A new method to measure electroretinograms (ERGs) elicited by temporal white noise

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Purpose: To provide the initial determination of the impulse response functions (IRF) of electroretinograms (ERG) derived from responses to temporal white noise (TWN) stimuli (TWN ERG) and to compare them with the conventional flash ERGs.

Methods: The TWN ERGs were recorded from nine participants with normal trichromatic vision. Temporal white noise stimuli had the property that amplitudes in the frequency domain (0-512 Hz) were constant, the phase at each frequency (0-359°) was randomly chosen and the luminance distribution around the mean was Gaussian. The IRF was obtained by cross-correlating the recorded response with the TWN stimulus. The TWN ERG responses were measured to full field (FF) and to 40° diameter stimuli at mesopic and photopic mean luminances and at different TWN contrasts. For comparison flash ERGs to FF and 40° stimuli were measured in three participants at a mean lumiance also used in the TWN ERG.

Results: The TWN ERG recordings were highly repeatable, with good signal-to-noise ratio and did not lead to blink artefacts. The TWN ERG resembled flash ERG waveforms and consisted of an initial negativity (N1) followed by a positivity (P1). These N1 and N1P1 components showed commonalities in implicit times with the a- and b-waves of the flash ERGs. The TWN ERGs lacked components similar to the oscillatory potentials (OPs). The TWN ERG depended linearly on stimulus contrast. There was a clear transition from rod to cone driven TWN ERGs at luminances around 1 photopic cd.m⁻².

Conclusions: The TWN represents a stimulation method that is measured with adaptation and contrast conditions similar to natural viewing environments. It is more convenient than flashes and thus less disturbed by blink artefacts. The TWN stimulus allows for independent variation of stimulus strength and mean luminance, which is not possible with flash ERGs. The compression of energy into a short time period in a stimulus flash may lead to non-linearities in flash ERGs (e.g. in the OPs) that are not present in the TWN ERGs. TWN ERGs may provide a new method to study the physiology of the retina.

Commercial Relationships: Jan J. Kremers, None; Beatris K. Feigl, None; Avinash Aher, None; Declan J. McKeefry, None; Neil R. Parry, None; John Maguire, None; Ian J. Murray, None; Andrew J. Zele, None

Support: DFG Grant KR1317/13-1; ARC Grant DP140100333
Program Number: 4278
Presentation Time: 11:30 AM–11:45 AM
Cycle-by-Cycle Electroretinography: Utilizing a Widely-Available Instrument to Assess Microvolt Signals in Usher Syndrome
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Purpose: 1. To investigate the validity of microvolt 30-Hz Flicker responses in patients with Usher syndrome
2. To share a protocol for cycle-by-cycle (CxC) Fourier analysis of a steady state signal obtained using a widely-available commercial electroretinography (ERG) console
Methods: Photopic ERGs were recorded from both eyes of twenty-one patients with a clinical diagnosis of Usher syndrome, enrolled under National Eye Institute protocol 05-EI-0096 (NCT00106743) or 15-EI-0128 (NCT02471287), between January and October, 2016. Cone flash and 30-Hz flicker responses were obtained according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards using a UTAS console (LKC, Gaithersburg, MD) and bipolar Burian-Allen electrodes (Hansen Ophthalmic Instruments, Iowa City, IA). A steady state 32-Hz flicker signal spanning 15 seconds (based on Sieving PA et al. IOVS 1998) was obtained using an Espion3 console (Diagnosys, Lowell, MA). Software developed for CxC analysis was used to calculate signal-to-noise ratio (SNR) and measurement uncertainty. The best 5 seconds interval was selected for final analysis and a low frequency filter was applied. Right eye data was used for statistical analysis.
Results: Patient age ranged from 16 to 75 years (mean 50, SD 18) and molecular testing was positive for biallelic USH2 mutations in 8 patients, MYO7A in 4, GPR98 in 2, USH1C in one; results were pending or inconclusive in six patients. ISCEV 30-Hz flicker ERG amplitudes ranged from 0.71 to 8 uV (mean 2.81, SD 1.77). Discrete Fourier transform failed to identify a peak at the stimulus frequency for any of these patients indicating these recordings were at noise-level. Statistical analysis of the CxC recordings (based on spread of single cycle Fourier components and also on the SNR threshold) identified 10 patients and three eyes where signal was not different from noise. In the other 19 eyes, the right eye amplitude ranged from 0.57 to 5.68 uV (mean 2.34, SD 1.28) and the SNR from 1.03 to 31.47 (mean 8.44, SD 6.68).
Conclusions: CxC ERG, recorded using a widely-available commercial instrument, is a valuable technique to assess the validity and SNR of microvolt signals common in Usher syndrome. It should be considered for any trials assessing the natural history and potential response to therapeutic intervention.
Commercial Relationships: Wadhit M. Zein, None; Antonello Fadda, None; Brett G. Jeffrey, None; Robert B. Hufnagel, None; Amy Turriff, None; Ramiro Maldonado, None; Brian P. Brooks, None; Benedetto Falsini, None; Paul A. Sieving, None
Clinical Trial: NCT00106743

Program Number: 4279
Presentation Time: 11:45 AM–12:00 PM
Full-field measures of visual function in X-linked retinoschisis: comparison of ERGs, luminance thresholds, and pupil responses
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Purpose: X-linked retinoschisis (XLRS) is typically characterized functionally by reduced full-field dark-adapted electroretinogram (ERG) b-wave amplitude, but focal dark-adapted luminance thresholds are generally normal or minimally elevated. To investigate this apparent discrepancy, visual function was assessed in XLRS patients using three full-field measures: ERG, luminance threshold, and pupil size.
Methods: Dark-adapted ERGs and pupillary light reflexes (PLR: percentage pupil constriction due to light stimulation) were recorded from 5 XLRS patients (19 to 40 years) and 5 normally-sighted subjects (27 to 39 years). ERGs and PLRs were obtained for a range of flash luminances (ERG: -3 to 1 log cd s m\(^{-2}\); PLR: -3 to 2.6 log cd m\(^{-2}\)) and these data were fit with Naka-Rushton functions to derive \(\hat{R}\) (maximum saturated response) and \(S\) (semi-saturation intensity; a sensitivity measure). PLR measurements were obtained with both 465 nm and 642 nm stimuli. Full-field dark-adapted luminance thresholds were also measured psychophysically at these wavelengths.
Results: Analysis of variance (ANOVA) indicated significant reductions in dark-adapted b-wave amplitude for the XLRS patients compared to the controls for all flash luminances (p < 0.05). Naka-Rushton fits indicated that the patients had \(\hat{R}\) reductions that ranged from 0.05 to 0.74 log units and increased \(S\) (sensitivity loss) that ranged from 0.45 to 1.6 log units. ANOVA also indicated that the dark-adapted, short-wavelength PLR was significantly reduced in the patients for low luminance flashes (-3 to 1 log cd m\(^{-2}\); p < 0.05) but not for high luminance flashes (2.0 and 2.6 log cd m\(^{-2}\); p > 0.05). Long-wavelength PLRs were significantly reduced for the patients at all flash luminances (p < 0.05). For both wavelengths, there were minimal reductions in pupil \(\hat{R}\) (less than 0.11 log units) and substantial increases in \(S\) (0.65 to 2.13 log units). Luminance thresholds were within the range of normal for all patients; mean patient and control threshold did not differ significantly (p > 0.08).
Conclusions: The results highlight the somewhat paradoxical finding that the presumed bipolar cell deficit that occurs early in the visual pathway, indicated by b-wave abnormalities, does not significantly affect psychophysical luminance threshold or maximum pupil response, but does significantly reduce sensitivity within the pupil pathway.
Commercial Relationships: J Jason McAnany, None; Jason C. Park, None; Frederick T. Collison, None; Gerald A. Fishman, None
Support: This research was supported by a Dolly Green Special Scholar Award (JM) and an unrestricted grant (UIC Dept. of Ophthalmology) from Research to Prevent Blindness, a National Institutes of Health Research Grant P30EY01792 (Dept. of Ophthalmology core), and the Pangere Family Foundation.
The photopic negative response (PhNR) is a slow negative wave. A selective increase in the slope of the PhNR intensity response function is the semi-saturation constant indicates elevated sensitivity of the response function of mTBI patients with a corresponding decrease in the semi-saturation constant indicates elevated sensitivity of the cellular generators of the PhNR in patients with mTBI. This finding may suggest a potential pre-cortical basis for photosensitivity in mTBI.

**Commercial Relationships:** Roa Al-Abdalla, None; Nabin Joshi, None; Jennifer Nguyen, None; Kenneth J. Ciuffreda, None; Suresh Viswanathan, None

**Support:** T35EY020481 SUNY College of Optometry

**Program Number:** 4282

**Presentation Time:** 12:30 PM–12:45 PM

**Changes in Retinal Function and Glial Reaction in an Impact Concussion Mouse Model**

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**Purpose:** A large proportion of enlisted men and women suffer traumatic brain injuries (TBIs) in accidents during training and deployment, in combat, and in “domestic” incidents. Since the retina is the most accessible part of the brain, the eye may serve as a literal window into TBI. Neuroinflammation and glial reaction have been observed in the brains of veterans (and athletes) with chronic traumatic encephalopathy consequent to TBI (see Goldstein et al.). Trauma-induced changes in the retina may both be concomitant to brain dysfunction and contribute to visual dysfunction. Therefore, we evaluated retinal changes in an impact concussion mouse model.

**Methods:** We examined 27 mice expressing green fluorescent protein in microglia one day before and three days after concussive head injury (HI, n=14) or sham (CT, n=13) treatment. We performed electoretinographic (ERG) testing in all mice (14 HI, 13 CT) and adaptive optics (AO) retinal imaging in a subset (5 HI, 9 CT). We recorded ERG responses to a >5 log unit range of full-field stimuli and obtained measures of the sensitivity and saturated amplitude of photoreceptor (a-wave), postreceptor (b-wave) and inner retinal cellular generators of the PhNR.

**Results:** For the PhNR intensity response function, n was significantly larger (p=0.03) and K was significantly smaller (p=0.036) in the mTBI group (n=1.6, K=0.13) relative to the control group (n=0.99, K=0.20). K also showed a significant negative correlation with n (r=0.18, m=-0.16, p<0.001). The V_max of the PhNR and all Naka-Rushton fit parameters of the b-wave were not significantly different between mTBI patients and control subjects.

**Conclusions:** A selective increase in the slope of the PhNR intensity response function of mTBI patients with a corresponding decrease in the semi-saturation constant indicates elevated sensitivity of the cellular generators of the PhNR in patients with mTBI. This finding may suggest a potential pre-cortical basis for photosensitivity in mTBI.
(OPs) activity. We expressed all values as the log change from baseline (ΔLogNormal) prior to statistical evaluation. We then used a semi-automated approach to label microglia in AO fluorescent scanning light ophthalmographs (AO-fSLOs) of a region near the optic nerve head (ONH).

**Results:** Following impact, we found modest but significant attenuation in photoreceptor (-0.12 log; -23%), postreceptor (-0.07 log; -15%), and inner retinal (-0.16 log; -31%) responses which did not occur in CT mice. We could readily resolve microglia and their ramifications in AO-fSLO images and, at the second test, found a marked increase in the number of microglia near the ONH in HI mice. Further, microglia were regularly distributed and in resting morphology at baseline but, after injury in HI mice, we observed marked microgliosis characterized by reactive phenotype with extensively overlapping ramifications and apparent perivascular reactivity.

**Conclusions:** Decrease in function, and corresponding glial reaction, occur in retinas following concussive injury. These retinal changes may be of prognostic value in TBI and may underpin some of the vision complaints of veterans with a history of TBI.

**Commercial Relationships:** James D. Akula, None; Olga Minaeva, None; R D. Ferguson, Physical Sciences, Inc. (E); Mircea Mujat, Physical Sciences, Inc. (E); Mark W. Wojnarowicz, None; Juliet A. Moncaster, None; Erich S. Franz, None; Andrew M. Fisher, None; Ivana Arellano, None; David G. Hunter, REBIScan, Inc. (P), REBIScan, Inc. (I); Anne B. Fulton, None; Lee E. Goldstein, REBIScan, Inc. (C), REBIScan, Inc. (P)

**Support:** DoD W81XH-14-1-0592, Massachusetts Lions Eye Research Fund, Boston Children's Hospital Ophthalmology Foundation