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ELECTRORETINOGRAPHIC RESPONSES OF THE GREAT HORNED OWL (*Bubo virginianus*)

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ABSTRACT.—Electroretinograms were recorded from four Great Horned Owls (*Bubo virginianus*). Two procedures, dark adaptation and flicker stimuli, were used to assess the contributions of rod and cone systems to electroretinograms. Recordings obtained after dark adaptation demonstrated scotopic (rod-generated) components. *B*-waves were broad and rounded and had a fairly long latency. When high intensity single-flash stimuli were used, *b*-waves had shorter latencies, and prominent *a*-waves were present, indicative of the addition of photopic (cone-generated) activity. Photopic activity was more clearly demonstrated with flicker ERGs. Scotopic fusion frequency was approximately 16 Hz. Photopic fusion frequencies were in the range of 35–45 Hz. The Great Horned Owl retina functions optimally during low luminance levels at night. However, the presence of a functional photopic system allows this owl to also function in brighter luminances of day.

The avian retina has been described by some as the ultimate in retinal organization (Walls 1942; Polyak 1957). Avian retinae, as with those of mammals, contain receptors for dim light (rods) and receptors for bright light and colors (cones). In general retinae of diurnal birds are dominated by cones while nocturnal birds possess retinae with a large number of rods and few cones (Walls 1942; Duke-Elder 1958). In owls which as a group are typically nocturnal, vision has retained the same importance as for their diurnal relatives. Owl retinae have been examined histologically (Bornshein and Tansley 1961; Hocking and Mitchell 1961; Oehme 1961; Fite 1973; Yew et al. 1977; Bowmaker and Martin 1978) and found to have high numbers of rods as would be expected for nocturnal animals. However, Fite (1973) and Oehme (1961) stress that in spite of the predominance of rods owl retinae contain approximately seven to eight percent cones, even in the most nocturnal species. Relative contribution of the cone component to retinal function has not been studied.

A preliminary study (Ault 1984) suggested that the Great Horned Owl retina produced ERGs that were qualitatively similar to those of other nocturnal vertebrates and dominated by scotopic (rod-generated) components, but under appropriate stimulus conditions some photopic (cone-generated) components were also present. However, the sample size of the study (N = 1) was too small to reliably evaluate ERG responses of the species. Martin (1982) suggested that eyes of owls and humans function similarly over a wide range of naturally occurring luminance levels as a result of their optics and structure. If so, then likely the Great Horned Owl ERG, as in the human, should reveal a duplicity of function possessing both scotopic and photopic components. Functional duplicity is also expected in light of the morphological confirmation of both rods and cones within the Great Horned Owl retina (Oehme 1961; Fite 1973). The objectives of this study were to record and quantitatively analyze ERGs from several Great Horned Owls in order to provide insight into the relative contributions of rods and cones to ERG response.

METHODS

Subjects. A total of eight retinae from four injured and unreleasable Great Horned Owls provided data for this study. Owls were sedated with 10 mg/kg acepromazine maleate (Ayerst Laboratories) administered intramuscularly and anesthetized with 80 mg/kg ketamine HCl (Ketaject®, Bristol Laboratories) administered intramuscularly. Anesthesia generally causes only slight reduction in amplitude of ERG components (Armington 1974). Eyes were examined ophthalmoscopically prior to testing and did not possess any significant ocular lesions. Subjects recovered fully after approximately 24 hr with no apparent after effects.

Apparatus and Procedures. Light source for dark adaptation tests was a Kodak Carousel projector with a 300 watt bulb. Single-flash stimuli were achieved by alternating opaque filters with empty slots in the carousel. Starting with an opaque filter, the carousel was rotated through an empty slot to the next opaque filter producing an intense flash of light of approximately 200 msec duration. Light was channeled through a slide holder and focused onto a 3 mm dia fiber optic light guide which was inserted into a black box containing the anesthetized owl. The light guide was brought to within a few millimeters of the cornea. Care was taken to insure that the light guide was aligned as closely as possible with the optical axis of the eye. Maximum luminance (i.e., intensity or brightness)

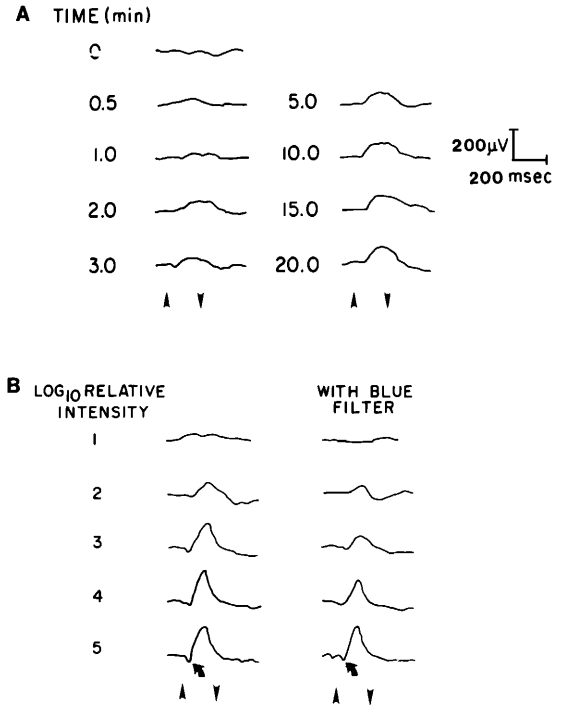

Figure 1. A) Representative Great Horned Owl electroretinograms during dark adaptation. Owl was pre-adapted to constant light of 1.9 cd/cm^2 for five min. Time indicates minutes after pre-adaptation light was shut off and dark adaptation began. Up arrowhead indicates stimulus on; down arrowhead indicates stimulus off. Stimulus: 1.9 cd/cm^2 attenuated with a 1 log unit neutral density filter and Wratten #47 blue filter. Note rise in *b*-wave amplitude as time progresses. B) Representative Great Horned Owl electroretinograms produced by single-flash stimuli without (left) and with (right) blue filter. Up and down arrowheads indicate stimulus as above. Relative intensity of five indicates maximum luminance of 1.9 cd/cm^2. Note *b*-wave increase with increase in intensity. *A*-waves (arrows) appear at high intensities.

of the light source measured by photometer at the cornea was approximately 1.9 cd/cm^2 and is roughly equivalent to the brightness of a clear sky at noon. Signals from the electrodes were channeled into a Grass 7P1-A preamplifier and tracings were recorded on a Grass Model 7 oscillograph.

Light source for flicker tests was a Grass Model PS33 photic stimulator interfaced with the fiber optic system described above. Maximum luminance measured at the cornea was approximately 0.45 cd/cm^2. The cornea was desensitized by topical application of Lidocaine HCl (Wyeth Laboratories) and a metal-plated mylar electrode was placed on the cornea (Chase et al. 1976). A needle reference electrode was inserted into skin of the ear canal immediately posterior to the eye and a needle ground electrode was inserted into skin of the wing. Pupil size was monitored throughout the recording session and remained sufficiently dilated to allow a maximum amount of light to enter the eye.

We recorded the responses of the retinae to both dark adaptation and flicker tests. The dark adaptation test was used to assess scotopic recovery of the retinae following exposure to bright light. In the dark adaptation test the retina was pre-adapted to constant light of 1.9 cd/cm^2 for five min. After five min, pre-adaptation light was shut off. Single-flash stimuli attenuated with a 1.0 neutral density filter and a Wratten #47 blue filter were delivered at various intervals to the retina. After owls were fully dark adapted (approximately 45 min), single-flash stimuli of increasing intensities (removal of neutral density filters), both with and without a blue filter, were delivered in succession.

The second test utilized flickering stimuli of various intensities and flicker frequencies to determine cutoff point between scotopic and photopic systems. Maximum luminance measured at the cornea was approximately 0.45 cd/cm^2. Various neutral density filters, but no color filters, were used in the procedure.

RESULTS

During dark adaptation, mean *b*-wave amplitude increased rapidly from an average of 7.8 μV at the beginning of dark adaptation to an average of 88.6 μV at five min into dark adaptation. After the first five min, *b*-wave amplitudes increased at a slower rate and eventually reached an average amplitude of 120.3 μV at approximately 20 min. Representative dark adaptation ERGs from one owl are shown in Figure 1A. *B*-waves were broad and rounded with

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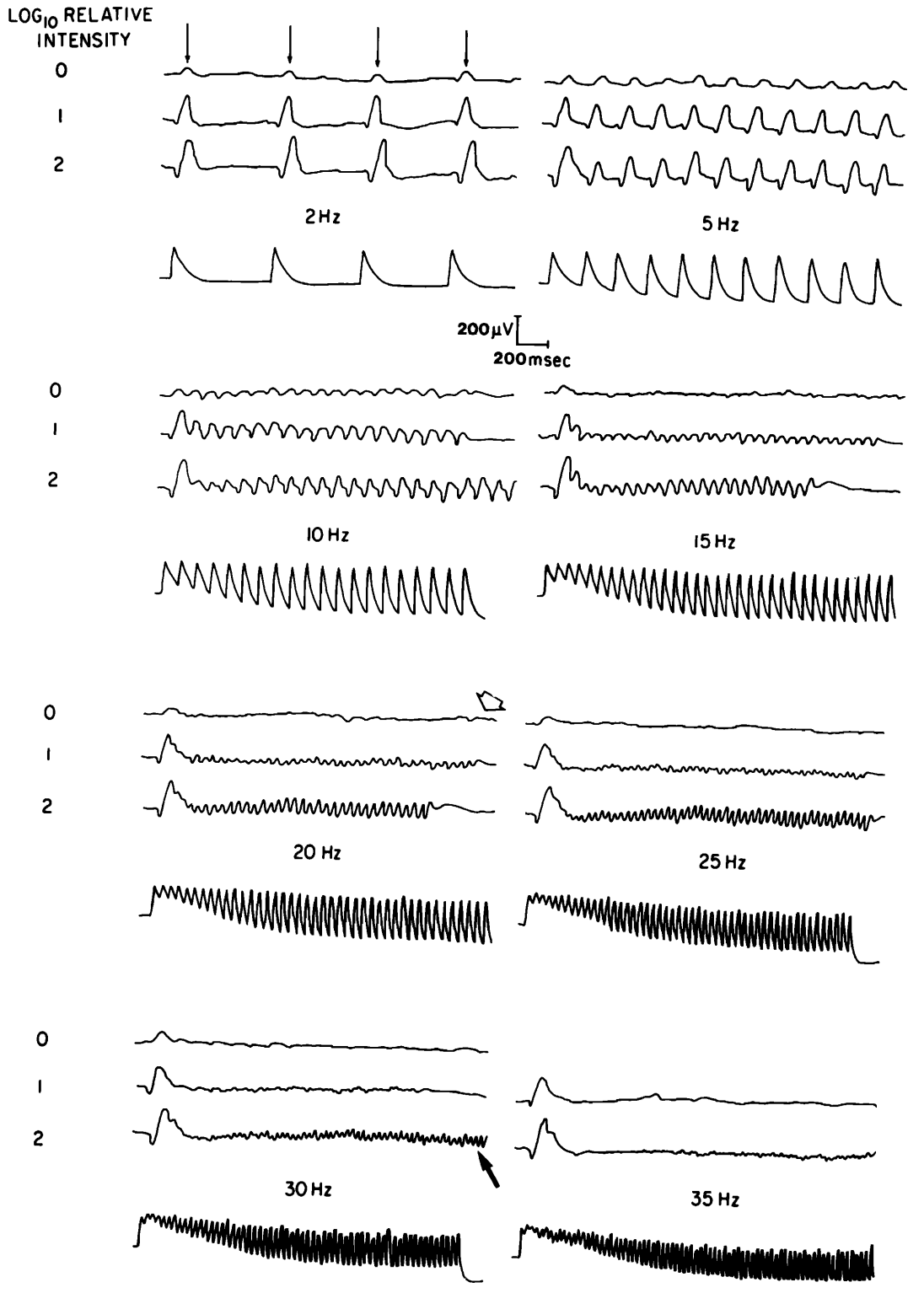
Figure 2. Representative Great Horned Owl flicker electroretinograms at various frequencies. Relative intensity of two indicates maximum luminance of 0.45 cd/cm^2. Stimulus tracings are shown below each frequency label. Note one-to-one correspondence of ERG response with two Hz stimulus at all intensities (thin arrows). As intensity and/or flicker frequency is increased, one-to-one response is reduced and eventually lost or "fused" (open arrow, for example). Note also one-to-one response at high intensity and high flicker frequency (thick arrow).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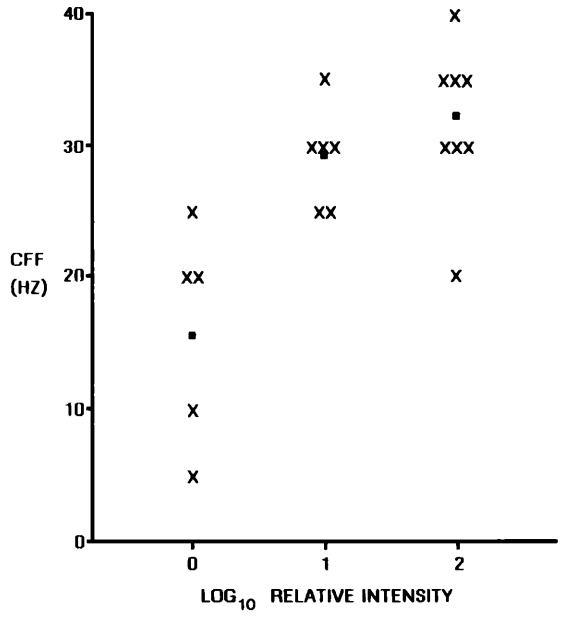

Figure 3. Critical fusion frequencies (CFF) plotted as a function of stimulus intensity. CFF was determined as the frequency at which there was no longer a one-to-one correspondence with the stimulus. Closed squares indicate the average CFF for each stimulus intensity; X indicates individual samples. A significant difference occurs between average CFF obtained at relative intensity of zero and average CFF values for relative intensities of one and two. Average CFF for relative intensities of one and two were not significantly different from each other (Dunnett's t-Test; $P < 0.01$ for all between group comparisons). Relative intensity of two represents maximum stimulus luminance of 1.9 cd/cm^2.

a fairly long latency (average latency = 113.2 msec) and a-waves were either very reduced or absent.

Figure 1B shows representative ERGs produced with single-flash stimuli of increasing intensity, both with and without a blue filter, at the end of dark adaptation. In the case without a blue filter b-wave amplitudes increased linearly from an average of 77.2 μV at lowest stimulus intensity to an average of 305.8 μV at highest stimulus intensity. The same pattern was evident when a blue filter was inserted. B-wave amplitudes also increased linearly from an average of 63.3 μV at lowest stimulus intensity to an average of 254.5 μV at highest stimulus intensity. Average b-wave latency decreased with increasing intensity both with and without a blue filter. In the case without a blue filter average latency ranged from 110 msec at lowest stimulus intensity to an average of 71 msec at highest stimulus intensity. In the case where the blue filter was inserted average latency ranged from 155 msec at lowest stimulus intensity to an average of 94 msec at highest stimulus intensity. A-waves also became more prominent as stimulus intensity was increased.

Figure 2 shows representative flicker ERGs from one owl. At low light intensities and low flicker frequencies ERG waveforms were evident when responding to stimuli on a one-to-one basis. An eventual loss of the one-to-one response occurred as intensities and flicker frequencies increased. Critical fusion frequencies (CFF) for each stimulus intensity were determined for all owls (Fig. 3). Analysis with Dunnett's t-Test showed that there was a significant difference between the average CFF obtained at 0.0 log units intensity (16.0 $\pm$ 3.7 S.E.) and average CFF values for 1.0 log units intensity (29.2 $\pm$ 1.5 S.E.) and 2.0 log units intensity (31.9 $\pm$ 2.1 S.E.). However, average CFF values for 1.0 and 2.0 log units intensity were not significantly different ($P <$ 0.01 for all between group comparisons).

Interestingly, the initial flicker ERG waveform changes shape as intensity and frequency are increased. Increase in b-wave amplitude and decrease in b-wave latency occurs, and a-waves also become more prominent.

DISCUSSION

Shape of the ERG waveform depends upon relative contributions of scotopic and photopic signals being propagated to inner retinal layers. In duplex retinae such as in the human, for example, the (b-wave is often composed of two components ($b1$ and $b2$), with different latencies; the short latency $b1$ component corresponds with photopic activity while the longer latency $b2$ component corresponds with scotopic activity (Brunette 1969). The $b1$ component can be isolated with the use of longer wavelength (i.e., red) stimuli while $b2$ components can be isolated with shorter wavelength (i.e., blue) stimuli. In the Great Horned Owl recovery during dark adaptation was dominated by scotopic processes manifested by slow rising b-waves which were fairly broad and had relatively long latencies. Since stimulus parameters used in dark adaptation generally elicit scotopic activity primarily, a photopic $b1$ component was not seen in this study during dark ad-

aptation. With different pre-adaptation and stimulus parameters a duplex retina ordinarily will demonstrate an early and transient photopic recovery indicated by *b*-waves with shorter latencies (dominated by the *b*1 component) followed by scotopic recovery.

Higher intensity single-flash stimuli without a blue filter produced ERGs with more prominent *a*- and *b*-waves. *B*-waves were also narrower and had a shorter latency, suggesting the presence of a photopic component which was contributing to overall response. Higher intensity stimuli were presumably able to initiate a cone response. Change in amplitude and latency of the *b*-wave and appearance of the *a*-wave as stimulus intensity is increased were similar to results obtained from cat (Brown 1968; Niemeyer 1976), rabbit (Ikeda 1966) and horse (Wouters et al. 1980) retinae. At low stimulus intensities the *b*-wave is broad and rounded and there is no *a*-wave, indicating primary activity produced by the scotopic system. At higher stimulus intensities the *b*-wave increases in amplitude and becomes steeper and more pointed. Also, *a*-waves become more prominent, indicating addition of a photopic component that contributed to overall response. In animals that have an essentially pure cone retina ERGs show an extremely high amplitude *a*-wave and very short latency *b*-wave composed almost exclusively of the *b*1 component (Tansley et al. 1961). Addition of a blue filter also increased average latency of the *b*-wave, suggesting that the photopic contribution was "filtered" out and the major contribution to the waveform was from the scotopic system.

Results from the flicker procedure more convincingly demonstrate the existence of a cone component in the retina of these owls. Large differences in CFF from low to high intensities is an indication of a shift from scotopic to photopic functions. A one-to-one response seen at low intensities and low flicker frequencies was produced primarily by scotopic activity. Rods were following the individual flicker, having not yet exceeded their critical fusion frequency. Loss or fusion of the one-to-one response occurred at fairly low flicker frequency. At higher intensities the one-to-one response fused at significantly higher frequencies, an indication of a switch to photopic activity since cones possess a higher critical fusion frequency than rods (Armington 1974; Fishman 1975). Such results are quite common in animals known to have mixed retinae, including humans (Armington 1974).

The ERG results confirm the expectation that the Great Horned Owl retina possesses a significant scotopic component. In addition a photopic component is present but is only evident with proper stimulus parameters. These results suggest that the Great Horned Owl retina is composed primarily of rods but also contains some cones as has been confirmed anatomically by previous light-microscopic observations (Oehme 1961; Fite 1973; Ault 1984).

The Great Horned Owl retina is dominated by scotopic processes which certainly impart an increased sensitivity to low light levels typically encountered. However, the owl is often active during the day and the few cones present may help mediate vision in more intense illumination of daylight hours. In a detailed study of optics and visual performance of the Tawny Owl (*Strix aluco*) Martin (1982) suggested that the resolving power of the eye of this owl and the pigeon (*Columba* sp.) were in fact very similar at photopic and mesopic luminances. The Tawny Owl has superior acuity to the pigeon at lower luminance levels, and although photopic acuity of both species are quite similar, acuity declines much faster in lower luminances for the pigeon than the owl (Martin 1982). Briefly stated, the pigeon by virtue of its optics and eye structure cannot function in lower luminance levels, while the owl by virtue of its optics and eye structure can function over a wide range of luminance from scotopic to photopic.

Additionally, specific neuroanatomical arrangements of photoreceptors contribute to spatial resolution and visual acuity in the Great Horned Owl. Foveal rods, which have a lower convergence ratio, give increased ability for point-to-point resolution at higher luminances, while non-foveal rods which have higher convergence ratios and higher absolute sensitivity may be serving this function at lower luminances (Fite 1973).

These observations, coupled with duplicity in retinal functioning revealed in this study, suggest that the Great Horned Owl is not only an effective nocturnal predator but is able to expand activity into the "diurnal realm" if needed.

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