Rod ERG Diurnal Rhythm in Some Patients
With Dominant Retinitis Pigmentosa

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Five patients with dominant retinitis pigmentosa who were monocularly entrained to a 14 hr light: 10 hr dark cycle showed an abnormal diurnal rhythm in the rod electroretinogram of the entrained eye. These patients as a group showed larger-than-normal reductions in b-wave sensitivity 1.5 hr and 8 hr after light onset relative to other times of day. The findings raise the possibility that these patients have an abnormality in rod photoreceptor function associated with the process of outer segment renewal. Invest Ophthalmol Vis Sci 29:494-498, 1988

Previous studies of normal human subjects who were monocularly entrained to a 14 hr light: 10 hr dark cycle showed that a diurnal rhythm existed in the rod electroretinogram (ERG) of the entrained eye.1,2 B-wave sensitivity was significantly lower 1.5 hr after light onset compared to other times of day. A diurnal variation in the rod ERG of normal, light entrained pigmented rats has been observed, with a-wave and b-wave sensitivities lowest at the time of day when phagosomes in the pigment epithelium were most numerous and rod outer segments were shortest.3 A normal rod ERG diurnal rhythm was recently reported in Royal College of Surgeons (RCS) pigmented rats with a defect in the capacity of the pigment epithelium to phagocytize outer segment discs; this finding suggested that the reduction in ERG sensitivity following light onset in humans was not directly due to a diurnal rhythm in phagosome frequency, but, instead, might occur as a consequence of an alteration in rod photoreceptor function.4 The presence of a diurnal rhythm in the rod ERG of normal humans, together with the findings in rats, raised the possibility of monitoring functional changes associated with rod outer segment renewal in patients with retinal diseases involving the rod photoreceptor. Initial studies were conducted on patients with dominant disease as these patients have abnormal, but often easily detectable, rod ERGs in the early stages.

Materials and Methods. Three siblings (ages 25, 26 and 32) from a family with dominant retinitis pigmentosa with complete penetrance, one patient (age 22) from a second family with dominant retinitis pigmentosa with complete penetrance, and one patient (age 54) from a family with dominant retinitis pigmentosa with reduced penetrance were studied. These patients had corrected visual acuities between 20/20 and 20/40, full or nearly full visual fields to a V-4e white test light with the Goldmann perimeter, and final dark-adapted rod thresholds that were elevated 0.5 to 2 log units above normal to an 11° white test light in all regions tested with a Goldmann-Weekers adaptometer. Full-field rod ERGs to blue light were reduced 50% to 90% below our lowest normal amplitude (normal range: 100-275 nV); full-field cone ERGs to 30 Hz white flicker were normal in amplitude in the three siblings and reduced 50% to 75% below our lowest normal amplitude in the other two patients (normal range: 50-125 μV).5 All patients had delayed rod b-wave implicit times (normal range: 71-108 msec); and the oldest patient also had delayed cone b-wave implicit time (normal range: 25-32 msec).5

In the three siblings rod loss exceeded cone loss based on both ERG testing with full-field stimuli and psychophysical testing with 2° blue and red stimuli; however, they did not admit to night blindness at the age of testing. The other patient with complete penetrance had comparably reduced rod and cone ERG responses and comparable elevations of her rod and cone psychophysical thresholds; she acknowledged nightblindness by age 11. The patient with reduced penetrance also had rod and cone responses that were comparably reduced, and she reported that night-
blindness began at age 35. All patients were considered to have generalized rod disease based on their delayed rod b-wave implicit times and their elevated rod psychophysical thresholds across the retina. Informed consent was obtained from all patients prior to measurement of their ERG diurnal rhythms.

Patients occluded the nondominant eye from light with eye patches between the hours of 10 PM and 8 AM for at least 3 days prior to ERG testing (2–3 days being sufficient to maximize the rod ERG diurnal rhythm in normal observers) and for the testing days as well to maintain entrainment. Full-field rod ERGs were obtained from the entrained eye 15 min before the time of light onset (ie, 7:45 AM), 1.5 hr after light onset (ie, 9:30 AM), 8 hr after light onset (ie, 4 PM), and 13 hr after light onset (ie, 9 PM) for 4 to 5 consecutive days; before the 9:30 AM, 4 PM, and 9 PM test sessions the entrained eye was dark-adapted for 1 hr. Patients were exposed for 10 min at 8 AM to a full-field white light of 32 cd/m² to trigger the rhythm as described previously for normal subjects. At all other times between recording sessions these patients were exposed to ambient room light and were encouraged to wear their sunglasses when outdoors. Pupils were maximally dilated prior to testing, and pupil diameters were measured following testing for the calculation of stimulus retinal illuminances. Results from the five patients were compared to those from five light entrained normal subjects (ages 18–35) tested at the same times of day.

Responses were monitored at the topically anesthetized cornea with a bipolar Burian-Allen contact lens electrode, differentially-amplified at a gain of 10,000 (3 db down at 2 Hz and 300 Hz), and computer-averaged (n = 16 for normal subjects and n = 32 for patients with retinitis pigmentosa). Blue (λmax = 440 nm, 40 nm half-bandwidth) 10 μsec flashes of varying integrated scotopic retinal illuminance (0.0 log scot td-sec maximum for the normal subjects and 0.4 log scot td-sec maximum for the patients as determined with an electronic photometer for pulse stimuli) were presented at 0.5 Hz to elicit rod-isolated b-wave responses. For the normals and patients, respectively, the maximum blue stimulus elicited rod-isolated responses because in each instance a red light (λ50% cut-off = 605 nm), photopically matched to the blue light, elicited no b-wave responses. Higher retinal illuminances were not used because they elicited a detectable, short latency cone contribution combined with the slower rod response to blue flashes which could have confounded the interpretation of results. Peak-to-peak b-wave amplitudes (ie, baseline to maximum cornea-positive peak in four patients and cornea-negative to maximum cornea-positive peak in one patient) and implicit times (ie, time interval between flash onset and cornea-positive peak) were derived from the digital readout of the computer.

For both the patients and the normal subjects the brightest stimulus did not elicit a maximum rod b-wave. It was, therefore, not possible to separate changes in Vmax, the asymptotic peak amplitude, from changes in σ, the semisaturation retinal illuminance, as a function of time of day according to the equation, V/Vmax = 1/(I/I0 + σ). The data were instead fitted to both a semilogarithmic model (ie, V = k log I, used previously for ERG diurnal rhythms in normal human subjects, and a linear model where I is much less than σ (ie, V = k I0), since the x-intercept did not change with time of day (see Results, Table 1); a change in slope at a particular time of day meant that response amplitudes to all stimuli were proportionally altered and is compatible with a change in total sensitivity (ie, Vmax/σ in the linear model). B-wave sensitivity was quantified for the semilogarithmic model with respect to slope (ie, ΔV/Δlog I), since the x-intercept did not change with time of day (see Results, Table 1).
Results. Rod ERGs from the light entrained eye of a representative normal observer on a single day of testing (Fig. 1A) illustrate b-wave amplitudes 10 to 15% smaller at 9:30 AM (ie, 1.5 hr after light onset) than at the other times of day. In contrast, rod ERGs from the light entrained eye of a representative patient with dominant retinitis pigmentosa on a single day of testing (Fig. 1B) illustrate b-wave amplitudes reduced 30% to 40% both at 9:30 AM and 4 PM relative to the two other times of day.

Log slopes (mean ± SEM) for data fitted to the semilogarithmic model (ie, $V = k \log I$) are graphed in Figure 2A for the normal group and for the patients with dominant retinitis pigmentosa at the four times of day. Analyses of variance showed significant effects of time of day for both groups (Table 1). Since mean log slope was not significantly different at 7:45 AM (ie, the end of the dark period) and 9 PM (ie, the end of the light period) for both the normal subjects and the patients with retinitis pigmentosa, slopes from these two times of day were averaged for each subject and considered as “baseline” for t-test comparison with the slopes at 9:30 AM (ie, 1.5 hr into the light period) and 4 PM (ie, 8 hr into the light period). At 9:30 AM mean slope was reduced 10% (±3%) below baseline for the normal subjects (paired t-test based on log slopes, $P = 0.01$) and 29% (±5%) below baseline for the patients (paired t-test, $P = 0.007$); the difference between means was significant (t-test, $P = 0.02$). At 4 PM mean slope was reduced 2% (±3%) below baseline for the normal subjects (paired t-test, $P = n.s.$) and 35% (±8%) below baseline for the patients (paired t-test, $P = 0.02$); and the difference between means was again significant (t-test with Welch’s correction for unequal variances, $P = 0.02$).

Log $I_{threshold}^{-1}$ (mean ± SEM) for data fitted to the linear model ($V = k I$) are graphed in Figure 2B for the normal group and for the patients with dominant retinitis pigmentosa at the four times of day. Again, analyses of variance showed significant effects of time of day for both groups (Table 2), and the values at 7:45 AM and 9 PM were comparable and could be combined as baseline. At 9:30 AM mean $I_{threshold}^{-1}$ was reduced 0.11 (±0.03) log unit (ie, 22%) below baseline for the normal subjects (paired t-test, $P = 0.02$) and 0.21 (±0.03) log unit (ie, 38%) below baseline for the patients (paired t-test, $P = 0.002$); this difference between means was also significant (t-test, $P = 0.03$). At 4 PM mean $I_{threshold}^{-1}$ was reduced 0.07
(±0.03) log unit (ie, 15%) below baseline for the normal
subjects (paired t-test, P = n.s.) and 0.20 (±0.05)
log unit (ie, 37%) below baseline for the patients
(paired t-test, P = 0.01); and the difference between
means was again significant (t-test, P = 0.04).

Figure 3 shows the mean data (solid circles) and
fitted regression line (solid line) for rod b-wave implicit
time vs. log amplitude at baseline (ie, mean of
7:45 AM and 9 PM) for a representative patient. The
increased implicit time expected if the amplitude re-
donstriction occurred at 9:30 AM and 4 PM combined
due to a loss of visual pigment is shown by the
open circle on the regression line. The implicit
time observed at 9:30 AM and 4 PM combined, indi-
cated by the solid square, was shorter than the im-
plexit time expected due to loss of visual pigment. The
mean (±SEM) values for expected minus observed implicit
times were 2.5 msec (±0.8 msec) for 9:30 AM based on all five normal subjects and 3.9 msec
(±1.2 msec) for 9:30 AM and 4 PM combined based on
all five patients with retinitis pigmentosa; both
differences were significant (paired t-tests, P = 0.01
and P = 0.03, respectively).

Discussion. This study demonstrates that some
light entrained patients with dominant retinitis pig-
mentosa have abnormally large reductions in rod
ERG b-wave sensitivity 1.5 hr and 8 hr after light
onset; the sensitivity of these patients as a group re-
covered to (or nearly to) the baseline level by 13 hr after light onset while the sensitivity of the normal subjects recovered to (or nearly to) the baseline level by 8 hr. Abnor-
mally reduced rod ERG sensitivity has been recently
reported at 8 hr following light onset in patients with
recessive and isolate forms of retinitis pigmentosa.9
Further studies are needed to determine whether ab-
normal rod ERG diurnal rhythms are characteristic
of all patients with progressive forms of retinitis pig-
mentosa.

Since comparable changes in the rod a-wave and
b-wave were reported previously for normal human1
and rat2 diurnal rhythms, it is likely that the changes
in the rod b-wave in the patients with dominant reti-
nitis pigmentosa occurred as a consequence of
tings in rod photoreceptor function, although
some change in inner retinal function cannot be ex-
cluded. Changes in the rod b-wave have also been
related to outer segment disc shedding in the rabbit.10
The present methodology could not distinguish re-
ductions in \( V_{\text{max}} \), the asymptotic peak amplitude,
from elevations in \( \sigma \), the semisaturation retinal illu-
miance, as contributing to the sensitivity losses fol-
lowing light onset. However, studies on the diurnal
rhythm of the pigmented rat showed only changes in
\( \sigma \), raising the possibility that changes in \( \sigma \) over the
day occurred in the human subjects.

**Table 2. Analyses of variance for log threshold of**

**V-I**

**functions for light-entrained normal subjects**

and patients with dominant retinitis pigmentosa**

<table>
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* Subjects were entrained to 8 AM light: 10 PM dark and tested at 7:45 AM, 9:30 AM, 4 PM, and 9 PM.

The reductions in rod ERG amplitudes in the pa-
ients at 9:30 AM and 4 PM and in the normal sub-
jects at 9:30 AM were associated with implicit times
that were shorter than those expected from loss of
visual pigment at these times of day (eg, Fig. 3). This
does not support the idea that the large loss in sensi-
tivity in the patients was simply due to reduction of
the normal amount of visual pigment from already
shortened rod outer segments. The absence of a diur-
nal variation in the x-intercept of the patient’s V-log I
functions is also consistent with this idea, since a re-
duction in optical density would have been expected
to increase the intercept (ie, shift the functions to the
right).

The cellular events that govern the reduction in
ERG sensitivity of light entrained humans and rats
following light onset are not known. They could in-

**Fig. 3.** Observed (filled square) and expected (open circle) rod
b-wave implicit times for the brightest stimulus at 9:30 AM and 4
PM combined for a 32-year-old patient with dominant retinitis pig-
mentosa. Expected implicit time was derived from the linear
regression of implicit time on log amplitude (solid line) for data at
7:45 AM and 9 PM combined (filled circles), taking into account
the mean reduction in amplitude at 9:30 AM and 4 PM.
The rat retina can be successfully grafted within a long time period which extends into the first 2 weeks of postnatal life. Postnatal grafts taken 1-2 days (PN 1-2) after birth demonstrated no significant differences in their ability to form successful grafts. However, grafting success begins to diminish gradually starting between PN 2-4 and reaches a low point in organization and survival by PN 14. PN 21 grafts rapidly degenerate by 1 to 2 days after transplantation. Although early postnatal retinal tissue can be successfully grafted, E 15 embryonic retinas make better grafts for their ability to form consistent laminae and to integrate with host tissue in a fresh lesion paradigm. Invest Ophthalmol Vis Sci 29:498–503, 1988

References


Donor Age Influences on the Success of Retinal Grafts to Adult Rat Retina

Robert Aramanr, Magdalene Seiler, and James E. Turner

The rat retina can be successfully grafted within a long time period which extends into the first 2 weeks of postnatal life. Postnatal grafts taken 1-2 days (PN 1-2) after birth demonstrate no significant differences in their ability to form successful grafts. However, grafting success begins to diminish gradually starting between PN 2-4 and reaches a low point in organization and survival by PN 14. PN 21 grafts rapidly degenerate by 1 to 2 days after transplantation. Although early postnatal retinal tissue can be successfully grafted, E 15 embryonic retinas make better grafts for their ability to form consistent laminae and to integrate with host tissue in a fresh lesion paradigm. Invest Ophthalmol Vis Sci 29:498–503, 1988

The eye has been successfully used numerous times as a recipient of tissue grafts. However, almost all intracocular graftings have dealt with nonretinal tissue grafts placed into the anterior chamber where the iris offers a rich vascular substrate. With the exception of one earlier report, only recently has the fetal retina been grafted into the anterior chamber where it survived and continued to differentiate. Although retinal grafting has been demonstrated to be a simple and highly successful technique, no attempts in the past have been made to graft these cells into an adult retinal penetrating lesion site in order to initiate repair.