ELECTRORETINOGRAMS AND RETINAL STRUCTURE OF THE EASTERN SCREECH OWL (*Otus asio*) AND GREAT HORNED OWL (*Bubo virginianus*)

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ABSTRACT — Electroretinograms (ERGs) were recorded from 2 species of owls: Eastern Screech Owl (*Otus asio*) and Great Horned Owl (*Bubo virginianus*). Dark adaptation and flicker stimuli were used to determine retinal activity and to infer retinal structure. The dark adaptation results showed typical patterns associated with retinas composed primarily of rods. This was indicated by the late regeneration of the scotopic b-wave. Flicker ERGs, however, also indicated a residual cone component. This was indicated by the one-to-one response at high luminance levels and high flicker frequencies. The ERG data confirm existing histological observations of high rod numbers and few cones in the retinas of nocturnal owls.

Owl retinas have been examined histologically by a number of investigators (Bornshein and Tansley 1961; Hocking and Mitchell 1961; Oehme 1961; Fite 1973; Yew et al. 1977; Bowmaker and Martin 1978). All reported retinas with high concentrations of rods, as would be expected for basically nocturnal animals. However, Fite (1973) and Oehme (1961) point out that owl retinas do possess very small concentrations of cones, even in the most nocturnal species.

Few electroretinographic studies have been performed on owls. Bornshein and Tansley (1961) obtained ERGs from Short-eared Owl (*Asio flammeus*) and compared response of the retina to that of the pigeon. These authors also correlated the ERG results with histological preparations of the owl and pigeon retinas. The ERGs and the histological examination revealed a retina composed predominantly of rods in the Short-eared Owl. Martin and Gordon (1975) recorded ERGs from the nocturnal Tawny Owl (*Strix aluco*) to determine its retinal spectral sensitivity. The ERG data supported earlier findings (Martin 1974; Martin and Gordon 1974) that the Tawny Owl possesses a retina with cone receptors that are present in large enough numbers to contribute to the visual response.

The purpose of this investigation was to record electroretinographic activity of the Eastern Screech Owl and Great Horned Owl, two species not previously investigated. Correlation of the ERG data with retinal structure of these species was made in an attempt to better define the relative role of the rods and cones in the visual process of these owls.

MATERIALS AND METHODS

Subjects and Anesthesia. — One Eastern Screech Owl and one Great Horned Owl were used for electroretinography. Both were anesthetized with an anesthetic mixture containing Ketamine (10 mg/ml), Acepromazine (0.1 mg/ml), and Xylazine (1.0 mg/ml). Dosage was 1 ml/kg body weight, administered IM. Average duration of anesthesia was approximately 1 h.

Each subject was placed into a light-tight, electrically grounded box, and a corneal electrode was placed on the eye. Space between the cornea and electrode was flooded with a saline (0.9% NaCl) conducting solution. A reference electrode was placed in the skin of the ear flap or in the skin of the ear canal. A ground electrode was inserted in the wing skin. A fiber optic light guide was hlaced a few mm from the cornea.

Electroretinograph. — The light source was a 300 watt tungsten-halogen lamp that could deliver steady, single-flash, or flickering stimuli. Flicker stimuli were produced by a motor-driven disc that interrupted the light to give equal time on and off. The light beam was focused upon a fiber optic light guide which delivered light to the subject's eye in Maxwellian view (Armington 1974). The light beam wavelength and intensity were adjusted by the use of various color and neutral density filters. Unfiltered light intensity from this apparatus was approximately 1.076×10^4 millilamberts (mL) (1 mL = 0.001 lumens/cm^2).

The recording electrodes used were silver pedestal corneal contact lens systems. Reference and ground electrodes were silver skin needle probes. Signals from the electrodes were channeled through a Tektronix TM 504 pre-amplifier. The signal was amplified and displayed on a Tektronix 5103N dual-trace storage oscilloscope. Traces were permanently recorded by Polaroid photography.

Procedure. — Two tests commonly used in electroretinography, dark adaptation and flicker stimuli, were used. Dark adaptation tests were used to observe changes in the ERG as the retina adjusted to darkness. The eye was first pre-adapted to light for 5 min to insure bleaching of the photopigments. Pre-adaptation retinal illuminance was approximately 1.076×10^3 mL for the Eastern Screech Owl and 1.076×10^1 mL for the Great Horned Owl. Different intensities were used for both owls to assess the effectiveness of such intensities for pre-adaptation, single-flash (20 msec duration) stimuli attenuated with a Kodak #2 neutral density filter and a Kodak #26 gel film red filter, were delivered at widely spaced intervals (see Figs. 1-3) to the eye to observe the retina's increasing sensitivity to darkness. After 20-30 min, full-intensity single-flash stimuli of red (Kodak #26 gel film), blue (Kodak #47, 47A, 47B gel film) and white (no filters) were given successively to assess the degree of photopic (cone) and scotopic (rod) recovery.

The second test utilized flickering stimuli of various intensities and flicker frequencies. Various neutral density filters, but no color filters, were used.

Histology. — The retinas of a Great Horned and Eastern

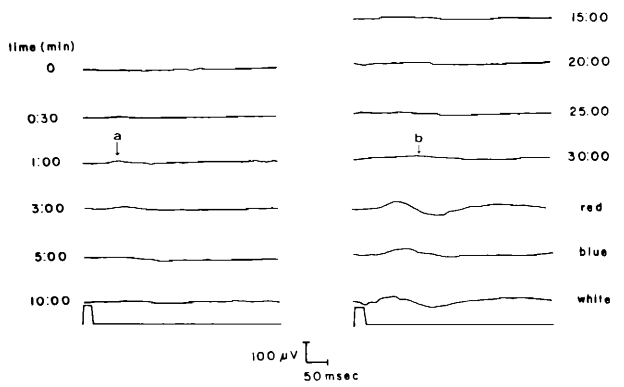

Figure 1. ERGs during dark adaptation in the Eastern Screech Owl. Time indicates minutes into dark adaptation. Stimulus: 1.076 x 10^4 mL attenuated with #2 neutral density filter and #26 red filter; 20msec duration. a) Slight regeneration of photopic b-wave after 1 minute into dark adaptation. b) Slight regeneration of scotopic b-wave after 30 minutes into dark adaptation.

Screech Owl were examined histologically. The Great Horned was the same animal used in the ERG study. The subjects were euthanized with lethal injection of Ketamine and enucleated. The posterior portion of the eye was cut away and fixed in Bouin's solution. The tissue was dehydrated in a graded ethanol series and cleared in cedarwood oil. Portions of peripheral retina were embedded in paraffin, sectioned meridionally at 5 m on a rotary microtome and stained with Hematoxylin and Eosin.

RESULTS

Dark Adaptation. — The ERGs from dark adaptation tests for both subjects showed very early low-amplitude responses which peaked at around 1-2 min into dark adaptation (Figs. 1a, 2a). Also, late appearing (between 20 and 30 min into dark adaptation) low-amplitude waveforms were observed (Figs. 1b, 2b). The final red, blue, and white stimuli produced waveforms of low amplitude.

Flicker Stimuli. — A change in waveforms were observed as the flickering stimuli were increased from low to high intensities and flicker frequencies; this was best demonstrated by the Eastern Screech Owl. At low intensities and low flicker frequencies, waves were evident as they followed the stimuli on a 1:1 basis (Fig. 3a). There was a fusion of this response as intensities and flicker frequencies increased, with a subsequent waveform taking over at high intensities and high flicker frequencies (Fig. 3b). The Great Horned Owl also displayed the above pattern, but with less clarity (Fig. 4).

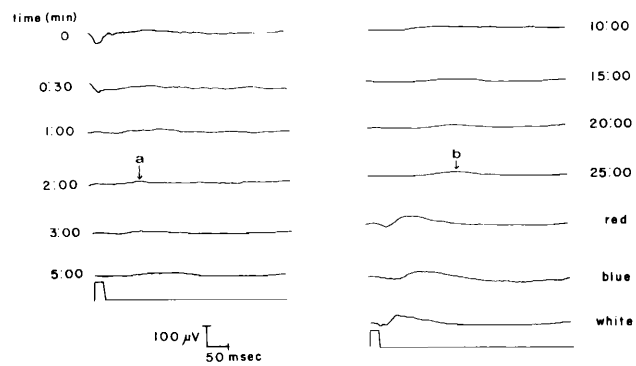

Figure 2. ERGs during dark adaptation in the Great Horned Owl. Time indicates minutes into dark adaptation. Stimulus: 1.076 x 10^4 mL attenuated with #2 neutral density filter and #26 red filter; 20 msec duration. a) Slight regeneration of photopic b-wave after 1-2 minutes into dark adaptation. b) Slight regeneration of scotopic b-wave after 20-30 minutes into dark adaptation.

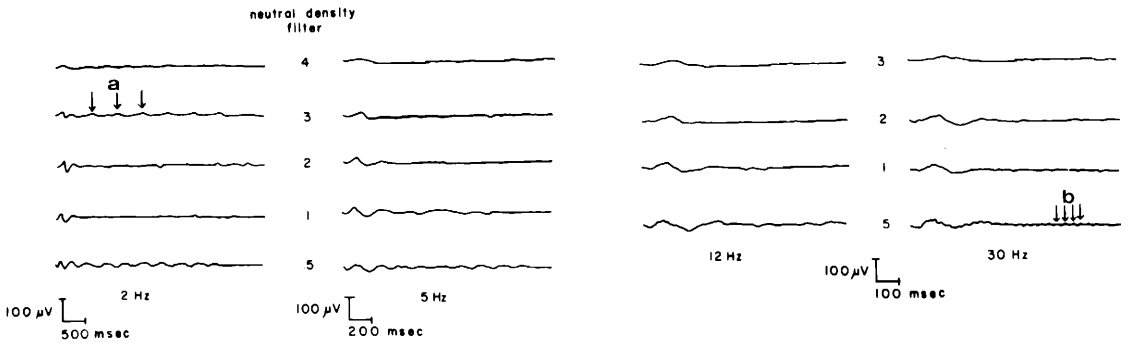

Figure 3. Flicker ERGs of the Eastern Screech Owl. a) Arrows indicate one-to-one response of waveforms to individual flickers at low intensity and low flicker frequency. b) Arrows indicate one-to-one response of waveforms to individual flickers at high intensity and high flicker frequency. 1.076 x 10^4 mL light source attenuated with indicated neutral density filters.

Histology. — The retinal layers of the Eastern Screech Owl could be clearly discerned histologically (Fig. 5). Retinas were composed primarily of rods; indicated by the elongated and cylindrical morphology of their outer segments in the receptor layer. The nuclei in the outer nuclear layer were also identified as rod nuclei because they were typically more elongated and were fairly evenly distributed throughout the outer nuclear layer (Walls 1942; Duke-Elder 1958). The rod nuclei of the Great Horned Owl were extremely elongated and closely packed.

A few cones were also seen in owl retinas. These were identified by their nuclei, which are typically rounder than rod nuclei and lie adjacent to the external limiting membrane. In all sections, cones were always few in number and were greatly outnumbered by the high density of rods.

DISCUSSION

The typical ERG waveform is composed of the a, b and c waves. The initial negative deflection (a-wave) is followed by a positive deflection (b-wave) normally of greater amplitude. The late-occuring

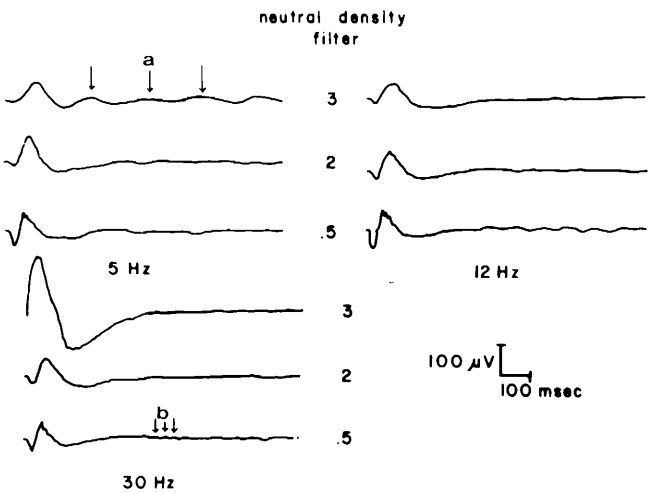

Figure 4. Flicker ERGs of the Great Horned Owl. a) Arrows indicate one-to-one response of waveforms to individual flickers at low intensity and low flicker frequency. b) Arrows indicate one-to-one response of waveforms to individual flickers at high intensity and high flicker frequency. 1.076 x 10^4 mL light source attenuated with indicated neutral density filters.

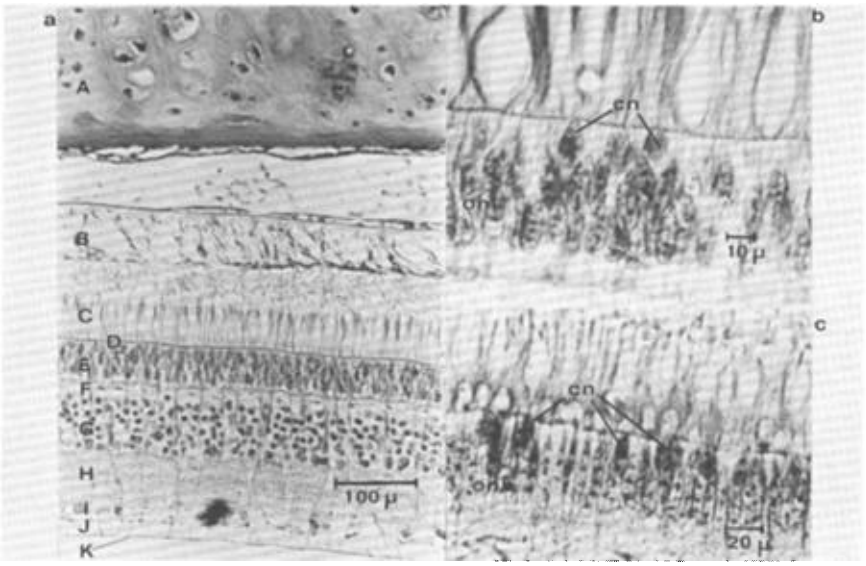

Figure 5. a). Layers of the Eastern Screech Owl retina. A) cartilaginous cup; B) pigment epithelium; C) receptor layer; D) external limiting membrane; E) outer nuclear layer; F) outer plexiform layer; G) inner nuclear layer; H) inner plexiform layer; I) ganglion cell layer; J) nerve fiber layer; and K) internal limiting membrane (200x); b) Eastern Screech Owl and c) Great Horned Owl retinas. cn = cone nuclei; onl = outer nuclear layer (primarily rod nuclei). (787.5x and 500x respectively).

positive deflection (c-wave) is not commonly evaluated in comparative studies. Brown (1968) explained that the a-wave is produced in the receptor cell layer, the b-wave through bipolar cell activity, and the c-wave by metabolic activity of the pigment epithelium. The a- and b-waves can be further subdivided into photopic (cone generated) and scotopic (rod generated) components. In general, the photopic components have shorter latencies and steeper slopes than scotopic components (Armington 1974). These patterns were evident in the owls studied (Figs. 1, 2)

The dark adaptation results revealed typical patterns associated with retinas composed predominantly of rods. The late regenerating wave forms were most likely scotopic b-waves suggesting that rods were regenerating after having been bleached during light adaptation. Latency of these b-waves suggested the response was from the scotopic system. Longer latency or implicit time (approximately 100 msec) is indicative of a scotopic rather than photopic b-wave (approximately 50-70 msec). Earlier low-amplitude responses were probably cone responses because of their early appearance during dark adaptation. Latency of these responses was also shorter than the scotopic responses, again suggesting generation by a cone component. As dark adaptation progressed, these early responses diminished and were replaced by the scotopic responses. After 25-30 min, there was still no complete regeneration of the scotopic responses in either owl, denoting that many of the rods were not yet adapted to the dark. The reduced effect of the blue light on the scotopic system also verified this since blue light is primarily a rod stimulator. These observations suggested a retina predominated by rods, but with a small cone component. However, it could mean that the initial light adapting intensity was too high. This seems unlikely since an absence of complete regeneration of the scotopic response was also observed in the Great Horned Owl which was exposed to a lower light-adapting intensity.

The shift from scotopic to photopic systems during the flicker procedures was indicated by a decrease in latency and an increase in amplitude of the initial b-wave as flicker frequencies and intensities were increased. The initial a-wave also became more prominent at higher flicker frequencies and intensities, providing further indication of the shift to the photopic system (Armington 1974). The rods and cones were also able to follow the individual flickering stimuli. At low intensities and low flicker

frequencies, rods were able to follow individual flickers, having not yet exceeded their critical flicker fusion frequency. As intensity and/or frequency was increased, rods "fused" the stimuli. Fusion occurred when the receptors could no longer respond to individual flickers on a 1:1 ratio but instead responded to them as if there was one constant stimulus. At high intensities and high flicker frequencies, cone response became dominant and was able to follow individual flickers since they possess a higher critical flicker fusion frequency than rods (Armington 1974). Histological results in combination with the ERG data indicated that the retinas were predominantly composed of rods. This supports the findings of previous workers (Bornshein and Tansley 1961; Hocking and Mitchell 1961; Oehme 1961; Fite 1973; Yew et al. 1977; Bowmaker and Martin 1978) who histologically demonstrated a retina composed predominantly of rods in the Great Horned Owl and other owl species. However, histological results and ERG data also demonstrated the presence of a cone component that was small but active.

My results and those of Bowmaker and Martin (1978) and Martin and Gordon (1975) verify that the retina of owls, even the most nocturnal species, possess cones in numbers large enough to contribute to the visual process. Existence of such a cone component could be the remnants of an ancestral cone-dominated retina. Nocturnal owls such as screech and Great Horned Owls are occasionally active during the day. It is reasonable to assume that the few cones that are present contribute to the owl's visual process in the brighter illumination of daylight hours.

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