1. Protocol for the photopic negative response (PhNR) of the full-field electroretinogram

2. Scope and applications

The photopic negative response (PhNR) of the light-adapted (LA) electroretinogram (ERG) is a negative-going wave that occurs after the b-wave in response to a brief flash. The PhNR reflects activity of retinal ganglion cells and their axons[1] and its amplitude can be reduced early in diseases that affect the inner retina. Photopic negative responses also occur in response to long duration flashes, following the b-wave at light onset, and d-wave at light offset, but most publications to date have used brief flashes. Only the brief flash PhNR will be addressed in this protocol.

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4. Patient population

This protocol for recording the PhNR can be used for testing patients in whom inner retinal integrity, and specifically signaling by retinal ganglion cells and their axons, may be compromised. For example, since 2000 reduced PhNR amplitudes have been reported in patients with glaucoma,[2-5] optic atrophy,[6, 7] central retinal artery occlusion,[8, 9] ischemic optic neuropathy,[10] diabetic retinopathy,[11] and idiopathic intracranial hypertension.[12] In some cases, the protocol may be useful for monitoring treatment effects in eyes with ocular hypertension or glaucoma.[13] Abnormal K^+ channel activity or other dysfunction of retinal glia may also be reflected in PhNR recordings.[14] This is because generation of the PhNR, which has a slow timecourse (Fig 1), is thought to involve glial potassium (K^+) currents that serve to remove the excess K^+ released into extracellular space during activation of retinal ganglion cells.[15]

5. Technical issues

The electrodes, and electronic recording equipment for this PhNR protocol are as described in the ISCEV Standard for full-field ERG.[16] The present protocol assumes full-field stimulation, while acknowledging that focal stimulation has been shown to be effective in assessing inner retinal function.[17] For the frequency bandwidth of the recording, the ISCEV Standard suggests a range of at least 0.3-300 Hz. For PhNR recordings, the bottom limit of the filtering could be even lower, and should not exceed 0.3 Hz, to minimize distortion and possible elimination of the slow negative wave. For spectral characteristics of the stimulus, whereas the ISCEV Standard recommends “visibly white” (broadband) stimuli, narrowband stimuli are recommended for recording the PhNR: specifically a red flash on a rod saturating

blue background. LED based stimulators typically provide 20 nm half bandwidth for the red and blue LEDs. The recommendation for narrowband stimuli is based on the outcome of studies that compared PhNR amplitudes using broad vs narrow band stimuli in nonhuman primates[18] and in glaucoma patients,[5, 18, 19] and more generally on a review of the literature which shows that most studies in patients have used red LED flashes on blue LED backgrounds. It should be noted that other narrowband combinations using blue flashes on yellow or orange backgrounds have also been reported to be effective for eliciting a robust PhNR.[19, 20]

6. Calibration

The stimulus strength for the brief flashes can be specified in photopic candela seconds per meter squared (phot cd.s.m^{-2}); the background in phot cd.m^{-2}. A spectroradiometer is required to determine the spectral characteristics of chromatic flashes. Care should be taken to measure a range of flash luminances as some Ganzfeld stimulators use different combinations and banks of LEDs for different luminance ranges and these may have different wavelength specifications. It is useful also to confirm that the background is strong enough to saturate rod photoreceptors, e.g. about 100 scot cd.m^{-2}. Blue backgrounds will saturate the rods while minimizing the photopic stimulus strength and hence the adapting effect of the background on cone-driven responses.

7. Protocol Specifications

The procedures for patient preparation and recording are as specified by the ISCEV Standard for the light-adapted ERG, including pupil dilation and 10 minutes of light adaptation if the patient was dark adapted for other testing prior to recording the light-adapted ERG. The following additional specifications are recommended.

a) The chromatic characteristics of the stimuli

b) Flash strengths and background luminance

c) Frequency of flash presentation

d) Signal averaging

- Background: steady, blue LED (450 - 485 nm); 100 scot cd.m^{-2}; ~10 phot cd/m^2
- Flash: <5 ms; red LED (630 - 660 nm); 1.0 – 2.5 phot cd.s.m^{-2}, or the stimulus strength that produces the largest PhNR amplitude, but does not exceed the initial stimulus strength producing amplitude saturation, or lead to the decline in response amplitude associated with the photopic hill.[21, 22] The dynamic range of the stimulus response function generally from ranges from ~ 0.01 to >2.0 phot cd.m^{-2}.
- Inter flash interval: 1 second. Some studies have used an interval of 500 ms, but this may not allow enough time for PhNR to fully recover to baseline (see Fig 1).
- Averaging of responses: there should be sufficient repetitions to provide good signal to noise, and many studies have used 20 trials or more. At least 8 – 10 trials or more for lower stimulus strengths if a range of stimuli are used that includes weak stimuli,

fewer may be necessary for saturated responses. Artifact rejection should be used if available. If single responses are saved, noisy responses can be removed during off-line analysis before averaging.

8. Response evaluation

As shown in Fig 1, the PhNR amplitude can be measured from baseline to the minimum point in the trough (BT). This is the most straight forward method of measurement. It also can be measured from the peak of the b-wave to the maximum amplitude in trough (PT), or, not shown, at a fixed time, e.g. 65 to 75 ms in the trough of the response. Using a fixed time could be helpful when responses in diseased eyes are small and the trough is difficult to locate. Note that the PT measurement is largely dominated by the b-wave amplitude, and a reduction in b-wave amplitude could therefore be misinterpreted as a reduction in PhNR amplitude. When measuring the PhNR it may be necessary to take account of the i-wave, or i-waves, positive deflection(s) of Off pathway origin[10] in the falling limb of the b-wave, and/or later in the trough (Fig 1). For responses to the suggested narrowband stimuli, such as those used for responses in Fig 1, the maximum trough amplitude generally occurs after the initial i-wave. Given the slow nature of the response, and the variety of amplitude criteria that have been used, peak time of the PhNR is generally not reported. The PhNR is moderately affected by age, so for the particular measure(s) chosen, appropriate age-matched normative data should be used.[3, 22] Comparisons of findings in patients to a normal range of PhNR amplitudes are also important, as the test-retest variability of PhNR amplitudes is generally greater than that of a- and b-waves.[21-24]

9. Reporting

Reporting of results of PhNR testing should include measurements of the a-wave, b-wave and PhNR and a computation of the PhNR:b-wave ratio. This helps to determine whether the origin of any change in PhNR amplitude is at the retinal ganglion cells themselves or a more distal location in the retina. The choice of method for measuring PhNR amplitude is open to the study and the site, but for comparison with other studies inclusion of the BT measure is advised. Some studies have compared the sensitivity of the ratio of PhNR to b-wave amplitude (i.e. PhNR normalized to b-wave) versus the simple BT measure for detecting glaucoma, and results were mixed.[4,25] Caution is needed as the ratio measure could be misleading in diseases where the b-wave is abnormal.

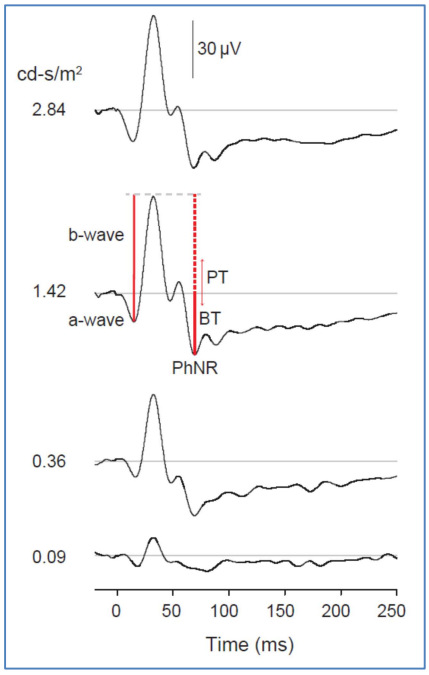

Figure 1: Illustration of the light-adapted ERG of a healthy subject (35 yrs) in response to a brief red LED flash (660 nm) on a blue background (460 nm) of 10 cd.m^{-2}. Figure shows PhNR amplitude measurements from baseline to PhNR trough (BT) and from b-wave peak to PhNR trough (PT). Adapted from reference[26] (the Association for Research in Vision and Ophthalmology is the copyright holder).

10. References:

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Part B. Justification for the protocol details and description of the consultation process

A systematic literature review was performed using PubMed to find publications that reported use of the PhNR from the period 1999 to 2017, The committee identified the relevant references listed above, discussed the methods used in the references to record PhNRs, and came to a consensus on the recommendations in the extended protocol.