ORIGINAL ARTICLE

Studying the Retinal Source of Photophobia by Facial Electroretinography

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ABSTRACT

Purpose. Photophobia is a debilitating clinical condition that disrupts the ability to use vision for everyday tasks in bright lighting conditions. The goal of the study is to develop a methodology to study the neural basis of photophobia and the contribution of the melanopic pathway to its etiology with differential chromatic responses by means of standard electroencephalographic recording equipment.

Methods. We introduce and validate the approach of recording wavelength-specific electroretinographic (ERG) responses from the face electrodes of the high-density whole-head electroencephalography recording system under light-adapted conditions.

Results. ERGs recorded in this way to whole-field chromatic stimuli exhibit striking differences between the photophobic and non-photophobic groups. The control responses were consistent with photopic intensity in peak time, and in the ordering of peak times as a function of wavelength condition, indicating a predominantly cone source of the signals. The photophobic responses, on the other hand, were substantially slowed relative to controls, with the peak times conforming to a different order as a function of wavelength condition than controls, implying that the cone response has been suppressed and that the responses derived from a different photoreceptor system consistent with mediation by melanopic retinal ganglion cells.

Conclusions. The results will be important for determining the neural pathways involved in photophobia and potential approaches to its treatment on the basis of this etiology.
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Key Words: electroretinogram, wavelength, photophobia, melanopsin, photopic

The objective of this study was the investigation to use the electroretinogram (ERG) to assess the respective contributions of the photoreception mechanisms to the conditions of photophobia in humans with mild traumatic brain injury (mTBI), providing biomarkers for the involvement of retinal mechanisms in photophobia (which may more properly be called "photalgia" with respect to the pain that is its primary symptom). Photophobia has been associated with abnormal ERG waveforms in one study of the PAX6 congenital nystagmus and photophobia,[1] showing a large reduction specific to the light-adapted cone ERG.

We introduce the use of a high-density multi-electrode electroencephalography (EEG) system to record the ERG simultaneous with whole-head EEG recording as an integrated approach to eye and brain response analysis (although the brain responses are not the topic of the present analysis).

To discriminate between different photoreceptor classes, ERGs were selectively stimulated by an appropriate choice of light wavelengths. To this end, we stimulated with each of the three color guns of a CRT monitor at maximum intensity, together with the combination of the three to produce white.

In an early study of the ERG under such wavelength-selective conditions, Motokawa and Mita[2] and Adrian[3] reported a striking anomaly of the waveforms in the long-wavelength region of the spectrum interpreted as reflecting a mixture of fast cone and slower rod responses (reproduced from Adrian[3] in Fig. 1). Light-adapted responses are small and fast, whereas dark-adapted responses are a mixture of two types of response at longer wavelengths and dominated by a slower response at shorter wavelengths.

METHODS

Participants

We tested two groups of participants who had experienced mTBI: one group with photophobia or light-induced pain symptoms and the second group with no photophobia symptoms.

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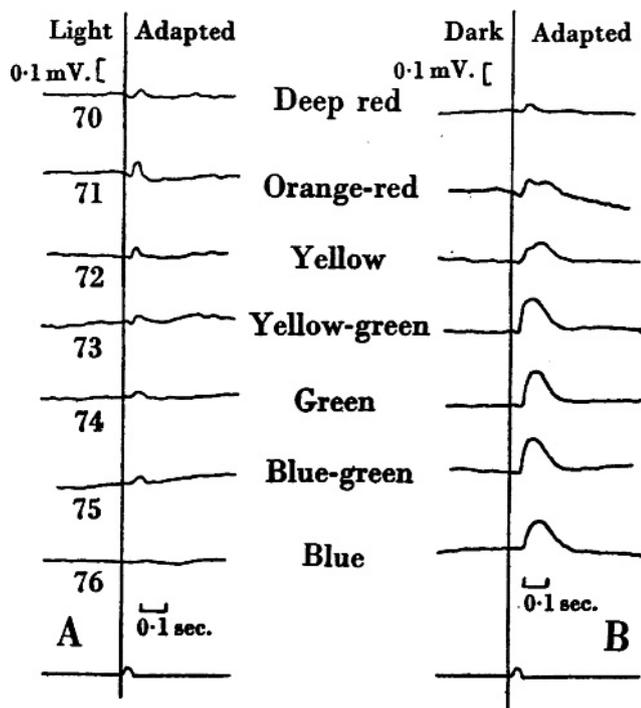
FIGURE 1.

Recordings by Adrian[3] of the human ERG as a function of light wavelength and adaptation state. Responses in the short-wavelength region of the spectrum are slow and rod-dominated, whereas those to long-wavelength are fast and cone-dominated, with a mixture in the "orange-red" spectral region.

The participants completed a *Validated Questionnaire of Photophobia*.[4,5] The research followed the tenets of the Declaration of Helsinki and was approved by the Smith-Kettlewell institutional review board, with informed consent obtained from the participants after explanation of the nature and possible consequences of the study.

Differences between them are interpreted as reflecting causes of the asthenopia (pain) in the photophobic group, which requires that we clearly separate these two groups. The individuals were assigned to the non-photophobic mTBI group if they experienced no discomfort on viewing the full-field flickering stimuli of the present study at their maximum intensities, and to the photophobia group if they did experience sufficient discomfort to require the use of a lower intensity for the recording procedures, as described in the following.

Recruitment

The study participants were recruited from a non-academic population via a social media website. Participants were included in the analysis if they met the criteria of letter acuity of 20/40 or better in both eyes (Bailey-Lovie chart, mean left eye denominator: 22 ± 5, mean right eye denominator: 23 ± 6), of having no visible ocular abnormalities, and of having had mTBI events defined as involving closed-head trauma resulting in a loss of consciousness for a period of 5 minutes or more, or loss of memory of the traumatic event per se.

Stimulation Conditions

Full-field recording was achieved by the use of a reflective rectangular hood in front of a ViewSonic monitor. At the viewing distance of 20 cm, the monitor screen had a visual angle of 90° horizontally and 60° vertically. The field was extended to 180° in both directions by means of the rectangular hood, which was lined with acrylic sheet mirror with an average reflectivity of 86%. There was a fixation target of four concentric circles of 3 arcmin width at 1, 2, 3, and 4° eccentricity,

Color selectivity was achieved by use of the color guns of the monitor, giving full-field stimulation conditions of R, G, B, and W (=R + G + B). The CIE color coordinates, peak wavelengths, and respective stimulation weights of the L, M, S, and melanopsin photoreceptor types for the three guns of the ViewSonic monitor, and their sum, are given in Table 1.

The time course of stimulation was alternation between 200 ms on/200 ms off periods, forming full-field 2.5 Hz square wave stimulation for a duration of 60 s (150 cycles). This form of stimulation has been used in a number of ERG studies under light-adapted conditions.[6–10] Because this was a study of photophobia, the usual approach of recording with dilated pupils at maximum stimulus intensity would have caused undue hardship. We therefore adopted the alternative nociphysical approach of stimulating with natural pupils at the highest intensity that was comfortable for the participant. Those with reported photophobia were shown the flickering stimulus at the lowest available intensity (3 log units below the max), which they observed for as long as needed to make a decision whether it was comfortable for them. If it was, the intensity was increased by a factor of 2 and the procedure repeated until their comfort level was exceeded. The highest intensity judged as comfortable was the one used for testing.

Intensity Estimation

Stimulus luminance was measured (in photopic cd/m^2) with a Minolta Chromameter. Because the ERGs were recorded with natural pupils, retinal illuminance was calculated on the basis of the product of screen luminance and pupil size, estimated according to the melanopsin spectral sensitivity function, which

TABLE 1.

Stimulation weights of the photoreceptor types for ViewSonic monitor color guns

Guns	x	y	Λ_{peak}	Rod	L-cone	M-cone	S-cone	MO	L + M + S
R	0.604	0.335	611.8	0.014	0.642	0.168	0.000	0.002	0.364
G	0.283	0.577	540.5	0.642	0.896	1.00	0.003	0.259	0.853
B	0.149	0.080	468.1	0.654	0.143	0.257	0.662	0.921	0.246

MO, melanopsin photoreceptors.

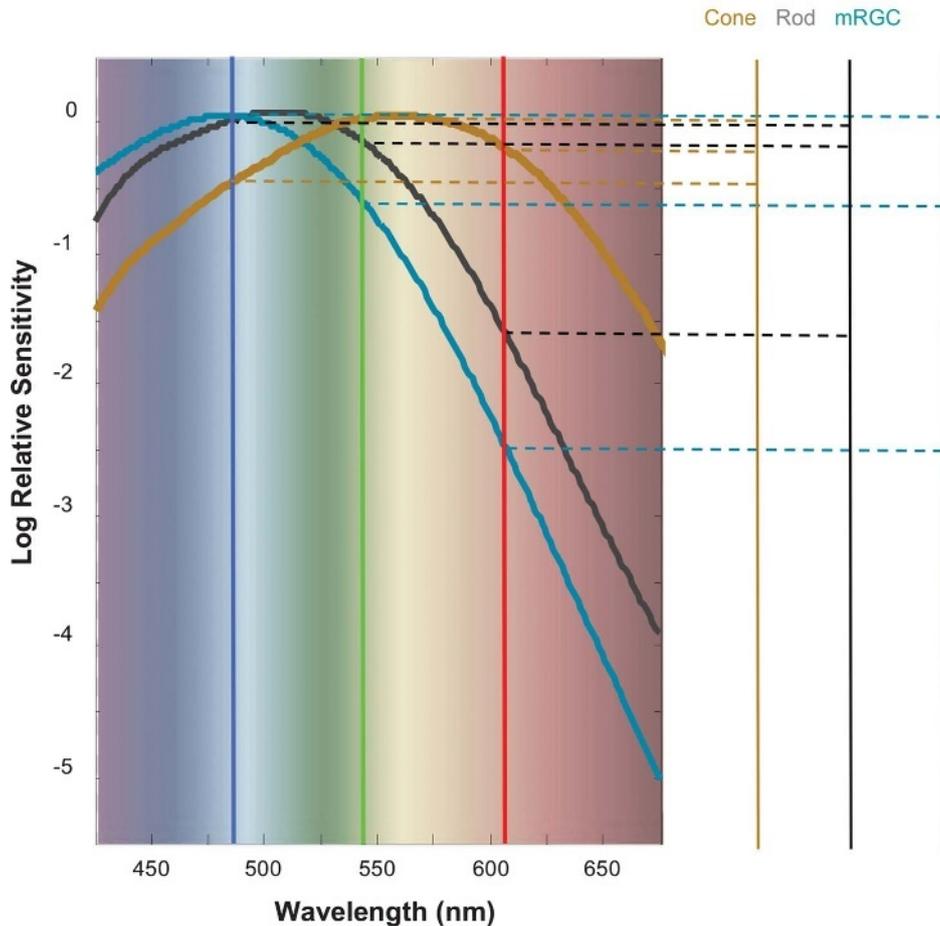## Relative Intensities for the Three Photopigment Classes

FIGURE 2.

Specification of relative intensities for the three photopigment classes—cones (beige curve), rods (black curve), and melanopsin retinal ganglion cells (turquoise curve). The intersection of each of these curves with the dominant wavelengths for the R, G, and B color fields specifies the relative photopic, scotopic, and melanopic spectral sensitivities for the respective photopigment classes.

has been shown to dominate the control of pupil size for long duration stimuli.[11,12] Although the stimuli consisted of short flashes, they were presented for a total duration of 1 minute, during which the slow pupil control system is expected to integrate the luminance in a manner that is dominated by the melanopsin spectral sensitivity, which peaks at 482 nm (see Fig. 2) and has a reciprocal control of pupil size in the high-intensity range,[11,12] On this basis, the average pupil sizes were 2.9, 2.4, 2.5, and 2.4 mm diameter for the R, G, B, and W color fields, respectively (based on the unified formula in Watson and Yellott[13]). These pupil sizes were used to specify the stimulus intensities under each of three definitions: photopic Td, scotopic Td, and melanopic Td, according to whether the physiological responses are assumed to be mediated by the cones, the rods, or the melanopsin retinal ganglion cells, respectively.
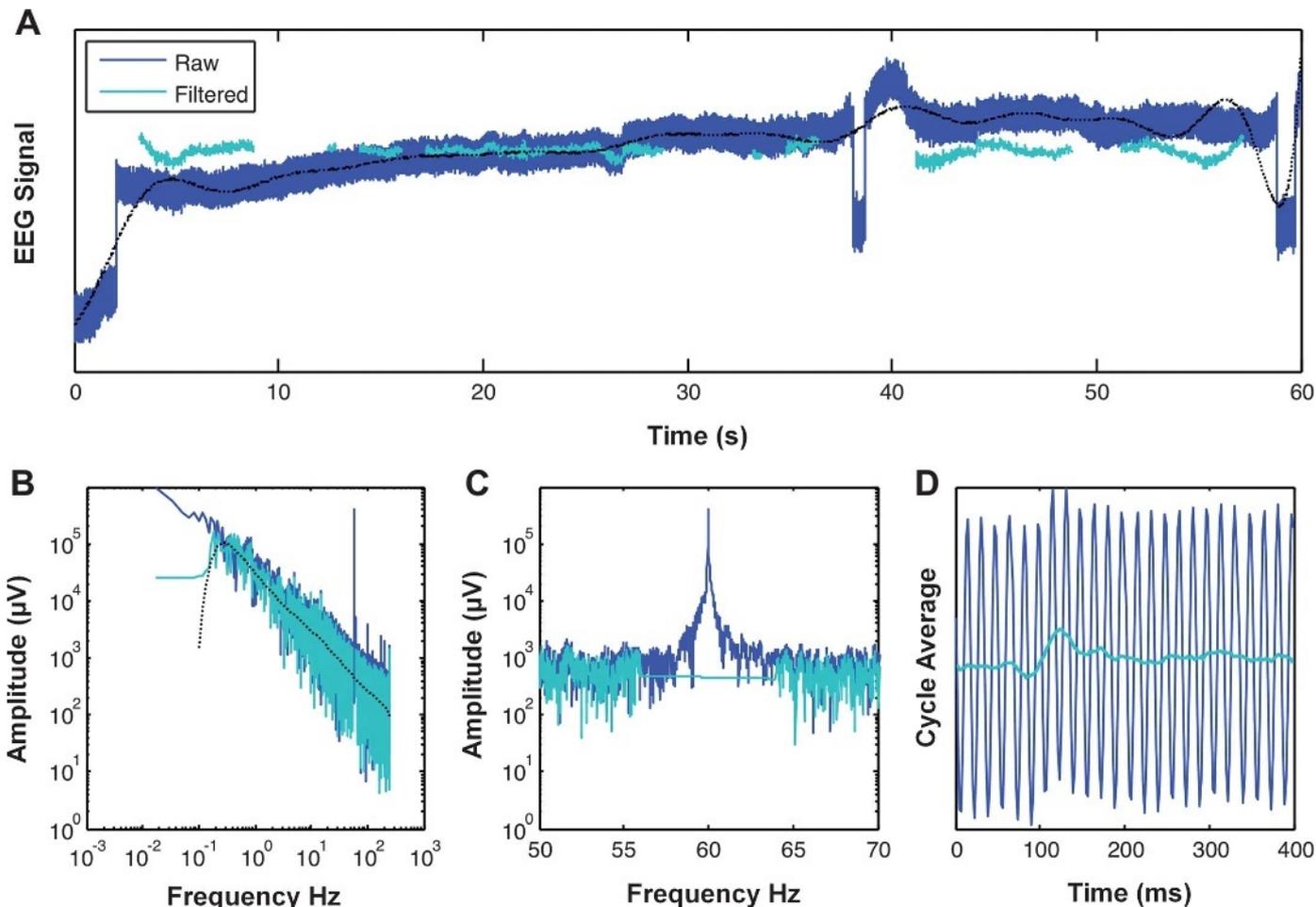
Recording

Electrical recording from the scalp and face was by means of the EGI Geodesic Netstation high-density, whole-head recording system (Electrical Geodesics, Inc., Eugene, OR), which incorporates 128 electrodes distributed around the head and face in addition to the scalp electrode. The ERG signal for each eye was derived from the differential signal between electrodes located immediately below the eye and the one at the temple adjacent to the eye. The recording bandwidth was 0.01–250 Hz.

Artifact Rejection

As shown in Fig. 3A, electrical artifacts caused by blinks and eye and head movements were removed by means of artifact rejection software that first applies a 0.2 Hz high-pass filter to each recording and then iteratively removes signal epochs that are more than 2 standard deviations beyond the statistical distribution of signal amplitudes at each electrode (including the face electrodes recording the ERG).

Electrical mains 60 Hz and monitor refresh 100 Hz pick-up were removed by means of the following procedure that we developed for the purpose. This innovation was based on the observation that, although the EEG spectrum falls off with temporal frequency, it does not obey an accurate 1/f rule but has both local and large-scale "bumps" relative to that approximation. Moreover, the electrical pick-up signals were not constant in amplitude but could vary with movement if caused by instability

FIGURE 3.

Illustration of the ERG/EEG noise filtering technique under (atypically) high 60 Hz pickup conditions. In each panel, the blue trace is the raw recording and the cyan trace is the result after frequency-normalization filtering and artifact rejection. (A) Sample 60 s trace from an EEG electrode. Note the large amplitude blink response artifacts and broad width of the trace reflecting the 60 Hz electrical pick-up. (B) 60 s amplitude spectrum in log-log coordinates, showing high-frequency fall-off in amplitude and narrowband spikes of oscillatory noise from electrical pickup, together with the eighth-order smoothing normalization of the amplitude spectrum (black dotted curve). (C) Expanded view of the 50–70 Hz region of the spectrum showing the 60 Hz pick-up frequency spike and the splatter around it from about 57–63 Hz. Cyan curve shows the region normalized by the filtering procedure. (Note that the width of the splatter region will vary with the form of the amplitude modulation due to unpredictable head movements, etc.) (D) Cycle-averaged waveform before (blue trace) and after (cyan trace) frequency-normalization filtering. Note the elimination of the 60 Hz electrical pick-up signal revealing the evoked response transient.

of an electrode (as is often the case in a 128-electrode net). Thus, the pick-up frequency could vary around its mean by an unpredictable amount due to the well-known effect of amplitude-modulation frequency splatter. Empirically, we found that the range of the frequency splatter was no greater than ±4 Hz, so this value was set as the limit for the frequency filtering.

By this means, rather than filtering the waveforms to below 60 Hz, which is the typical approach to reduce the 60 Hz pickup in EEG recordings, we are able to analyze the average cycle waveform up to the full bandwidth of 250 Hz (Fig. 3), which is particularly important for proper resolution of the fast waveform components such as the ERG.

RESULTS

Assessment of the waveforms of the ERG signals from the face electrodes led to a concern that they may have been subject to contamination from EEG signals from the forehead. Various electrode configurations were assessed, and the one that best isolated the ERG was found to be the differential signal from the cheek to the anterior temple electrode. An example of the average ERG from five control participants is shown in Fig. 4, with a gray box indicating the 200 ms stimulation period and labels indicating the a-, b-, d-waves and photopic negative response.[14] Under these conditions, the a-wave peaks at about 20 ms, the b-wave at about 45 ms, and the photopic negative response at about 80 ms. The d-wave is an off-response and peaks at about 70 ms after the stimulus offset. In the standard interpretation, the a-wave reflects predominantly the photoreceptor current, the b-wave is a product of outer plexiform layer activity, the photopic negative response reflects amacrine and ganglion cell and activity,[14] with the d-wave representing the off-equivalent of the on b-wave. (The slower c-wave from the pigment epithelium is not manifested at this time scale.)

Fig. 5 shows ERGs averaged for the left and right eyes for the four uniform-field color conditions (R, G, B, and W chromaticities)

Control white (W) ERG response
5-subject average
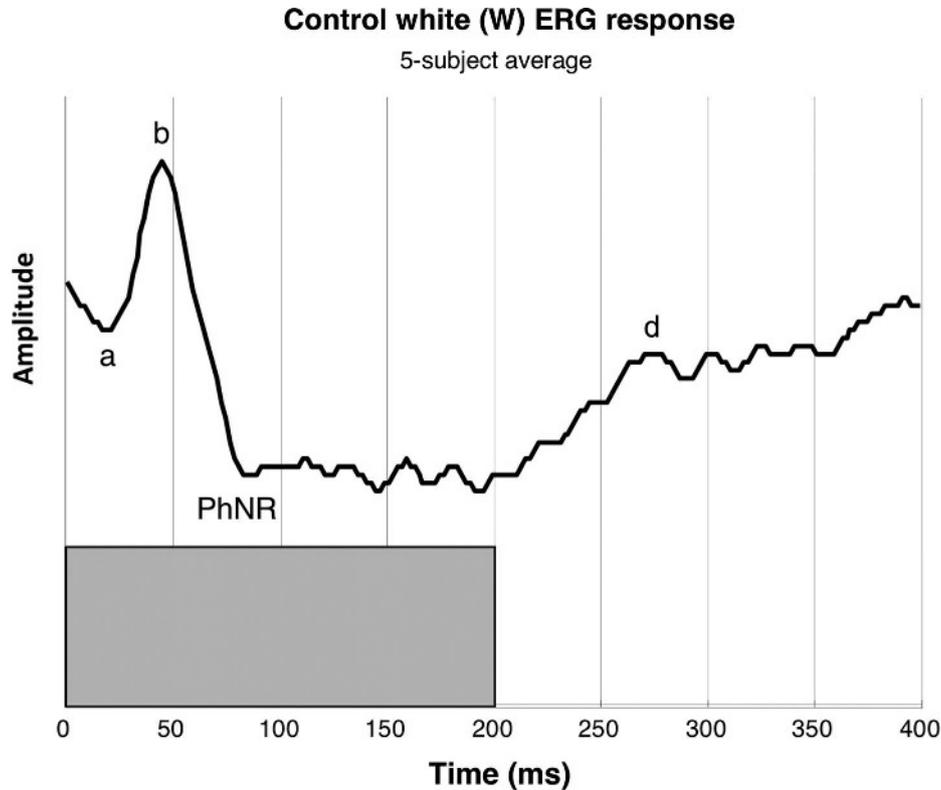
FIGURE 4.

The light-adapted ERG for white light in control participants, showing the time course of stimulation (gray box) and the a, b, photopic negative response and d peaks of the typical ERG waveform.

for the individual participants, with the vertical position scaled in terms of log photopic luminous intensity. As indicated by their positions on the ordinate, the intensities were set lower for the photophobic participants in accordance with their tolerance level for each color field. In the non-photophobic cases, the peak times line up well at equivalent intensity levels, with the highest intensities at about 60 ms peak times and the B fields with lower intensities at 70–90 ms.

For the white light conditions (160 phot Td at full intensity), the initial portion of the mean ERG waveform for the non-photophobic group shows the classic ERG form, with the b-wave peaking around 50 ms, though a little slower than for the controls exemplified in Fig. 4. (The differential waveforms of the four-color conditions are analyzed in more detail in relation to Fig. 6.) In the photophobic cases in the lower panels of Fig. 5, the peak times for the b-waves for W, G, and B are much longer, at 80–140 ms, even where the intensities are similar to those of non-photophobic responses (first four lower panels). The exception is again the R responses, which are much weaker around the peak times of the other responses, and have even later peaks around 150–250 ms in most cases, consistent with a delayed version of the second peaks in Fig. 5, upper panels.

To assess the behavior of the ERG in more detail, average responses for the non-photophobic and the four moderate photophobic participants, those with the highest intensity settings, are plotted in Fig. 6, with error bands. Each dataset has been scaled in terms of its vertical position on the ordinate according to the log of the best of three candidate scalings specified in Fig. 2—in terms of

log intensities defined by the photopic, scotopic, or melanopic spectral sensitivity functions in Fig. 6B, C, and D, respectively.

The first point to note in Fig. 6A is that the non-photophobic W, G, and B responses have similar waveforms whereas the R response is strikingly discrepant, with a similar early peak but instead of falling back to (or beyond) the baseline level, it rises up to a second peak deflection in a manner reminiscent of the Adrian[2] long-wavelength response in Fig. 1. The photophobic responses in Fig. 6B by comparison are markedly slower. Again, the W, G, and B responses have similar waveforms but now there is no discernable peak at a similar time in the R response, but only a noticeably later peak. The natural conclusion is that the photophobia is characterized by two ERG effects, an overall slowing of the time to peak and a loss of the early peak or x-wave, in the R response. Specifically, the mean time to peak for the highest intensity condition (W) is 54 ms for the non-photophobic group and 106 ms for the photophobic group.

The statistics of the parameters can be assessed at several levels. At the first level, we may ask whether parameters, peak times, or amplitudes differed significantly from those for the W condition within any of the groups. None of these differences was significant for either group. The next level is whether any of the parameters differed for either color condition in one of the mTBI groups relative to the control group, which was indeed that case for the photophobic mTBI group. The peak times (τ) were significantly longer at $P < 0.01$ on the t-test for all four color conditions (W, G, R, B) than for the control group (τ_C = 53, 51, 67, 71 ms; τ_{Ph} = 117, 124, 184, 114 ms; t_{diff} = 6.19, 8.13, 6.19, 11.88). None of the

Non-photophobic TBI

Photophobic TBI
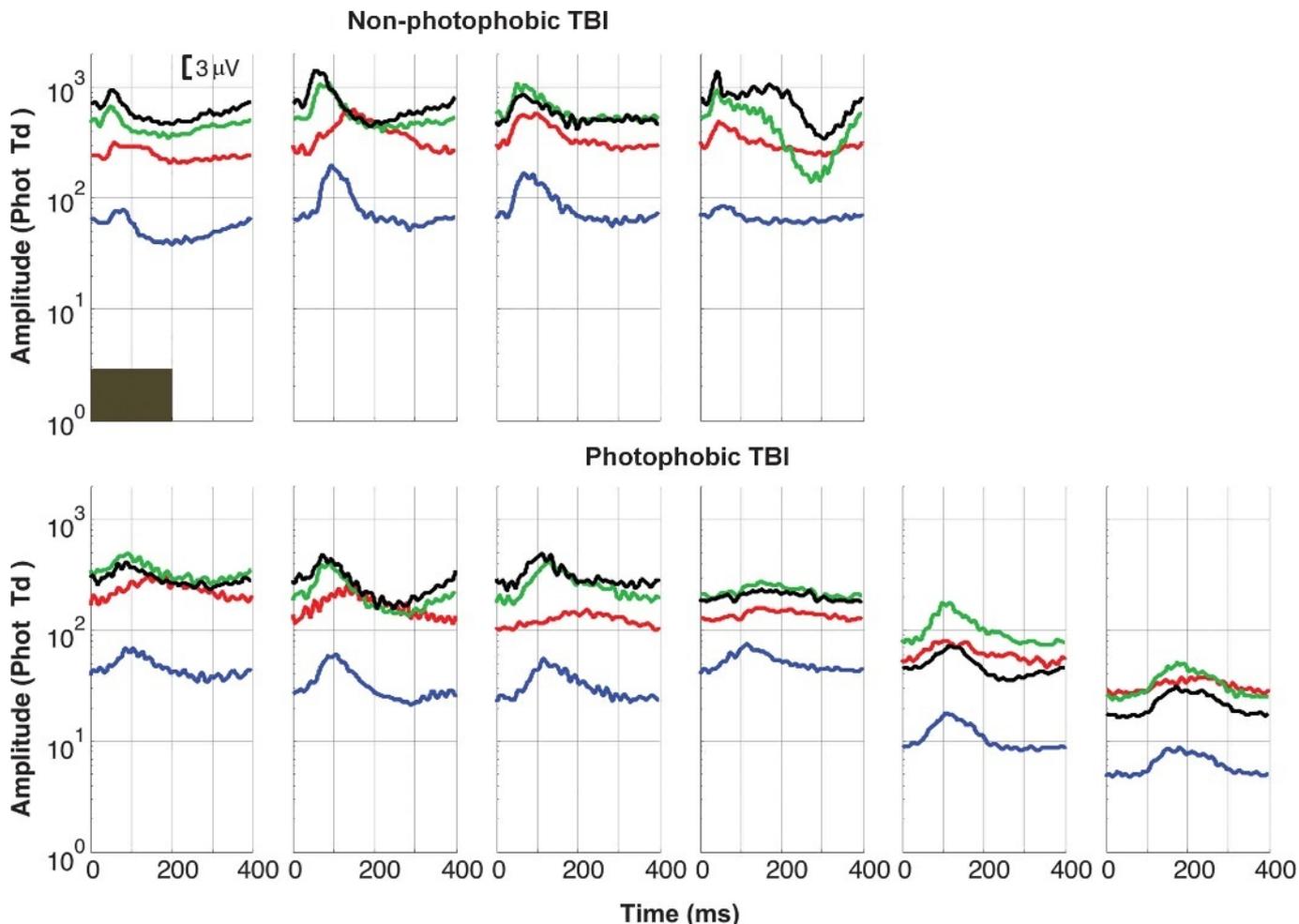
FIGURE 5.

ERGs for each participant (averaged over repeats and for the left and right eyes) for the four uniform-field color conditions (R, G, B, and W chromaticities, color coded, with black for W). Response position on the abscissa is scaled in terms of photopic luminous intensity. Upper panels: non-photophobic participants. Lower panels: photophobic participants. Black block in upper left panel indicates stimulus duration.

amplitudes or the peak times for any other comparisons with the control were significant. The third level is to compare the parameters for the photophobic versus non-photophobic mTBI groups, which were not significantly different for any color condition.

The interpretation of these results, and the analysis in relation to Fig. 6C and D, is considered in Discussion, but here it should be noted that the intensity settings for these results for the four mildest photophobes, though lower than the non-photophobes, fall in an overlapping range that does not support an interpretation that the degree of slowing of the peak times is attributable to the intensity reductions. Even a 1 log unit reduction would only slow the response by about 20 ms, whereas the mean intensity reduction for the mild non-photophobes is only about one-third of a log unit, corresponding to a less than 10 ms effect.

This qualitative analysis of the peak times relative to intensity units in Fig. 6 is provided in Table 2 in terms of the degree of adherence of the peak times τ to a linear function of log intensity, I, in the form $\tau(I) = a.\log10(I) + k$, where a and k are scaling constants. The relative Pearson R^2 values for these fits in log values give some indication as to the relative alignment of the responses under different intensity regimes (see Fig. 2). Although with only four values the R^2 values do not reach statistical significance after

correction for multiple applications of the statistical test, they give an idea of the relative ordering of the intensity alignments. Thus, for the non-photophobic group, the alignment on photopic intensity has the highest R^2, consistent with the responses being generated by cones. For the photophobic group, the photopic alignment is given no support, and the form that comes closest to significance is the melanopic alignment. (However, it should be borne in mind that these are not fully independent tests because if it is aligned by one form of intensity function, it will necessarily by misaligned by another.)

DISCUSSION

The main result of this study is that the average responses in the moderate photophobic TBI group (Fig. 6B) have much later peak times than those of the non-photophobic TBI group (Fig. 6A), to an extent that is not compatible with the small intensity differences resulting from the photophobic settings. One possible interpretation is that the ERG signals are predominantly cone responses that have been substantially delayed (by about a factor of 2). This interpretation is, however, not supported by the pattern of peak times, which in the non-photophobic group are about the
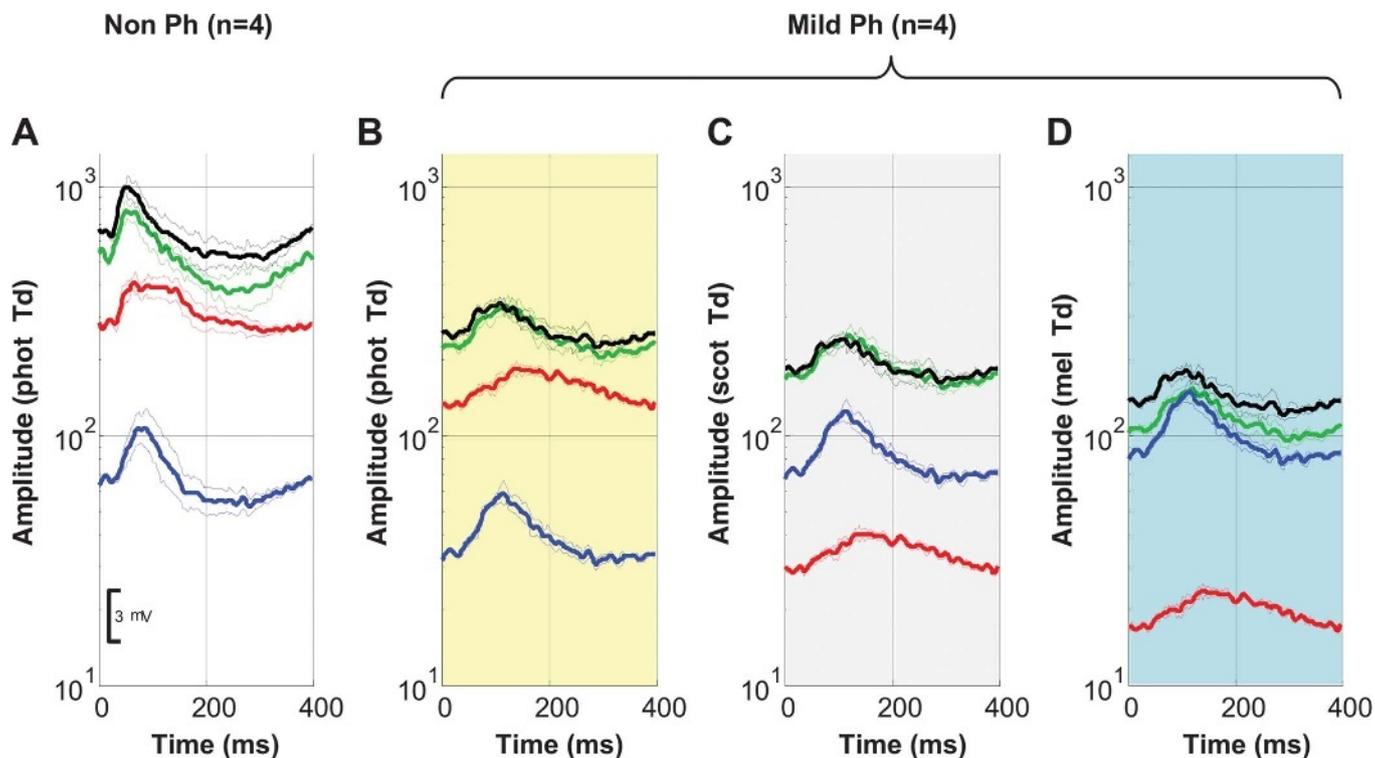
FIGURE 6.

Average amplitude-normalized responses for the photophobic (Ph) and the four highest-intensity non-photophobic (non-Ph) datasets, emphasizing the difference between the two groups according to the scaling of relative intensities (in terms of retinal illuminance in Trolands; Td). Dashed lines show 1 SEM around the means. (A) Non-Ph control responses with baseline level scaled to photopic intensity (5-subject average). (B) Ph responses with baseline level scaled to photopic intensity (5-subject average). Note that the responses are considerable slower to all stimuli, but that the ordering does not produce a convincing progression of peak times with intensity. (C) Ph responses with baseline level scaled instead to scotopic (scot) intensity (4-subject average), which gives the appropriate ordering but with a large jump in the R peak time relative to B. (D) Ph responses with baseline level rescaled to melanopic (mel) intensity, for which the peak times now progress appropriately with intensity.

same for R, G, and W but later for B, switching in the photophobic group to being about the same for G, B, and W, and much later for R.

This pattern is what would be expected on another possible interpretation, which is that the cone responses have been attenuated by the mTBI so as to be effectively invisible, revealing underlying rod responses, which should then conform to a progressive scaling in terms of the scotopic spectral sensitivity function (see Fig. 2). This possibility is evaluated in Fig. 6C. However, the order of the peak times is not fully compatible with their being pure rod responses because the response to the G stimulus, which has the highest scotopic intensity, has a slightly later peak time than the that for the B stimulus, whereas the response for the R stimulus is much later, even though its intensity is only a little less than that of the B stimulus.

As a result, we assessed the more radical hypothesis that the peak times might be controlled by a scaling in proportion to the spectral sensitivity of the melanopsin-expressing retinal ganglion cells (Fig. 6D).[11,12] Replotting the responses this way (see Fig. 2) produced a scaling of the peak times fully consistent with control by the melanopic intensities of the stimulus fields. The functional significance of this result is nevertheless unclear. The simplest interpretation is that the responses at these high intensities in the photophobic are derived from the melanopic retinal ganglion cells, which have been reported to drive ERGs in normal adults under spectral isolation conditions.[15,16] However, the melanopic cell population is only a very small proportion (~0.2%) of the total number of ganglion cells,[17] so it would be surprising if they could dominate the ERG response in such a fashion.

CONCLUSIONS

In conclusion, our novel approach of recording ERG responses from the scalp electrodes of the whole-head EEG recording system is showing clear and well-resolved ERG responses, which are exhibiting striking differences between the photophobic and non-photophobic

TABLE 2.

Goodness of fit of the ERG intensity scaling according to the applicable form of photopigment intensity scaling

TBI Category	Non-Photophobic			Photophobic		
Alignment	Photopic	Scotopic	Melanopic	Photopic	Scotopic	Melanopic
R^2	0.95	0.44	0.15	0.00	0.64	0.90

groups. The non-photobic control responses are consistent in peak time and in their ordering as a function of wavelength condition, suggesting a predominantly cone source of the signals. The photophobic responses, on the other hand, are substantially slowed relative to controls, with the peak times conforming to a different order as a function of wavelength condition than controls, implying that the cone response has been suppressed and that the responses derive from a different photoreception system. Evaluation of the hypotheses that the photophobic responses are under the control of rods or of melanopic retinal ganglion cells suggests that the evidence is in favor of the latter in some form, despite the relative paucity of such cells in the human retina. Further investigation of these responses over the full intensity range is therefore warranted before any conclusion could be seriously considered. The results will be important for determining the neural pathways involved in photophobia, a debilitating clinical condition that disrupts the ability to use vision for everyday tasks in bright lighting conditions.

ACKNOWLEDGMENTS

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