

Video Article

Visual Evoked Potential Recording in a Rat Model of Experimental Optic Nerve Demyelination

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Abstract

The visual evoked potential (VEP) recording is widely used in clinical practice to assess the severity of optic neuritis in its acute phase, and to monitor the disease course in the follow-up period. Changes in the VEP parameters closely correlate with pathological damage in the optic nerve. This protocol provides a detailed description about the rodent model of optic nerve microinjection, in which a partial demyelination lesion is produced in the optic nerve. VEP recording techniques are also discussed. Using skull implanted electrodes, we are able to acquire reproducible intra-session and between-session VEP traces. VEPs can be recorded on individual animals over a period of time to assess the functional changes in the optic nerve longitudinally. The optic nerve demyelination model, in conjunction with the VEP recording protocol, provides a tool to investigate the disease processes associated with demyelination and remyelination, and can potentially be employed to evaluate the effects of new remyelinating drugs or neuroprotective therapies.

Video Link

The video component of this article can be found at <https://www.jove.com/video/52934/>

Introduction

Optic neuritis is one of the most common form of optic neuropathy, causing complete or partial loss of vision¹. Histologically, it is featured by inflammatory demyelination, retinal ganglion cell axonal loss and varying degrees of remyelination in the optic nerve². Optic neuritis is usually the manifest onset of multiple sclerosis. The visual evoked potential (VEP) is a non-invasive tool for investigating the function of the visual system. It reflects the post-retinal function from the retina to the primary visual cortex and is affected in many optic nerve disease conditions³. The VEP has been predominantly used in optic neuritis patients to assess the integrity of the visual pathway⁴.

The latency of VEP, which reflects the velocity of signal conduction along the visual pathway, is considered to be an accurate measurement of the level of myelin associated changes in the optic nerve⁵; while the amplitude of VEP is believed to be closely correlated with axonal damage of the retinal ganglion cells (RGC)⁶. This hypothesis has been fairly well established using the rat model of lysolecithin-induced optic nerve demyelination⁵.

Here, we explicate a comprehensive protocol of optic nerve microinjection technique in rodents, which can minimise the surgical manipulation-related damage to the nerve *per se* as well as to the adjacent tissues such as extraocular muscles and blood vessels. Also, the skull electrode implantation surgery has been described for VEP recording in animals⁷. The VEP recordings can be repeatedly carried out on animals over a period of time to assess demyelination/remyelination related changes as well as impact on axonal integrity in the optic nerve.

Protocol

Ethics Statement: All procedures involving animals were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Animal Ethics Committee of Macquarie University.

1. VEP Electrode Implantation

1. Anesthetize the animal with an intraperitoneal injection of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg).

Note: Following induction of anaesthesia, observe the withdrawal reflexes (pinch test, corneal and palpebral reflex, etc.) and their absence as an indication to begin surgery. Continually monitor animals throughout the surgery and administer additional anaesthetic drug (10% of initial ketamine dose per top-up) if the reflexes are present. Adult rats (>12 weeks) are used in the experiments.

2. Shave the skin of the surgical area. Place the animal on a warming pad (37 °C) to maintain body temperature during the surgery. Prepare skin through topical application of povidone-iodine. Apply surgical draping. Apply topical ophthalmic ointment to prevent corneal dryness under general anaesthesia. Maintain asepsis by using sterile instruments.
3. Make a longitudinal skin incision on the midline of the head skin. Clear the connective tissue to achieve good exposure of the skull.
4. Carefully, drill small burr holes manually using a micro hand drill at 7 mm behind the bregma and 3 mm lateral to the midline.
5. Implant screw electrodes through the skull into the cortex (area 17), penetrating the cortex to a depth of approximately 0.5 mm. Implant a reference screw electrode on the midline 3 mm rostral to the bregma. Apply dental cement to encase and fix the screws (not always required).
6. Suture the skin of the head, administer antibiotic ointment onto the skin and allow the animal to recover from anaesthesia on a warming pad. Note: Alternatively, electrodes can be left exposed, so that skin doesn't need to be reopened in each recording. Immediately upon cessation of surgery but prior to anaesthetic recovery, administer a non-steroidal anti-inflammatory drug (NSAID) (if not administered pre-operatively) or an opioid analgesic. Monitor the animals constantly until full recovery from anaesthetic and fully ambulatory.
7. Allow at least 1 week for the animals to recover from the surgery prior to VEP recording.

2. Optic Nerve Injection

1. Anesthetize the animal, prepare the skin and apply draping as above (1.1 and 1.2).
2. Make a 1- to 1.5-cm incision in the skin above the orbit of a randomly selected eye. Open the subcutaneous tissue to reach the orbital cavity using fine iris scissors. Open the conjunctiva and anterior Tenon's capsule under the operating microscope.
3. Retract the extraocular muscles and intraorbital lacrimal glands to expose approximately 3 mm length of the optic nerve. Open the dura and arachnoid matter layers around the optic nerve longitudinally using an ophthalmic blade.
4. Insert the glass pipette into the optic nerve at a distance of 2 mm posterior to the globe. The glass micropipette is attached to a Hamilton syringe.
5. Inject 1% lysolecithin (0.4 - 1.0 µl, with 0.02% Evan's Blue which doesn't have effects on myelination) slowly into the nerve approximately over a period of 30 sec.
6. Suture the skin incision. Apply antibiotic ointment to prevent infection. Fellow eyes can be served as internal controls for electrophysiology recordings.
7. Place the animals on a warming pad to recover from anaesthesia.

3. VEP Recording

1. Anesthetize the animal and prepare the skin as 1.1 and 1.2.
Note: Lower dose of anaesthetics can be used for electrophysiological recording (ketamine 40 mg/kg and medetomidine 0.25 mg/kg).
2. Place the rat in a dark room and allow it to adapt to darkness for 5 - 30 min. In some cases rats can respectively be dark adapted O/N for scotopic or light adapted for photopic VEP recordings⁸.
3. Maintain the body temperature at 37 ± 0.5 °C by the homeothermic blanket system with a rectal thermometer probe.
4. Dilate the pupils with 1.0% tropicamide eye drops. Open the skin over the skull to access the pre-placed *in situ* screw electrodes.
5. Connect the screw over the contralateral visual cortex of the stimulated eye and the reference screw to the amplifier. Insert a needle electrode into the tail as the ground. Measure and maintain the electrode impedance below 5 kΩ.
6. Place a mini-Ganzfeld stimulator directly on the skin around the eyelids to provide superior eye isolation⁷. The illumination of the stimulator needs to be calibrated beforehand by a photometer.
7. Deliver photic stimulation through light flashes 100 times at a frequency of 1 Hz, with low and high band-pass filter settings of 1 and 100 Hz, respectively. The signal sampling rate is at 5 kHz.
Note: Signals should be sampled at least at about 250 - 300 Hz to ensure that more than two samples are collected during each cycle.
8. Suture the skin back and keep the animals on a warming pad to recover from anaesthesia. Recording can be repeatedly recorded on individual animals to monitor functional changes over a period of time.
9. At the end-point, administer an overdose injection of sodium pentobarbitone (100 mg/kg, i.p.) to euthanize the animal. Confirm euthanasia by cardiac arrest, respiratory arrest and decrease of body temperature.

4. Tissue Preparation and Histology

1. Remove the optic nerves from euthanized animals under the microscope and fix the tissue in 1% paraformaldehyde O/N.
2. Wash the tissue thoroughly with saline. Treat the tissue in an automatic tissue processor and embed in paraffin. Cut sections (5 - 10 µm) using a rotary microtome.
Note: For immunohistochemistry study, fix tissue in 1% paraformaldehyde, wash with saline and incubate with 30% sucrose O/N. Embed tissue in OCT embedding medium and make cryosections using a cryostat.
3. Incubate sections in 0.1% fast blue solution such as Luxol (in 95% ethanol) O/N at 56 °C. Differentiate the sections in 0.05% lithium carbonate for 30 sec and then 70% ethanol for another 30 sec. Finally, counterstain in 0.1% cresyl violet solution for 30 sec before mounting the sections. Use the fast blue staining to identify myelin in the optic nerve⁵.

Representative Results

Reproducible intra-session VEP traces are shown in **Figure 1** and a significant delay in N1 latency can be seen after the optic nerve injection. Partial optic nerve lesions of demyelination can be observed on histological sections using Luxol fast blue staining⁵. **Figure 2** shows a representative section with a small focal demyelinated lesion in the centre of the optic nerve. Note that cross section does not represent total volume of lesion. The demyelinated area can be measured on each consecutive cross-section of the nerve to deduce the lesion volume by using

three-dimensional reconstruction. We have demonstrated a strong correlation between latency delay and lesion volume using this model in our previous studies and there was no VEP latency delay in the saline-injected controls⁵.

It is believed that early VEP components following the flash illumination are more stable⁷ and principally affected by the excitation of the primary visual cortex via retino-geniculate fibres. Our study has shown that N1 delay has the strongest linear relationship with demyelination in the optic nerve⁵. Hence, we recommend that N1 latency should be used for data analysis and for longitudinal VEP monitoring in assessing the impact of remyelinating therapies. The amplitude of VEP, although more variable compared to the latency, is more indicative of function of the axons in the optic nerve⁶. Electroencephalogram-based scaling can be considered for amplitude analysis⁹.

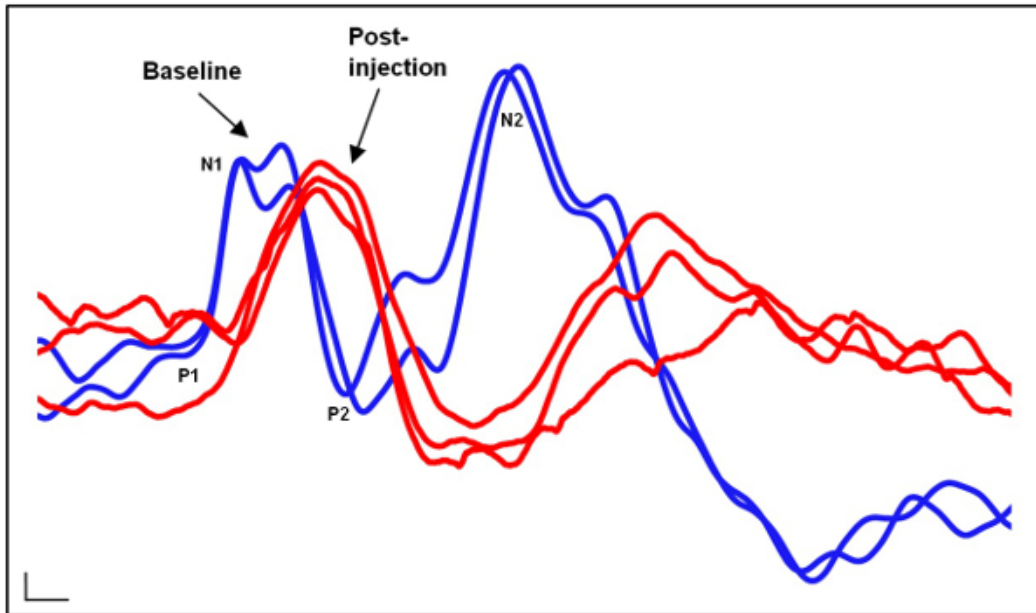


Figure 1. VEP delay after optic nerve injection. Representative VEP traces from an individual rat before and 2 days after the optic nerve microinjection (0.8 μ l lysolecithin). VEP recordings were repeated on the each day (intra-sessional traces are shown in the same colour) to demonstrate the reproducibility of this VEP recording protocol. (vertical scale bar: 10 μ V; horizontal scale bar: 10 msec). [Please click here to view a larger version of this figure.](#)

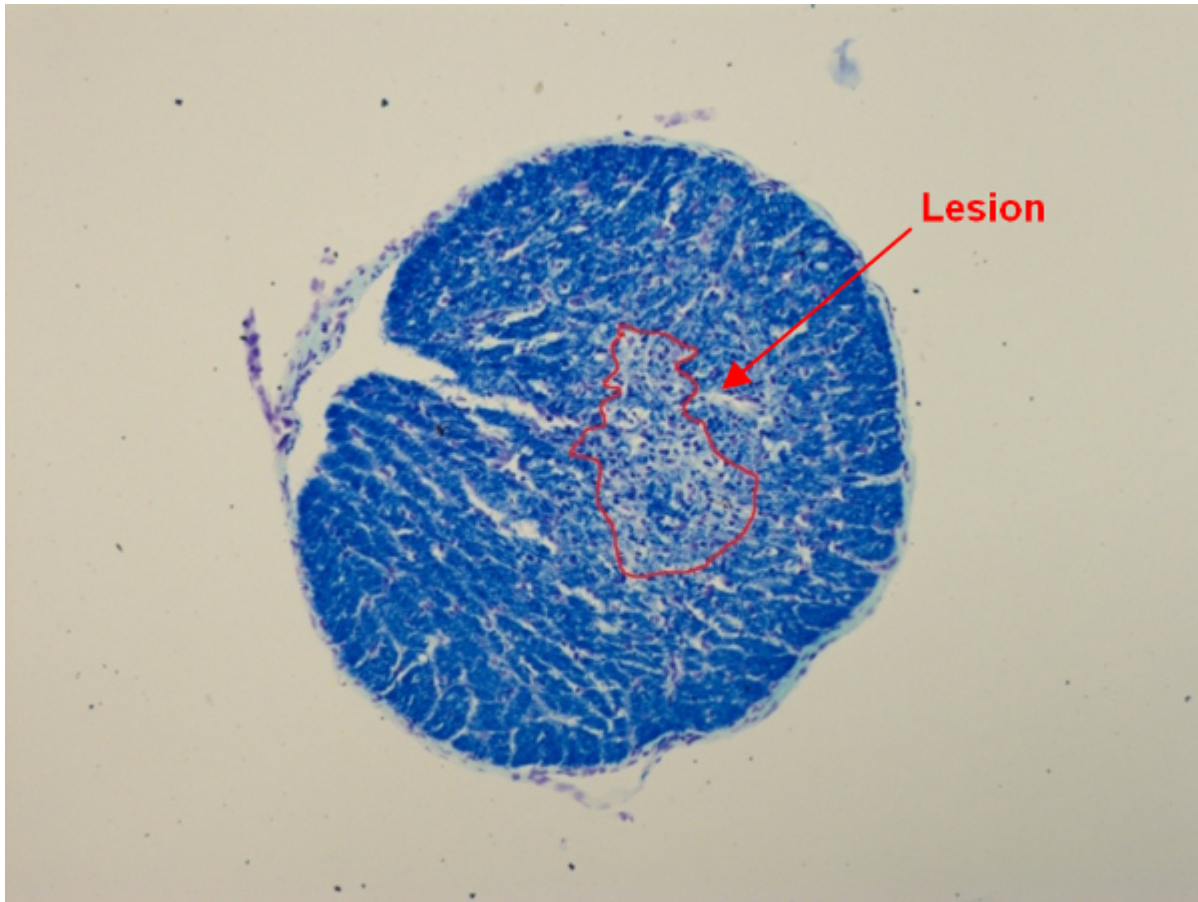


Figure 2. Demyelination in the optic nerve. Representative cross-section of the optic nerve from a rat after lysolecithin microinjection. The myelin component is stained blue by using luxol fast blue. A small focal lesion of demyelination can be seen in the centre of the section. Demyelinated area can be measured on longitudinal serial cross-sections to estimate the lesion volume in a three-dimensional scale. [Please click here to view a larger version of this figure.](#)

Discussion

The optic nerve is very susceptible to mechanical damage. Optic nerve crush injury over a duration of 1 s can lead to about 75% loss of RGC over a period of 2 weeks¹⁰. Therefore, extreme care is required while performing the surgical procedures. According to the authors' experience, it is much better to adapt a blunt dissection approach to expose and make way through the tissues around the optic nerve along the orientation of the nerve, rather than penetrating in a perpendicular orientation to the optic nerve. Also, the dura and arachnoid mater should be unwrapped longitudinally to avoid any axotomy-like damage to the RGC axons. Evan's blue is usually used to facilitate the microinjection, as presence of the dye makes the nerve light blue in colour at the injection site.

The reproducibility of VEP recording is critical for this experiment. Different types of anaesthetics and the depth of anaesthesia can affect the VEP waveforms¹¹. We do not recommend using inhaled isoflurane anaesthesia for the VEP study, as isoflurane can lead to burst suppress on the electroencephalogram response¹², probably due to GABAergic activity, resulting in more variable VEP traces¹³. Combination of ketamine/medetomidine anaesthesia provides stable anaesthesia that can quickly be reversed by administration of an alpha receptor antagonist such as atipamazole (2.5 mg/kg i.p). Body temperature can also have a significant impact on the speed of nerve conduction, especially in demyelinated nerves. Temperature-dependant changes in visual evoked potentials have been observed in rat VEPs and latencies were observed to be significantly longer as body temperature was lowered^{14,15}. Hence, it is important to maintain a uniform body temperature using a homeothermic blanket system during the VEP recordings.

Also, it is important to understand that the pathogenesis of lysolecithin-induced demyelination differs from that in optic neuritis or multiple sclerosis. Lysolecithin-induced demyelination results from the detergent like action of the toxin upon myelin lipids rather than immune-mediated inflammatory reaction, which is usually seen in the real disease scenarios¹⁶. If the study aims to investigate the mechanisms of demyelination/axonal damage in multiple sclerosis, an immune-mediated demyelination model, such as experimental autoimmune encephalomyelitis (EAE)¹⁷, should be considered. The advantage of using the toxin-induced demyelination model is that a focal lesion in the optic nerve can be produced and monitored longitudinally. In contrast to the brain lesions, the effects of demyelinated lesions on the optic nerve are more clinically apparent and measureable (lesion size can be accurately determined by VEPs or MRI imaging). Although remyelination in the optic nerve appeared to be not as pronounced as that observed previously in the case of spinal cord¹⁸, this optic nerve injection model, in conjunction with the VEP recording technique, is able to provide a superior tool to monitor the process of demyelination and remyelination *in vivo*, and to potentially evaluate new remyelinating therapies.

Disclosures

None of the authors have competing interests or conflicting interests.

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