Video Article

The Rodent Model of Nonarteritic Anterior Ischemic Optic Neuropathy (rNAION)

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Abstract

Nonarteritic anterior ischemic optic neuropathy (NAION) is a focal ischemic lesion of the optic nerve that affects 1/700 individuals throughout their lifetime. NAION results in optic nerve edema, selective loss of the retinal ganglion cell neurons (RGCs) and atrophy of the optic nerve. A rodent model of NAION that expresses most NAION features and sequelae has been developed, which is applicable to both rats and mice. This model utilizes a focal laser application of 532 nm wavelength to illuminate a photoactive dye, Rose Bengal (RB), to cause capillary damage and leakage at the targeted anterior optic nerve (the laminar region). After rNAION induction, there is an early optic nerve ischemia, optic nerve edema, and intraneural inflammation, followed by selective RGC and axonal loss. Since the optic nerve is a CNS white matter tract, the rNAION model is applicable to mechanistic studies of selective white matter ischemia, as well as neuroprotective analyses and short and long-term mechanisms of glial and neuronal response to ischemia.

Video Link

The video component of this article can be found at https://www.jove.com/video/54504/

Introduction

Nonarteritic anterior ischemic optic neuropathy (NAION) is a focal ischemic lesion of the anterior portion of the optic nerve (ON)[1]. NAION is the most common cause of sudden optic nerve related vision loss in individuals over the age of 50[2]. The mechanism is believed to be a compartment syndrome that results in intraneural edema, and causes compression of the capillaries supplying the axons within the optic nerve[3].

Since the ON is actually a central nervous system (CNS) tract, the rodent NAION (rNAION) model can be used to study the mechanisms and responses to isolated CNS white matter strokes. The rNAION model may therefore be useful in dissecting many problems associated with stroke related damage to white matter. It can be used to evaluate different neuroprotective strategies and agents in white matter stroke.

One of the most attractive features of the model is that it is a painless, noninvasive procedure. The laser power can be adjusted to produce various degree of ischemic damage. Another feature is that it relies on laser induced superoxide radicals to damage the capillary endothelium, producing a progressive capillary dysfunction. It is this dysfunction and progressive edema that is believed to be remarkably similar to the mechanism that causes NAION. Research has shown that it does not cause direct capillary clotting, but works through at least two mechanisms: superoxide induced death and stripping of some of the capillary endothelial cells[4], and NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) associated inflammatory up regulation in remaining endothelium, with increased fluid transport across the cell membranes into the interstitium[5] . The closure of the optic nerve capillaries and compression caused by interstitial fluid accumulation result in optic nerve head ischemia. A schematic picture is shown in **Figure 2**. The rNAION model can be used in both rat and mouse species[6,7], and can be varied in the level of its severity, from a mild lesion to a complete, but painless destruction of the optic nerve and the retina, such as central retinal artery occlusion(CRAO).

Protocol

This protocol was approved by the University of Maryland Institutional Animal Care and Utilization Committee (IACUC; Baltimore, MD, USA)

1. Experimental Set-up

1. Make a custom designed contact lens from a clear optical grade circular 7 mm in diameter Plexiglas, of 3 mm thickness. Cut the circular lenses with a drill press. Use a standard drill bit to make the inner curve, and finally polish the outer and inner curves using a contact lens polisher of ultrafine grit (1000/3000).

2. Prepare 2.5 mM Rose Bengal (RB) in pH 7.4 phosphate buffer saline (PBS) in advance, filter sterilize with a 0.45 micron syringe filter and store 1 ml aliquots in -20 °C in a light-tight container for up to 6 months.
   NOTE: The use of albino outbred male animals, such as Sprague Dawley minimizes the strain dependent response differences, enables increased ease of induction and reduces the variability that can occur with estrus cycling female.
3. Set up the frequency doubled Neodymium-yttrium aluminum garnet (Fd-YAG) ophthalmic medical laser, which generates a 532 nm laser light. Mount the laser on a Haag-Streit ophthalmic slit lamp using a standard ophthalmic laser adapter. This commercially available device enables simultaneous visualization of the animal's eye and laser spot application. The medical laser also has an aiming beam for proper focusing and centration using same spot size as laser induction. Laser power parameters are as follows:
   1. For rat rNAION induction: use 500 µm spot size/50 mW laser power/1,000 msec duration/1,000 msec interval.
   2. For mouse rNAION induction: change the spot size to 300 µm for smaller optic disc and leave other parameters the same as the rat setting.
   3. Routinely use a laser power meter to assure the laser power output.

2. Experimental Procedure

1. Turn on the laser power and set up the appropriate laser parameter. Warm the laser for at least five minutes before use.
2. Weigh the animal to determine the appropriate dose for ketamine/xylazine and RB dye. Anesthetize the animal by intraperitoneal injection of a 1 ml/kg mixture of 80 mg/ml ketamine and 4 mg/ml xylazine.
3. Leave the animal in a heated cage until fully anesthetized. Check for no response to aversive stimuli (tail or toe pinch). Check animals for depth of anesthesia every 10 min.
4. Dilate the animal's pupils with 1% tropicamide and anesthetize the surface of the eye with 0.5% proparacaine. If using pigmented animals, such as Long Evans, a 2.5% neosynephrine ocular drops will increase pupil dilation.
5. Use scissors to cut the whiskers close to the muzzle on the side to be induced to avoid blocking the view.
6. Put a drop of 1% methylcellulose or other ophthalmic coupling drop to the inside of the custom-made contact lens, and then apply the lens onto the rat eye.
7. Place the animal on a platform adjusted to the height of the slit lamp. Set the animal's head at a 45° angle so that the eye is perpendicular to the slit lamp and laser beam.
8. Visualize the eye through the ophthalmic slit lamp. Make sure the aiming beam is the right size as well as focused and centered directly on the visualized optic nerve. Photograph the retinal fundus using a digital camera with high ASA (1,200 - 2,000) speed mounted on one of the eye pieces of the slit lamp with a custom made adapter.
   NOTE: The ability to see the choroidal vessels reveals the transparency of the retina. This is an important sign in order to be able to detect later retinal ischemia, which can confound the interpretation if it occurs. rNAION is optic nerve ischemia, which results in isolated RGC loss, while retinal ischemia results in retinal damage affecting all of the cells of the inner retinal layers.
9. Optionally, further image the retina and optic nerve using a spectral domain-optical coherence tomograph (SD-OCT[8]) to evaluate the un-induced eye. Scan *en face* (**Figure 3B**) as well as 7 cross-sectional scans through the retina (**Figure 3C**). The SD-OCT imaging uses the same contact lens used for laser induction.
10. Inject 1 ml/kg RB intravenously through tail vein, and wait for 30 sec, then activate the laser power. This time delay enables the RB to distribute evenly throughout the circulation. We use a 50 mW laser pulse at one sec/pulse. Greater energies (≥ 60 mW) can damage the retina or cause retinal vascular ischemia.
    Caution: Make sure everyone has a pair of laser safety filtering glasses of the appropriate blocking wavelength to prevent stray laser light entering investigator's eye.
    NOTE: The laser administration must be given rapidly after the IV RB injection since the dye is eliminated quickly from the circulation. The longer the laser induction, the more severe the optic nerve ischemia. Generally, animals are given 7 - 12 sec pulses in a rapid succession. The contact of the laser light with the circulating dye gives the optic nerve vessels a beautiful golden glow that can be seen through the slit lamp (**Figure 4B**). This proves the dye was injected systemically and distributed into the blood stream. If the glow is faint, or none at all (**Figure 4A**), the dye was not injected intravenously. In this case, do not give a second injection immediately, since the animal will need to recover for at least two days before reinjection.
11. Immediately after induction, remove the contact lens. Cover both eyes with ophthalmic triple antibiotic (Neosporin/polymyxin/bacitracin) ointment with dexamethasone, and place the animal on a 37 °C warming pad in a single housed cage under close observation until full recovery.
    1. Clean the contact lens with distilled water and wipe dry with a non-abrasive cleaning cloth for future use.
    2. Two days after the induction evaluate the optic nerve edema by both fundus color photography and SD-OCT analysis[8].
       NOTE: Comparing the degree of optic nerve edema gives an estimate of the severity of the optic nerve ischemia. At two days, the optic nerve disk margin is blurred, and the retinal veins are slightly dilated, compared with the contralateral (un-induced) eye. Perform electroretinography (ERG) and flash visual evoked potential (VEP[11]) at two and four weeks post induction for electrophysiology analysis.

Representative Results

The contact lens enabled central retina visualization (**Figure 1**). The focal laser spot illuminates the optic disc at the back of the retina (**Figure 2**). The normal un-induced retina is shown imaged by slit lamp bio-microscope (**Figure 3A**) and by SD-OCT (**Figure 3B and 3C**). During laser induction, when no dye is present in the circulation, laser light does not result in vessel and disk fluorescence (**Figure 4A**). Intravenous RB and the laser light illumination on the optic disk results in a golden color fluorescence on the optic nerve (**Figure 4B**). Two days following rNAION induction, the optic nerve is swollen (**Figure 5A**). *En face* and cross-sectional SD-OCT reveal disk swelling (**Figure 5B**) and expansion of the optic nerve (**Figure 5C**).

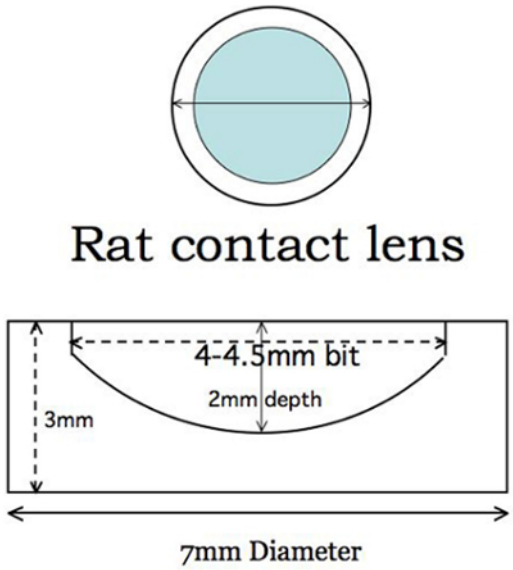

Figure 1. Schematic of the Rat Planoconcave Contact Lens. The custom made contact lens is made from 3 mm Plexiglas (design shown), with outer diameter 7 mm, inner diameter 5 mm. This lens is designed to fit over the cornea of the rat eye, and enable direct visualization of the retina. Please click here to view a larger version of this figure.

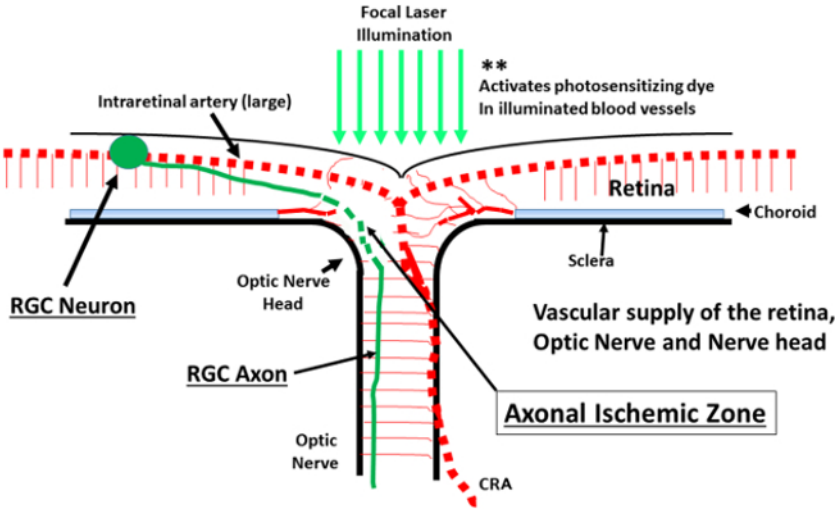

Figure 2. Schematic of rNAION Model. Longitudinal cross section through the back of the eye. The laser spot is centered on the optic disc (green arrows). The green cell and axon represents the retinal ganglion cell neuron. Immediately following intravenous RB administration, the RB circulates through the vasculature in the back of the eye and optic nerve. The laser beam is used to illuminate the disc for 7 - 12 sec depending on the ischemic severity desired. The laser activates the photosensitizing RB to generate superoxide radicals, which causes capillary closure in the anterior ON head (axonal ischemia; seen on the left side of the nerve as loss of small red lines) while sparing the larger intraretinal vessels emerging from the ON head into the eye. The focal ischemia produces localized axonal dysfunction (detached green line). Please click here to view a larger version of this figure.

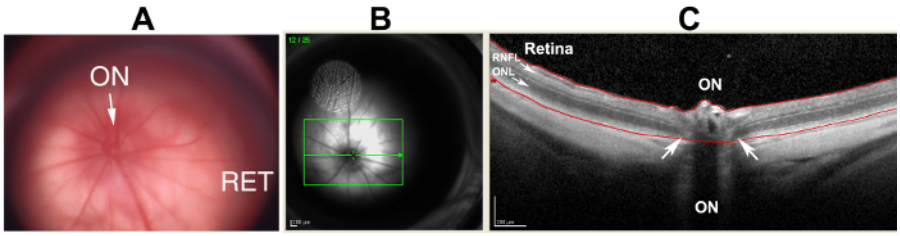

Figure 3. Baseline Normal Retina (RET) and Optic Nerve (ON) Analysis using Color Photography and SD-OCT. A. Picture of a normal Sprague-Dawley rat retinal fundus by slit lamp fundus photography. The retinal vessels emerge from the optic disc to supply the inner layers of the retina. The disc has a clear border and a reddish surrounding hue (choroidal flush). The retinal vessels are thin and evenly distributed. The optic nerve disk margin is usually well demarcated before rNAION induction. **B.** Picture of a normal fundus by *en face* SD-OCT imaging. Individual cross sectional images of the retina shown in panel C are generated from within the green box. Scan direction is shown with a green arrow. **C.** Single SD-OCT cross section scan of normal retina and optic disc showing the retinal layers. The retinal nerve fiber layer (RNFL, small white arrow) has a greyish look, but is lighter than the underlying outer nuclear layer (ONL, small white arrow). The RNFL is flat against the optic nerve. The optic nerve shadow is narrow (indicated by two arrows). ON: optic nerve. RNFL: retinal ganglion cell/nerve fiber layer. ONL: Outer nuclear layer. Scale bar: 200 µm. Please click here to view a larger version of this figure.

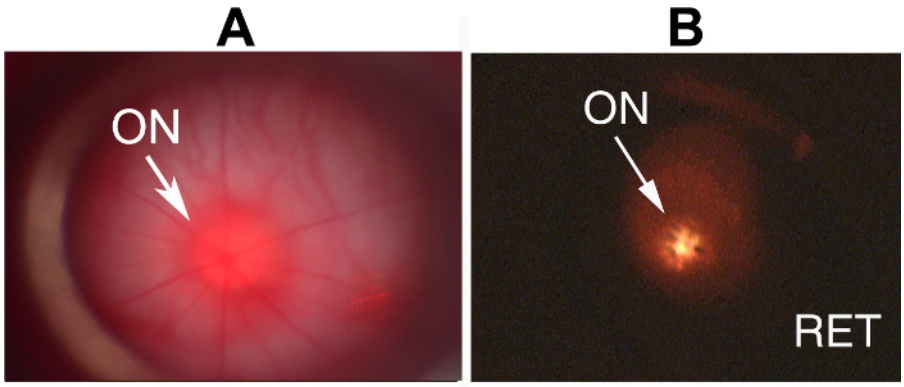

Figure 4. rNAION Induction Appearance. A. 532 nm/500 µm spot laser illumination of the optic nerve disc and retina without systemic RB injection. The optic nerve disc and retina are dark. **B.** 532 nm laser illumination 30 sec following systemic RB injection. The ON shows a golden glow within the vessels emerging from the disk at the laser spot site, indicating systemic RB in the blood stream illuminated by the green laser light. Please click here to view a larger version of this figure.

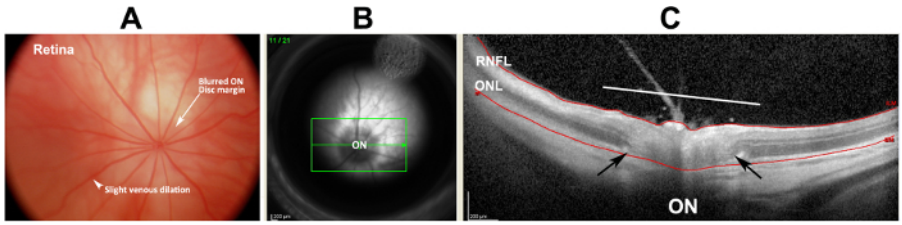

Figure 5. Retinal and Optic Nerve Analysis 2 days after rNAION Induction. A. Color fundus picture shows ON swelling and optic disc pallor, with loss of the surrounding choroidal flush. The veins are usually enlarged and curved, sometimes with boxed (interrupted flow) veins. **B.** *En face* SD-OCT picture post-induction shows disc edema and venous dilation. Individual cross section is generated from within the green box. Scan direction is shown with a green arrow. **C.** Cross section shows ON disk edema, as evidenced by increased thickness of the RNFL and reduced grey intensity (more white, consistent with higher water content). The diameter of the intra-retinal ON (indicated between the black arrows) is increased. Please click here to view a larger version of this figure.

Discussion

While there are a number of models of optic nerve damage (optic nerve crush[12], optic nerve transection[13], and PION[14]), the rNAION model is humane, adaptable to both rats and mice. It more closely resembles the human clinical condition of NAION. This condition includes progressive anterior optic nerve edema, an anterior optic nerve compartment syndrome, focal axonal ischemia, isolated retinal ganglion cell axonal damage and loss over a prolonged time course. The current report gives the appropriate steps for rNAION induction, discusses potential problems during induction, and described early post induction analyses that can be used to evaluate the quality of the induced lesion prior to data inclusion. The advantage of the rNAION model is that with practice, relatively consistent levels of damage can be achieved. Typically 10 - 11 sec exposure results in 40 - 65% RGC loss. The success rate can vary from individual to individual, depending on experience and skill, but an experienced investigator can achieve nearly a 100% induction rate.

In addition to its ease of induction, the severity and time course of the lesion can be easily monitored. An important early part of the quality control in the rNAION model, which can add to model advantages, is early post induction analysis. We normally evaluate the animals two days after the induction, when the optic nerve edema is maximal. The rNAION is characterized by optic nerve edema that resolves over a five day

period (roughly 5 times faster than that which occurs in humans), followed by optic nerve pallor and isolated retinal ganglion cell loss. One can identify retinal ischemia (as distinct from optic nerve ischemia) by loss of retinal transparency and whitening of the retina. Retinal ischemia is functionally confirmed by loss of inner retinal signal with electroretinography (ERG). If there is widespread loss of retinal transparency and whitening of the retina, it suggests diffuse retinal ischemia, which is consistent with central retinal vein occlusion, and not rNAION alone. If the induction results in severe retinal ischemia in a number of animals (characterized by sectional or total retinal whitening), the induction parameters can be reduced by one or two seconds exposure time. This way one can optimize both the degree of optic nerve damage, and get consistency of induction in animals. Animals with significant loss of ERG signal should be eliminated from the study. Isolated loss of optic nerve function can be confirmed by flash visual evoked potential measurement (VEP).

There are a number of variables to be kept in mind when using the rNAION model. Individual animals can differ in the overall severity of the lesion, so that multiple animals need to be used in the neuroprotective assays, and a power analysis should be performed to determine the minimum number of animals needed to achieve a statistically valid results, particularly when a more modest protective effect is seen or predicted. We have found that 10 - 15 animals are required for determining a 25% RGC protective effect in rats, and 15 - 20 animals when mice are used. Since the RB dye used in the induction is rapidly eliminated, once the animal is injected, variability can also be dependent on the speed of the time required to perform the induction. Slight differences on the focus on the optic disc, differences in the angle of laser illumination, and a difference in the speed of induction can also affect the outcome. One dedicated individual should be selected to perform the technique in each lab, to further reduce variability. Roughly 10 - 15% of induced animals may need to be eliminated after early post-induction evaluation due to severity of induction (central or branch retinal vein occlusion). This report does not discuss myriad other intrinsic variables that may influence outcome, such as from sex differences, circadian rhythm, and age or strain differences. These questions must be satisfied by the individual investigator. There are other modified parameter settings recently reported, such as 80 mW laser power[15]. These modifications used different contact lens or laser wavelength but resulted in similar outcomes.

It is important to realize that despite the similarities of rNAION to many aspects of clinical NAION, rNAION is a model, and no model is a perfect duplicate of a human disease, since the actual causative factors in NAION are unknown, and the vascular and inflammatory physiological control of the rodent retina and optic nerve are different in many ways from the human and nonhuman primate. Findings generated by the model need to be interpreted in light of these differences. Regardless of this, the rNAION model is a valuable method of rapidly dissecting many of the potential pathophysiological mechanisms responsible for visual loss and approaches to neuroprotection in a living mammalian system.

Disclosures

Authors have nothing to disclose.

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References

1. Banik, R. Nonarteritic Anterior Ischemic Optic Neuropathy: An Update on Demographics, Clinical Presentation, Pathophysiology, Animal Models, Prognosis, and Treatment. *J. Clin. Experimental Ophthalmol.* **10**, 1-5 (2013).
2. IONDT study group. Characteristics of patients with nonarteritic anterior ischemic optic neuropathy eligible for the Ischemic Optic Neuropathy Decompression Trial. *Arch Ophthalmol.* **114**, 1366-1374 (1996).
3. Tesser, R.A., Niendorf, E.R., Levin, L.A. The morphology of an infarct in nonarteritic anterior ischemic optic neuropathy. *Ophthalmology.* **110**, 2031-2035 (2003).
4. Bernstein, S.L., Johnson, M.A., Miller, N.R. Nonarteritic anterior ischemic optic neuropathy (NAION) and its experimental models. *Prog Retin Eye Res.* **30**, 167-187 (2011).
5. Nicholson, J.D., *et al.* PGJ2 Provides Prolonged CNS Stroke Protection by Reducing White Matter Edema. *PLoS One.* **7** (12) (2012).
6. Goldenberg-Cohen, N., *et al.* Oligodendrocyte Dysfunction Following Induction of Experimental Anterior Optic Nerve Ischemia. *Invest Ophthalmol Vis Sci.* **46**, 2716-2725 (2005).
7. Bernstein, S.L., Guo, Y, Kelman, S.E., Flower, R.W., Johnson, M.A. Functional and cellular responses in a novel rodent model of anterior ischemic optic neuropathy. *Invest Ophthalmol Vis Sci.* **44**, 4153-4162 (2003).
8. Berger, A., *et al.* Spectral-Domain Optical Coherence Tomography of the Rodent Eye: Highlighting Layers of the Outer Retina Using Signal Averaging and Comparison with Histology. *PLoS One.* **9** (5) (2014).
9. Huang, T.L., *et al.* Protective effects of systemic treatment with methylprednisolone in a rodent model of non-arteritic anterior ischemic optic neuropathy (rNAION). *Exp Eye Res.* **131**, 69-76 (2015).
10. Mantopoulos, D., *et al.* An Experimental Model of Optic Nerve Head Injury. *Invest Ophthalmol Vis Sci.* **57**, 6222 (2014).
11. You, Y., *et al.* Visual Evoked Potential Recording in a Rat Model of Experimental Optic Nerve Demyelination. *J. Vis. Exp.* (101), e52934 (2015).
12. Templeton, J.P., Geisert, E.E. A practical approach to optic nerve crush in the mouse. *Mol Vis.* **18**, 2147-2152 (2012).
13. Magharious, M. M., D'Onofrio, P. M., and Koeberle, P. D.. Optic Nerve Transection: A Model of Adult Neuron Apoptosis in the Central Nervous System **.** *J Vis Exp.* (51): e2241 (2011).
14. Wang, Y., *et al.* A Novel Rodent Model of Posterior Ischemic Optic Neuropathy. *JAMA Ophthalmol.* **131** (2), 194-204 (2013).

15. Huang, T.L., Chang, C.H., Chang S.W., Lin, K.H., Tsai, R.K. Efficacy of Intravitreal Injections of Antivascular Endothelial Growth Factor Agents in a Rat Model of Anterior Ischemic Optic Neuropathy. *IOVS April 2015.* **56**, 2290-2296 (2015).

