A standardized method to create peripheral nerve injury in dogs using an automatic non-serrated forceps

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Abstract
This study describes a method that not only generates an automatic and standardized crush injury in the skull base, but also provides investigators with the option to choose from a range of varying pressure levels. We designed an automatic, non-serrated forceps that exerts a varying force of 0 to 100 g and lasts for a defined period of 0 to 60 seconds. This device was then used to generate a crush injury to the right oculomotor nerve of dogs with a force of 10 g for 15 seconds, resulting in a deficit in the pupil-light reflex and ptosis. Further testing of our model with Toluidine-blue staining demonstrated that, at 2 weeks post-surgery disordered oculomotor nerve fibers, axonal loss, and a thinner than normal myelin sheath were visible. Electrophysiological examination showed occasional spontaneous potentials. Together, these data verified that the model for oculomotor nerve injury was successful, and that the forceps we designed can be used to establish standard mechanical injury models of peripheral nerves.

Key Words
oculomotor nerve; forceps; instrument; nerve injury; model; quantitation; cranial nerve; peripheral nerve; neural regeneration

Research Highlights
(1) A method to create oculomotor nerve injury was reported.
(2) A forceps that can be quantitated (0–100 g) and timed (0–60 seconds) was designed.
(3) Quantitative injury of dog oculomotor nerve verified that the designed forceps can be used to establish mechanical injury models of peripheral nerves.

INTRODUCTION
The oculomotor nerve of dogs is a very vulnerable structure. Trauma to the oculomotor nerve can be caused by either fractures of the skull base or operations to treat cavernous sinus tumors1). Recently, scientists and doctors have begun to explore the functional recovery and regeneration of injured oculomotor nerves2-3). We think that standardization of the compression methods used to damage peripheral nerves is necessary to ensure accurate comparisons across studies that investigate the regeneration of injured cranial nerves. However, because access to the oculomotor nerve is difficult due to its depth and narrowness of the skull base, investigators cannot readily create a crush injury without damaging other cranial nerves and brain areas. There are several methods used to
induce peripheral nerve crush injuries\cite{4-5}, including historical methods such as ligatures of suture or stainless steel, tight-fitting arteries, metal spring clips, compression clamps, and tubes of silicone and polyethylene\cite{6-8}. Although these models are widely used in studies of peripheral nerve regeneration, the main drawback facing most of these methods is that the injury cannot be quantified at the site of the trauma\cite{7, 9-10}. Recently, several investigators have attempted to create a standardized method to cause crush injuries to optic nerves, but in multiple cases, a preset pressure of either 0.6 N or 1.82 N was required for 10 seconds\cite{11-12}. Owing to differences in pressure-application method and non-comparable pressure levels, comparing results obtained by different investigators is often difficult\cite{10, 13}. This study describes a standardized method that not only generates an automatic and standardized crush injury in the skull base, but also provides investigators with option to choose from a range of varying pressure levels.

RESULTS

Quantitative analysis of experimental animals
Five dogs (beagles) initially included entered the final analysis.

General condition of animals following oculomotor nerve injury
All procedures used were well tolerated by the dogs, and did not result in either lasting wounds or infection. The oculomotor nerves were well exposed. Immediately after acute compression injury, complete flaccid paralysis of the oculomotor nerve was observed in all animals following the procedure, including a significant increase in pupil diameter, abolition of the light reflex, ptosis of the involved eyeball, and infraduction. No dog exhibited any recovery of eyeball movement, pupil diameter, or light reflex within 2 weeks after surgery.

Histological changes in the injured oculomotor nerve
Two weeks after surgery, disordered oculomotor nerve fibers, and thin myelin sheaths were visible. However, there were still a few normal axons (Figure 1). No significant difference in axonal number was observed between dogs.

Neuro-electrophysiological changes in the injured oculomotor nerve
Spontaneous electromyograms of the interior oblique muscle were at isoelectric levels, and spontaneous potentials only occasionally appeared at 2 weeks postoperatively (Figure 2). No significant electrophysiological difference was seen between dogs, including spontaneous electromyogram amplitudes and action potential duration.

DISCUSSION

Oculomotor nerve dysfunctions are usually associated with severe head trauma or operations on the base of the skull\cite{1}. As a result of direct or indirect damage to the third cranial nerve, ptosis of the involved eyeball and infraduction are frequently observed. Therapy and outcome after oculomotor nerve injury are still very poor\cite{1-2}. Previously, we developed an animal model for electrophysiological research by completely transecting and reconstructing oculomotor nerves anatomically\cite{3, 14}. Developing an animal model to investigate oculomotor-nerve crush injury is critical. Many methods have been developed and adapted to assess sensory-motor recovery in rat models of sciatic- and optic-nerve crush injury\cite{15-18}. However, without a standard method, the unavoidable variation by investigators from all over the world creates a significant disadvantage\cite{19-20}. Additionally, many of the methods used to induce injury are also difficult to perform and
maintain in the limited space of skull base. This study described a simple and automatic device, the experimental standardized forceps, to create oculomotor nerve injury. The most important advantage of this device is that it can be used for generating graded compression injuries. We used the forceps to create 10 g compressions in dog oculomotor nerves for 15 seconds, and both histological examinations and electrophysiology revealed that the crush injury was precise and reproducible, thus making it satisfying the conditions of standardization.

Additionally, comparing results of nerve compression-injuries obtained by different investigators is difficult due to differences in method of pressure application and comparable pressure levels\cite{21-23}. In the present study, we described a new method to crush the oculomotor nerve by using a specially designed and automatically injured forceps. We have created a new standardized forceps and developed an oculomotor nerve-compression model using beagles. The forceps is precise, standardized, and automatic. With precise electronic control, a fixed pressure can be preset and applied to the nerve trunk for a variable time of 0–60 seconds according to the needs of the researcher.

Here, we demonstrated a device that can be used for standardization. Research for a practical, reproducible, and sensitive test to analyze recovery of function after a peripheral nerve lesion is critical because it would allow quantification of the injury to the nerve on which the functional reinnervation of target organs relies.

We have successfully established a standardized, automatic, precise nerve crush model in dogs. It can be used to investigate peripheral nerve axonal degeneration and regeneration.

MATERIALS AND METHODS

Design
A study of establishing animal models of peripheral nerve injury.

Time and setting
This experiment was performed at the Experimental Center, Shanghai Jiao Tong University School of Medicine, China, from August 2007 to April 2009.

Materials
All surgical procedures were performed in five 24-month-old adult female beagles weighing 9.8–10.3 kg (Permit No. SCXK (Hu) 2007-0004), obtained from School of Medicine, Shanghai Jiao Tong University, China. Animals were handled and cared for in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, issued by the Ministry of Science and Technology of China\cite{24}.

Methods

**Design and manufacture of the experimental standardized forceps**

The standardized forceps used in the present study to cause oculomotor nerve crush injuries were reconstructed from a pair of bipolar coagulation forceps (Shanghai Medical Instruments (Group) Ltd., Corp. Surgical Instruments Factory, Shanghai, China). A pair of bipolar coagulation forceps can be used as an instrument for resection and coagulation in the skull base. The thin and long forceps arms can be easily manipulated in a deep and narrow space. Because the tip of bipolar forceps is fine-alloy material and the inner surfaces of the forceps tip is smooth, protective, and non-serrated, it was used to crush oculomotor nerves in our study.

Based on the lever principle, and combined with automatic control theory and sensor techniques, the standardized oculomotor nerve injury forceps included three components: entity, dynamic, and automatic controlling facets.

**Entity portion**

After the right arm of the forceps was cut off 17 cm away from the tip, and the proximal and distal ends were connected through a metal hinge, a simple and flexible structural joint was made (Figure 3). Then, a micropressure Honeywell sensor (Honeywell, Shanghai, China) was placed on the surface 1.7 cm lateral to the joint. The instrument was fixed to the opposite arm of the forceps through a metal frame and was equipped with a trapezoidal chock and a hydraulic proximal piston (self-made; supplementary Figure 1 online). The distal end of the piston was fixed closely to the ipsilateral arm laterally. The piston and trapezoidal chock were connected via damping factors resulting from interactive motion. The amount of tension in the piston can be varied by adjusting the amount of hydraulic energy, resulting in changes of resistance in the trapezoidal chock and the sensor, and then ultimately at the tip of the forceps.

**Dynamic portion**

Control of kinetic energy was technically difficult, but achieved in the following manner. A micromotor
equipped with a decelerating device (Shanghai Micromotor Factory, Shanghai, China) was integrated into the belt-driving system (self-made). This system can drive a nut, and then make screwing motion (Figure 4). Resulting tension then drives another piston, and the hydraulic pressure energy in the drive system is returned to the original level. Based on the lever principle, this is a way to realize the energy transfer process. Eventually, tension occurring at the tip of the forceps made it possible to crush the oculomotor nerve.

**Automatic controlling portion**
Internal circuitry allowed the forceps to carry out automatically the steps required to generate the oculomotor nerve crush injuries. The Honeywell sensor exhibits good sensitivity to pressure measured at the output port of the sensor. A dynamic compensation unit (self-made) was connected to this output port in series for improving the characteristics and compensating for dynamic measurement error of the sensors.

Calibration of the forceps was carried out using different quality of weights under constant voltage direct-current. In this manner, the precise relationship between the output values of the sensor and the different forces could be obtained. The output signal from the sensor was then used as input to an amplification system, allowing us to set a comparative value to the amplifier circuit. We were then able to control precisely the amount of force we wished to apply to the nerve by adjusting this comparative value. Additionally, we included a component that controls the duration of the compression (0–60 seconds) that assures an equal amount of force will be applied for each separate crush lesion.

**Establishment of oculomotor nerve injury model**
General anesthesia was performed by intramuscular injection of a mixture of ketamine (10 mg/kg, Jiangsu Hengrui Medicine, Jiangsu Province, China), neotachosleep (0.1 mL/kg; Institute of Veterinary Medicine of China Academy of Military Medical Sciences, Jilin Province, China), diazepam (1 mg/kg, Shanghai Xudonghaipu Pharmaceuticals, Shanghai, China) and atropine (0.05 mg/kg, Shanghai Hefeng Pharmaceuticals, Shanghai, China). Atropine was administered to inhibit saliva secretion. Maintenance of anesthesia for approximately 5–6 hours was maintained by periodic intramuscular administration of a lower dosage of the same anesthetic mixture as needed during the operation.

Improved Pterional Approach was adapted\(^ {25} \), and the posterior segments of the right oculomotor nerve in the cavernous sinus were exposed in all Beagle dogs after craniotomy (Figures 5, 6). For inducing the crush injuries, the entire oculomotor nerve was crushed with the standardized forceps by applying 10 g of pressure to the nerve trunk for 15 seconds. The crush lesion was made with the microsurgical forceps about 5 mm distal to the midbrain. Care was taken to ensure that the crush did not compromise the peripheral tissue. After the crush, the wound was sutured. Then, antibiotic was applied, and the animals were allowed to recover from the surgery.
General, histological and electrophysiological observation
The animals were carefully observed during the whole study period for eyeball movement, pupil diameter and light reflex. Additionally, the oculomotor nerve trunk at the posterior segment of the cavernous sinus was stained with toluidine blue (Amresco, Solon, Ohio, USA). Variation in axons was determined by histological examination using light microscope (Olympus, Tokyo, Japan) and spontaneous electromyographic activity was recorded intramuscularly (Powerlab system, AD Instruments Pty Ltd., Castle Hill, Australia) from the right interior oblique muscle at 2 weeks post-surgery.

Author contributions: Xuhui Wang wrote the paper. Liang Wan participated in forceps design and manufacture. Xuhui Wang and Xinyuan Li established animal models. Youqiang Meng participated in electromyogram monitoring. Ningxi Zhu and Baohui Feng performed histological slicing and analysis. Min Yang performed statistical analysis. Wenchuan Zhang drafted and validated the manuscript. Shugan Zhu advised the study. Shiting Li was in charge of funding, and acted as the study instructor.

Conflicts of interest: None declared.

Ethical approval: The experiment was given full approval from the Animal Ethics Committee of Shanghai Jiao Tong University in China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application disputations.

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REFERENCES

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