


Diurnal Rhythm in the Human Rod ERG

Relationship to Cyclic Lighting

David G. Birch,* Michael A. Sandberg, and Elior L. Berson

Five normal subjects were monitored with periodic full-field ERG testing during entrainment to a 14L:10D lighting cycle. On the second day of entrainment, mean ERG threshold was significantly higher 1.5 hr after light onset than at other times of day and was comparable to a previously reported threshold elevation following 7 days of entrainment. This diurnal rhythm was not detectable in eyes entrained for 3 days that were then maintained in darkness for 1 day. The findings support the idea that 2 days of entrainment are required to maximize the diurnal rhythm in the human rod ERG and that an adapting light may be required to trigger this rhythm. Invest Ophthalmol Vis Sci 27:268–270, 1986

Six normal adult subjects who were monocularly entrained for 7 days to a 14-hr light:10-hr dark (14L:10D) lighting cycle showed a diurnal rhythm in their dark-adapted, full-field rod ERG. An analysis of variance showed that time of day had a significant effect on the rod ERG in the entrained eye, with b-wave threshold 0.13 log-unit higher 1.5 hr after light onset than at other times of day. No effect of time of day was seen in the unentrained eye, suggesting that this phenomenon was regulated within the eye. The amplitude of the a-wave was significantly smaller 1.5 hr after light onset in the entrained eyes, indicating that the diurnal rhythm was present in the rod photoreceptors and not due to possible diurnal fluctuations in synaptic transmission or post-receptor function. The increase in b-wave threshold in this electrotoretinographic study in humans was compatible with anatomical studies in the rat, frog, cat, and monkey that have shown a daily burst of rod outer segment (ROS) disc shedding in light-entrained animals 1.5–2 hr after light onset. The minimum number of days of entrainment required to detect this diurnal rhythm in the human ERG was not evaluated.

Previous studies in the rat suggested that the daily burst in ROS disc shedding followed a circadian rhythm since it persisted in continuous darkness for at least 2 wk without a light trigger. In other species (eg, Rana pipiens) the diurnal disc shedding rhythm has appeared directly related to light onset and had a weak or absent circadian component.

The present study was done in part to determine the minimum number of days of cyclic (14L:10D) illumination necessary to produce a diurnal rhythm in rod b-wave threshold comparable to that measured previously following 7 days of entrainment. A second goal was to determine whether the diurnal rhythm in the rod ERG of entrained human subjects persists in continuous darkness and thus fulfills one criterion for a circadian rhythm.

Materials and Methods. Five adult subjects (ages 18 to 35) with normal ophthalmic examinations and normal rod and cone full-field ERGs were tested following at least 1 hr of dark-adaptation at 7:45 AM, 9:30 AM, and 4:00 PM and/or 9:00 PM for 5 successive days with different light–dark cycle protocols. Informed consent was obtained from all subjects after the details of the following procedures had been explained fully. On day 0 (control day) subjects were repatched immediately following the 7:45 AM test session, with no additional light-exposure prior to the 9:30 AM test session. With the exception of 1 hr of dark-adaptation prior to the 4:00 PM and 9:00 PM test sessions, subjects were exposed to ambient illumination for the remainder of the day. Subjects were then monocularly patched from 10:00 PM until 8:00 AM in order to begin to establish a 10-hr dark:14-hr light cycle. The same eye was patched from 10:00 PM to 8:00 AM for 3 additional nights. On days 1–3 (entrainment days) the entrained eye was exposed for 10 min to a steady 34 cd/m² background at 8:00 AM and then to ambient illumination for the remainder of the day. Testing was
conducted at 7:45 AM (15 min prior to light onset), at 9:30 AM (1.5 hr after light onset), and at 4:00 PM (8 hr after light onset), and/or 9:00 PM (13 hr after light onset), with 1 hr of dark adaptation prior to the 9:30 AM, 4:00 PM, and 9 PM test sessions, respectively. On day 4 (darkness) the eye was tested at 7:45 AM, 9:30 AM, 4:00 PM and/or 9:00 PM but otherwise remained patched throughout the day.

Eyes were topically anesthetized with 0.5% proparacaine hydrochloride and pupils were then maximally dilated with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride (administered under dim red illumination). Rod ERGs were elicited with 10 μsec short-wavelength (λ<sub>max</sub> = 440 nm, half-band-width = 47 nm) full-field flashes that were varied in retinal illuminance in approximately 0.3 log-unit steps from −1.3 log scot td-sec to 0.0 log scot td-sec. ERG responses were monitored at the cornea with a bipolar contact lens electrode, amplified, recorded on magnetic tape, and signal-averaged. Rod ERG b-wave thresholds were calculated from the b-wave amplitude vs log retinal illuminance function from each test session. A response half the amplitude of that elicited by the brightest flash was selected for each subject and used as the threshold criterion throughout the study. Regression lines were computed for those values falling within the linear range of the amplitude vs log retinal illuminance function to determine the log retinal illuminance necessary to produce the threshold criterion amplitude for each test session. A log threshold difference for each subject was determined on each day by subtracting the mean of the log thresholds for the 7:45 AM, and 4:00 PM, and/or 9:00 PM test sessions from the log threshold obtained at 9:30 AM. Results for entrainment days (ie, days 1–3) and for day 4 in darkness were compared statistically with results for day 0.

**Results.** The mean log threshold difference at 9:30 AM based on rod ERG testing of 5 normal subjects is shown in Table 1 for the 3 light–dark cycle protocols (ie, control, entrainment, and darkness). The mean log threshold difference observed at 9:30 AM on the control day (day 0) was not significantly different from zero. Relative to the control day, the mean difference for the first day of entrainment (day 1) was not significantly elevated. However, significant threshold elevations were obtained on the second and third days of entrainment; individual differences on day 3 were fewer than those on day 2 as reflected in the standard errors. In contrast, on day 4 in darkness the mean log threshold difference observed at 9:30 AM was not significantly different from the mean log threshold difference measured on the control day (or from zero).

**Discussion.** The results of this study demonstrate that the diurnal rhythm in the rod b-wave of normal subjects is not present without prior entrainment to a fixed lighting schedule but does become detectable during the second day of entrainment. The magnitudes of the effect on the second and third days of entrainment (0.15 and 0.14 log-unit, respectively) are comparable to that previously reported in 6 subjects tested following 7 days of entrainment (ie, 0.13 log–unit). As noted in our previous report, the size of this threshold elevation is larger than what would be expected (ie, < 0.04 log–unit) from an autoradiographic study in the monkey whereby on average about 10% of each rod outer segment was shed each day. The reason for this difference remains to be defined.

In the absence of a light-trigger at 8:00 AM on day 4, the threshold at 9:30 AM was comparable to those at other times of day. Since persistence of a rhythm without an environmental trigger is a necessary characteristic of a circadian rhythm, the absence of a rise in threshold at 9:30 AM on day 4 supports the idea that the diurnal rhythm in the human rod ERG is not circadian. This contrasts with our recent finding in pigmented rats, light-entrained for 25 days, which showed a significant elevation of ERG threshold 1.5 hr after expected light onset on the first circadian day. We cannot exclude the possibility that a phase shift and/or broadening of the rhythm may be occurring on day 4 in our human subjects, as has been observed in histological studies of disc shedding in the rat after several days in constant darkness and in the tadpole on the first day in darkness. Alternatively, a circadian rhythm in the rod ERG in humans may require more than 3 days of prior entrainment or may appear only after more than 1 day of darkness following entrainment. The lack of a detectable rhythm on day 4 also indicates that the full-field ERG test flashes at 7:45 AM were not in themselves triggering a threshold increase ~1.5 hr later.

Since a diurnal rhythm can be detected in the normal human ERG following only 2–3 days of entrainment to cyclic light rather than 7 days as previously reported, this abbreviated period of entrainment should facilitate

**Table 1. Effect of daily protocol on full-field rod ERG threshold**

<table>
<thead>
<tr>
<th>Day</th>
<th>Protocol</th>
<th>Mean log threshold difference* (± S.E.)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>control</td>
<td>−0.04 ± 0.06</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>entrainment</td>
<td>0.04 ± 0.05</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>entrainment</td>
<td>0.15 ± 0.08</td>
<td>0.039</td>
</tr>
<tr>
<td>3</td>
<td>entrainment</td>
<td>0.14 ± 0.04</td>
<td>0.018</td>
</tr>
<tr>
<td>4</td>
<td>darkness‡</td>
<td>0.01 ± 0.05</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* Mean log threshold difference ± S.E. at 9:30 AM for 5 normal subjects; see methods for calculation.
† One-tailed t-test comparing days 1 to 4, respectively, to day 0; N.S. = not significant (p > 0.05).
‡ Continuous patching throughout day with no adapting light at 8:00 AM.
management of similar studies in patients with retinal degenerations. Preliminary data have revealed that a rhythm in the ERG can be detected in a family with dominantly inherited retinitis pigmentosa after 3 days of entrainment.¹¹

Key words: rod, electroretinogram, circadian, diurnal rhythm, disc shedding, entrainment, retinitis pigmentosa

From the Berman–Gund Laboratory for the Study of Retinal Degenerations, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts. *Present address: Retina Foundation of the Southwest, 8220 Walnut Hill Lane, Suite 012, Dallas, Texas 75231, and Department of Ophthalmology, Southwestern Medical School, Dallas, Texas 75235. Supported in part by National Eye Institute Project Grant EY00169 and in part by the National Retinitis Pigmentosa Foundation, Baltimore, Maryland. Reprint requests: Michael A. Sandberg, PhD, Berman–Gund Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114.

References


Rod and Cone ERGs and Their Oscillatory Potentials

P. Ewen King-Smith,* David H. Loffing, † and Ronald Jones*

Normal human ERGs were recorded from a dark-adapted subject using white and colored test flashes. Oscillatory potentials (OPs) were studied after high-pass digital filtering. When blue and red responses were compared at equivalent photopic intensities, OPs were visible at much lower intensities for the blue flashes. The sensitivity of the rod outer segments was reduced from maximum, the first (negative) wave for red flashes maintained a latency of 20–25 msec before being lost in noise, whereas the first wave for blue flashes increased its latency progressively from 25 to 60 msec. These differences between photopically matched red and blue responses are interpreted to be due to rod-generated responses. When blue, orange, and white responses were compared at equivalent scotopic intensities, the latency of the largest negative wave was found to be similar for all three colors. The authors interpret this wave to be the beginning of the rod-generated OPs, so that the preceding waves (particularly evident for orange flashes) are cone-generated OPs, and they propose that the existence of separate rod and cone OPs should be borne in mind when investigating clinical changes in OPs. Invest Ophthalmol Vis Sci 27:270–273, 1986

The oscillatory potentials (OPs) of the human electroretinogram (ERG) are high frequency wavelets which occur during the rising phase of the b-wave.¹³

Considerable clinical interest in OPs has been generated by the demonstration that they may be selectively reduced in diseases of the inner retina.²³ Despite this interest, there is still much uncertainty about the physiological origins of the OPs. For example, there is considerable disagreement about whether they are derived only from cones, or whether rod responses can also generate OPs.¹ We believe that the results reported here unequivocally demonstrate that both rods and cones contribute to the OPs.

Materials and Methods. Potentials were recorded using a gold foil electrode⁴ (EL50, SC Electronics); and amplified by a Data Inc. 2124 amplifier (Fort Collins, CO) with low and high frequency cuts at 0.2 and 500 Hz. This amplified signal was sampled at 1-msec intervals and averaged by a North Star Horizon computer (San Leandro, CA). Oversize responses due to blinks or eye movements were rejected. Averages of 50 to 200 responses (to 1 flash per sec) were stored on magnetic disk and were later digitally high-pass filtered to emphasize the OPs by convolving the average with a weighting function w(t) consisting of a delta function minus a gaussian function defined by