

Interactive report

# Diurnal daylight phase affects the temporal properties of both the b-wave and d-wave of the human electroretinogram<sup>☆</sup>

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Accepted 14 November 2000

## Abstract

Aspects of the anatomy and physiology of the cone pathway are known to vary according to the phase of the natural light cycle. Using a prolonged flash stimulus (~200 ms), we have examined the human electroretinogram (ERG) over a 24 h period. We report that whilst the a-wave of the photopic ERG does not alter, there are profound effects upon the implicit times of both the b-wave and d-wave components. Both components are significantly slower in the night-time period and systematically become faster (15–22% reduction in implicit time), reaching a peak at around midday. The daily variation in the temporal properties of the ERG is abolished by constant light, but is retained during constant darkness. The data suggest that the changes in the temporal properties of the cone pathway affect both cone-ON and cone-OFF pathways. This suggests that the diurnal effect is presynaptic to the second order neurones, and most likely resides in the cone synapse. © 2001 Elsevier Science B.V. All rights reserved.

*Theme:* Sensory systems

*Topic:* Retina and photoreceptors

*Keywords:* Electroretinogram; ERG; Diurnal; d-wave; b-wave; a-wave

## 1. Introduction

It has become increasingly evident that important aspects of retinal physiology and morphology are regulated by the activity of a local retinal clock [7]. Thus it has been shown in numerous studies that the anatomical and physiological properties of retinal rod and cone pathways can be regulated according to the phase of diurnal cycle, and many of these changes are maintained in the absence of external light cues. In this way, it has been shown that the temporal properties of cone pathways are affected by prolonged dark adaptation [5]. Furthermore, photopic flash electroretinogram (ERG) recording throughout the 24-h natural light cycle using a brief xenon flash stimulus (~50

μs), has established that the implicit time of the cone driven b-wave varies throughout in opposition to the changes in the ambient light level [2]. The previous studies also established that these changes in b-wave kinetics are not associated with changes in the cone light response itself, because cone adaptation was constant and these changes were not accompanied by any variation in the ERG a-wave. Cone photoreceptors contact three types of second order neurones; ON-bipolar cells, OFF-bipolar cells and horizontal cells. Whilst it is now generally accepted that the b-wave response to a brief flash is largely a reflection of ON-bipolar activity, currently we cannot say which of the second order neurones are modulated with respect to the diurnal cycle.

In order to address this question we have examined the steady state light-ON light-OFF ERG to a prolonged photopic stimulus (~200 ms), during the normal ambient 24-h diurnal light cycle. Measuring the ERG in this way, using a prolonged light stimulus permits a detailed analysis

<sup>☆</sup>Published on the World Wide Web on 15 December 2000.

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of the a-wave, b-wave (light-ON) and d-wave (light-OFF) components.

## 2. Materials and methods

In these studies ERGs were measured from 4 observers (MWH, ABM, NPD, AJ) aged 26–36, all of whom had normal vision. ERGs were recorded using skin electrodes (Biomed Ltd.) attached bilaterally beneath the eye. These were chosen, as in previous studies [2], to facilitate observer comfort over the prolonged experimental periods. The ERG stimulus was provided by a custom built Ganzfeld dome, built largely according to Sieving's design [10]. The stimulus consisted of a white light flash, 225 cd

$m^{-2}$  (duration 188 ms) presented upon a constant rod saturating blue/green background ( $30 \text{ cd m}^{-2}$ , Kodak 11,  $\lambda_{\text{max}}=511 \text{ nm}$ ), both of which evenly illuminated the entire globe. For each recording the stimulus was presented 50 times with 800 ms refractory period and the resulting evoked response averaged. ERGs were recorded using a CED (Cambridge Electronic Design) isolated amplifier (CED 1902) and digitised (CED  $\mu$ 1401). The recordings were bandwidth filtered (0.5–200 Hz) and post-processed using commercial software (Signal, CED). Additional post-recording digital high-cut filtering (150 Hz), was occasionally applied to the records as and when appropriate to reduce background noise.

In the experiments the observers were kept under the natural ambient lighting in a rural location (Imperial College, Silwood Park). The light levels were logged throughout using a photometer (Macam). ERGs were measured at typically hourly intervals, at which time the observers entered the lab and were dark adapted for 10 min prior to recording. This period was chosen to ensure constant short-term adaptation of the cone photoreceptors [2]. In constant light experiments, observers were maintained in a brightly lit laboratory ( $>250 \text{ lux}$ ), but were still dark adapted (10 min) prior to the recordings. In constant dark experiments, the observers had their experimental eye patched at dusk the previous day; they were then brought into the dark laboratory at midnight and kept there for the duration. During this time they were only occasionally exposed to a dim red safe-light (Kodak 1A). A typical evoked ERG waveform is illustrated in Fig. 1a. The amplitudes and implicit times of the principal components (a-wave, b-wave and d-wave) were measured for each observer.

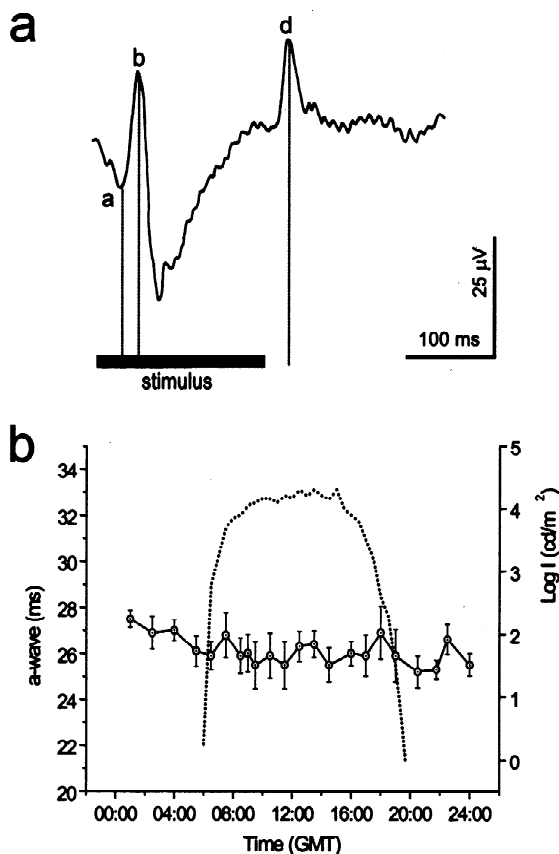


Fig. 1. (a) Typical averaged ERG waveform recorded from an observer in this study. Presentation of the light stimulus ( $225 \text{ cd m}^{-2}$ , 188 ms) is depicted by the solid bar beneath the recording. Following light-ON the ERG comprises of an initial a-wave (a) and b-wave (b). At light-OFF the ERG is characterised by a transient d-wave component (d). In these studies the amplitudes and implicit times of the three components were measured for each recording. a-wave and b-wave implicit times were measured from the onset of the stimulus, whilst the d-wave was measured from the cessation of the stimulus. (b) ERG a-wave implicit time (open symbols, mean  $\pm$  1 S.E.M.) for the four observers plotted as a function of time (GMT, Greenwich Mean Time–h). Also plotted is the mean ambient luminance (dotted line). Note that despite the large variation in ambient light and retinal light history the a-wave response remains constant throughout.

## 3. Results

Analysis of the a-wave components revealed no temporal or amplitude variation in the ERG a-wave in any of the observers through the 24-h cycle. The mean a-wave implicit time is plotted as a function of time in Fig. 1b, together with the plot of the ambient daylight. Similarly analysis of the amplitude of the b-wave component again revealed no systematic variation related to the diurnal light cycle. In contrast, the implicit time ( $\Delta t$ ) of the b-wave was found to vary significantly in 5 of the 6 eyes studied. The b-wave implicit times recorded during the daytime period were consistently shorter than those recorded at night. In order to reduce observer variation in the baseline implicit times, we normalised the responses from each eye according to the longest recorded night-time b-wave implicit times, then averaged the b-wave changes ( $\Delta t$ , ms) and plotted them as a function of time of day (Fig. 2a). The results show that the implicit time of the b-wave response varies systematically throughout the 24-h recording period and is shortest during the middle of the daylight hours.

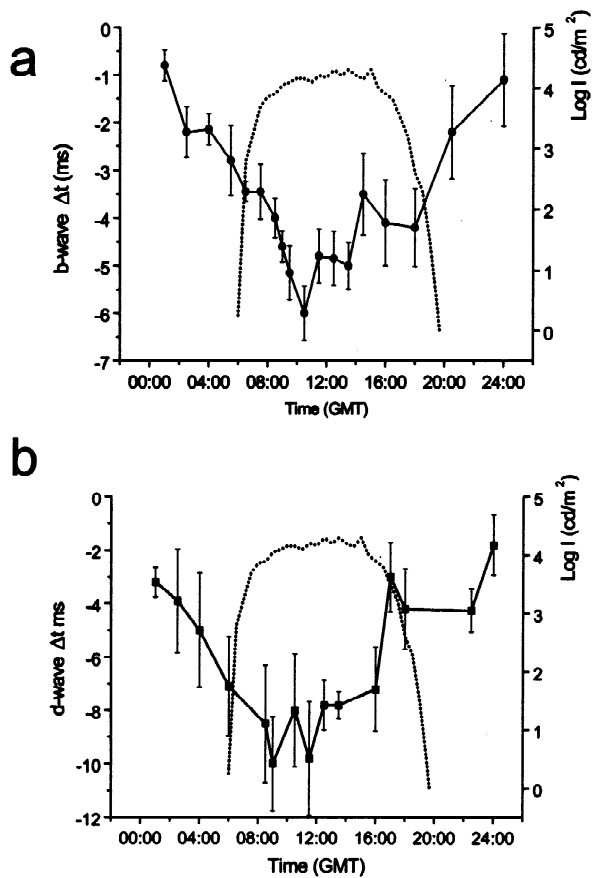


Fig. 2. (a) ERG b-wave normalised implicit time ( $\Delta t$ , ms  $\pm 1$  S.E.M.) plotted as a function of time (GMT, h) for the four observers. Note that the b-wave implicit time varies systematically through the 24-h cycle. The b-wave is slowest during the dark/night period and is fastest around the mid-day/light period. (b) ERG d-wave normalised implicit time ( $\Delta t$ , ms  $\pm 1$  S.E.M.) plotted as a function of time (GMT, h) for the four observers. The d-wave implicit time (measured from the stimulus-OFF) varies systematically through the 24-h cycle. Again the temporal changes are in opposite phase to the ambient light level. Fastest responses were attained in the middle day periods for all the observers.

When we compared the b-wave implicit times recorded during the middle 4 h of daylight (11:00–15:00) with those recorded in the middle 4 h of subjective night (23:00–03:00) we found that the mean b-wave implicit time differed by some 7 ms. Similarly, the differences between the fastest and slowest b-wave responses for the individual observers ranged from 6.5 ms to 7.8 ms. The mean daytime value ( $\pm 1$  S.E.M.) was  $42.4 \pm 0.34$  ms, and during the night it was  $49.7 \pm 0.61$  ms, and this difference was statistically significant (*t*-Test,  $P < 0.001$ ).

We also examined the properties of the d-wave response at light-OFF, again measurements of the amplitude of the d-wave response revealed no systematic variation in any of the observers. However, we did find a consistent variation in the d-wave implicit time, as measured from the cessation of the light stimulus. Again the slowest responses were measured in the night-time recordings and the d-wave response was fastest in the middle of the day. The mean

data for our observers, again normalised in respect to the longest implicit times, is shown in Fig. 2b. Examining the mean implicit times for the day and night periods (11:00–15:00 and 23:00–03:00) established a mean daytime value of  $27.3 \pm 0.51$  ms, compared to  $35.0 \pm 0.37$  ms at night, and this difference was statistically significant (*t*-Test,  $P < 0.001$ ).

The magnitude of the change in d-wave implicit time ( $\sim 22\%$ ) was greater than that observed for the b-wave ( $\sim 15\%$ ). Furthermore, comparisons of the temporal oscillations suggest that the d-wave changes are marginally phase advanced compared to that seen in the b-wave responses, however, preliminary cosinor analysis of the data failed to establish that this difference was significant. It is also evident that the b-wave and d-wave changes are not just simply phase locked to the ambient light level. In two of the four observers a small amplitude secondary light-OFF response ( $d_2$ -wave) was variably observed. However, this  $d_2$  wavelet was of small amplitude and it proved impossible to resolve any systematic variation in this ERG component.

We examined further the dependence of the temporal changes upon the natural ambient light cycle using a constant light study. In this case the responses of 4 subjects (4 eyes) were examined over a 12-h period beginning in late morning. The subjects were maintained in a constant bright environment throughout. Recordings were taken every 2 h until around midnight. The results showed that the implicit time of the b-wave and d-wave responses were effectively clamped at a normal daytime level throughout the experimental constant light period (Fig. 3a). In three subjects (3 eyes) the effects of constant darkness were examined in experiments that began at midnight through to early afternoon (Fig. 3b). At the start of the experiments the ERG b-wave and d-wave began at typical night-time latencies. Then, even though the subjects were held in complete darkness, the ERG b-wave and d-wave times began to shorten and approach the typical daytime values during the period of expected day. These changes were not accompanied by any significant change in the photoreceptor a-wave response.

#### 4. Discussion

In these experiments we have used a prolonged-flash stimulus (188 ms) to examine the daily variation in the a-wave, b-wave and d-wave components of the photopic ERG. The experiments were performed in a Ganzfeld dome stimulator in the presence of a rod-saturating background. The observers experienced a natural light cycle during the course of the experiments and their short-term cone adaptation was held constant using a 10 min dark adaptation prior to the recordings. We have shown that in these conditions, both the amplitude and implicit time of the a-wave component is constant throughout the 24-h

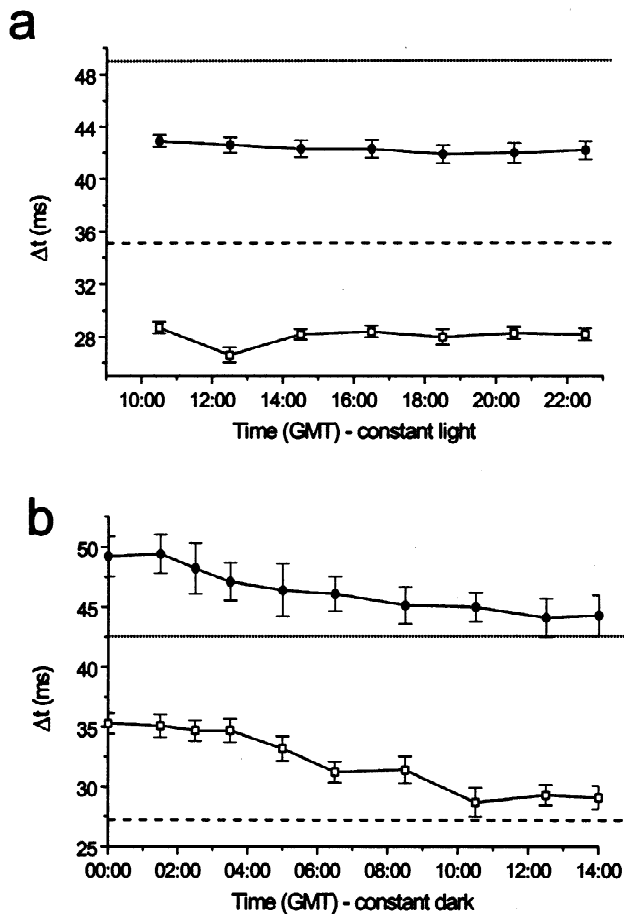


Fig. 3. (a) Effects of constant light on the b-wave and d-wave implicit times. Observers ( $n=4$ ) were maintained in a constant bright environment ( $>250$  lux) from the beginning of the experiment at 10:00 GMT for around 12 h. The ERGs were measured at 2 h intervals and the mean implicit times ( $\pm 1$  S.E.M.) of the b-wave (●) and d-waves (□) are plotted against time. Note that the implicit times of both components remain relatively constant ('normal' night-time values for b- and d-wave are denoted by the horizontal dotted and dashed lines respectively) (b) Effects of constant darkness. Observers ( $n=3$ ) were maintained in constant darkness from 00:00 h for 14 h. The mean implicit times of the b-wave (●) and d-waves (□) are plotted against time. Note that in the absence of normal daylight cues, both components approach the mean normal daytime implicit times (dotted line and dashed line) at around midday.

cycle (Fig. 1b). These results are very similar to those seen with brief-flash stimuli using similar observer light history protocols [2], and imply there is little or no temporal variation in the human cone light response. We have however, established that the temporal properties of both the b-wave and d-wave components vary systematically throughout the normal light cycle (Fig. 2a,b). In both cases the implicit time appears to oscillate, with the fastest responses occurring in the middle of the daylight hours. We have shown that these temporal oscillations can be abolished by constant light (Fig. 3a) and our results show that if the observers are maintained in bright light from mid-morning onwards, the normal retardation of the b-

wave and d-wave that occurs during the onset of expected night can be blocked. In contrast, when the subjects were maintained in complete darkness from 00:00 h, the ERG b-wave and d-waves still approached their normal daytime values in the absence of daylight cues (Fig. 3b). These findings are significant in defining the underlying mechanism for the diurnal variation in the kinetics of the photopic ERG. That the rhythm appears to free run in constant darkness, suggests that it may be driven by an ocular circadian clock. Interestingly, a local retinal clock in many species (including mammals) appears to involve the circadian release of local melatonin [13,14]. Furthermore, it has also been shown that retinal melatonin release is abolished in constant light and free runs during constant darkness. It should also be considered that in other studies some diurnal variations, for example changes in photopic threshold, remain even in constant light [8]. This raises the possibility of multiple regulatory systems acting to control photopic visual function during the normal diurnal cycle.

Prolonged flash stimuli, as used in this study, have been reported to differentiate the cone photoreceptor contribution to ON- and OFF-pathways in the primate photopic ERG [1,11]. Whilst it remains to be fully resolved, it appears that the b-wave response to a brief xenon flash of light may represent a rather complex algebraic sum of responses to both light-ON and light-OFF [3,9]. In contrast, the prolonged flash stimulus can partially separate these components. Thus, it has been suggested that with a prolonged flash stimulus the b-wave is principally initiated through ON-bipolar cell activity (additionally shaped by the activity of hyperpolarizing second order neurones), whilst the d-wave principally involves activity in the cone OFF-bipolar pathway [11,12]. Certainly, pharmacological agents that block ON-bipolar cells appear to suppress the b-wave and potentiate the d-wave response at light-OFF [6,12]. We have therefore provided the first evidence that diurnal phase affects both the ON- and OFF-cone pathways in the human retina.

In their initial study Hankins et al. [2], suggested that long-term light history during the natural diurnal cycle was likely to be modulated through a rod-cone interaction. This conclusion was based upon measuring an action spectra for the light exposure (light-history) required to drive the ERG b-wave kinetics from the dark level to the light level. The data revealed that the spectral response ( $\lambda_{\max} \sim 500$  nm) failed to match the human cone photopigments. More recently, it has been suggested that a population of novel non-rod/non-cone retinal pigments may function as retinal irradiance detectors [4], and it is tempting to speculate that non-image forming light detectors may play a role in this regulatory retinal phenomena.

Whilst there was no evidence for a temporal variation in the light response of cone-photoreceptors themselves, previously the effect could only be localised non-specifically at the second order neuron level using a brief-flash stimulus. We have now established that the temporal

properties of both the b-wave and d-wave components oscillate in opposition to the variation in ambient light level. This suggests that the temporal changes in the cone-pathway affect both ON- and OFF-bipolar systems. The most likely explanation is therefore that the temporal response changes occur somewhere presynaptic to the entire second order pathway — most likely within the cone synapse. Interestingly, anatomical evidence suggests that the density of synaptic ribbon structures in the terminals of cone photoreceptors appears to change according to the day/night cycle [15,16]. Whilst the precise function of synaptic ribbons remains unclear, that such anatomical variation occurs might be functionally related to the physiological changes we have reported here.

The concept that the entire postsynaptic cone pathway is temporally regulated according to the diurnal cycle is of considerable interest. Whilst it is now evident that long-term light history [5] and diurnal phase [2] can regulate the speed of cone-driven responses, the only visual advantage to such a mechanism would be to match the temporal disparity between rod- and cone-signals under mesopic light levels. It appears that to achieve this the cone-signal is slowed somewhere within the cone-second order synapse.

### Acknowledgements

This work was supported in part through a Wellcome Trust Prize PhD studentship (SRJ).

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