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14. ABSTRACT

The objective of the project is the investigation of two proposed mechanisms of mTBI-related photophobia: 1) that mRGC damage is a primary mechanism in photophobia and 2) that mTBI-induced edema can be the causal mechanism of photophobia. The project uses ERG (Aim 1), high-density EEG (Aim 2), functional MRI (Aim 3), and tensor-based morphometric MRI (Aim 4) to assess the respective contributions of these mechanisms of photophobia in humans with mTBI, providing biomarkers for the involvement of these mechanisms. The goal for the present period was to complete the analysis techniques for all four Aims and begin testing the photophobic and non-photophobic participants.

For Aim 1, a novel approach of recording ERGs through the EGI net was validated and the latency and amplitude assessment technique for each color condition developed. For Aim 2, a component analysis for the high-density EEG was developed and shown to elicit responses with the predicted mRGC-specific spectral characteristics. In Aim 3, the fMRI techniques for determining subcortical brain structures activated by mRGC-specific stimuli were validated. In Aim 4, the tensor-based morphometry methodology was developed to assess brain tissue edema or shrinkage relative to controls. The testing of participants on all four modalities is on schedule.

15. SUBJECT TERMS
Traumatic Brain Injury (TBI), Photophobia, ERG, EEG, fMRI, MRI, Tensor-Based Morphometry (TBM), Melanopsin, Brainstem

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1. INTRODUCTION

The objective of the project is the investigation of two proposed mechanisms of mTBI-related photophobia: 1) that mRGC damage is a primary mechanism in photophobia and 2) that mTBI-induced edema can be the causal mechanism of photophobia. The project will use ERG, high-density EEG, tensor-based morphometric MRI and functional MRI to assess the respective contributions of these mechanisms of photophobia in humans with mTBI, providing biomarkers for the involvement of these mechanisms.

In Aim 1, selective stimulation of mRGCs in humans will elicit ERGs by an appropriate choice of light wavelengths. In Aim 2, high-density EEG recording will determine photophobic-specific signal in the scalp surface distribution of electric responses. In Aim 3, fMRI will determine if there is a functional deficit in key brain structures activated by mRGC-specific stimuli. In Aim 4, tensor-based morphometry will test the causal role of brain tissue edema or shrinkage in photophobia.

2. KEYWORDS

Traumatic Brain Injury (TBI), Photophobia, ERG, EEG, fMRI, MRI, Tensor-Based Morphometry (TBM), Melanopsin, Brainstem

3. ACCOMPLISHMENTS

a. What were the major goals of the project for Year 1?

The projects scheduled for completion or initiation in Year 1 were as follows:

Task 1. Finalization of experimental protocol for submission to DoD Surgeon General (months 1-5, Oct 2014 - Feb 2015) [*Completed*]

Task 2. Development of integrated suite for tetramodal ERG/EEG/fMRI/TBM analysis (months 6-11, Mar-Aug 2015) [*Completed*]
2a. Implementation of wavelength-selective stimulation systems for the three functional modalities – ERG/EEG/fMRI (months 6-7, Mar-April 2015)
2b. Development of stimulus software for presentation of mRGC and control wavelength-selective stimulation across the three functional modalities – ERG/EEG/fMRI (month 8, May 2015)
2c. Refinement of ERG and EEG artifact rejection software for signal pre-processing (month 9, June 2015)
2d. Development of amplitude and latency analysis software for ERG and EEG (month 10, Jul 2015)
2e. Development of PCA spatial component analysis software for high-density EEG (month 11, Aug 2015)

Task 3. Recruitment of subjects into a group of 10 with mTBI associated with photophobia symptoms (mTBI/P) and 10 with mTBI without photophobia symptoms (mTBI/nonP) (months 10-15, Jul - Dec 2015) [*70%]*

Task 4. Electrophysiological testing (months 10-20, Jul 2015 - May 2016) [*35%*]
4a. ERG testing of the mTBI/P and mTBI/nonP groups
4b. Individual analysis of the ERG data
4c. EEG testing of the mTBI/P and mTBI/nonP groups
4d. Individual analysis of the EEG data

Task 5. MRI testing (months 10-20, Jul 2015 – May 2016) [*10 - 25%]*
5a. fMRI testing of the mTBI/P and mTBI/nonP groups
5b. Individual analysis of the fMRI data
5c. TBM testing of the mTBI/P and mTBI/nonP groups
5d. Individual analysis of the TBM data

b. **What was accomplished under these goals?**

The project is proceeding successfully in meeting the planned schedule for the tetramodal assessment of the photophobic and non-photophobic participants. Despite the delayed start due to regularatory human subject documentation approval, recruitment, developments and testing are on schedule. The early results of the individual analysis of this exploratory project have been remarkably successful in idenfiying melanopsin-specific responses in the ERG, EEG and fMRI signals. In each case, this is the first time that melanopsin-specific responses have been identified in these signals in a form where rod responses can be excluded. We are therefore well-placed to go forward with the evaluation of the melanopsin pathway involvement in photophobia. Moreover, the tensor-based morphometry methodology developed, was successful in showing brainstem tissue shrinkage relative to controls, thus implying a degenerative effect of brainstem edema following TBI.

Task 1. Finalization of experimental protocol for submission to DoD Surgeon General

Following the award, the experimental studies protocol was given a programmatic review by the Scientific Panel. The human subjects documents (Study Protocol, Consent Form, Recruitment Advertisement, Photo Release Form, Bossini Questionnaire, Clinical Screening Form) have been reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with all applicable DOD, US Army, and USAMRMC human subjects protection requirements. We received the final approval from the USAMRMC ORP HRPO on March 17th, at the end of the second quarter.

Importantly, we were able to fully compensate for the administrative delay enforced by late approval of the Human Subject documents.

Task 2. Development of integrated suite for tetramodal ERG/EEG/fMRI/TBM analysis

We have all the planned experimental apparatus components, and we have designed, programmed and run the requisite initialization studies to validate the details of the EEG and ERG protocols. The main experimental procedure and initial results are outlined below. We have also continued to monitor the literature to optimize details of the design of the multimodal ERG/EEG/fMRI assessment protocol, which had suggested some modifications of the experimental protocol**.**

Electroretinographic strategy

One innovation that we have proposed was to use skin electrodes from the high-density EEG system for the ERG recordings. Thus, rather than employing a separate ERG recording system in a separate session, we investigated and validated the methodology of recording ERGs via differential signals from the face electrodes already available in the whole-head EEG system. In this way the EEG and ERG modalities can be recorded simultaneously in the same session, providing a much more efficient use of the limited resources for this project.

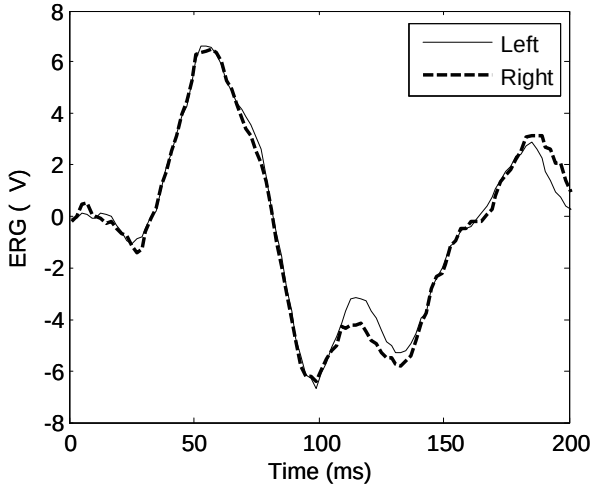


FIGURE 1. An example illustrating our ability to successfully record ERG through our innovative approach using skin electrodes from the high-density EEG system. ERGs for the left (solid line) and right (dashed line) at white square-wave alternation rate of 2.5 Hz. Note similarity of the signals for the two eyes.

The initial studies showed that we are able to successfully record the ERG. Figure 1 is showing an example of a B-wave elicited by sinusoidal stimulation with a similar response in the two eyes (blue vs red traces), indicating the feasibility of recording ERG from the EEG electrodes. Such an integrated EEG/ERG approach would ensure a much higher experimental efficiency. This approach has the additional advantage of providing simultaneous ERG/EEG recordings for applicability to event-related paradigms.

2a. Implementation of wavelength-selective stimulation systems for the three functional modalities – ERG/EEG/fMRI

Experimental Procedure Development

ERG/EEG

The key issue in the software development was to determine the optimal stimulation rates for identifying the melanopsin retinal ganglion cells (mRGC) pathway response. Since the ERG is recorded simultaneously with the EEG from the face electrodes of the 128-electrode EGI net, and since it has the similar timing properties as the EEG, it is considered in concert with the EEG stimulation. The following stimulation rates were assessed based on standard ERG and EEG protocols and on considerations from previous studies on the neurophysiology and pupil responses of the mRGC pathway:

30 Hz (sine), 25 Hz (sine), 5 Hz (sine & square), 2.5 Hz (sine & square), 0.5 Hz (sine & square), 0.25 Hz (square), 0.05 Hz (sine) wave modulation of each color channel with respect to black.

These forms of stimulation were all assessed for the spatio-temporal-chromatic ***discriminativity*** among photopigment sources based on the scalp distributions for each of the three color channels of the monitor (red, R; green, G; and blue, B), and for all three together to make white (W).

In the preliminary studies, the 5 Hz responses showed extensive discriminative structure of differential sources but were too fast for a clear identification of the component latencies within the 200 ms cycle time (due to the inevitable phase ambiguities). This frequency was therefore halved to 2.5 Hz to give a 400 ms cycle time in order to resolve the phase ambiguities. Moreover, the sine/square comparison revealed that the square wave stimulation generated a much stronger response at the lower frequencies. Finally, the 0.05 Hz stimulation was too slow to generate any significant responses due to length of time required to obtain a sufficient number of the 20 s cycles and the difficulty of suppressing blink response for the full 20 s cycle time.

The net result of the pilot studies was to determine that the optimal stimulation conditions for ERG/EEG component identification are 2.5 Hz and 0.25 Hz square wave conditions for R, G, B and R+G+B (=W).

2b. Development of stimulus software for presentation of mRGC and control wavelength-selective stimulation across the three functional modalities – ERG/EEG/fMRI

We have finalized the design of the stimuli for the ERG/EEG/fMRI studies based on the pilot results from this period. The fourth imaging modality, tensor-based morphometry of the subcortical melanopsin pathway, is based on structural MRI, so no stimulus development is required for this modality.

The **ERG/EEG** experiment consisted of uniform-field stimulation in a factorial design consisting of two temporal frequency conditions (2.5 and 0.25 Hz square alternation) crossed with four wavelength conditions. The wavelength conditions are fields of narrowband stimulation with the R (610 nm), G (540 nm) or B (470 nm) color guns, and one broadband condition consisting of the sum of the three narrowband stimulus to make white (W). The outputs of the three color guns are approximately equal in ***radiance***, which means that they have differential ***luminances*** according to the spectral luminosity function. The net result is that their mean luminances measured 16, 120 and 48 cd/m^2 for the 470, 540 and 610 nm guns, respectively, comfortably within the photopic range. To ensure enough samples, the 2.5 Hz conditions were programmed to run for 60 s, and the 0.25 Hz conditions for 120 s, each repeated twice, for each of the four color conditions (R, G, B and W).

FMRI Stimuli

The four fMRI runs consisted of 6 cycles of:

1. 20 s of 2.5 Hz Blue/Black Flicker alternating with 20 s Steady Black

2. 20 s of 2.5 Hz Red/Black Flicker, alternating with 20 s Steady Black
3. 20 s of Steady Blue alternating with 20 s Steady Red
4. 20 s of Steady Blue alternating with 20 s Steady Green

The preliminary studies confirmed that we can set all flicker frequencies at 2.5Hz to match the frequency used for the EEG/ERG studies.

2c. Refinement of ERG and EEG artifact rejection software for signal pre-processing

ERG/EEG artifacts due to blinks and eye and head movements have been 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 are removed by means of the following novel procedure that we developed for the purpose. This innovation was based on the observation that, while 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 due to instability 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 $\pm$ 4 Hz, so this was set as the limit for the frequency filtering.

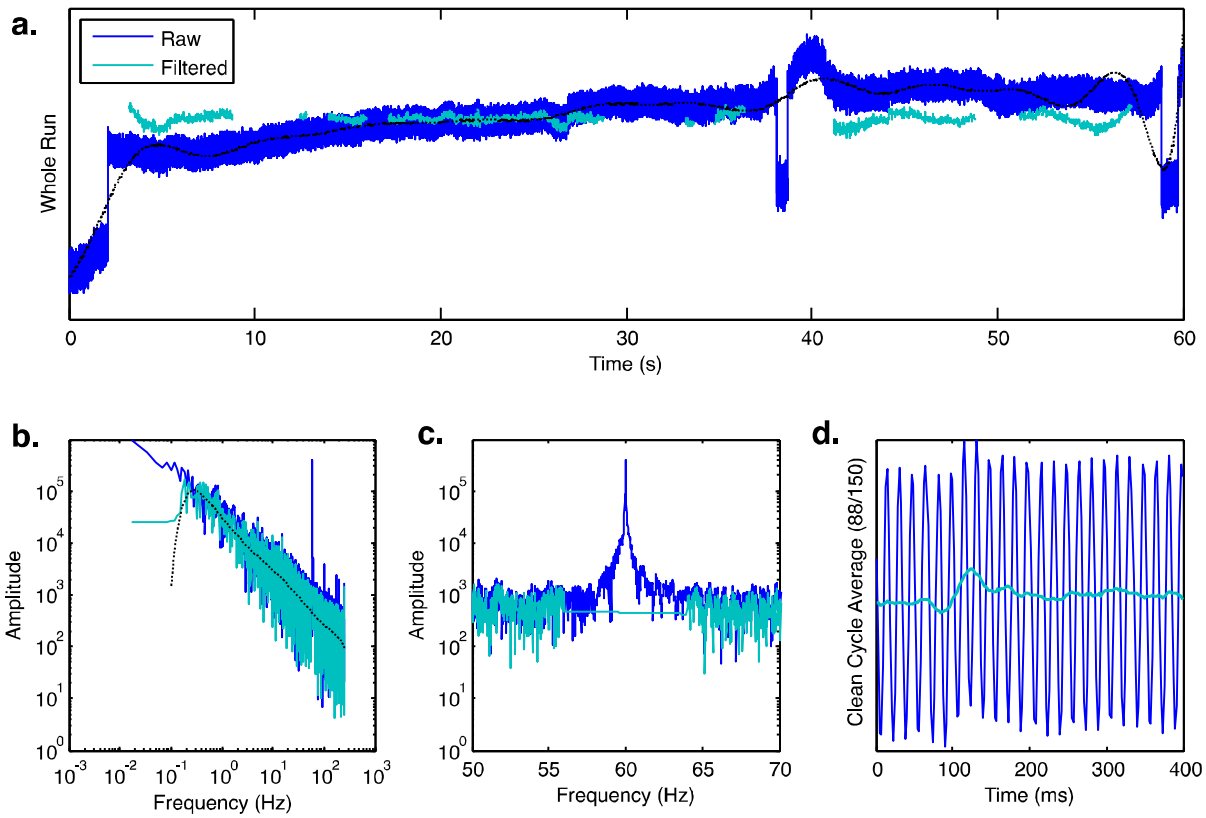


FIGURE 2. Illustration of the ERG/EEG noise filtering technique. In each panel, the blue trace is the raw recording and the cyan trace is the result after frequency-normalization filtering and artifact rejection. **a.** Sample 60 s trace from an EEG electrode**.** Note the large amplitude blink response artifact and broad width of the trace reflecting the 60Hz 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 8th-order smoothing normalization of the amplitude spectrum. (black dashed 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.) **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 clean evoked response transient.

Main steps of the ERG/EEG noise filtering technique:

1. Provide drift correction for whole EEG record for each channel (60 s or 120 s, depending on the stimulus condition) with a 0.2Hz digital high-pass filter.
2. Calculate the amplitude (A) spectrum at each electrode as a function of frequency (f).
3. Fit an 8th-order polynomial to the log-log amplitude spectrum excluding ±4Hz bands around harmonics of electrical main (60Hz) and display refresh (100Hz) frequencies.
4. For all frequencies within ±4 Hz of the known noise frequencies of 60 and 100 Hz and their low order harmonics (120, 180 and 200 Hz), scale the complex component to have the amplitude of the polynomial while preserving phase.
5. Revert to the time domain with an inverse Fourier transform.
6. For each color condition, break the waveform into segments at the stimulus repetition cycle.
7. Average the response waveforms over good cycles, defined as those with ERG variance within a robust estimate of 3 from the mean standard deviation across the cycle.
8. For channels with impedances > 150 kΩ, the signal was omitted from the analysis.

Thus, rather than filtering the waveforms to below 60 Hz, as is done in most EEG recordings to reduce the 60 Hz pickup, we are able to analyze the average cycle waveform up to the full bandwidth of 250 Hz, which is particularly important for proper resolution of the fast waveform components such as the ERG.

2d. Development of amplitude and latency analysis software for ERG and EEG

A goal of the project was to record ERG under the same conditions as, and simultaneous with, the EEG recordings using the facial electrodes incorporated within the EGI 128-electrode nets. We were successful in this, as reported in the July Progress Report. Follow up assessment of the responses across the array of facial electrodes revealed that the analysis procedure can be further improved, as the initial approach of taking the differential signal between the electrodes immediately above and below the eyes was missing a substantial proportion of the early signal attributable to the ERG sources. We therefore elected to take the alternative approach of taking the average signal of a subset of 14 EEG channels that are distributed bilaterally around the eyes.

We developed the amplitude and latency analysis software for ERG and EEG as follows:

To provide a robust estimate of the peak amplitudes and latencies of the ERGs, they were filtered with a low-pass filter and the waveform maximum used to define the amplitude relative to the baseline of the first 20 ms and its corresponding latency (see Figure 3). Note that, although the amplitudes of ERG signals from skin electrodes are substantially smaller than those from scleral electrodes, the facial skin electrode component has comparable signal-to-noise ratio.

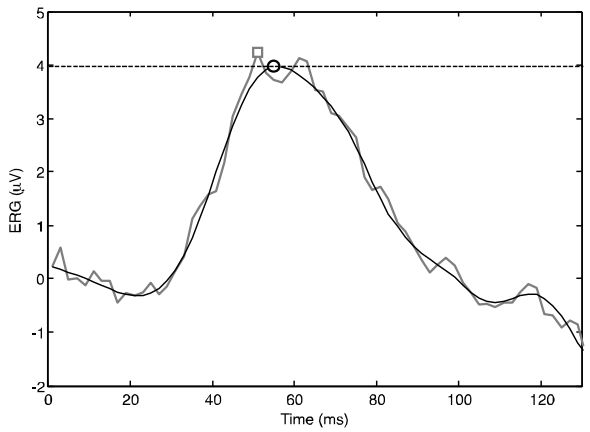


FIGURE 3. Example of the ERG waveform peak analysis. The cycle-averaged ERG (gray curve was smoothed by low-pass filtering (black curve) and the amplitude and latency determined from its maximum value (circle). This approach provides a more robust estimate of the peak amplitude and latency than simply taking the maximum of the raw waveform (square).

2e. Development of PCA spatial component analysis software for high-density EEG

We developed the PCA spatio-temporal-chromatic component analysis software for high-density EEG by:

1. taking the 114 non-facial electrode channels of the EGI net and running a temporal PCA on the matrix of time series concatenated across color conditions,
2. transforming the matrix to run a spatial PCA on the resultant right-singular vector matrix,
3. combining the primary spatial component with each temporal component, and obtaining the least-squares solutions for the component weights across color conditions (R, G, B, W).

Sample results for the EEG component analysis are shown in terms of scalp maps of the strength of activation in Fig. 4. These component weight distributions across color stimuli may then be used to identify the functional role of the component with respect to chromatic processing. In particular, weights matching the photopic spectral sensitivity function with a maximum for the G stimulus will be taken as deriving from the R,G,B cone pathway, components with a maximum weight for the B stimulus as deriving from the melanopsin pathway, and components with maximum weight across both B and G channels as from the rod pathway. As in Fig. 4, these three main options are well-represented in our datasets, together with other distributions representing other (presumably higher cortical) aspects of color processing.

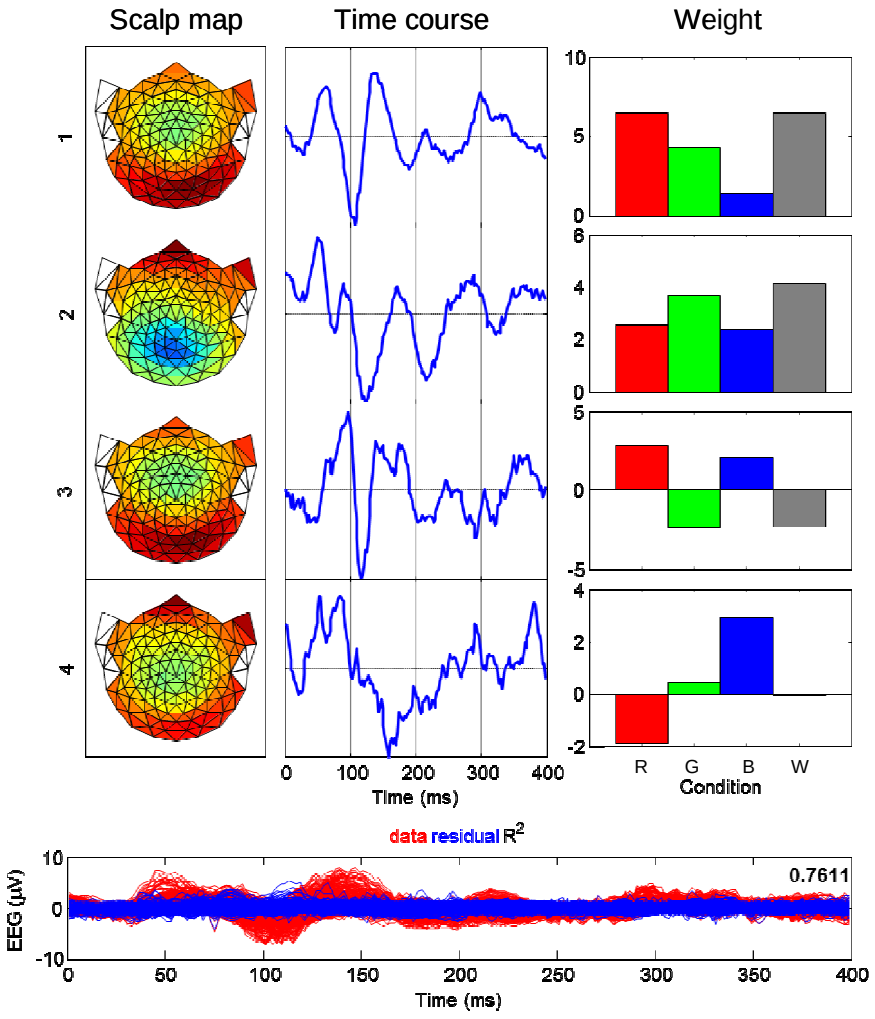


FIGURE 4. EEG component analysis. **Upper panel:** ***Left column:*** Scalp sensor distribution of four PCA components; ***Middle column:*** Time courses of the same PCA components; ***Right column:*** Weights for the same PCA components across two repeats of the four color conditions (R,G,B and W). **Lower panel:** 'Butterfly' plot of the individual cycle responses across the 125 of the 128 EEG sensors (red curves) and residual after accounting for the PCA components (blue curves), with the proportion of variance accounted for by the PCA model (inset value; note the high proportion accounted for: 86%).

Task 3. Recruitment of subjects into a group of 10 with mTBI associated with photophobia symptoms (mTBI/Ph) and 10 with mTBI without photophobia symptoms (mTBI/nonPh)

Subject recruitment has been successfully initiated. We have already recruited 6 photophobic and 8 non-photophobic individuals in preparation for the testing phase of the project. Half of these recruited subjects have already undergone most of the individual testing.

Task 4. Electrophysiological testing

4a. ERG testing of the mTBI/Ph and mTBI/nonPh groups

The integration of the ERG with the EEG recording protocol allows us to record ERGs simultaneously with all the EEG stimulus conditions. This was a successful strategy. Some representative results are reported in the following section.

4b. Individual analysis of the ERG data

The stimuli are short- (200 ms) and long-duration (2000 ms) pulses, designed to assess the early ERG response recorded from the 14 face electrodes of the EGI net at high signal-to-noise ratio with many (150) repetitions and the slow development of the response over several seconds, respectively.

Short-duration condition

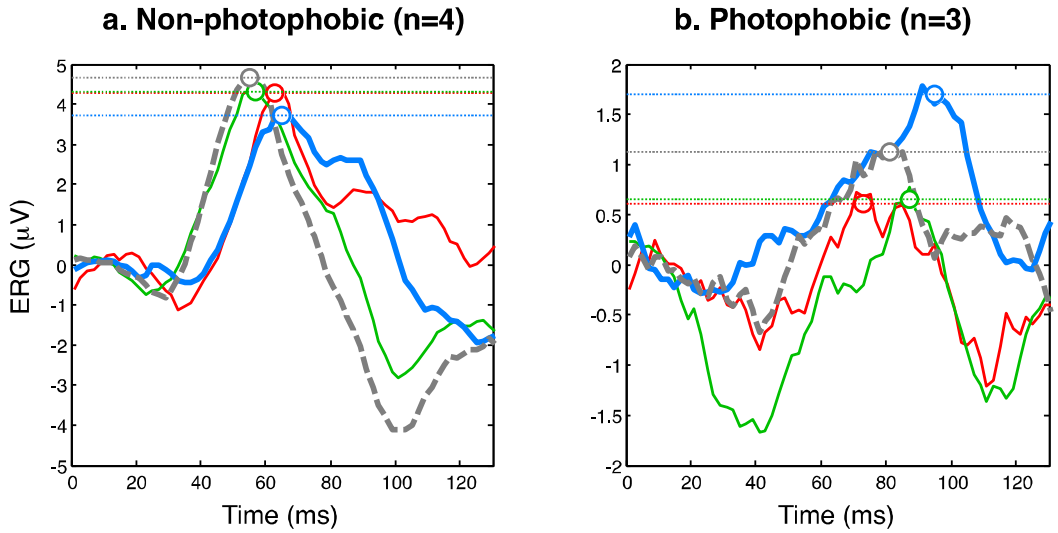


FIGURE 5. Interim average ERG responses for the short-duration condition for illustrating the distinctive behavior of the melanopsin component and the differences between the TBI/nonPh **(a)** and TBI/Ph **(b)** groups tested so far. Color coding: R (red curve), G (green curve), B (cyan curve) and combined white W (black dashed curve). Circles show peak amplitude and latencies measures. **a.** Note delayed response to the B color-field in the TBI/nonPh group, representing the response dominated by the melanopsin pigment signal (although this is only marginally evident in the peak latency index). **b.** Note noisy, low amplitude, delayed responses in the TBI/Ph group, with the melanopsin (B) component replicating the extra delay relative to the other three color conditions but with amplitude significantly above even the W response.

Table 1. Latencies and Amplitudes of the Four Color ERGs for the Two TBI Groups

	R	G	B	W	
TBI/nonPh	63	57	65	55	ms
	4.25	4.20	3.69	4.57	V
TBI/Ph	73	87	95	79	ms
	0.64	0.67	1.64	1.10	V

On the one hand, in the TBI/nonPh group, the analysis procedures described in Section 2d gave rise to ERG responses with the typical characteristics that, for the short-latency regime, the B response was typically about as large as the G response, and that the W response was typically not significantly larger than the G or B response (Fig. 5, a). The G, W responses typically had similar latencies, while the B response was typically slower by about 10 ms relative to W. On the other hand, the photophobic, TBI/Ph, group had much noisier waveforms, much reduced amplitudes with the B response switching from being the weakest of the four in the non-photophobic to become the strongest of the four color responses; similarly, in the latencies were all increased, with B maintaining the longest latency of all colors in the TBI/Ph (Fig. 5, b).

Long-duration condition

For the slow waveform regime of the 4 s scans, the goal was to identify a slow ERG component that showed differential behavior between the B and G conditions reflective of melanopsin activity. As seen in the interim results in Figure 6 (left panel), such a melanopsin characteristic while not evident in the TBI/nonPh group, emerged in the TBI/Ph group at about 100 ms and was maintained for most of the light-on epoch. Conversely, following light offset, a strong melanopsin component emerged in the TBI/nonPh group (right panel) over the time period where the other responses were recovering from an initial offset transient. The light offset responses in the TBI/Ph group were weak, but showed a similar relative ordering. Note that the B responses should not show much contribution from S cones because of their low density across the retina and reduced sensitivity at 470 nm relative to their peak at 430 nm, which again is evidence that the measured responses originate from the melanopsin RGCs instead.

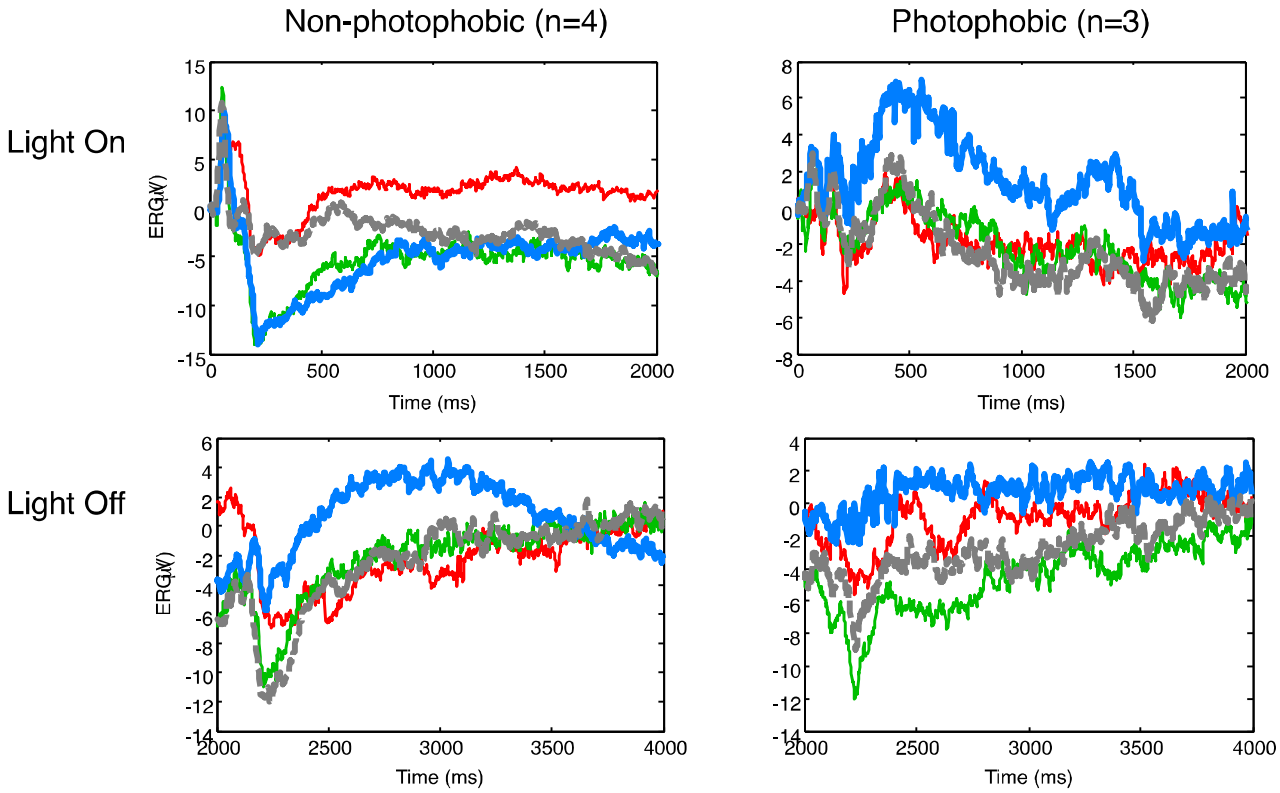


FIGURE 6. Averaged ERG responses to R, G, B and W color fields (coding as in Fig. 5), shown separately for the 2000 ms light on and light off epochs (upper and bottom panels) for the interim groups of TBI/nonPh (left panels) and TBI/Ph (right panels) participants, demonstrating a dramatic difference between the non-photophobic and the photophobic groups for both the light-on and light-off phases. Note in particular the profound difference in the blue response as a function of group.

4c. EEG testing of the mTBI/Ph and mTBI/nonPh groups

EEG testing has proceeded in parallel with the ERG testing on the EGI system. The results, however, are not amenable to waveform averaging due to the variety of resulting temporal waveforms.

4d. Individual analysis of the EEG data

The PCA component analysis described in Section 2e resulted in 3-5 components with significant weights for each participants. The goal of the study was to identify components with a spectral weighting corresponding to the selectivity of the melanopsin system, namely those with a stronger response to the B (470nm) than the other color conditions. This criterion was successfully met in 6 of the 7 participants tested so far. The B response was stronger than the G response in all cases, indicating that it was not mediated by rods, and the pattern of activation strengths was inconsistent with mediation by S-cones, as illustrated in a representative case in Fig. 7. We therefore conclude that it represents the first evidence of EEG activation of the melanopsin pathway in the brain. The ring-like spatial pattern of activation around the lowermost electrodes was consistent with an early subcortical source in the melanopsin pathway.

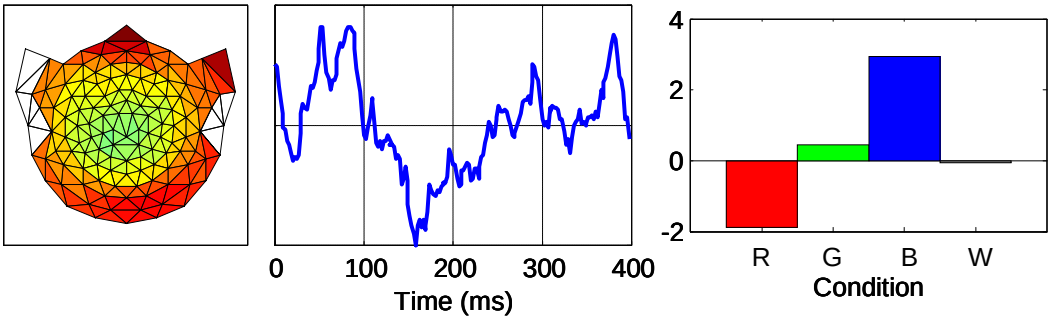


FIGURE 7. EEG component showing the strongest response to the B stimulus, forming a candidate melanopsin-specific component. Red outline in upper row indicates the stimulus timecourse. Left panel: scalp distribution of the 4th component in Figure 3; Middle panel: Temporal component; Right panel: Component weight across R, G, B and W.

Task 5. MRI testing

5a. fMRI testing of the mTBI/P and mTBI/nonP groups

The key to the fMRI studies for this project is the high-resolution (1.6 x 1.6 x 1.6 mm) functional imaging prescription targeting the brainstem and subcortical regions of the trigeminal pathway. The initial data are already showing that this prescription makes it possible to identify color-related activation from several key nuclei at various levels of the trigeminal complex and other melanopsin associated structures. Figure 8 shows an example of the midline and bilateral activation sites identified by this technique: the trigeminal nuclei; the principal nucleus of the trigeminal complex, the mammillary bodies, the pituitary body, and the pineal gland.

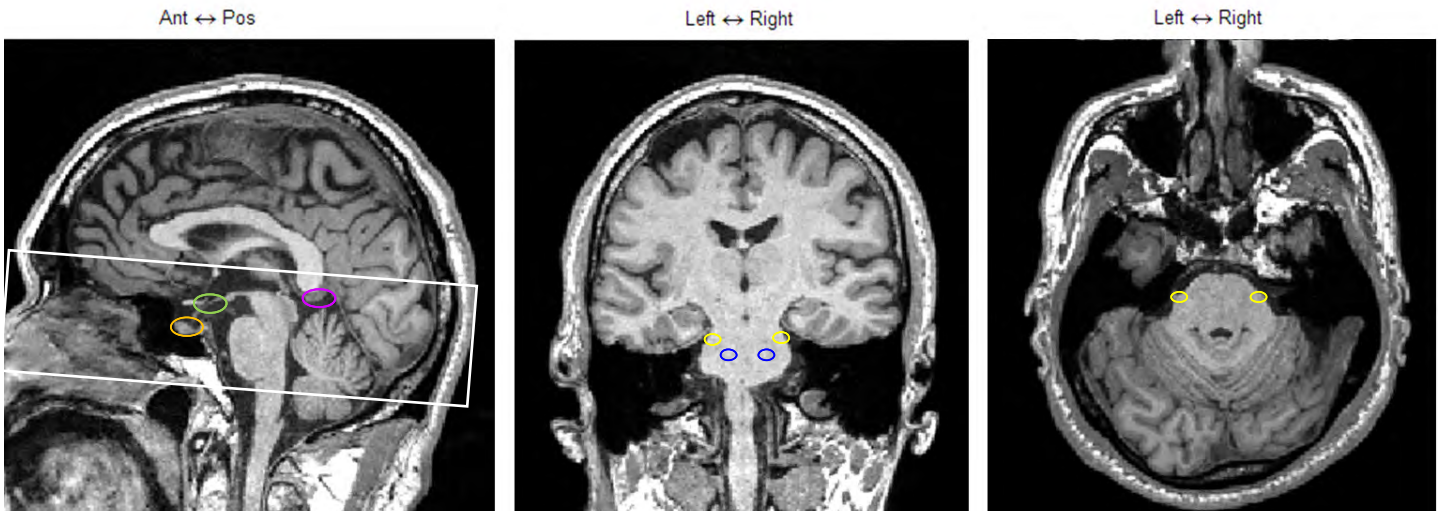


FIGURE 8. Color stimulus brainstem activation sites related to the trigeminal pathway: pituitary body; mammillary bodies; pineal body; trigeminal nuclei; principal nucleus of the trigeminal complex. White box indicates the range of the high-resolution brainstem prescription.

5b. Individual analysis of the fMRI data

The responses of each of these structures to the color-specific conditions of our stimulus battery are shown in Fig. 9. The stimuli are of two types: i) 20 s of color flicker vs 20 s of a black field, and ii) the interchange between a 20 s steady field of blue and a 20 s steady field of some other color.

The data show not only strong responses in these traditionally non-visual subcortical structures, but these responses were well differentiated for the different color conditions. The block design [20 s / 20 s] of color stimuli generated a range of remarkable responses in these non-visual brainstem structures (see Fig. 8). The responses are positive to the blue onset for some subcortical structures, negative for others, and the sign of the change can be in the opposite direction for the B/R flicker stimulus relative to the B/G color change stimuli. Finally, the B flicker and B/G color change stimuli give approximately similar patterns of temporal response across the set of subcortical structures.

All these features indicate the capability of our procedures and experimental design to identify a high specificity of a range of subcortical response regions related to the melanopsin pathway for the color change stimuli, which will be fully analyzed as the project progresses. At this point, the main message is that the preliminary studies are successful in recording color-specific responses from good sample of trigeminal and melanopsin-related subcortical structures that are candidates for structures that may be responsible for photophobic reactions. We are therefore well-positioned to assess the role of these structures in the mediation of photophobia in the participant groups for the main project.

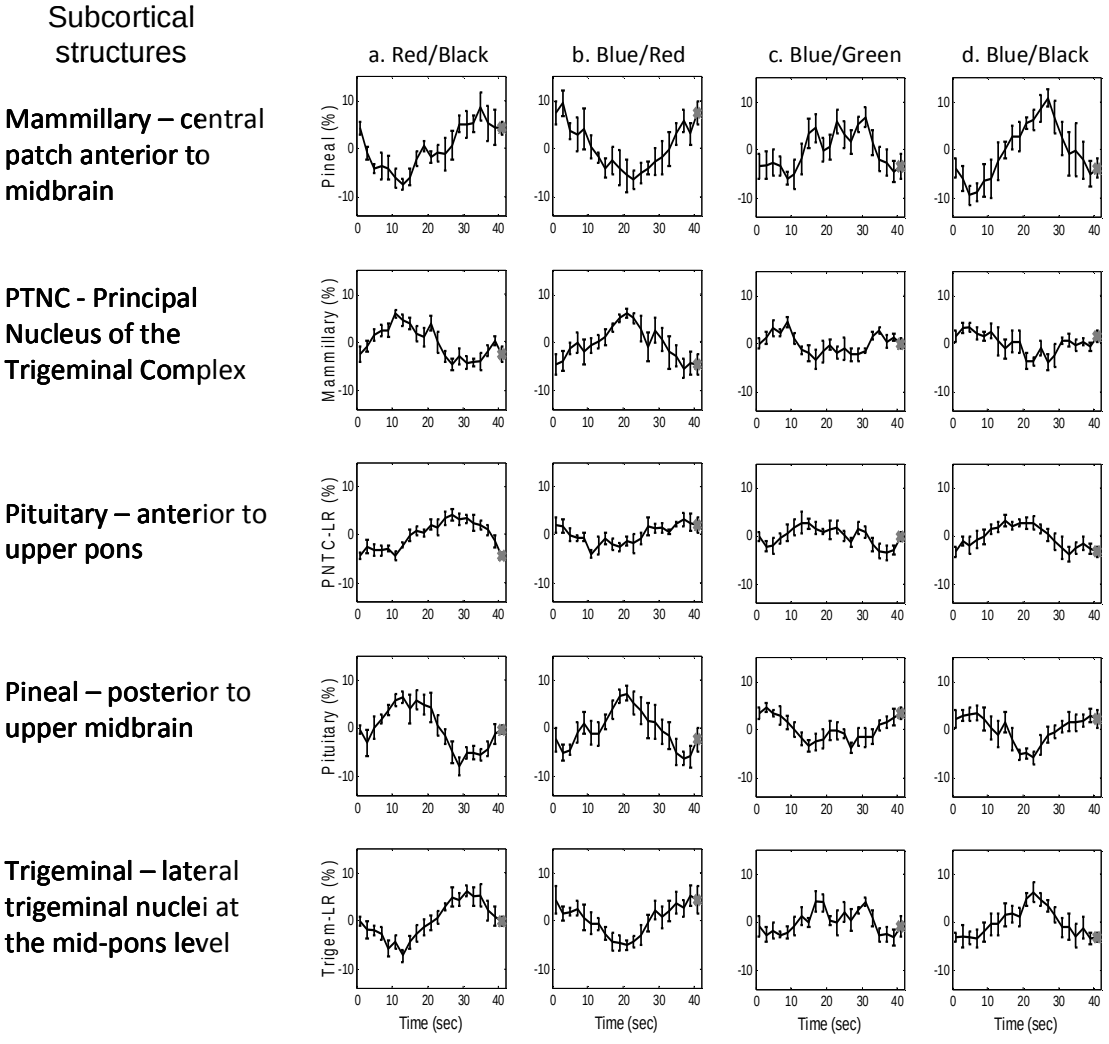


FIGURE 9. Response waveforms for the five nuclei shown Fig. 8 across 4 different 20s/20s color conditions: **a:** red flicker vs black; **b:** steady blue vs steady green; **c:** steady blue vs steady green; **d:**.blue flicker vs black. Note radical difference among response waveforms for different color conditions in the same nuclei.

5c. TBM testing of the mTBI/P and mTBI/nonP groups

The T1 MRI scans required for TBM have been obtained on 5 participants to date, so it is still early for running a meaningful group comparison between the TBI/nonPh and TBI/Ph groups. We, however, are providing an overview of the individual results in the form of a comparison between those participants with TBI and prior data for control non-TBI individuals.

5d. Interim analysis of the TBM data

Figure 10 shows an interim comparison using the tensor-based morphometry (TBM) developed for the MR anatomy data. Brainstem surfaces are aligned with a 12-parameter affine transformation. The colored structure includes (from top to bottom) the segmented midbrain, pons and medulla. Color coding represents the tensor difference between this averaged TBI brainstem and the control average brainstem, with the statistical significance of each difference coded in the lower set of brainstem images. The most significant effect was shrinkage in a bilateral region of the upper posterior brainstem in the TBI group relative to controls, which may indicate neural degeneration in these structures.

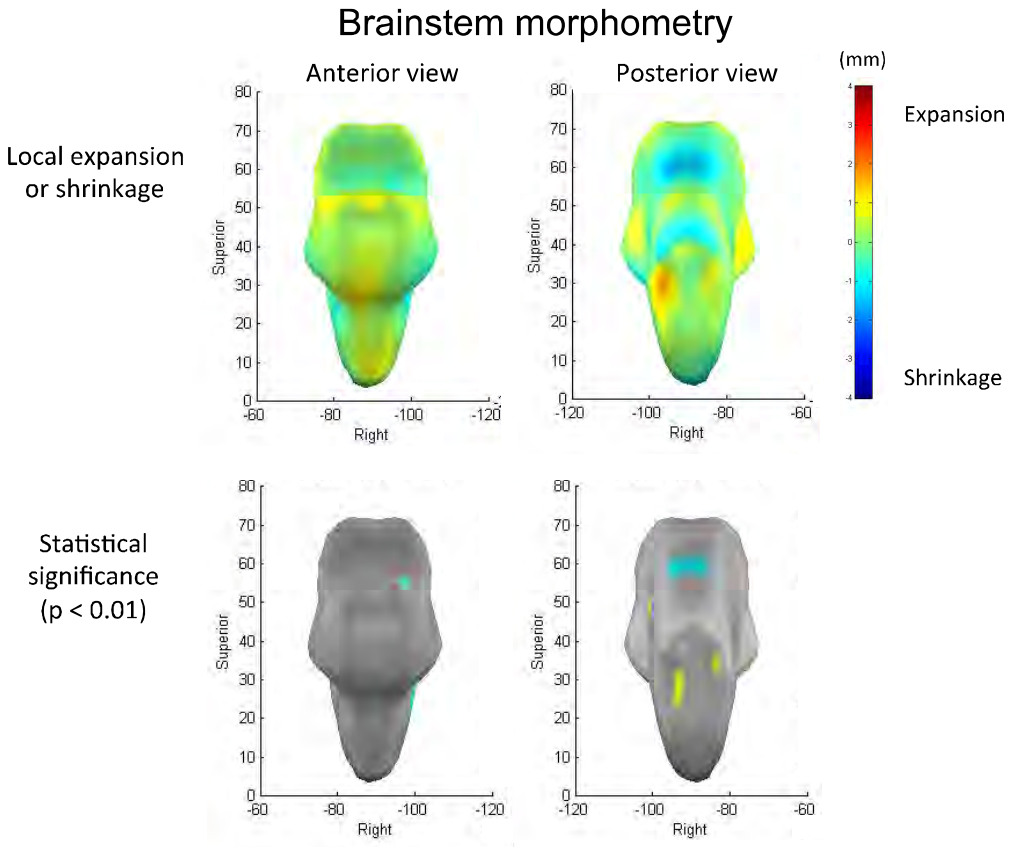


FIGURE 10. Interim analysis of brainstem morphometry for the TBI group relative to previous controls. Upper panels: anterior and posterior views of the human brainstem, with color map representing the deviations (expansion or shrinkage) from average control brainstem structure. Lower panels: statistical significance of the deviations in the upper panels. ($p < 0.01$, uncorrected).

c. **What opportunities for training and professional development has the project provided?**

Nothing to Report.

d. **How were the results disseminated to communities of interest?**

Nothing to Report.

e. **What do you plan to do during the next reporting period to accomplish the goals?**

The tasks are specified in relation to the respective Aims specified in the SoW.

Task 3. Recruitment of subjects into a group of 10 with mTBI associated with photophobia symptoms (mTBI/Ph) and 10 with mTBI without photophobia symptoms (mTBI/nonPh) (months 10-15, Jul - Dec 2015)
We expect to complete the recruitment of the remaining 6 participants by the end of the coming quarter. However, if any of the recruited participants withdraw or prove unsuitable for inclusion in the study (for example, due to excessive blinks or eye movements during testing), we will continue recruiting through the end of the experimental testing period (to May, 2016) to complete the requisite participant numbers.

Task 4. Electrophysiological testing (months 10-20, Jul 2015 - May 2016)
4a. ERG testing of the mTBI/Ph and mTBI/nonPh groups
4b. Individual analysis of the ERG data
4c. EEG testing of the mTBI/Ph and mTBI/nonPh groups
4d. Individual analysis of the EEG data
The combined ERG/EEG testing of the recruited participants will be continued through the coming quarter. The individual analyses will be performed as the data become available, and the effectiveness of analysis protocol reviewed periodically to determine whether it is optimal or could be improved.

Task 5. MRI testing (months 10-20, Jul 2015 – May 2016)
5a. fMRI testing of the mTBI/Ph and mTBI/nonPh groups
5b. Individual analysis of the fMRI data
5c. TBM testing of the mTBI/Ph and mTBI/nonPh groups
5d. Individual analysis of the TBM data
The fMRI and TBM measures from the recruited participants will be continued through the coming quarter. The individual analyses will be performed as the data become available, and the effectiveness of analysis protocol reviewed periodically to determine whether it is optimal or could be improved.

Task 6. Group data analysis and write-up of results (months 21-24, June – Sept 2016)
5a. Group data analysis of ERG/EEG/fMRI/TBM data for mTBI/P and mTBI/nonP groups
5b. Write-up of results for publication
In the last four months, we will run group data analysis on all four brain imaging modalities – from the ERG and EEG to the fMRI and TBM. We will run comparisons of the photophobic with the non-photophobic TBI participants to meet the goals of the project. The findings will be written-up for publication in high-rank scientific journals.

4. **IMPACT:**

a. **What was the impact on the development of the principal discipline(s) of the project?**

1) This project is the first to develop a tetramodal brain-imaging capability (ERG, EEG, fMRI & TBM) in both the field photophobia (in either TBI or normal controls) and in the field of studies of the recently discovered intrinsically photosensitive/melanopsin Retinal Ganglion Cells (mRGCs), which provides the potential for full-spectrum investigation of the underlying brain mechanisms as a basis for the development of effective therapeutic approaches.

2) One innovation that we have developed is the use of skin electrodes from the high-density EEG system for recording ERGs. Such an integrated ERG/EEG approach ensures a much higher experimental efficiency and has the additional advantage of providing simultaneous ERG/EEG recordings for applicability to event-related paradigms.

b. **What was the impact on other disciplines?**

Nothing to report at this juncture.

c. **What was the impact on technology transfer?**

Nothing to report.

d. **What was the impact on society beyond science and technology?**

Nothing to report for now.

5. CHANGES/PROBLEMS:

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations:

Three talks on the results of the exploratory procedures developing the methodologies for identifying melanopsin-specific responses at the perceptual, pupillometric and EEG levels of analysis have been presented at international meetings, with acknowledgements to the funding agency. The pupillometric analysis was developed in the process of validating the response ratio approach to the identification of melanopsin-specific signals in the ERG, EEG and fMRI responses.

Presentation at the Vision Sciences Society, St Petersburg, FL, May, 2015
Tyler CW. “Contourless Color Field Induction”

Presentation at the Society for Neuroscience, Chicago, Il, October, 2015:
Likova, LT., Tyler CW. “Differential cerebral sources of human color responses”

Presentation at the Pupil Colloquium, Oxford, UK, September, 2015
Tyler CW, Likova, LT, Nicholas, SC. “Validation of the pupillometric index of melanopsin response”

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: Lora T. Likova, Ph.D.
Project Role: Principal Investigator
Nearest person month worked: 12%
Contribution to Project: Dr. Likova has been responsible for the overall guidance of all activities on the project, preparation of all documents, coordination the collaboration with the UWV, oversight of the grant personnel, design of the experimental protocols, following literature developments, ERG/EEG/fMRI/TBM results analyses and design optimization, organizing and leading team discussions, accepted conference presentation for the Society for Neuroscience, progress report preparation.

Name: Christopher W. Tyler, Ph.D., D.Sc.
Project Role: Co-PI
Nearest person month worked: 6%
Contribution to Project: Dr. Tyler has been involved in all design and implementation of the experimental protocols, updating the literature review, ERG/EEG/fMRI/TBM results analyses and design optimization, in all discussions and progress report preparation, accepted conference presentation for the Society for Neuroscience.

Name: J. Vernon Odom, Ph.D.
Project Role: Co-PI
Nearest person month worked: 5%
Contribution to Project: Dr. Odom has been involved in the design and optimization of the ERG experimental protocols and the review of new literature in the domains of the project.

Name: Kristyo Mineff, MS
Project Role: Research Assistant
Nearest person month worked: 35%
Contribution to Project: Mr. Mineff has been responsible for subject recruitment, subject consent, running all behavioral tests, as well as he has been involved in the testing and implementation of all experimental protocols for the brain imaging modalities, lab organization and participation in the literature review in the domains of the project.

Name: Spero Nicholas, MS
Project Role: Sr. Programmer/Analyst
Management of collaborative aspects of the project with the Smith-Kettlewell Brain Imaging Center, design and programming of the stimulus software, design and programming of the ERG, EEG and fMRI data analysis software.

b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

There has been no change in other support for key personnel.

c. What other organizations were involved as partners?

1. **Organization Name:** University of West Virginia
2. **Location of Organization:** Morgan Town, West Virginia
3. **Partner's contribution to the projec**t:

Collaboration: Dr. Odom has been involved in the design and optimization of the ERG experimental protocols and the review of new literature in the domains of the project at 5% during periodic visits to The Smith-Kettlewell Eye Research Institute in San Francisco, where all the work on the project has been done.

8. Special Reporting Requirements: Quad Chart

See next page.

Mechanisms of Photophobia in Traumatic Brain Injury: Therapeutic Implications

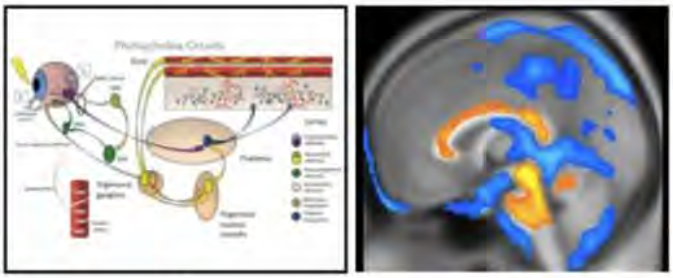
PI: Lora T. Likova, Ph.D. **Org:** Smith-Kettlewell Eye Research Insititute **Award Amount:** $250,000

Study/Product Aim(s)

- The primary goal is the mitigation and treatment of intractable photophobia due to mild Traumatic Brain Injury (TBI)
- A critical need for this goal is to determine the neural mechanisms of such photophobia
- The multimodal methodology is designed to assess two hypothesized mechanisms: that the mTBI photophobia is mediated by melanopsin-containing retinal ganglion cell (mRGC) pathway, and/or that it is due to tissue edema in deep brain structures

Approach

A high proportion of mTBI cases suffer from intractable light sensitivity (photophobia). We will take a four-pronged approach to identifying the key brain locus of the light-induced pain: ocular ERG, whole-head EEG, MRI morphometry and functional MRI of subcortical pathways. This multi-level tetramodal assessment protocol will determine the neural mechanisms of the photophic disability.



A B

A: Diagram of the mRGC pathway for photophobia from the retina to the thalamic and brainstem nuclei and cortex (from Noseda & Burstein, 2011).
B: Average pattern of chronic tissue swelling (blue) and shrinkage (orange) determined by MRI morphometry following TBI incidents (from Sidaros et al., 2007).

Timeline and Cost

Activities / CY	14	15	16	
Finalize protocol & IRB approvals				
Integrated tetramodal development				
ERG & EEG testing				
MRI and fMRI testing				
Analysis and write-up of results				
Estimated Budget ($K)	**$45K**	**$130K**	**$75K**	**$000**

Updated: Oct 2015

Goals/Milestones

CY14 Goal – Protocol Finalization
☐ Validation of experimental protocol & DoD Surgeon General approval
☐ Development of integrated tetramodal hardware/software suite

CY15 Goals – Experimental Studies
☐ Finalization of integrated tetramodal hardware/sortware suite
☐ mTBI recruitment, and ERG and EEG testing
☐ FMRI and TBM testing, and individual analyses

CY16 Goal – Production readiness
☐ Group tetramodal analysis (ERG, EEG, MRI, fMRI) for assessing the neural sources of photophobia
☐ Write-up of results

Budget Expenditure to Date
Projected Expenditure: $250,000
Actual Expenditure: $113,114