

# Morphological and functional changes of the optic nerve following traumatic optic nerve injuries in rabbits

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**Abstract.** The aim of the present study was to investigate the morphological changes of the optic nerve following traumatic injuries and decompression at different times after injury, and to observe the changes of the visually evoked potentials, to identify the relevant associations between surgical opportunity and the clinical effect of traumatic optic nerve injuries. Rabbits were chosen as the animal model for the study. All the rabbits were randomly divided into five groups (A-E), representing the normal control, decompression in 48 h, in 1 week, in 2 weeks and non-decompression groups, respectively. The pattern reversal visual evoked potentials (P-VEP) and morphological changes of the optic nerve were observed. The P-VEP of each healthy rabbit revealed typical NPN contours, while NPN waves in the injured rabbits were low and flat. The latent period of the P-wave was lengthened and the amplitude was reduced. The differences of the latent period and amplitude pre- and post-trauma were statistically significant. The morphological changes were also assessed. In the normal control group, the astrocytes of the optic nerve exhibited a cylindrical form and were arranged evenly on the vertical section. The neural fibers were arranged neatly, were even following application of a dye, and the cross section exhibited a normal configuration of the blood vessel. For the 48-h decompression group, the arrangement of the astrocytes was even on the vertical section, and vacuoles, slight swelling of the nerve, exudation around the blood vessel and a small amount of astrocytic hyperplasia were observed in the damaged area. In the non-decompression group there were large areas of necrosis, clear nerve demyelination, serious exudation around the blood vessel and astrocytic hyperplasia were observed. In conclusion, the optic nerve decompression is beneficial to protect the visual function in indirect optic nerve injuries. Visual function may be improved by decompression in 48 h compared to 2 weeks. In order to

prevent secondary axon injury and to protect visual functions, the decompression should be performed as soon as possible.

## Introduction

Traumatic optic nerve injury is one of the complications of head trauma, and is also the main cause of head trauma causing permanent vision loss. The optic nerve is located at the back of the eyes. Head or orbital trauma can cause optic canal fracture fragments, which may cut off or oppress the optic nerve. Optic nerve sheath membrane hemorrhage due to the fracture of the skull base can cause subdural or subarachnoid hemorrhage, and optic nerve avulsion can be caused by orbital trauma. All these can cause traumatic optic nerve injury (1). Visual evoked potential examination has an extremely important value for the diagnosis of traumatic optic neuropathy. Currently, it is a widely used clinical objective evaluation method for visual function following injury. In the present study, through an established animal model of indirect optic nerve injury, visual evoked potential and the morphological changes of the optic nerve tissue were observed following injury, as well as the decompression changes at different times of the trauma model, in order to study the mechanism of traumatic optic nerve injury and the association between the surgical time and curative effect, to offer a theoretical foundation for the selection of a suitable surgical time for clinical treatment of traumatic optic neuropathy.

## Materials and methods

**Experimental materials and groups.** A total of 30 healthy adult male New Zealand white rabbits, with a body weight of 2.0-3.0 kg, were provided by the Animal Experiment Center of Nanjing General Hospital of Nanjing Command (Nanjing, China). Upon examination, all rabbits had normal refractive media, equal and round pupils, and normal reaction to light, without fundus oculi abnormalities. All the rabbits were randomly divided into five groups (A-E), with 6 rabbits in each group, representing the normal control, 48-h decompression, 1-week decompression, 2-week decompression (1) and non-decompression groups, respectively. The left eye of each animal was selected for analysis.

**Establishment of the optic nerve crush injury model.** Rabbits were anesthetized by subcutaneous administration of 50 g/l ketamine (100 mg/kg). The Tenon's capsule was incised from

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the supraorbital rim, the superior and medial rectus were separated and clipped, and the optic foramen was separated and exposed along the temporal sclera. To block the optic nerve and lead to a crush injury of the optic nerve, a conical soft silica gel with a 2 mm diameter at the thin end was tucked in the optic hole. Two days after surgery, rabbits with pupils >4.5 mm, with disappearance of direct light reflex and no clear extruded eyeballs were regarded as success models. Optic nerve decompression surgery was carried out to remove the silica gel (2).

**Pattern reversal visual evoked potentials (P-VEP).** All the examinations were performed using a NeuroMax 1004 machine (Xltek, Oakville, ON, Canada) in a dark room at 18-25°C, with all ketamine rabbits anesthetized. Recording electrodes were placed ~5 cm away and back from the midpoint of the ears, the reference electrode was subcutaneously placed at the midpoint of the eyes, and the ground electrode was placed at the tip of the ipsilateral ear. The eyes were 30 cm away from the screen, to flip stimulate (alternate light and shade of the stimulus) the full-field monocular vision with an all wild checkerboard pattern, whilst the contralateral eye was masked. Stimulation parameters were as follows: The checkerboard was of size 30' (half light and half shade), with an average luminance 50 CD/m<sup>2</sup>, 90% contrast, and the flip frequency was 1.1 Hz. Stimulus-caused signals were filtered (with a passband of 0.5-100 Hz), amplified (2x10<sup>4</sup> times) and input onto a microcomputer for superposition. Superposition was for 128x, and analysis for 300 msec. The P-VEP waveforms of 1 h pre- and post-trauma, 1 h pre-decompression, and 2 weeks post-decompression were recorded, and the latency and amplitude of the main wave (P-wave) were determined, with each stimulus repeated ≥3x. For the normal group, a set of data were collected only as a control, and data of the non-decompression group were collected in accordance of the check time of the 2 weeks decompression group. The data and waveform were collected and statistical processed by a microcomputer.

The P-VEP observation indexes included the absolute incubation period and amplitude of the NPN contours main wave (P-wave). The absolute incubation period is the time from the beginning of stimulation to the peak of the P-wave, and the irregular P-wave shape can be measured from the intersection of an extension cord of the rise and fall branch of the P-wave peak. Absolute volatility is the range from a previous negative peak to the amplitude of the P-wave. The latent period and amplitude mentioned in the following are all absolute incubation period and amplitude.

**Optic nerve morphology.** All the experimental animals of the post-trauma decompression group were sacrificed 2 weeks after the decompression, and the optic nerves were fixed in 10% formaldehyde solution, stained with haematoxylin and eosin, and observed under a light microscope. The non-decompression group was sacrificed 4 weeks after trauma, with the same treatment as before. The optic nerve specimens in the normal control group were collected as the control. Morphological observations included swelling of the optic nerve, axonal degeneration, myelin depigmentation, hyperplasia of astrocytes, and vascular lesions within the optic nerve.

**Statistical analysis.** The data are presented as the mean ± standard deviation. All statistical analyses were performed using SPSS 10.0 (SPSS, Inc., Chicago, IL, USA). Independent sample t-test was used in self-comparison of pre- and post-decompression, and one-way analysis of variance was used to compare the P-VEP of multiple groups 2 weeks post-decompression. Differences between groups were compared using the least significant difference test when homoscedasticity was assumed. P<0.05 was considered to indicate a statistically significant difference.

## Results

**P-VEP changes.** The P-VEP of each healthy rabbit revealed typical NPN contours. The NPN contours in the injured rabbits were low and flat. The latent period of the P contour was lengthened and the amplitude was reduced (Tables I and II). The differences between the latent period and amplitude pre- and post-trauma were significant statistically (P<0.05). The comparisons between P-VEP pre- and post-decompression were as follows: Comparisons of the amplitude and latent periods prior and subsequent to decompression in the i) 48-h decompression group were significantly different (P<0.05), ii) in the 1-week decompression group exhibited no differences (P>0.05), and iii) comparisons of amplitude prior and subsequent to decompression in the 2-week decompression group exhibited no differences (P>0.05), while the latent period was significantly different (P<0.05).

The comparisons of P-VEP 2 weeks post-decompression were as follows: i) The differences of the latent period between two groups among B, C and D were significantly different (P<0.01), those of amplitude between groups B against C and D were significant (P<0.01), while those between groups C and D were significant (P<0.05). ii) The differences of the latent period and amplitude between groups B and C with group E were significant (P<0.01), while those between groups D and E exhibited no significance (P>0.05). iii) There was no significance in the differences of amplitude and latent period between groups A and B (P>0.05), while there were significant differences among group A and groups C-E (P<0.01).

**Optic nerve morphological changes.** In the normal control group, the astrocytes of the optic nerve exhibited a cylindrical form and were arranged evenly on vertical section (Fig. 1) the neural fibers were arranged neatly and were stained evenly, and the cross section showed a normal configuration of the blood vessel.

For the 48-h decompression group, the arrangement of the astrocytes was even on the vertical section, with vacuoles, slight swelling of the nerve, exudation around the blood vessel and a small amount of astrocytes hyperplasia observed in the damaged area (Fig. 2).

In the 1-week group, the arrangement of the astrocytes was inordinate on the vertical section. Numerous vacuoles of different sizes, demyelination of the nerve, uncovered axons, clear exudation around the blood vessel and evident astrocytes hyperplasia were observed in the majority of areas.

In the 2-week group, the cylindrical arrangement of the astrocytes disappeared completely on the vertical section.

Table I. Latent period of the P-wave in the different periods.

Groups	Pre-trauma, msec	1 h post-trauma, msec	1 h pre-decompression, msec	2 weeks post-decompression, msec
A	63.67±4.74	NA	NA	NA
B	62.03±8.79	71.77±7.96	86.47±14.28	71.25±8.51
C	65.91±12.78	80.58±9.38	94.72±7.13	106.43±11.79
D	63.50±7.69	74.02±6.14	116.35±17.13	158.73±15.16
E <sup>a</sup>	63.80±7.60	75.85±5.75	114.42±19.57	169.75±16.25

<sup>a</sup>Detection time of group E was the same as group D. NA, not applicable; A, normal control group; B, 48-h decompression group; C, 1-week decompression group; D, 2-week decompression group; E, non-decompression group.

Table II. Amplitude of the P-wave in the different periods.

Groups	Pre-trauma, μV	1 h post-trauma, μV	1 h pre-decompression, μV	2 weeks post-decompression, μV
A	5.35±1.17	NA	NA	NA
B	5.93±1.09	4.82±0.50	4.42±0.42	5.25±0.78
C	5.58±1.47	4.05±0.82	3.05±0.71	2.88±0.76
D	5.00±0.85	3.85±0.62	2.53±0.78	1.83±0.96
E <sup>a</sup>	5.31±0.83	4.20±0.81	2.60±0.92	1.58±0.63

<sup>a</sup>Detection time of group E was the same as group D. NA, not applicable; A, normal control group; B, 48-h decompression group; C, 1-week decompression group; D, 2-week decompression group; E, non-decompression group.

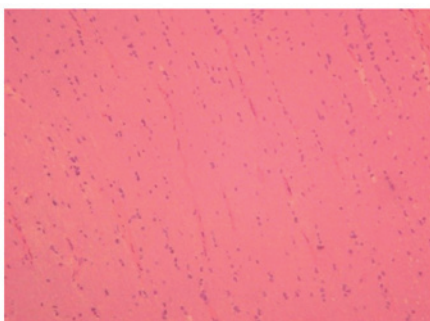


Figure 1. Normal optic nerve. The vertical section shows that astrocytes are arranged evenly, and the neural fibers arranged neatly and were evenly stained (haematoxylin and eosin; magnification, x100).

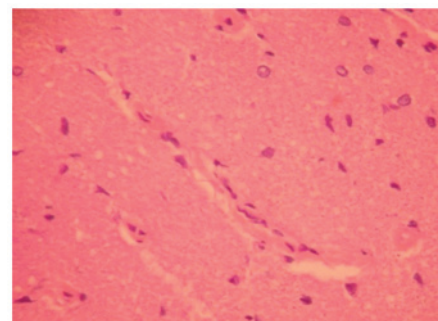


Figure 2. Optic nerve of the 48-h decompression group. The arrangement of the astrocytes was even on the vertical section, and vacuoles were observed in the damaged area (haematoxylin and eosin; magnification, x200).

Evident astrocytic hyperplasia filled the field of view, and clear nerve demyelination and local necrosis was observed, while exudation around the blood vessel was reduced, with inflammatory infiltration in some of the blood vessels (Fig. 3).

In the non-decompression group, large areas of necrosis, evident nerve demyelination, serious exudation around the blood vessel and astrocytic hyperplasia were observed.

## Discussion

Optic nerve injury is a type of severe head trauma combined injury, and numerous studies have established a series of optic nerve injury animal models, such as optic nerve transverse,

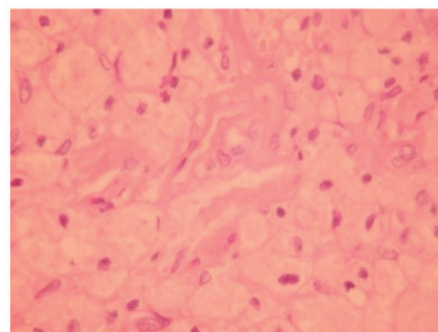


Figure 3. Optic nerve of the 2-week decompression group. The cylindrical arrangement of astrocytes disappeared completely and local necrosis was apparent on the vertical section (haematoxylin and eosin; magnification, x200).

tractive and crush injury (3-5); each has its advantages and disadvantages. Optic nerve transection injury is established by cutting off the optic nerve at any position prior to the optic chiasma, leading to all retinal optic ganglion cell axons to rupture completely, which can guarantee all experimental animals at the same degree of wounding and is convenient for a control study. The common characteristics of the optic nerve crush animal model are to crush the optic nerve at any position prior to optic chiasma with different instruments, to maintain the integrity of the epineurium. This model is simple, easy to operate, and can exactly establish the optic nerve injury with a high injury rate, as exposure of the optic nerve from the conjunctival approach results in lighter trauma to the experimental animals, with a low animal mortality, improved repeatability, and is similar to clinical practice, therefore, it is widely used. However, the injury degree is operator dependent, as the optic nerve damage is variable under different operators, and the damage degree is difficult to quantitate precisely. The optic nerve tractive injury animal model includes parallel and vertical optic canal axial traction. The former can exactly lead to diffuse axonal injury, the latter is similar to optic canal fracture caused by a cutting injury. The optic nerve crush injury animal model can imitate the optic nerve damage that extrudes the surrounding tissue of the orbital neoplasm and clinical indirect optic nerve injury. This simple surgery is easy to operate, can cause exact optic nerve damage in experimental animals with less trauma, and postoperative nursing and further observation is simple. All the aforementioned models have limitations on quantifying the injuries. In the present study, a circular cone soft silicone was adopted to block the optic nerve to establish an optic nerve crush injury, and the size of the smaller end and the depth of extrusion were controlled. The P-VEP and pathological results showed that the optic nerve crush injury was incomplete, and the injury degree was consistent.

Clinically, indirect optic nerve injury caused by head trauma is difficult to examine and evaluate. Visual electrophysiological examination is an objective visual function assessment method, and can clinically improve early diagnosis ability of indirect optic nerve injury (6,7). VEP can develop from visual information through the visual nerve system. At the later stages of visual formation are the mass responses to visual stimuli that are recorded from electrodes placed on the cerebral cortex, which can reflect the total functional status from the retinal ganglion cells to the visual cortex. VEP, mainly on behalf of the electrical activity complicated within the process of visual production in the 10-20° of the central vision, from the synapse, axon, optic nerve to the occipital visual cortex, can sensitively reflect the integrity and function of the neuron axon and myelin sheath in each optic nerve district. It has been shown that following all wild stimulation in one eye of the rabbit, the occurrence rate of the P-wave in the NPN contours recorded from the cortex was 100%, and the latent period was the most stable, with a higher amplitude and relatively variable N-wave (8). Therefore, in the present experiment, only the wave latency and amplitude of the P-wave were used for analysis. There are numerous types of visual evoked cortical potential. As a stable waveform with small individual variation, P-VEP is widely used to evaluate the optic nerve diseases. In optic nerve tractional injury, Bain *et al* (9)

reported that the electrophysiological changes began to appear when the optic nerve stretch to 5.5 mm, while the morphology change appeared following a stretch to 6.8 mm, suggesting that electrophysiological changes are more sensitive compared to morphology. Therefore, P-VEP was used as the main observation index in the present study.

Compared with the normal control group, the incubation period of the P-wave was evidently prolonged in the early stage of trauma, with a significantly lower amplitude, suggesting that visual function is not completely lost in the direct mechanical effect caused partly by axon injury. Subsequently, the incubation period continued to prolong, and the amplitude continued to reduce. Following direct injury, the blood circulation obstacle and tissue edema of the surrounding environment caused secondary axonal injury leading to the further decline in visual function. The 2-week post-decompression P-VEP results showed that when decompression was implemented 48-h after injury, the incubation period will be significantly shorter compared to pre-decompression, and can return to normal levels, with no evident difference to group A. When decompression occurs 1 or 2 weeks after injury, there were no evident improvements on the incubation period, and the incubation period of the 2-week post-decompression group was markedly prolonged compared to the 2-week pre-decompression. These results indicate that following optic nerve injury, early decompression can protect the visual function better than later decompression, and to a certain extent it can reverse the damage on visual function, as early decompression (1 week) can prevent axon secondary injury, avoiding further visual function decline, whereas decompression 2 weeks after is not ineffective. From 108 cases of clinical superciliary arch fractures, Matteini *et al* (1) concluded that the best surgical intervention must be carried out within 7 days, and <12 days, which is consistent with the present experimental results.

Swelling, myelinoclasia and axon changes were the main observations following indirect optic nerve injury. In the present experiment, due to the difference of decompression time after injury, the optic nerve morphology change was different at 2 weeks after decompression. In the 48-h decompression group, vacuoles, slight swelling of the nerve, exudation around the blood vessel and a small amount of astrocytes hyperplasia were observed in the damaged area, whereas the arrangement of astrocytes was even on the vertical section. As the decompression time prolonged, myelinoclasia aggravated gradually, accompanied by necrosis of the damaged area, and inordinate arrangement and evident hyperplasia of the astrocytes. The present results showed that as the decompression time prolonged, the optic nerve crush injury appeared to exhibit mild to severe local pathological changes, and evident myelinoclasia changes and astrocytes proliferation appeared in the non-decompression trauma group 4 weeks after injury. Certain studies (10,11) quantitatively analyzed retinal optic ganglion cells (RGCs) following optic nerve crush injury and reported that RGCs reduced rapidly in the first 2 weeks, reduce to ~50% at the end of 2 weeks, and decreased slowly at the latter 2 weeks. Thus, direct optic nerve damage can lead to part of the axon injury and induce a series of changes, such as RGC acute loss, impaired RGC release cytotoxic compounds, causing intracellular calcium overload, free radicals and

glutamic acid increase, followed by an intact axon or the original RGCs on the damage edge in axons suffered from secondary degeneration (12). Therefore, positive and effective measures following optic nerve injury can reduce and delay the secondary injury of RGCs, otherwise, the RGCs will eventually degenerate (13).

The pathogenesis of traumatic optic nerve injury remains to be elucidated. Maxwell *et al* (14) proposed that due to space limitations of the optic canal, progressive blood circulation disorder and hematoma of the indirect optic nerve injury may turn into a vicious cycle, which leads to further loss of the original recovery potential of the injured optic fiber. The optic nerve secondary damage theories are the theoretical basis of optic nerve decompression. There are controversies on the treatment of traumatic optic nerve injury, and the choice of clinical treatment is largely based on personal experience (15). Goldberg and Steinsapir (16) proposed that optic nerve decompression is beneficial to the recovery of indirect optic nerve injury regardless of whether there are optic canal fractures. Cook *et al* (17) retrospectively analyzed the treatment results of 244 patients reported in 46 studies, and identified that the treatment group recovered better than the no treatment group. No significant difference was apparent between the curative effect of surgical decompression, hormone therapy and surgery plus hormone treatment, and visual recovery was associated with the extent of the initial injury. Recovery of patients without fractures was better than with fractures, and anterior ethmoid fracture was better than posterior ethmoid fracture.

The present study established a rabbit optic nerve crush injury animal model, and systematically observed visual function changes following optic nerve injury in the different periods after decompression. Neuron secondary injury is one of the main reasons for the progressive decline of the optic nerve function, and therefore, protecting neurons without primary damage from secondary damage is an important aspect of optic nerve injury research. Optic nerve decompression can reduce the indirect optic nerve injury, and early decompression following optic nerve injury can better protect the visual function compared to later decompression. To a certain extent, this can avoid further decline of visual function and reverse optic nerve function damage (18).

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