Video Article

Implantation and Recording of Wireless Electroretinogram and Visual Evoked Potential in Conscious Rats

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Abstract

The full-field electroretinogram (ERG) and visual evoked potential (VEP) are useful tools to assess retinal and visual pathway integrity in both laboratory and clinical settings. Currently, preclinical ERG and VEP measurements are performed with anesthesia to ensure stable electrode placements. However, the very presence of anesthesia has been shown to contaminate normal physiological responses. To overcome these anesthesia confounds, we develop a novel platform to assay ERG and VEP in conscious rats. Electrodes are surgically implanted sub-conjunctivally on the eye to assay the ERG and epidurally over the visual cortex to measure the VEP. A range of amplitude and sensitivity/timing parameters are assayed for both the ERG and VEP at increasing luminous energies. The ERG and VEP signals are shown to be stable and repeatable for at least 4 weeks post surgical implantation. This ability to record ERG and VEP signals without anesthesia confounds in the preclinical setting should provide superior translation to clinical data.

Video Link

The video component of this article can be found at https://www.jove.com/video/54160/

Introduction

The ERG and VEP are minimally invasive *in vivo* tools to assess the integrity of retinal and visual pathways respectively in both the laboratory and clinic. The full-field ERG yields a characteristic waveform which can be broken down into different components, with each element representing different cell classes of the retinal pathway[1,2]. The classic full-field ERG waveform consists of an initial negative slope (a-wave), which has been shown to represent photoreceptor activity post light exposure[2-4]. The a-wave is followed by a substantial positive waveform (b-wave) which reflects electrical activity of middle retina, predominantly the ON-bipolar cells[5-7]. Furthermore, one can vary luminous energy and inter-stimulus-interval to isolate cone from rod responses[8].

The flash VEP represents electrical potentials of the visual cortex and brain stem in response to retinal light stimulation[9,10]. This waveform can be broken down into early and late components, with the early component reflecting activity of neurons of the retino-geniculo-striate pathway[11-13] and the late component representing cortical processing performed in various V1 laminae in rats[11,13]. Therefore simultaneous measurement of the ERG and VEP returns comprehensive assessment of the structures involved in the visual pathway.

Currently, in order to record electrophysiology in animals, anesthesia is employed to enable stable placement of electrodes. There have been attempts to measure ERG and VEP in conscious rats[14-16] but these studies employed a wired setup, which can be cumbersome and may lead to animal stress by restricting animal movement and natural behavior[17]. With recent advances in wireless technology including improved miniaturization and battery life, it is now possible to implement a telemetry approach for ERG and VEP recording, decreasing the stress associated with wired recordings and improving long term viability. Fully internalized stable implantations of telemetry probes have proven to be successful for chronic monitoring of temperature, blood pressure[18], activity[19] as well as electroencephalography[20]. Such advances in technology will also assist with repeatability and stability of conscious recordings, increasing the platform's utility for chronic studies.

Protocol

Ethics statement: Animal experiments were conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (2013). Animal ethics approval was obtained from the Animal Ethics Committee, University of Melbourne. The materials herein are for laboratory experiments only, and not intended for medical or veterinary use.

1. Preparing Electrodes

Note: A three channel transmitter is used for surgical implantation which enables 2 ERG and 1 VEP recording to be conducted simultaneously. The three active and three inactive electrodes need to be pre-fashioned into a ring shape before implantation in order to attach to the eye. For identification purposes, the manufacturer has enclosed active electrodes in half white, half colored plastic sheaths while inactive electrodes are covered in full colored sheaths. The ground electrode (clear plastic sheath) is left unaltered. For all active and inactive electrodes conduct steps 1.1, 1.2, 1.3 and 1.7.

1. Untwist the double stranded stainless steel electrode with two fine tipped pliers.
2. Trim one of the stainless steel strands (approximately 1 cm from the tip), leaving a single longer straight strand remaining to shape the ring electrode.
3. Fold the single stainless steel strand back onto itself and twist, forming a smooth ring at the tip of the electrode.
4. For the ERG active electrodes fashion this loop ~ 0.2 - 0.5 mm in diameter by twisting the base of the loop (for the purpose described here, shape two active electrodes in this way to record ERG from both eyes), and for the ERG inactive and VEP electrodes make the loop diameter ~ 0.8 mm diameter (in this example, do this for one active VEP electrode and all three inactive electrodes).
5. Hook the circular VEP active electrode around a stainless steel screw (diameter 0.7 mm, length 3 mm) so the electrode rests against the screw head.
6. Hook the 3 inactive electrodes (2 ERG, 1 VEP) around a second stainless steel screw (diameter 0.7 mm, length 3 mm).
7. Pull the plastic sleeve forward over the sharp ends of the two stainless steel strand to reduce irritation.
8. Sterilize the telemetry transmitters by soaking in 2% glutaraldehyde for more than 10 hr at approximately 25 °C. Then rinse the transmitter with sterile saline 3 times.

2. Transmitter Implantation

1. **Animal Preparation**
    1. Disinfect the surgical area prior to experimentation by cleaning with 70% ethanol. Autoclave all surgical equipment before use and maintain equipment in chlorhexidine when not in use during surgery. Cover the animal with a surgical drape during surgery to maintain a sterile environment. Ensure all experimenters wear surgical masks, sterile gloves and gowns.
    2. Induce anesthesia with 1.5 - 2% isoflurane, at a flow rate of 3 L/min and maintained at 1.5 - 2 % at 2 L/min throughout the surgery. Confirm sufficient depth of anesthesia by absence of a pedal reflex upon pinching the muscle between the toes.
    3. Shave a 40 mm x 30 mm area over the abdomen from above the groin to the sternum.
    4. Shave a 30 mm x 20 mm area over the forehead, posterior to the eyes and anterior to the ears.
    5. Disinfect the two shaved areas. For the forehead area disinfect 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). Over the abdomen disinfect with 10% povidone iodine and 70% ethanol.
    6. Apply 1 drop of proxymetacaine to the cornea for additional topical anesthesia.
    7. Apply 1 drop of carboxymethylcellulose sodium to the cornea to prevent drying of the eyes.

2. **Surgical implantation**
    1. Make a 10 mm incision on the head along the vertical midline between the ears with a surgical scalpel.
    2. Make a 5 mm incision on the abdomen through the skin layer along the midline below the sternum.
    3. Tunnel a 5 mm diameter cannula subcutaneously from the abdomen incision to the head incision.
    4. Feed the electrode wires (3 active and 3 inactive) of the transmitter through the cannula from the abdomen to the head.
    5. Leave the reference electrode with the transmitter base and cover the electrode tip with aseptic gauze.
    6. Cover the electrode tips (3 active and 3 inactive) with aseptic gauze.
    7. Secure the rat's head to a stereotaxic platform.
    8. Extend the forehead incision to 30mm in length with surgical scissors.
    9. Expose surgical area by retracting loose skin with 2 sutures (3 - 0) at ~ 3 and 9 o'clock.
    10. Scrape off the periosteum overlying the skull using sterilized gauze to expose bregma, lambda and midline sutures.
    11. Drill two holes through the skull at the VEP active (7 mm ventral to bregma 3 mm lateral to midline) and inactive (5 mm rostral to bregma on the midline) stereotaxic co-ordinates.
    12. Attach VEP active and inactive electrodes with pre-attached stainless steel screws (diameter 0.7 mm, length 3 mm) to the skull with a small screw-driver to ~ 1 mm in depth into the premade holes. This anchors the screw to the bone without damaging the underlying cortical tissue.
    13. To implant the ERG active electrodes use an 8 - 0 suture to temporarily retract the upper eyelid.
    14. Insert a 16 to 21 G cannula subcutaneously from behind the eye through to the superior conjunctival fornix.
    15. Remove the guiding needle.
    16. Feed the active electrode through the shortened plastic catheter from the forehead towards the eye. Then remove the plastic catheter.
    17. Use a temporary suture (8 - 0), which is threaded through the electrode loop, to prevent the electrode from retracting back into the tunnel.
    18. Make a 0.5 mm incision on the superior conjunctiva at 12 o'clock, 1 mm behind limbus. Use blunt dissection to expose the underlying sclera.
    19. Implant an 8 - 0 or 9 - 0 suture immediately behind the limbus at half scleral thickness.
    20. Remove temporary suture from ERG active electrode.
    21. Anchor the ERG active electrode to the half scleral thickness suture by tying 3 consecutive knots ensuring the tip of the electrode is situated close to the limbus.
    22. Close the conjunctival flap using 1 to 2 interrupted sutures (8 - 0 to 9 - 0). Ensure that the conjunctiva completely covers the ERG electrode to improve comfort.
    23. Remove the eyelid retracting suture.

24. Repeat the procedure for the contralateral eye.
25. Apply cyanoacrylate gel over the skull to secure all stainless screws and electrode wires. Ensure the ERG active electrodes are not pulled too tight before securing to enable eye movements.
26. Close the head wound using a non-absorbable 3 - 0 suture.
27. Rotate rodent to expose abdominal area. Lengthen the abdominal dermal incision to 40 mm along the linea alba with surgical scissors.
28. Make a 35 mm incision through the inner muscle wall to expose the inner abdominal cavity.
29. Using two sutures (3 - 0) attach the transmitter body to the animal's right hand side inner abdominal wall. Avoid contacting the liver.
30. Loop the ground electrode and secure in this shape with a suture (3 - 0). Place it free-floating in the abdominal cavity.
31. Close the peritoneum using a continuous suture (3 - 0).
32. Close the skin incision using interrupted sutures (3 - 0).

3. **Post-operative care**
    1. Monitor the animal until it has regained sufficient consciousness to maintain sternal recumbency. House the animal singly following the surgery.
    2. Administer carprofen subcutaneously for analgesia (5 mg/kg) once a day for 4 days.
    3. Add prophylactic oral antibiotics (Enrofloxin, 5 mg/kg) to the drinking water for 7 days post-surgery.
    4. Apply an anti-inflammatory ointment to skin incision sites to reduce irritation for the first 7 days post-surgery.

3. Conduct ERG and VEP Recordings in Conscious Rats

1. Dark adapt animal for 12 hr prior to ERG and VEP recordings
2. Conduct all experimental manipulations under dim red illumination (17.4 cd.m^{-2}, λ_{max} = 600 nm)
3. Apply topical anesthesia (0.5% proxymetacaine) and dilating (0.5% tropicamide) drops to the cornea.
4. Guide the conscious rodent into a custom made, clear restrainer.
   Note: The length of this plastic tube can be adjusted to accommodate different sized rats with the overall diameter fixed at 60 mm. The front end of the device is tapered to minimize head movement and contains perforations to enable normal breathing. This tapered front allows alignment and stabilization of the rat's head and eyes to the opening of the Ganzfeld sphere. Note that the rodent has been acclimatized to the restrainer (3 to 5 occasions) prior to surgery.
5. Place the rodent in front of the Ganzfeld bowl with the eyes aligned with the opening of the bowl.
6. Turn on indwelling transmitter by passing a magnet within ~ 5 cm of the transmitter. Verify that the transmitter is on by checking the LED status light on the receiver base.
7. Collect signals over a range of luminous energies (*i.e.,* -5.6 to 1.52 log cd.s.m^{-2}) as described previously[21]. Briefly, average more signals at the dimmer light levels (~ 80 repeats) and less at the brighter luminous energies (~ 1 repeat). Gradually lengthen the interstimulus interval from 1 to 180 seconds from dimmest to the brightest light level.
8. To isolate the ERG rod and cone responses utilize a twin-flash paradigm[8]. For example, initiate two flashes at 1.52 log cd.s.m^{-2} with a 500 msec inter-stimulus interval in-between.
9. To record VEP signals, average 20 repeats at the brighter luminous energies (*i.e.,* 1.52 log cd.s.m^{-2}, 5 sec inter-stimulus interval).
10. To evaluate implant stability, which is assessed by signal variability over time, conduct ERG and VEP recordings 7, 10, 14, 21 and 28 days post-surgery.
11. Following experimental period, euthanize rats via intracardial injection of pentobarbiturate (1.5 ml/kg) after ketamine:xylazine anesthesia (12:1 mg/kg).

Representative Results

The photoreceptor response is analyzed by fitting a delayed Gaussian to the leading edge of the initial descending limb of the ERG response at the top 2 luminous energies (1.20, 1.52 log c.s.m^{-2}) for each animal, based on the model of Lamb and Pugh[22], formulated by Hood and Birch[23]. This formula returns an amplitude and a sensitivity parameter, (**Figure 1C** and **1D**, respectively). A hyperbolic function was fitted to the luminous energy response of rod bipolar cells for each animal, which also returned an amplitude and a sensitivity parameter, (**Figure 1E** and **1F** respectively). Cone bipolar cell amplitude was analyzed as peak response of the waveform (top waveform of **Figure 1A** and **1B**), with implicit time taken as the time it took to reach peak response. For further details please see Charng *et al*. [24].

Figure 1A and **B** shows ERG waveform ± SEMs (n = 8) in conscious rats at day 7 and 28 post-surgery. The waveforms appear to be slightly larger at day 28 compared to day 7, but linear mixed model analysis revealed no significant time effect (p = 0.14 to 0.67) for photoreceptor (dark-adapted PIII) amplitude (**Figure 1C**) and sensitivity (**Figure 1D**); rod bipolar cell (dark-adapted PII) amplitude (**Figure 1E**) and sensitivity (**Figure 1F**); cone bipolar cell (light-adapted PII) amplitude (**Figure 1G**) and implicit time (**Figure 1H**). Similarly, VEP waveform SEMs (n = 8, **Figure 2A**) appear comparable at 7 and 28 days post-surgery, with amplitude (**Figure 2B** and **2C**) and timing (**Figure 2D - 2F**) parameters showing no significant time effect (p = 0.20 to 0.93). These results indicate robust ERG and VEP signal stability.

Average signal-to-noise (SNR, n = 8) ratio of both ERG (**Figure 3A**) and VEP (**Figure 3B**) returned good stability over the five conscious recording sessions. In this scenario, ERG signal is defined as the as the amplitude of the ERG P2 response while noise is the maximal peak to trough amplitude calculated from a 10 msec pre-stimulus interval. In the VEP, P2-N1 amplitude is deemed as the signal while noise is also returned by the peak to trough of the 10 msec pre-stimulus interval. There was no significant time effect across the SNR of both ERG and VEP (p = 0.49 and 0.62 respectively).

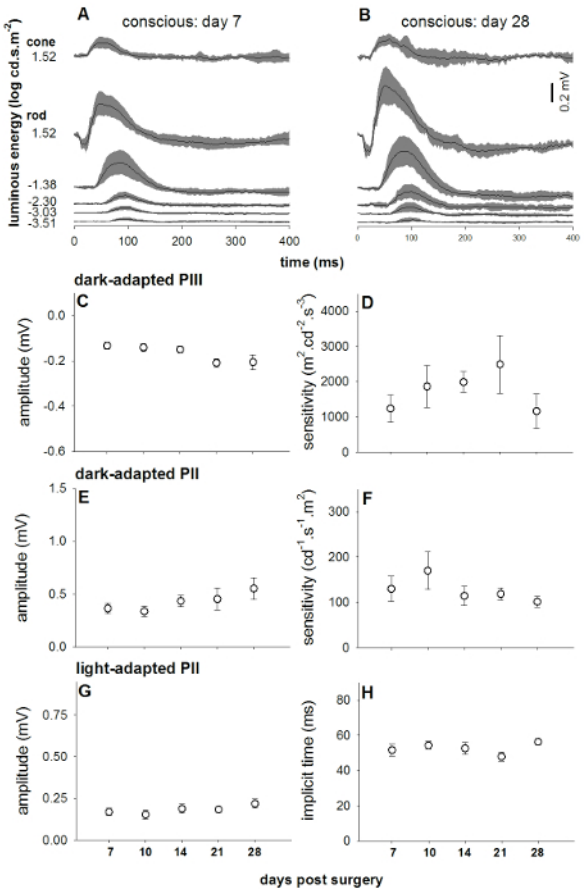

Figure 1: Conscious Electroretinograms Exhibit Characteristic Waveforms and Repeatable Measurements. (**A**-**B**) ERG waveforms ± SEMs (n = 8) across a wide range of luminous energies at day 7 (**A**) and 28 (**B**) post-surgery. (**C**-**F**) Rod and cone ERG parameters are plotted against time after implantation. Rod (dark-adapted PIII) photoreceptoral amplitude (**C**) and sensitivity (**D**), rod bipolar cell (dark-adapted PII) amplitude (**E**) and sensitivity (**F**), and cone bipolar cell (light-adapted PII) amplitude (**G**) and implicit time (**H**) all showed stable recordings over the 5 sessions. All symbols indicate average value (± SEM). This figure has been modified from Charng *et al.*[24] Figure 4. Please click here to view a larger version of this figure.

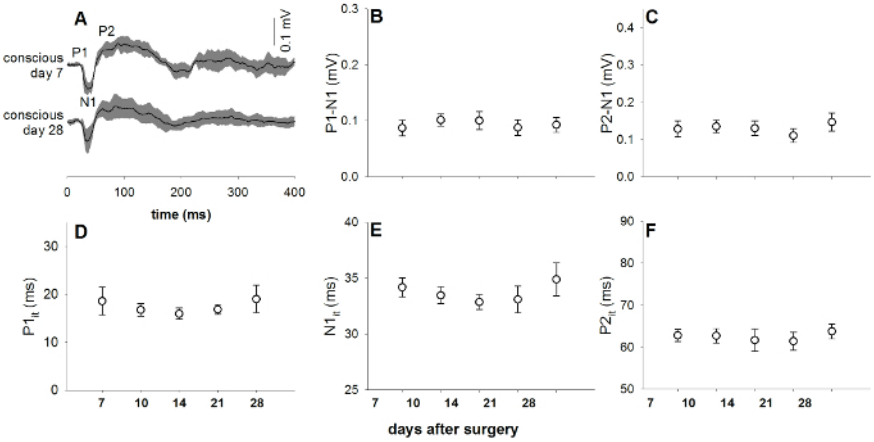

Figure 2: Conscious Visual Evoked Potentials Exhibit Characteristic Waveforms and Repeatable Measurements. (**A**) VEP waveforms ± SEMs (n = 8) are plotted at day 7 and 28 post surgery. (**B**-**F**) VEP amplitude and timing parameters are assessed over 1 month after implantation. P1-N1 (**B**) and P2-N1 (**C**) amplitude as well as P1 (**D**), N1 (**E**) and P2 (**F**) implicit time parameters were all stable over the 5 recording sessions. All symbols indicate average value (± SEM). This figure has been modified from Charng *et al.*[24] Figure 6. Please click here to view a larger version of this figure.

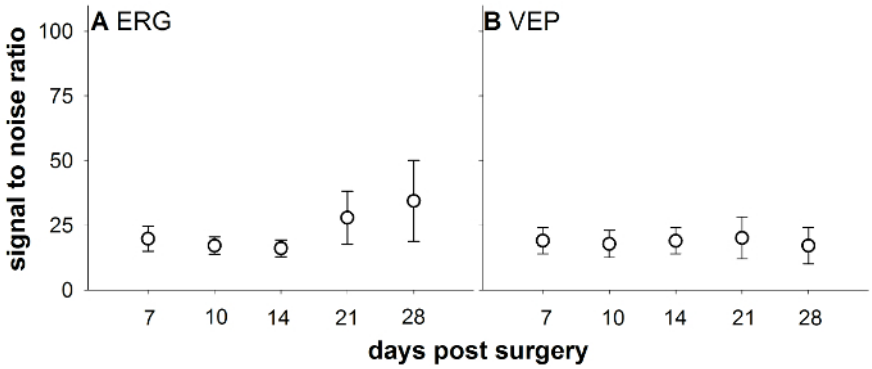

Figure 3: The Telemetry System Demonstrates Stable Signal-to-Noise Ratio Over Time. The signal-to-noise ratio of the (**A**) ERG and (**B**) VEP were not significantly altered over time (n = 8). All symbols indicate average value (± SEM). This figure has been modified from Charng *et al.*[24] Figure S1. Please click here to view a larger version of this figure.

Discussion

Due to the minimally invasive nature of visual electrophysiology, ERG and VEP recordings in human patients are conducted under conscious conditions and only require the use of topical anesthetics for electrode placement. In contrast, visual electrophysiology in animal models is conventionally conducted under general anesthesia to enable stable electrode placement by eliminating voluntary eye and body movements. However, commonly used general anesthetics alter the ERG and VEP responses as shown by our previous publication[24] and others[25-27]. As such development of a conscious ERG and VEP platform in a rodent model provides superior representation of physiological responses in animal models, which may in turn afford better translatability from preclinical to clinical findings. Another disadvantage of using anesthesia is that it limits the duration of an experiment. More specifically, the use of prolonged anesthesia as well as repeated administration of anesthetics can increase chance of adverse effects such as drug build up and associated respiratory problems[28].

This study showed that the telemetry system in conscious rats returned robust ERG and VEP signal stability for at least 28 days post-surgery. Our group is the first to conduct conscious wireless ERG and VEP responses simultaneously[24] and this manuscript details the surgical and recording procedures involved. Comparison to other surgical procedures conducted with wired conscious ERG and VEP recordings illustrate superior stability in the ERG and equivalent repeatability in VEP recordings over a 1 month period[15].

The surgical techniques and subsequent conscious recordings have the potential to be applied to various animal models. The platform has potential utility in multiple applications where it is beneficial to avoid confounds associated with anesthesia[29]. These include drug discovery, improved translation to human studies, and chronic or longitudinal experiments.

Possible modifications to the technique include altering the number of biopotential channels implanted and simultaneously recorded. This can vary from 1 to 4 biopotential leads and thus could measure visual evoked electrophysiology from between 1 eye to 2 eyes and 2 visual cortices. Note that the alteration in the number of biopotentials channels also leads to modification of the band-width recorded which will have implications for high frequency electrophysiological signals. For example the 3 channel biopotential transmitter used in this study (F50-EEE) was chosen to show that it is possible to simultaneously record visually evoked responses from the retina and visual cortex of a conscious rat. However, these 3 channel transmitters have a band-width of 1 - 100 Hz, which can faithfully record ERG a- and b-waves but will alter oscillatory potentials due to their higher frequency[24]. In contrast, if it was of interest to the study to record oscillatory potentials then a transmitter with less recording channels (*i.e.*, broader band-width) could be employed. It is also possible for the light stimulus to be altered, for example instead of conducting full-field ERG and VEP, visual physiology in response to flicker stimuli may also be utilized.

One major limitation in translating this technique to other animal models is the size of the animal's eye. One should have no problem implanting the ocular electrodes to animals bigger than rats. However, it would be challenging to implant the ERG electrode onto a mouse eye due to the smaller working area. The cortical implantation, on the other hand, should be relatively straightforward to perform in most laboratory animals.

There are several aspects of the surgery that need to be closely observed to ensure successful implantation. It is imperative that the ERG electrode ring is formed into a smooth ring due to irritation that may be induced by any rough edges on the loop. The implantation of ERG active electrodes is facilitated by two concurrent experimenters, one to stabilize the eye while the other attaches the electrode to the sclera. Particular care has to be taken to ensure the scleral suture (2.2.19) is only half-thickness, as a full thickness scleral suture will puncture the eyeball and cause vitreous leakage. The implantation of electrodes onto the skull (VEP active and ERG/VEP inactive electrodes) is less technically demanding than that of ERG electrodes. Nevertheless, it is imperative that once the electrodes are anchored to the skull, the wires are allowed to uncurl naturally to reduce any unnecessary tension. Acclimatization to the recording restrainer prior to surgical implantation is advantageous to reduce excessive movements during ERG and VEP recordings.

Disclosures

RG and RF are employees to the commercial funder of this research (Pfizer Neusentis and Pfizer Global Research). MI was an employee of Pfizer Global Research during this research and is currently an employee of Proteostasis Therapeutics (Cambridge, USA).

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