Request for input from the ISCEV membership: ISCEV standard EOG (2017) Update -- October 2016 Draft

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Overall there has been no change to the testing protocol of the Standard EOG, 15 minutes dark then 15 minutes light with testing of 10 saccades each minute. The Fast Oscillation is a recommended test that uses continuous recording with 60 second intervals of light and dark for a minimum of four light-dark cycles.

Summary of Major Changes

1. Updated and extended sections covering the mechanism of the EOG and FO are given in this new draft based on the developments in our understanding of bestrophin and as a background to the different mechanism of these electrophysiological tests of RPE function.
2. The new draft allows of stopping the EOG once a light-rise has been obtained so that the standard EOG ratio can be calculated, in an effort to minimize the length of the procedure.
3. The new draft suggests a method for smoothing the physiological response using a weighted average of the standing potential amplitudes –although other methods for smoothing the EOG plot are acceptable.
4. A move away from the Arden Eponym, with the 'Arden ratio' noted but now referred to as 'the standard EOG ratio'
5. Figures have been updated:
    a. Figure 1 Replaced with real data showing 0.1 Hz high pass filter
    b. Figure 2- New figure showing blink artefact and over/undershoot with correct positioning of cursers.
    c. Figure 3- Unchanged image of the electrode positions but modified caption.
    d. Figure 4- Raw and smoothed trace of the standard EOG indicating the Dark Trough and Light Peak
    e. Figure 5- Trace of the Fast Oscillation.: Replaces figure 4 schematic of the FO in the 2011 Standard.
6. Reporting of the Dark Trough amplitude is recommended as before but an explanation is given now that this is an indication of the degree of the strength of the tight-junctions between the RPE cells that is proportional to the epithelial potential that is recorded as the 'standing potential' with the EOG.

Summary of Minor Changes

1) Electrode placement and preparation are consistent with the VEP standard
2) a.c and d.c changed to AC and DC – this is the convention
3) Fast Oscillations are singular as this is a single oscillation

Areas for Comments/Discussion:

1. Pre-Adaptation:

a. The committee discussed the addition of a suggestion that there be a period of 2-5 minutes under 30 cd/m^2 ahead of the dark phase to standardize the adaptive state of the retina.

a. *Do any labs have evidence that this may assist with the development of the dark trough or improve reliability of the recordings?*

2. Recording of Saccades:

a. The committee was inclined to remove the section that prohibited an auditory cue ahead of each saccade as we were unsure of the impact of allowing auditory cues during the recordings.

i. Line 255: “However, there should not be auditory cues before every alternation of the fixation lights, as this may prompt erroneous saccades before the fixation lights change.”

a. *Should the above sentence be retained, removed or modified?*

3. Impedance and filtering:

a. The committee discussed the possibility of increasing the low pass filter to 100Hz as the 30 Hz low pass may mask poor recording?

b. The committee also discussed whether the impedance could be < 10 kΩ with up to 3-4 kΩ between electrodes given the 0.1-30 Hz will reduce any line interference.

c. *Ultimately the committee left the recording parameters unchanged although we would welcome comments on these points from the membership.*

4. Normative Data:

a. Despite several recent studies reporting normative values for the EOG and FO, the committee recommends lab based normative data be used.

b. *Given the standardisation of the EOG with respect to duration, electrode use and illumination the committee suggests that a normative database could be established for the EOG with reference ranges available in a future standard?*

c. *This may be possible through a meta-analysis of published data or across labs that would be willing to share data sets?*

Consultation Draft October 2016

ISCEV Standard for Clinical Electro-oculography (2017 update)

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This update was approved by ISCEV. This document is available on the ISCEV website: http://www.iscev.org.

The authors represent the International Society for Clinical Electrophysiology of Vision.

Abstract

The clinical electro-oculogram (EOG) is an electrophysiological test of the outer retina and retinal pigment epithelium (RPE) in which changes in the electrical potential across the RPE are recorded during successive periods of dark and light adaptation. This document presents the 2017 EOG Standard from the International Society for Clinical Electrophysiology of Vision (ISCEV: www.iscev.org). This standard has been reorganized and updated, but without substantive changes to the testing protocol from the previous version published in 2011. It describes methods for recording the EOG in clinical applications and gives detailed guidance on technical requirements, practical issues, and reporting of results. It is intended to promote consistent quality of testing and reporting within and between clinical centers.

Key words Arden ratio, ISCEV standards, Clinical Electrophysiology, Electro-oculogram (EOG), Light adaptation, Retinal pigment epithelium (RPE), Fast Oscillation (FO)

Abbreviations

DT – Dark Trough
EOG – Electro-oculogram
ER – Endoplasmic Reticulum
ERG – Electroretinogram
FO – Fast Oscillation
ISCEV – International Society for Clinical Electrophysiology of Vision
LP – Light Peak
RPE – Retinal Pigment Epithelium
TEP – Trans Epithelial Potential

This Standard is one of a series of ISCEV Standards and Guidelines for clinical electrophysiology of vision [1-7] developed by ISCEV and listed here: iscev.org/standards [8]. This Standard supersedes previous versions of the ISCEV Standard for Clinical Electro-oculography which was first published in 1993 [1] and subsequently revised and last published in 2011 [2]. This Standard contains updated information and has been reorganized for greater clarity and for consistency with other ISCEV Standards, without substantive changes to the 2011 testing protocol.

This ISCEV Standard describes basic procedures that allow reproducible recordings that are comparable across laboratories. It is intended that the ISCEV Standard EOG protocol be used widely, but not to the exclusion of other tests or protocols that are not covered by this Standard. Electrophysiologists are encouraged to extend the EOG as required when clinically relevant to maximize the diagnostic value of the standard EOG and FO recordings for their patients and for clinical trials.

Clinical and research users of the EOG are encouraged to use the most recent Standard, to achieve consistency of results within and between test centers. Reports of standard EOG recordings performed in this manner should cite this 2017 Standard. When a method is used which deviates from the Standard, the deviations should be fully described.

The Electro-oculogram

Electrophysiology of the outer retina in dark and light

There is a difference in electrical potential between the anterior and posterior of the eye, known as the standing potential of the eye. The standing potential is an indirect measure of the trans-epithelial potential (TEP) of the retinal pigment epithelium (RPE). The TEP is equal to the difference in the membrane potential of the basolateral and the apical membranes, which are electrically coupled through the tight-junctions of the RPE. Changes in the resistance between the apical or basolateral membranes or membrane potentials alter the amplitude of the TEP and thus the recorded standing potential of the eye.

A change in the standing potential can be induced by retinal adaptation to ambient illumination by alternating intervals of dark and light. The standard EOG utilizes the RPE's response to changing illumination to assess the function of the outer-retina and RPE. There are two clinical tests of the standing potential of the eye. The standard EOG is recorded during 15 minutes of dark adaptation followed by 15 minutes of light adaptation. The Fast Oscillation (FO) is a different additional EOG procedure which is performed during alternate 1 minute dark and light periods. The changes in the standing potential for the standard EOG and the FO are derived from different mechanisms.

Mechanism of the standard EOG

During the 15 minute period of dark adaptation there is a fall in the recorded standing potential typically reaching a minimum at 10–15 minutes, and this is referred to as the Dark Trough (DT). The DT amplitude is stable and thus is an indirect measure of the integrity of the RPE based on the TEP, which is maintained by the tight junctional proteins between the apical and basal membranes. For this reason the amplitude of the DT should be noted.

Following light onset there is an increase in the TEP of the RPE producing the light-rise of the EOG. The mechanism initiating the light-rise is unknown but it requires a normally functioning interface between the rod photoreceptors and RPE. The light-rise is ultimately the result of an increase in intracellular free calcium, which is released from the endoplasmic reticulum (ER), regulated by an interaction between ER bestrophin and L-type calcium channels associated with the basolateral membrane. Intracellular calcium gates the opening of a basolateral calcium-dependent chloride channel. Increased chloride conductance depolarizes the basolateral membrane which increases the TEP, recorded as an increase in the standing potential of the eye. The light-rise usually reaches a maximum at 7–12 minutes after light onset and is known as the Light Peak (LP). The LP is the first of several peaks (the damped oscillation) which become progressively smaller for up to 90 minutes during light exposure.

The standard clinical EOG provides an indirect measure of the minimum amplitude of the standing potential in the dark (at the DT), and then again at its maximum amplitude in the light (at the LP). This is expressed as the standard EOG light peak to dark trough (LP : DT) ratio, also known as the “Arden ratio”.

Mechanism of the Fast Oscillation

The FO is an optional additional test that has a different mechanism to the standard EOG owing to the shorter dark and light intervals used. At light onset there is a fall in potassium in the sub-retinal space that causes a strong outward potassium current across the apical membrane of the RPE and subsequent hyperpolarization, and c-wave, of the ERG. The fall in sub-retinal potassium also slows the apical NaK2Cl co-transporter and reduces intracellular levels of chloride which in turn hyperpolarizes the basal membrane and reduces the TEP and creates the trough of the FO after 35–45 seconds after light onset. The TEP returns to normal, as ionic homeostasis is restored and a peak is recorded during the subsequent dark period after in a further 35–45 seconds. The alternation between dark and light at one minute intervals establishes a continuous oscillation that is dependent on changes in ionic permeability at the apical and basal membranes and the electrical coupling between these membranes by tight junctions.

Diseases affecting the light response of the EOG

The light response of the EOG is affected in diffuse disorders of the RPE and disorders of the photoreceptor layer of the retina including acquired retinopathies and retinal dystrophies characterized by rod dysfunction or chorio-retinal atrophy. In most of these disorders EOG abnormalities are proportional to the severity of rod-mediated electroretinogram (ERG) abnormalities. Notable exceptions include disorders of the bestrophin gene (*BEST1*). These include Best vitelliform macular dystrophy (Best disease), autosomal recessive bestrophinopathy (ARB), and autosomal dominant vitreoretinochoroidopathy (ADVIRC). In Best disease the ERG is usually normal and the standard EOG ratio abnormal. A normal EOG may distinguish Best disease from

other autosomal dominant retinal disorders with similar fundus features including some cases of adult vitelliform macular dystrophy and pattern dystrophy. In ARB and ADVIRC the ERG is often abnormal but the EOG is severely or disproportionately abnormal. An abnormal EOG, not explained by ERG reduction, may also be associated with some toxic and other retinopathies.

Principles of the clinical EOG measurement

The standing potential of the eye may be assessed using skin electrodes attached either side of each eye to record successive horizontal saccadic eye movements. The patient tracks alternating lights separated by a fixed angle, to enable constant eye movement excursions which are recorded as a series of positive and negative deflections or potentials that coincide with ocular rotation. The magnitude of the eye movement potential (at a fixed angle) is a fixed proportion of the standing potential. During a dark/light cycle, the magnitude of the potential changes proportionate to changes in the TEP across the RPE.

The Standard Method

Technologic requirements

Electrodes

Skin electrodes such as sintered silver–silver chloride, standard silver–silver chloride or gold cup electrodes are recommended for recording EOGs. The skin should be prepared by cleaning, and a suitable paste or gel used to ensure good, stable electrical connection. The electrode-skin contact impedances should be below 5 kΩ as measured between 20 and 40 Hz.

Stimulator

The EOG should be performed using a full-field (Ganzfeld) dome. The Ganzfeld should have a comfortable head/chin rest, and two red fixation lights located

15° left and right of center. The fixation lights should be bright when the light adapting background is on, and dim (to be just visible) in the dark.

Light and dark

The dark phase should take place in total darkness, with the fixation lights dimmed to the minimum necessary to enable fixation.

The light phase requires even lighting throughout the dome. The adapting light should appear white and have a luminance of 100 photopic cd/m^2 measured at the position of the eye. To account for minor variability in equipment and calibration the acceptable range within the Standard for the light adapting background is 90 to 110 photopic cd/m^2. Calibration of the Ganzfeld stimulator should be carried out periodically. Modest room lighting may be turned on during the light phase as long as ambient luminance is less than that in the bowl.

Note that adapting light sources of different types, such as tungsten, halogen, LED, and fluorescent, have different spectral characteristics and the color may change with brightness. This makes the definition of standard lighting inherently imprecise, although for practical purposes most “white” light of the correct luminance will give very similar results.

Amplification and filtration

Amplifiers should have a band pass of either 0 to 30 Hz (DC), or 0.1 to 30 Hz (AC), to make recordings of saccadic eye movements appear as square waves (figure 1). For a 30° saccade, the typical standard EOG amplitude is between 250 and 1000 μV with an essential frequency content of 0 to 30 Hz. In theory, the ideal recording technique is DC amplification but this is generally impractical because of baseline drift. Thus, we recommend AC recording with a 0.1 Hz high pass filter. If a higher frequency filter is used (e.g. 0.5 Hz), it will distort the square waves, making identification of overshoot and stepped saccades difficult.

_____Insert Figure 1 Near Here_____

Manufacturers should allow the examiner to have access to all of the raw data for each 10 second recording epoch so that the examiner is able to visually inspect the individual saccadic records to enable accurate cursor placement around any artefacts to measure the standing potential as shown in figure 2.

_____Insert Figure 2 Near Here_____

The operator should be able to see the saccadic recordings as they occur, to ensure that there are no artefactual signals, problems such as amplifier saturation (that can be corrected by adjusting amplifier gain), and that patients are compliant with the task.

Preparation of the patient

Pupils

The pupils should be dilated maximally before testing and their size recorded. If full pupil dilation is impossible or undesirable, an attempt should be made to increase the adapting luminance so that an equivalent retinal illumination is approximated. The amount of light passing through the pupil, when measured in Trolands, is the product of luminance (in cd/m^2) and pupil area (in mm^2). For example, to produce the same effect upon the retina, twice as much dome luminance is required with a 5-mm diameter pupil (roughly 20 mm^2) than with a 7-mm diameter pupil (roughly 40 mm^2). The report should describe any deviations from the Standard.

Electrode placement

After suitable skin preparation, recording electrodes should be placed close to the canthi of each eye as in Figure 3. The electrodes from each eye are connected to

separate channels of a differential amplifier. A separate electrode should be attached and connected to the ground. Convenient and commonly used ground electrode positions include the forehead, vertex, mastoid and earlobe. The impedance between any pair of electrodes should not exceed 5 kΩ. The electrodes, amplifier and impedance meter must be approved for medical use.

_____Insert Figure 3 Near Here_____

Pre-exposure to light

The patient should be in stable indoor lighting before the test, to stabilize the EOG, for at least 30 minutes. Indirect ophthalmoscopy, fundus autofluorescence and/or photography and other strong illumination changes must be avoided during this period. As near as practical, the pre-test light exposure should be the same for all patients.

Explanations

The procedure should be explained to the patient, noting that head position must not change, as this is one common artefact source, and that the eyes should only move left and right. The patient should be instructed not to anticipate the onset of the alternation of the fixation lights but to move their eyes only when the lights change. Practice the procedure with the recording system prior to dark adaptation, to familiarize the patient with the task and to check on the stability and quality of the recorded EOG.

Clinical recording

Recording of saccades

Fixation lights should alternate once per second, for 10 seconds out of every minute. The EOG potentials are recorded during these 10 second periods. There

should be a warning, verbal or automatic, of the impending start of each recording period to ensure readiness of the patient and operator. However, there should not be auditory cues before every alternation of the fixation lights, as this may prompt erroneous saccades before the fixation lights change.

Dark phase

For the dark phase total darkness should be maintained for 15 minutes, except for the dim fixation lights. EOG recordings should be made once a minute for 10 seconds, as specified above. The operator should have a concurrent view of the recordings to check for patient compliance, and for errors such as noise or overshoot.

Light phase

For the light phase a dome background light of 100 photopic cd/m^2 should be turned on to "trigger" the initial light phase response and should remain on for the duration of the light phase. However, the luminance can be increased gradually over a short period (e.g. 20 seconds) to minimize patient discomfort. Longer ramps (e.g. lasting minutes), will alter the responses. Continue the recording for 10 seconds out of every minute (as above) for at least 15 minutes to register the presence or absence of the LP. If the LP can be clearly identified during the recordings then the test may be stopped before 15 minutes. It may be necessary to extend the light phase to fully characterize abnormal responses but a delay in the LP should be reported. The patient should remain positioned in the headrest of the stimulator bowl throughout the procedure, with eyes open to maintain retinal illumination.

Patient compliance

Patients will have difficulty performing saccadic eye movements if they cannot fixate reliably because of poor central vision, diplopia or ocular motility problems (including nystagmus). Patients with diplopia may be advised to look between the pair of images, or one eye can be patched if the suspected retinal disorder

is bilateral. Patients who are very young or those with a physical or learning disability may not be able to perform the standard EOG. In young children with suspected Best disease it may be possible to test their parents, since a carrier of Best disease will have an abnormal EOG light-rise, irrespective of whether the fundus is normal.

Patient compliance can vary due to fatigue or inattention. Common problems are drifting of the head back from the stimulator, head turning, and irregular eye movements during the recording or eye closure during the light phase. These can be minimized by having a real-time view of the EOG recording via an infrared camera. In most cases, gentle coaching and reminders will minimize the effects of poor compliance.

Analysis and reporting

Saccadic amplitude

The EOG amplitudes should be measured in microvolts (μV) either manually or by a computer algorithm after visual inspection. Care must be taken to avoid measuring the effects of overshoot (see Figure 2) or irregular (artefactual) saccades. The average of the amplitudes within each 10-second recording epoch should be measured, excluding outliers, artifacts or responses consistent with poor fixation. If a computer algorithm is used, it is important to ensure that the values returned represent true EOG amplitudes and not artefactual records. Particular causes of unreliability are overshoot, stepped saccades, missing saccades, inverse saccades (eyes going in the opposite way to the fixation lights), and eccentric fixation in which the saccade length switches between two or more values.

Dark trough and light peak: Smoothing

The average EOG amplitude calculated from each 10 second epoch should be plotted. However, there is always “noise” in real-life recordings, and the goal of the EOG measurement is to record the physiological DT and LP, rather than the lowest or highest values. Thus, the first critical step is that the underlying physiologic curve be

recognized and drawn, in order to derive the DT and LP amplitudes. This can be achieved by using a curve fitting algorithm, a spline (curve fitting ruler) or the weighted average of each point where the Amplitude (*A*) at time point (*t*) is given by equation 1;

$$A_t = \frac{\left(A_t + \left(0.5A_{(t-1)}\right) + \left(0.5A_{(t+1)}\right)\right)}{2} \qquad \text{equation 1}$$

Figure 4 shows raw data plotted and the subsequent smoothing of the data using equation 1. It is helpful if uncertain or artefactual values can be identified and marked at the time of recording, so that they can be ignored later when curve-fitting.

_____Insert Figure 4 Near Here_____

Light peak to dark trough ratio calculation

The ratio of the LP : DT amplitudes is the standard EOG ratio and also known as the “Arden ratio”. The LP and DT amplitudes are derived from the smoothed (physiologic) EOG record. It is important to note that a normal ratio does not imply a normal DT and that the DT amplitude should also be reported.

Reporting

Clinical reports should state the standard EOG ratio, DT amplitude (in microvolts), the time from the start of the light phase to the LP (if present), the pupil size, and the type of adapting light source. The report should also describe any difficulties encountered during testing that may affect confidence in the results, such as patient compliance or inconsistent eye movements.

Normative data

Each center must establish its own set of normative values for the EOG and FO. The median value (not the mean) should be used to define reference values and the actual values on either side of the median that bracket 90 percent of the reference ranges (5^{th} – 95^{th} centile) should be determined by direct tabulation of the reported results. The normal standard EOG ratio is typically between 1.7 and 4.3 with a LP time ranging from 7-12 minutes.

Interaction between eyes

The EOG potentials from one eye can contaminate the response from the other. The magnitude of this effect is approximately 15% with electrodes placed on each side of the nose close to the inner canthi. It rises to about 40% if the electrodes come close together and touch (i.e. become a common central electrode on the bridge of the nose). This interaction can give misleading results in cases of an electrically inactive eye (e.g. total retinal detachment), or absent eye, because the defective eye will appear to have the same standard EOG ratio as the fellow eye, albeit with a much smaller standing potential. If the eyes have similar standing potentials but different standard EOG ratios the measured ratio from the better eye is enhanced at the expense of that from the weaker eye, which can in fact appear to have a standard EOG ratio of less than 1.0, solely due to interaction.

Deviation from the Standard

This Standard represents a basic and core procedure for the assessment of generalized function of the RPE/photoreceptor interface. If a center chooses techniques which vary from the Standard, it is critical to cite this document and specify any deviations, such as differences in the luminance level for the adapting light or in the duration of the dark or light adapting intervals. The standing potential can also be used to monitor eye-movements in studies unrelated to retinal and RPE pathophysiology.

Fast Oscillation

Some centers measure the fast oscillation (FO), in conjunction with the standard EOG. The FO has the opposite polarity to the standard EOG. Light onset causes a decrease in the standing potential, while in darkness there is an increase in the standing potential.

The FO is recorded using the same technical specifications as the standard EOG (amplifier, electrode placement, fixation targets, background luminance and 1 per second saccades). However, the saccades and the recording should be continuous without interruption for the duration of the test. Light and dark are alternated every 60 seconds to induce the FO, which have a near sinusoidal appearance (figure 5). During each light interval a trough develops and begins to rise again after 35-45 seconds. The subsequent interval of darkness results in a peak at 35-45 seconds following the onset of darkness. The total number of light-dark intervals should be at least 4 with 60 second intervals of light and dark, making a total test time of 8 minutes. Pre-adaptation does not affect the FO, so this test can be performed either independently or in conjunction with the standard EOG.

Figure 5 shows a FO recording formed by dark and light intervals. The FO amplitude can be calculated from the average of the peak to trough ratios from each of the dark/light cycles. The time to the peak or trough should be calculated from the time of light offset or onset respectively and averaged for the number of light/dark cycles in the recording.

Acknowledgements

References

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Figures and Captions

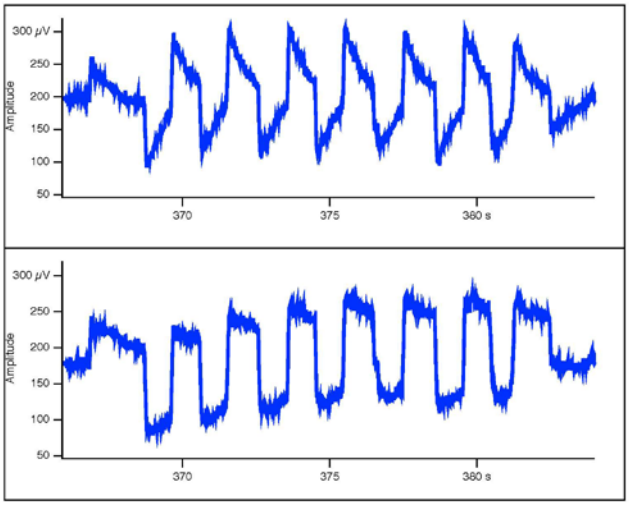

Figure 1 Upper trace shows filtering with 0.1–30 Hz, lower trace with post-hoc DC restoration by digital integration, rendering it similar to direct DC recording. DC recording (or restoration) makes it easier to perform the plateau measurements.

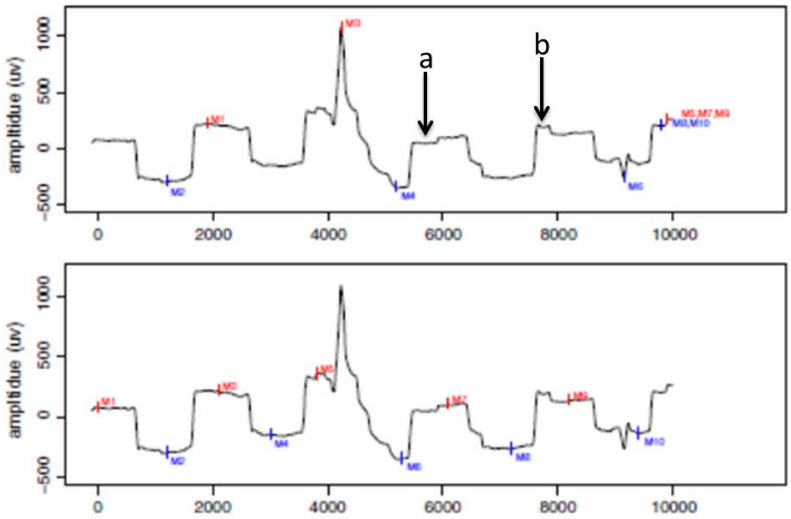

Figure 2 Examples of 10-second saccadic records with a blink artefact at approximately 4000 ms. Arrow ‘a’ indicates an initial undershoot and arrow ‘b’ an overshoot of the fixation target visible by the step in the plateau of the EOG recording (upper trace). The lower trace shows the manual placing of markers at the peak and trough of the EOG recording as the eye performs horizontal saccades for ten seconds at one second intervals.

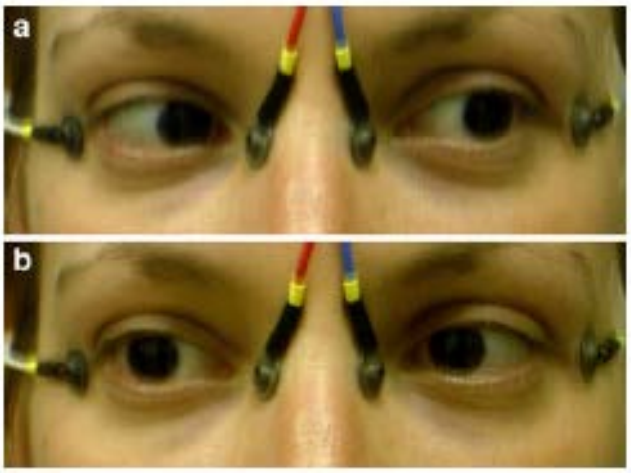

Figure 3 Recording electrode positions located near the inner and outer canthi of each eye. As the eyes perform horizontal saccades: left (a) then right (b) the amplitude of the standing potential is recorded across the active electrodes.

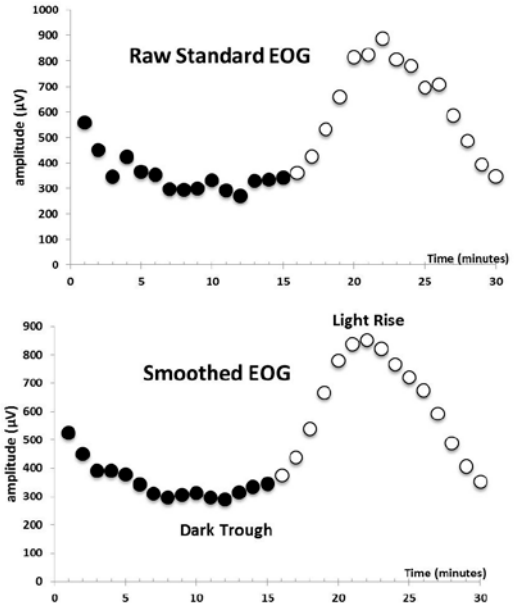

Figure 4 Upper figure shows the raw standing potential values for an EOG with 15 minutes of dark (black circles) and 15 minutes of light (white circles). Smoothing of the data using equation 1 helps to define the physiological response and the DT and LP points from which to calculate the standard EOG light rise to dark trough ratio (Lower figure). Computer algorithms or fitting with a spline rule may also be utilized.

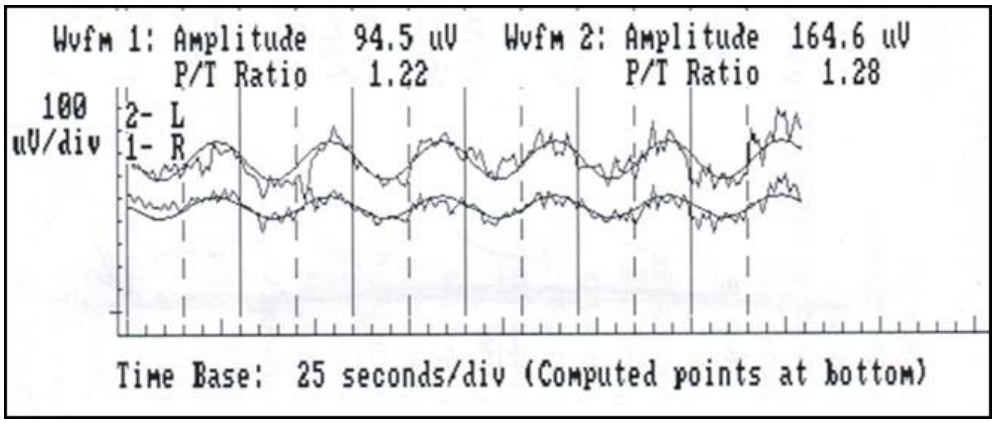

Figure 5. Typical Fast Oscillation recording with six cycles of 75 second light and 75 second dark intervals generating a trough in the light and a peak in the dark interval for the Left (upper) and Right (lower) waveforms. Parameters were derived from the fit of the sine wave to the raw data. Amplitude was calculated from the difference between the absolute amplitudes of the peak and trough of the sine wave and the P/T (Fast Oscillation ratio) is the ratio of the peak to trough amplitude.