Video Article

Simultaneous Recording of Electroretinography and Visual Evoked Potentials in Anesthetized Rats

Christine T. Nguyen[1], Tina I. Tsai[1], Zheng He[1], Algis J. Vingrys[1], Pei Y. Lee[1], Bang V. Bui[1]

[1]Department of Optometry and Vision Sciences, University of Melbourne

Correspondence to: Bang V. Bui at bvb@unimelb.edu.au

URL: https://www.jove.com/video/54158
DOI: doi:10.3791/54158

Keywords: Neuroscience, Issue 113, Electroretinogram, visual evoked potential, electroretinography, electrophysiology, visual evoked response, retinal function, optic nerve function

Date Published: 7/1/2016

Citation: Nguyen, C.T., Tsai, T.I., He, Z., Vingrys, A.J., Lee, P.Y., Bui, B.V. Simultaneous Recording of Electroretinography and Visual Evoked Potentials in Anesthetized Rats. *J. Vis. Exp.* (113), e54158, doi:10.3791/54158 (2016).

Abstract

The electroretinogram (ERG) and visual evoked potential (VEP) are commonly used to assess the integrity of the visual pathway. The ERG measures the electrical responses of the retina to light stimulation, while the VEP measures the corresponding functional integrity of the visual pathways from the retina to the primary visual cortex following the same light event. The ERG waveform can be broken down into components that reflect responses from different retinal neuronal and glial cell classes. The early components of the VEP waveform represent the integrity of the optic nerve and higher cortical centers. These recordings can be conducted in isolation or together, depending on the application. The methodology described in this paper allows simultaneous assessment of retinal and cortical visual evoked electrophysiology from both eyes and both hemispheres. This is a useful way to more comprehensively assess retinal function and the upstream effects that changes in retinal function can have on visual evoked cortical function.

Video Link

The video component of this article can be found at https://www.jove.com/video/54158/

Introduction

Measurement of the electroretinogram (ERG) and visual evoked potential (VEP) provide useful quantitative assessments of the integrity of the visual pathway. The ERG measures the electrical responses of the retina to light stimulation, while the VEP measures the corresponding functional integrity of the visual pathways from the retina to the primary visual cortex following the same light event. This manuscript describes a protocol for the recording and analysis of ERG and VEP responses in a commonly used laboratory model, the rat.

The ERG provides an index of the functional integrity of a number of key retinal cell classes by quantifying the retina's gross electrical response to a flash of light. A coordinated series of ionic fluxes initiated by light onset and offset, produce detectable changes in voltage that can be measured using surface electrodes placed outside the eye. The resultant waveform represents the combination of a series of well-defined components, differing in amplitude, timing and frequency. A substantial body of research has shown that these components are relatively well conserved across many vertebrate retinae and that the components can be separated from each other. By judiciously selecting the stimulus (flash stimulus, background, interstimulus interval) conditions and choosing specific features of the composite waveform to analyze one can be confident of returning a measure of a specific group of retinal cells[1,2]. These characteristics underlie the utility and hence the widespread applications of the ERG as a non-invasive measure of retina function. This manuscript focuses on the methodology for measuring the ERG and analyzing its features to return information about some of the major cell classes in the retina, namely photoreceptors (the PIII component), bipolar cells (the PII component) and retinal ganglion cells (the positive scotopic threshold response or pSTR).

The VEP provides an assay of the cortical response to light; first originating from the retina and thereafter communicated serially via the optic nerve, optic tract, thalamus (lateral geniculate nucleus, LGN) and optic radiation to area V1 of the cortex[3]. In rodents, the majority (90 - 95%) of optic nerve fibers from each eye decussate[4] and innervate the contralateral mid-brain. Unlike the ERG, it is as yet not possible to attribute different components of the VEP to specific cell classes,[5] thus changes anywhere along the visual pathway could affect the VEP waveform. Nevertheless, the VEP is a useful non-invasive measure of visual performance and visual pathway integrity. The VEP, when used in conjunction with the ERG, can provide a more complete assessment of the visual system (*i.e.,* retina/visual pathway).

ERG and VEP recordings can be conducted in isolation or in combination, depending on the application. The methodology described in this paper allows simultaneous assessment of retinal and cortical visual evoked electrophysiology from both eyes and both hemispheres in anesthetized rats. This is a useful way to more comprehensively assess retinal function and the upstream effects that changes in retinal function can have on visual evoked cortical function.

Protocol

All experimental procedures were conducted according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, set out by the National Health and Medical Research Council in Australia. Ethics clearance was obtained from the University of Melbourne, Science Faculty, Animal Ethics Committee (approval number 0911322.1).

1. Pre-implantation of Chronic VEP Electrodes

Note: If concurrent ERG and VEP signals are to be collected animals must be surgically implanted with VEP electrodes at least 1 week prior to signal collection.

1. Sterilize the surgical bench prior to experimentation by cleaning with chlorhexidine (0.5% in 70% ethanol). Autoclave all surgical equipment before use. Cover the animal with a sterilized surgical drape. Ensure all experimenters wear surgical masks, gowns and sterilized gloves.
2. Induce anesthesia with 3 - 3.5% isoflurane with O_2 at a flow rate of 3 L/min. Maintain anesthesia at 1.5% and 2 L/min throughout the surgery. Ensure sufficient depth of anesthesia by the absence of paw pinch reflex.
3. Apply 1% carboxymethylcellulose sodium on the cornea to prevent drying of the eyes.
4. Shave a 30 mm x 30 mm area over the forehead, posterior to the eyes and anterior to the ears.
5. Place animal on a heat pad (37 ˚C) to maintain body temperature and stabilize animals' head with a stereotaxic frame.
6. Disinfect the shaved area with 10% povidone-iodine three times. Avoid the use of alcohol-based antiseptics for area near the eye, being consistent with the Standard of Practice set out by the Association of Surgical Technologists.
7. Make a median-sagittal incision on the head with a scalpel and from this excise a ~ 20 mm diameter circle of dermal tissue to expose the cranial bone.
8. Remove underlying periosteum by scraping and drying with gauze to expose the coronal and sagittal cranial sutures.
9. Using a dental burr attached to a drill, trepan two holes (0.7 mm diameter, depth ~ 1 mm) through the skull on both hemispheres at the stereotaxic co-ordinates: 7 mm caudal to bregma, 3 mm lateral to midline.
10. Screw in stainless steel screws (diameter 0.7 mm, length 3 mm, sterilized with chlorhexidine) into the two pre-made holes up to a depth of ~ 1 mm (2 mm of screw exposed) to allow firm anchorage. This contacts the dura without damaging the underlying cortical tissue.
11. Prepare surgical area for dental amalgam by drying the cranial bone with gauze, and retracting loose skin with two 3 - 0 sutures at ~ 4 and 8 o'clock.
12. Spread dental amalgam over the exposed skull to secure the screw electrodes (stainless steel screws described in step 1.10) in place. Ensure ~ 1.5 mm of the screws remain exposed for recording.
13. Remove the retraction sutures.
14. Inject 0.5% carprofen subcutaneously (5 mg/kg) for analgesia and saline (sodium chloride 0.9%, 1.5 ml) subcutaneously for fluid replacement.
15. Allow animal to recover in separate cages. Do not leave animal unattended until it has regained sufficient consciousness to maintain sternal recumbency.
16. Do not return animal to the company of other animals until it has fully recovered from surgery (minimum 5 days).
17. Continue to administer 0.5% carprofen subcutaneously for analgesia (5 mg/kg) once a day for 4 days.
18. Record ERG and VEP 1 week following surgery.

2. ERG and VEP Recording

1. Data collection preparation
    1. Use computer software to simultaneously trigger stimulus and acquire data[2] according to the settings recommended below.
        1. Amplify the signals[3] (ERG: ×1,000, VEP: x10,000) with the gain internally set by an isolated pre-amplifier and amplifier, and with both eyes matched for impedance.
        2. Set sampling rate for the ERG to 4 kHz over a 650 msec recording window (2,560 points), To do this, click the tab for "time base" in the data acquisition software (for name and version of software see Table for Materials), select "2,560" for Samples, and "500 ms" for time which will return a 650 msec recording window.
            1. Use the same method to set the sampling rate for the VEP to 10 kHz over a 250 msec epoch. Allow a 10 msec pre-stimulus baseline for both ERG and VEP recordings. To do this, click the "Setup" tab; select "Stimulator" to bring up a new dialogue window; in that window select "pulse" from the drop down list for "Mode"; and set the value for "delay" to "10 ms".
        3. Set ERG band-pass filtering to 0.3 - 1,000 Hz (- 3 dB). This is done by clicking "Bio Amplifier" in the data acquisition software. Then set the value for "High Pass" to "0.3Hz", and the value for "low pass" to "1 kHz".
        4. Using the abovementioned method in 2.1.1.3, set VEP band-pass settings to 0.1 - 100 Hz (- 3 dB) as recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV) for human VEP recordings[6].

2. Electrode preparation
    1. Custom-make the ERG active/inactive and VEP active/inactive electrodes by attaching silver wire or an alligator clip to an electrode lead, respectively[2]. Commercially obtain ground electrode.
    2. For the 4 custom-made electrodes, cut the male end from the electrode lead extension. Remove 1 cm of the outer polytetrafluoroethylene insulation coating with a scalpel blade ensuring the inner wire is not damaged.
    3. Pre-fashion the ERG inactive electrodes by cutting a 70 mm length of silver wire (0.3 mm thickness) and forming a loop ~ 8 mm in diameter to encircle the rat eye. Prepare a uniform circle by shaping the loop on a 1 ml pipette tip.

4. Pre-fashion VEP inactive electrodes by cutting a 70 mm length of silver and forming an ellipse ~ 8 mm in length-wise diameter to hook onto rat incisors.
5. Pre-fashion the ERG active electrodes by cutting a 30 mm length of silver wire and forming a small loop to gently contact the rat cornea (~ 1-2 mm in diameter)
6. Securely attach electrodes (2 ERG active, 2 ERG inactive, 1 VEP inactive) to the electrode lead by entwining the silver with the exposed inner wire.
7. Insulate excess exposed metal with masking tape to reduce photovoltaic artifacts.
8. On the ERG inactive electrodes stick a small piece of hook-and-loop fastener (~ 5 mm × 20 mm) to the masking tape to enable stable attachment to the rodent neck strap.
9. Attach alligator clip to the inner wire of the electrode leads to make the VEP active electrodes.
10. Prior to recordings, electroplate the exposed surfaces of the silver wires (*i.e.,* the inactive ring and active tip) with chloride using a 9 V DC source for 20 sec to improve signal conduction.
    1. To do this, immerse the silver tip of the ERG electrode wire (acting as the anode of a primary cell) into normal saline; connect the other end of this electrode wire to the positive terminal of a 9 V battery.
    2. Connect another wire (the cathode) to the negative terminal of the battery, and immerse the other end into saline as well. Disconnect after 20 sec and observe the sliver tip of the ERG electrode wire to be coated evenly in white color.
    Note: Prepare new ERG electrodes for each experimental session (~ up to 8 hr) to ensure patency of the chloride coating.

3. Animal preparation
    1. Dark-adapt the animals overnight (≥ 8 hr) prior to recordings in a light tight room. Ensure maximal dark adaptation by turning off room lights, closing all doors and blinds. Minimize light leakage by placing light-proof materials around junctions of doors/windows and placing computer screens outside thick black curtains.
    2. Conduct animal preparation in a dark room with the aid of dim red light-emitting diode (LED; 17.4 $cd.m^{-2}$, λ_{max} = 600 nm) to sustain rod sensitivity.
    3. Anesthetize rat by injecting ketamine/xylazine (60 : 5 mg/kg) intramuscularly. Confirm sufficient depth of anesthesia by the absence of a paw pinch reflex.
    4. To maintain sedation, administer a further dose of anesthesia (50% of initial dose) after 50 min if necessary.
    5. For additional topical anesthesia apply one drop of 0.5% proxymetacaine to each eye, and blink off excess fluid.
    6. For pupil dilation apply one drop of 0.5% tropicamide to each eye, then dry off excess fluid.

4. ERG and VEP electrode positioning
    1. Place animal on the ERG platform in front of the Ganzfeld bowl situated in the Faraday cage. Avoid using an electrical heating pad, as it can introduce electrical noise into the electrophysiological recordings. Note: The platform is attached to a circulated heated water platform to maintain body temperature.
    2. Secure animal to platform with a strip of hook-and-loop fastener placed firmly but not tightly around the nape.
    3. Hook the inactive VEP electrode around bottom incisors of anesthetized rat.
    4. Position the ERG inactive electrodes by encircling the scleral ring non-invasively around the eye's equator. Stabilize this by attaching electrodes to the hook-and-loop fastener strip around the nape. Repeat for the contralateral eye.
    5. Fasten VEP active electrodes by attaching alligator clips to stainless-steel screws pre-implanted on the skull.
    6. Place a small drop of 1% carboxymethylcellulose sodium on cornea prior to placement of the ERG active electrode to improve signal quality. Note: The viscose fluid also helps maintain corneal hydration throughout experimentation to minimize the formation of desiccation-type cataract in rodents[7].
    7. Place a small drop of 1% carboxymethylcellulose sodium on the lower incisors to improve contact of the VEP inactive electrode and thus signal quality.
    8. Position the ERG active electrodes to lightly touch the central corneal surface using a micromanipulator attached to a custom-built stereotaxic arm.
    9. Insert 2 - 5 mm of the ground needle electrode (stainless steel) subcutaneously into the tail.
    10. If necessary dry any excess fluid from the inferior eyelid prior to recording to improve signal quality.
    11. Slide platform closer to the Ganzfeld bowl ensuring the animal's eyes align with the opening of the bowl to enable even illumination of both retinas (see step 2.4.1).
    12. Close the Faraday cage to reduce extraneous noise.

5. Data collection
    1. Use a dim test-flash (- 0.52 log $cd.s.m^{-2}$) to assess whether electrode placement is satisfactory[2]. Under control conditions this would result in an ERG amplitude of ~ 800 µV and an inter-eye variability of no greater than 10%. If required reposition electrodes.
    2. Following the test-flash allow animals to dark-adapt for 10 min in complete darkness prior to recording.
    3. Present flashes of light stimuli using a Ganzfeld bowl whilst collecting ERG and VEP signals simultaneously over a ~ 500 msec time-window. Progress from dimmer to brighter light levels in order to maintain sufficient dark-adaptation for particular waveforms.
    4. Collect signals over a range of luminous energies to elicit STR, b-wave and a/b-wave waveforms of the ERG. Average more signals at the dimmer light levels (20 repeats) and less at the brighter luminous energies (1 repeat). Gradually lengthen the inter-stimulus interval from 1 to 180 sec from dimmest to the brightest light level. See **Table 1** for an example protocol.
    5. To isolate the ERG rod and cone responses, utilize a paired-flash paradigm[8]. Initiate four flashes at 1.52 log $cd.s.m^{-2}$ with a 500 msec inter-stimulus interval[2] in-between. Digitally subtract the cone waveform (3^{rd} or 4^{th} flash) from the mixed waveform (1^{st} flash) to derive the putative rod response.
    6. To record VEP signals, average 20 repeats at the brighter luminous energies (*i.e.*, - 0.52 to 1.52 log $cd.s.m^{-2}$, 5 sec inter-stimulus interval). Note that the first flash in this sequence returns the conventional dark-adapted ERG response.
    7. Allow 1 - 3 min for re-adaptation after (20) VEP sweeps before the next brighter ERG step, depending on the luminous energy.

8. After completion of data collection, euthanize the anesthetized animal with intracardiac injection of pentobarbital sodium (325 mg/ml, 3 ml).

Waveform	Stimulus light energy (log cd.s.m^{-2})	Number of repeats	interstimulus interval (sec)
STR	-6.24	20	2
STR	-5.93	20	2
STR	-5.6	20	2
STR	-5.33	20	2
Rod b-wave	-4.99	10	2
Rod b-wave	-4.55	10	2
Rod b-wave	-4.06	5	5
Rod b-wave	-3.51	5	5
Rod b-wave	-3.03	1	15
Rod b-wave	-2.6	1	15
Rod b-wave	-1.98	1	15
Mixed a-/b-wave	-1.38	1	30
Mixed a-/b-wave	-0.94	1	30
Flash 1: Mixed a-/b-wave Average of 20: VEP	-0.52	20	5
			(90 sec before next)
Flash 1: Mixed a-/b-wave Average of 20: VEP	0.04	20	5
			(120 sec before next)
Flash 1: Mixed a-/b-wave Average of 20: VEP	0.58	20	5
			(180 sec before next)
Flash 1: Mixed a-/b-wave Average of 20: VEP	1.2	20	5
			(180 sec before next)
Flash 1: Mixed a-/b-wave Average of 20: VEP	1.52	20	5
			(180 sec before next)
Cone a-/b-wave	1.52	4	0.5

Table 1. ERG and VEP Recording Protocol Using a Range of Stimulus Energy. Stimulus presentations progress from dim (top) to bright (bottom) flashes, with sufficient inter-stimulus interval to ensure dark adaption. At the end of protocol, repetition of four flashes with short interval is presented to elicit the cone mediated response.

3. Analysis of ERG Waveforms

Note: ERG and VEP analysis has been described in detail previously.[3,9,10] The following sections provide a brief overview.

1. Export signals in digital voltage-time format to a spreadsheet software for data analysis.
2. Rod photoreceptoral function
    1. Model the leading edge of the a-wave PIII with a delayed Gaussian (Equation 1)[11].
    PIII (i,t) = Rm_{PIII}· [1 - exp(-i· S (t - t_d)^2)] for t > t_d (Equation 1)
    2. Optimize the fit over an ensemble of two brightest luminous energies[12,13] (*i.e.*, 1.22 and 1.52 log cd.s.m^{-2}).
    3. Model up to 90% of the a-wave amplitude to avoid post-receptoral intrusions[14].
    Note: The model returns the saturated amplitude (Rm_{PIII}, µV), sensitivity (S, m^2.cd^{-1}.s^{-3}) and delay (t_d, msec) of the photoreceptoral response.

3. Rod bipolar cell function
    1. Digitally subtract the PIII model (see above) from the mixed waveforms to return the mixed PII with overlying oscillatory potentials.
    2. To extract the rod PII from the mixed PII, digitally subtract the cone response (3rd or 4th flash at 1.52 log cd.s.m^{-2}) from the mixed PII (1st flash at 1.52 log cd.s.m^{-2}).
    3. Then, apply a low-pass filter to the waveform (46.9 Hz, -3 dB, Blackman window) to remove oscillatory potentials. The remaining waveform is the rod PII response[10].
    4. Extract the rod PII peak amplitude and plot it against all stimulus intensities (below -2 log cd.s.m^{-2} and rod-isolated PII at 1.52 log cd.s.m^{-2})[10].
    5. Model these data using a hyperbolic function (Equation 2), which provides a measure of inner retinal cell integrity.

$V_{(i)} = V_{max} (i^n/(i^n + k^n))$ (Equation 2)
Note: This equation returns maximal PII response (V_{max}, µV), 1/sensitivity (k,log cd.s.m-2) and the slope of the function (n)[15].

4. Cone bipolar cell function
   Note: As the cone response is taken at a single intensity (1.52 log cd.s.m^{-2}) the amplitude and timing are retuned at this light level.
   1. Extract maximal cone PII response[2,16].
   2. Extract implicit time to which this maximal response corresponds[2,16].

5. Ganglion cell function
   1. As the STR is a small signal, apply a low pass filter with 50 Hz notch to waveform to eliminate high frequency and line noise (46.9 Hz, -3 dB, Blackman window).
   2. Extract maximal pSTR response[3,17].
   3. Extract implicit time to which this maximal response corresponds[3,17].

4. Analysis of VEP Waveforms

1. Extract maximum and minimum components of the VEP (P1, N1 and P2). For detail see references[3,6].
2. Express amplitudes as trough-to-peak amplitudes from their preceding peak or trough (P1N1 and N1P2)[3,6].
3. Extract implicit time ($_{it}$) to which this maximal response corresponds ($P1_{it}$, $N1_{it}$, $P2_{it}$)[3,6].

Representative Results

The ERG a-wave (> -1.38 log cd.s.m^{-2}), b-waves (> - 4.99 log cd.s.m^{-2}) STRs (< - 4.99 log cd.s.m^{-2}) and the VEPs (> - 0.52 log cd.s.m^{-2}) were recorded simultaneously (**Figure 1 and 3**). At very dim flashes, a positive STR (pSTR) is seen at approximately 110 msec after the flash, and a negative STR (nSTR) at approximately 220 msec (**Figures 1 and 2**). An ERG with a large b-wave, peaks between 50 to 100 msec after the onset of a moderate flash which can be analyzed for its PII response (**Figures 1 and 2**). At this stimulus energy, the negative a-wave before the peak is negligible. At brighter luminous energies the negative deflection a-wave becomes more prominent which can be quantified with the PIII response (**Figure 2**). The scotopic VEP waveform shows a negative response (P1N1; 15 - 70 msec window) followed by a positive deflection (N1P2; 30 - 100 msec) (**Figures 3 and 4**).

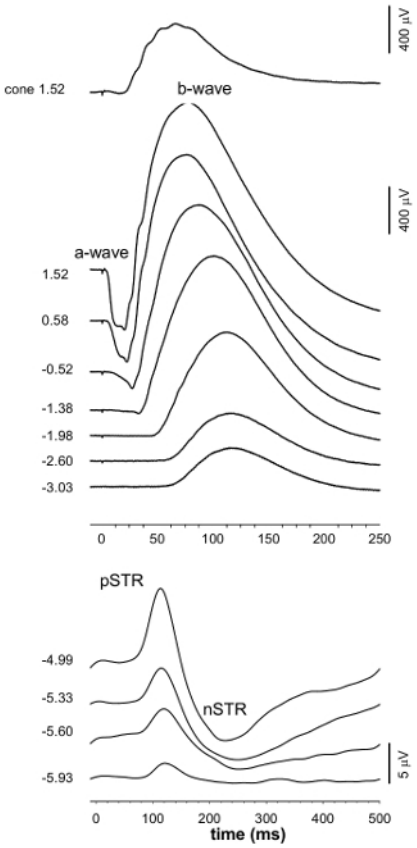

Figure 1. Group Average ERG Waveforms. The ERG alters with increasing stimulus intensity. Numbers to the left of waveform indicate the luminous exposure used to elicit the waveform. Note the different amplitude and time scales for each panel. At dimmer luminous energies the positive and negative components of the scotopic threshold response can be elicited (pSTR, nSTR). As stimulus energies get brighter, the a and b-wave response can be assayed, and a paired-flash paradigm allows the cone response to be measured. Please click here to view a larger version of this figure.

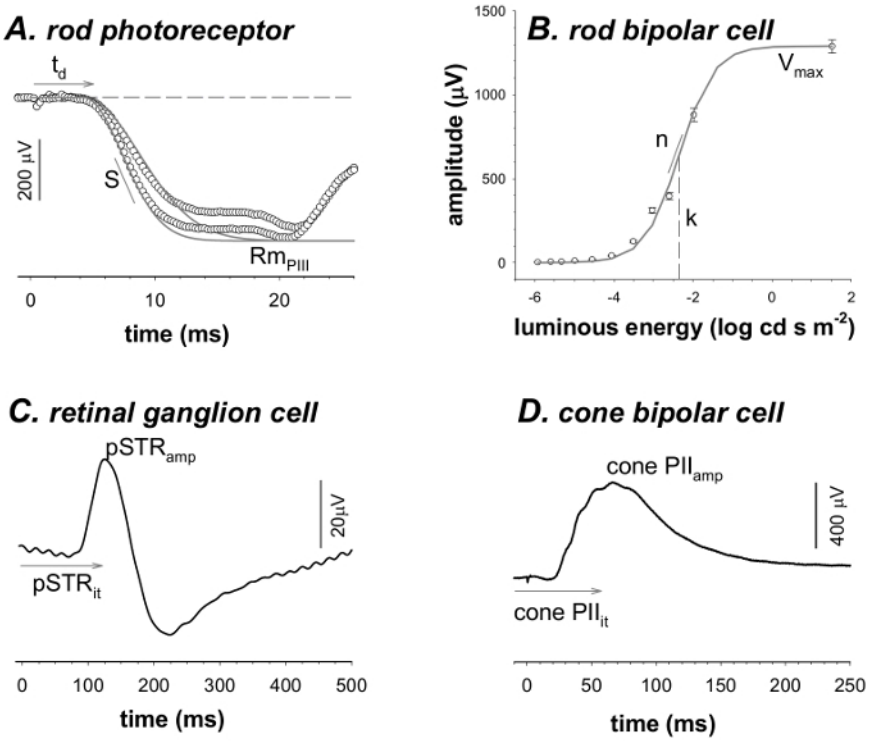

Figure 2. ERG analysis. (A) Rod photoreceptor function can be assayed by using a PIII to model the a-wave. A-waves at 1.22 and 1.52 log cd.s.m^{-2} (unfilled circles, ○) are fit as an ensemble with a PIII (grey lines, Equation 1) to 90% of the minimum which returns Rm_{PIII} (saturated amplitude, µV) S (sensitivity, $m^2.cd^{-1}.s^{-3}$) and td (timing delay, msec) parameters. **(B)** Rod bipolar cell function (mean ± SEM) can be assayed by modelling the intensity response series of the rod PII (unfilled circles ○) with a Naka-Rushton function (grey line). This returns V_{max} (saturated amplitude, µV), k (1/sensitivity, log cd s m^{-2}) and n (slope). **(C)** Retinal ganglion cell function is assayed at dim luminous energies and quantified by pSTR peak amplitude ($pSTR_{amp}$) and timing ($pSTR_{it}$). **(D)** Cone bipolar cell function is elicited with a paired-flash paradigm quantified by cone PII peak amplitude (cone PII_{amp}) and timing (cone PII_{it}). Please click here to view a larger version of this figure.

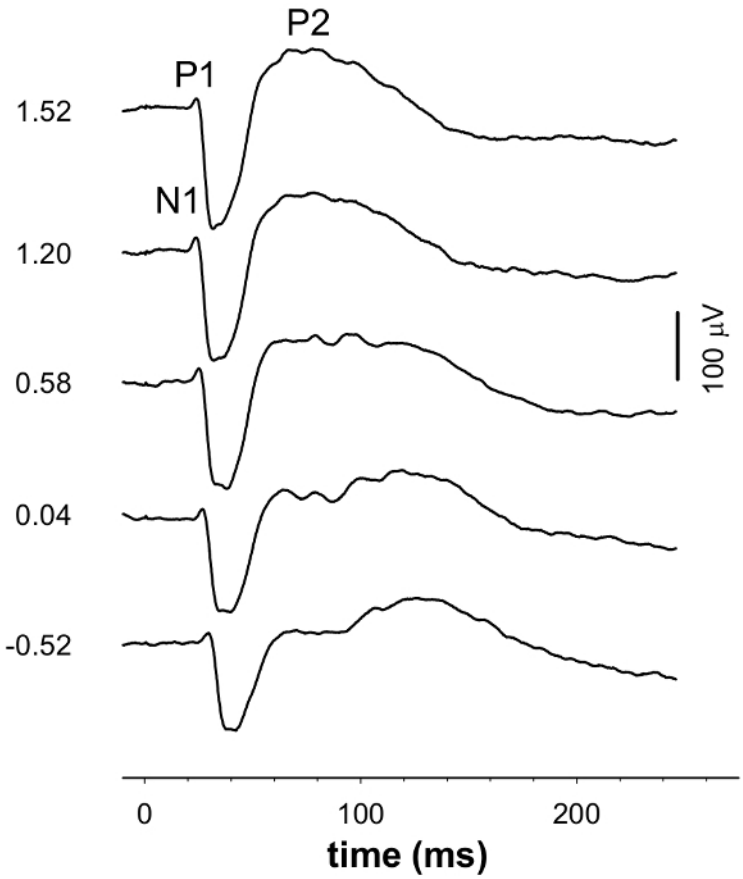

Figure 3. Group Average VEP Waveforms. The shape of the VEP waveform alters with increasing stimulus energy. Numbers to the left of the waveform indicate the luminous exposure used to elicit the waveform. Please click here to view a larger version of this figure.

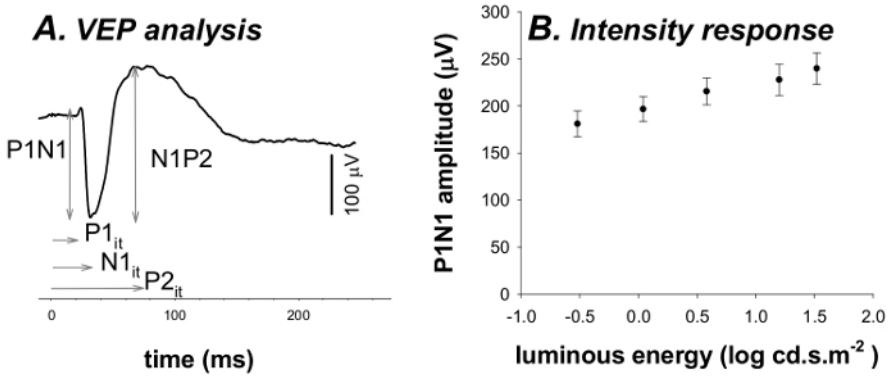

Figure 4. VEP Analysis and Intensity Response Function. (A) Amplitude analysis of the VEP is taken as peak to trough (P1N1) and trough to peak (N1P2) amplitudes. The implicit times ($_{it}$) of these responses is also returned ($P1_{it}$, $N1_{it}$, $P2_{it}$). **(B)** The VEP P1N1 amplitude (mean ± SEM) increases with increasing stimulus energy. Please click here to view a larger version of this figure.

Discussion

The ERG and VEP are objective measures of visual function from the retina and cortex, respectively. The advantage of simultaneous recording is that a more comprehensive view of the entire visual pathway is afforded. Specifically, the complementary information from their concurrent assessment could provide a clearer delineation of the site of injury in the visual pathway (*e.g.,* for disorders with overlapping ERG yet distinct VEP manifestations[18], when optic neuropathy may co-exist with primary cerebral atrophy[19,20], or when VEP loss may be confounded by manifestation of injuries at several locations in visual pathway[21,22]). By measuring the ERG and VEP concurrently, an index of the gain between retinal and cortical response can also be derived. This may provide a useful tool to detect subtle pathological changes. The current protocol allows for ERG and VEP measurement in commonly used laboratory rats but can readily be adapted to other mammalian species[23-25]. ERG and VEP waveforms from rodents provide a reasonable preclinical surrogate for responses observed in human eyes[26-28].

By designing a specific stimulus protocol, both ERG and VEP response can be obtained during a single recording session. **Table 1** shows a progression in light levels with appropriate consideration of the recovery time between consecutive flashes. This protocol provides a balance between the need to maximize signal-to-noise characteristics and to limit recording time within the anesthetic window provided by a single dose of ketamine:xylazine. Therefore, this technique may be useful for an objective quantitative measure of visual function for research into basic physiology and disease.

A comprehensive assessment of the visual system can be achieved by concurrently assessing bilateral retinal responses and visually evoked cortical responses. However, each technique can also be conducted in isolation and monocularly instead of binocularly to simplify the procedure. The current protocol describes scotopic ERG and VEP signals chosen to isolate the rod-pathway given that rats have a rod-dominated retina. If light adapted responses are of greater interest to the study, it is also possible to conduct photopic ERG and VEP signals by pre-adaption to a background light.

One major limitation of this technique is the need to conduct the procedure under anaesthetized conditions to enable stable electrode placement. Nevertheless this approach provides robust signal-to-noise characteristics enabling detection of subtle treatment changes.

Due to the small amplitude of the STR and its sensitivity to light adaptation, several steps need to be closely observed to ensure successful recording of this response. Firstly, sufficient dark adaptation needs to be implemented, which includes overnight dark adaptation (≥ 8 hr), electrode placement under dim red lighting (17.4 $cd.m^{-2}$, λ_{max} = 600 nm), and re-dark adaptation following a dim test-flash (10 min for - 0.52 log $cd.s.m^{-2}$). Furthermore, the signal-to-noise characteristics of the STR can be improved by averaging over multiple signals (*i.e.,* 20 signals) collected with short inter-stimulus intervals (*i.e.*, 2 sec). One of the advantages of this comprehensive assessment of both eyes and cortices is to enable comparison to the contralateral recording[3]. As such, particular care should be taken in electrode making (*i.e.*, same sized and shaped electrodes), to ensure minimal inter-eye and inter-cortical variability.

Given the extensive usage of both ERG and VEP techniques to provide *in vivo* measures of the visual pathway and its disease-related processes, it would be useful to collate other pathway-specific protocols (*e.g.*, ON/OFF or cone sub-type specific), and perform simultaneous ERG/VEP recordings with different stimulus modalities (*e.g.*, flicker, pattern, sawtooth) to extend the application of this technique in clinical diagnoses. Another logical step of this application in the future would also be to record the ERG and VEP concurrently from conscious[29], freely-moving animals to avoid anesthetic influences on neural physiology[30].

Disclosures

The authors have no disclosures relevant to this work.

Acknowledgements

Funding for this project was provided by the National Health and Medical Research Council (NHMRC) 1046203 (BVB, AJV) and Melbourne Neuroscience Institute Fellowship (CTN).

References

1. Nguyen, C. T. O., Vingrys, A. J., & Bui, B. V. Dietary omega-3 fatty acids and ganglion cell function. *Invest Ophthalmol Vis Sci*. **49**, 3586-3594 (2008).
2. Weymouth, A. E., & Vingrys, A. J. Rodent electroretinography: methods for extraction and interpretation of rod and cone responses. *Prog Retin Eye Res*. **27**, 1-44 (2008).
3. Tsai, T. I., Bui, B. V., & Vingrys, A. J. Effect of acute intraocular pressure challenge on rat retinal and cortical function. *Invest Ophthalmol Vis Sci*. **55**, 1067-1077 (2014).
4. Cowey, A., & Franzini, C. The retinal origin of uncrossed optic nerve fibres in rats and their role in visual discrimination. *Exp Brain Res*. **35**, 443-455 (1979).
5. Weinstein, G. W., Odom, J. V., & Cavender, S. Visually evoked potentials and electroretinography in neurologic evaluation. *Neurol Clin*. **9**, 225-242 (1991).
6. Odom, J. V. *et al.* Visual evoked potentials standard (2004). *Doc Ophthalmol*. **108**, 115-123 (2004).
7. Ridder, W. H., Nusinowitz, S., & Heckenlively, J. R. Causes of cataract development in anesthetized mice. *Exp Eye Res*. **75**, 365-370, (2002).
8. Nixon, P. J., Bui, B. V., Armitage, J. A., & Vingrys, A. J. The contribution of cone responses to rat electroretinograms. *Clin Experiment Ophthalmol*. **29**, 193-196 (2001).
9. Bui, B. V. *et al.* Using the electroretinogram to understand how intraocular pressure elevation affects the rat retina. *J Ophthalmol*. **2013**, 262467 (2013).
10. Nguyen, C. T., Vingrys, A. J., & Bui, B. V. Dietary omega-3 fatty acids and ganglion cell function. *Invest Ophthalmol Vis Sci*. **49**, 3586-3594 (2008).
11. Hood, D. C., & Birch, D. G. A quantitative measure of the electrical activity of human rod photoreceptors using electroretinography. *Vis Neurosci*. **5**, 379-387 (1990).
12. Birch, D. G., Hood, D. C., Locke, K. G., Hoffman, D. R., & Tzekov, R. T. Quantitative electroretinogram measures of phototransduction in cone and rod photoreceptors - Normal aging, progression with disease, and test-retest variability. *Arch Ophthalmol*. **120**, 1045-1051 (2002).
13. Bui, B. V., & Vingrys, A. J. Development of receptoral responses in pigmented and albino guinea-pigs (Cavia porcellus). *Doc Ophthalmol*. **99**, 151-170 (1999).
14. Robson, J. G., Saszik, S. M., Ahmed, J., & Frishman, L. J. Rod and cone contributions to the a-wave of the electroretinogram of the macaque. *J Physiol*. **547**, 509-530 (2003).
15. Severns, M. L., & Johnson, M. A. The care and fitting of Naka-Rushton functions to electroretinographic intensity-response data. *Doc Ophthalmol*. **85**, 135-150 (1993).

16. Bui, B. V., & Fortune, B. Origin of electroretinogram amplitude growth during light adaptation in pigmented rats. *Vis Neurosci*. **23**, 155-167 (2006).
17. Bui, B. V., & Fortune, B. Ganglion cell contributions to the rat full-field electroretinogram. *J Physiol*. **555**, 153-173 (2004).
18. Tremblay, F., Laroche, R. G., & Debecker, I. The Electroretinographic Diagnosis of the Incomplete Form of Congenital Stationary Night Blindness. *Vision Res*. **35**, 2383-2393 (1995).
19. Bayer, A. U., Keller, O. N., Ferrari, F., & Maag, K. P. Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. *Am J Ophthalmol*. **133**, 135-137 (2002).
20. Wostyn, P., Audenaert, K., & De Deyn, P. P. An abnormal high trans-lamina cribrosa pressure difference: A missing link between Alzheimer's disease and glaucoma? *Clinical Neurology and Neurosurgery*. **110**, 753-754 (2008).
21. Yucel, Y. H., Zhang, Q. A., Weinreb, R. N., Kaufman, P. L., & Gupta, N. Effects of retinal ganglion cell loss on magno-, parvo-, koniocellular pathways in the lateral geniculate nucleus and visual cortex in glaucoma. *Prog Retin Eye Res*. **22**, 465-481 (2003).
22. Gupta, N., & Yucel, Y. H. What changes can we expect in the brain of glaucoma patients? *Survey of Ophthalmology*. **52**, S122-S126 (2007).
23. Kong, Y. X. *et al.* Impact of aging and diet restriction on retinal function during and after acute intraocular pressure injury. *Neurobiol Aging*. **33**, 1126 e1115-1125 (2012).
24. Bui, B. V., Sinclair, A. J., & Vingrys, A. J. Electroretinograms of albino and pigmented guinea-pigs (Cavia porcellus). *Aust N Z J Ophthalmol*. **26 Suppl 1**, S98-100 (1998).
25. Jobling, A. I., Wan, R., Gentle, A., Bui, B. V., & McBrien, N. A. Retinal and choroidal TGF-beta in the tree shrew model of myopia: isoform expression, activation and effects on function. *Exp Eye Res*. **88**, 458-466 (2009).
26. Robson, J. G., & Frishman, L. J. Dissecting the dark-adapted electroretinogram. *Doc Ophthalmol*. **95**, 187-215 (1998).
27. Robson, J. G., & Frishman, L. J. The rod-driven a-wave of the dark-adapted mammalian electroretinogram. *Prog Retin Eye Res*. **39**, 1-22 (2014).
28. Hudnell, H. K., & Boyes, W. K. The comparability of rat and human visual-evoked potentials. *Neurosci Biobehav Rev*. **15**, 159-164 (1991).
29. Charng, J. *et al.* Conscious wireless electroretinogram and visual evoked potentials in rats. *PLoS One*. **8**, e74172 (2013).
30. Hetzler, B. E., & Berger, L. K. Ketamine-Induced Modification of Photic Evoked-Potentials in the Superior Colliculus of Hooded Rats. *Neuropharmacology*. **23**, 473-476 (1984).

