.[3] It would be expected that these receptors might manifest in gross ERG recordings. However, the rod : cone ratio of approximately 100:1[10] will mean that the cone response should make only a small contribution to the rat ERG. The a-wave amplitudes observed in the present study are consistent with the fewer numbers of cones. However, the large and robust cone b-wave amplitude was unexpected. It implies that the cone system has significant amplification at post-receptoral levels for its contribution to increase five-fold in the b-wave (cone contribution to a-wave approximately 10%, b-wave approximately 45%).

To our knowledge, no literature describes the isolated rat rod and cone responses. Hence the findings of our study will be compared to humans and mice.[14,15] Hood and Birch found in humans that the leading edge of the cone a-wave can be used to quantitatively measure cone receptor activity.[16] Coloured stimuli were utilized to isolate receptoral responses in the dark-adapted retina and revealed that cones had a significant contribution to both the leading edge of the human a-wave and the b-wave at higher intensities.[16] However, Ekesten *et al.* report that murine cones do not contribute to the leading edge of the a-wave, in contrast to the human ERG, and that they only produce a modest b-wave.[15]

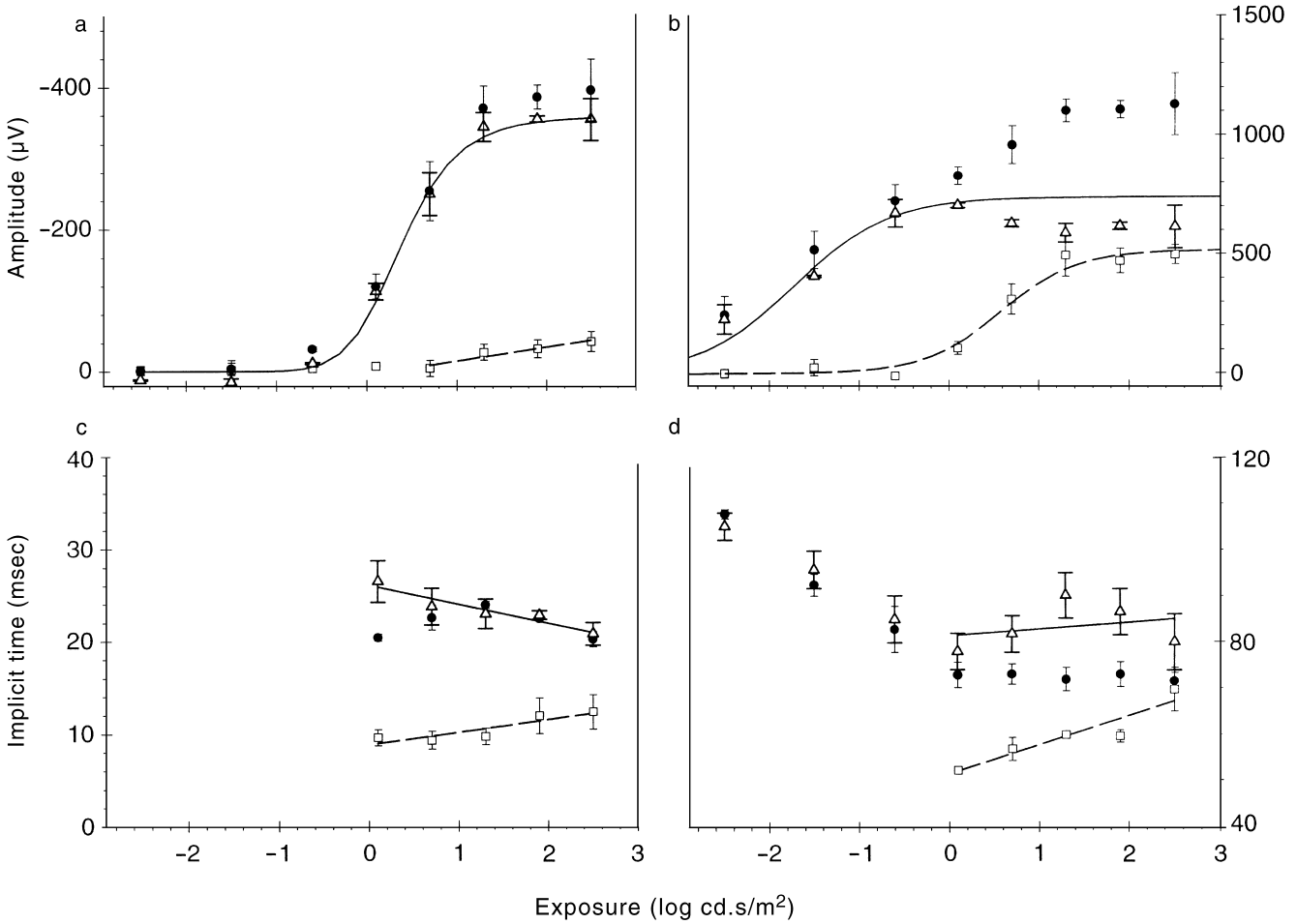

Figure 3. Average (± SEM) intensity versus amplitude response functions of the mixed and isolated (a) a-waves and (b) b-waves. (c,d) Implicit times for the waveforms. Mixed rod/cone outcomes are shown by filled symbols whereas rod and cone isolated responses are shown by unfilled symbols. Rod: △, ——; cone: □, ----. The lines give either the best fitting linear or Naka–Rushton relationship.

Our finding for a small but robust a-wave at high light levels in rat is more in keeping with human physiology than mouse. We also find that the cone system of the rat contributes significantly to the dark-adapted post-receptoral b-wave (507 μV) at photopic stimulus exposures in contrast to the mouse data of Ekesten (100 μV).[15]

Short interstimulus intervals, as used in the twin-flash paradigm, are useful methods for extraction of rod and cone responses in both humans[12,16] and small laboratory animals.[15] This method isolates cone signals by silencing rod inputs and is based on the slower temporal response of rods. This technique does not require any a priori assumptions of spectral response characteristics, inherent to techniques involving chromatic isolation. Additionally, as this method utilizes short interstimulus intervals it may be readily incorporated into existing ERG protocols. In the rat, we showed that exposures < 0.1 $\log_{10}$ cd.s/m^2 produce little cone contribution to either the a-wave or b-wave. At higher light levels, the cone contribution to the a-wave is minimal, but a significant contribution is found for the b-wave implying substantial post-receptoral amplification in the cone system.

References

1. Szel A *et al. J. Opt. Soc. Am. A* 2000; **17**: 568–79.
2. Granit R. *J. Physiol. (Lond.)* 1933; **77**: 207–39.
3. Cicerone CM. *Science* 1976; **194**: 1183–5.
4. Green DG. *J. Physiol.* 1973; **228**: 781–97.
5. Govardovskii VI *et al. Vision Res.* 1992; **32**: 19–27.
6. Ernst W *et al. Nature* 1975; **258**: 170–71.
7. Jacobs GH *et al. Vision Res.* 1975; **15**: 375–8.
8. Neitz J, Jacobs GH. *J. Comp. Psychol.* 1986; **100**: 21–9.
9. Perlman I. *J. Physiol.* 1978; **278**: 161–75.
10. Szel A *et al. Exp. Eye Res.* 1992; **55**: 47–52.
11. National Health and Medical Research Council. *Australian Code of Practice for the Care and Use of Animals in Scientific Research*. Canberra: Australian Government Publishing Service, 1990.
12. Birch DG *et al. Invest. Ophthalmol. Vis. Sci.* 1995; **36**: 1603–14.
13. Press W *et al. Numerical Recipes in C*. Cambridge: Cambridge University Press, 1992.
14. Hood DC, Birch DG. *Vision Res.* 1993; **33**: 1605–18.
15. Ekesten B *et al. Doc. Ophthalmol.* 1998; **97**: 23–31.
16. Hood DC, Birch DG. *Vis. Neurosci.* 1993; **10**: 857–71.