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# Screen-imaging guidance using a modified portable video microscope for middle cerebral artery occlusion<sup>☆</sup>

Xingbao Zhu<sup>1</sup>, Junli Luo<sup>1</sup>, Yun Liu<sup>1</sup>, Guolong Chen<sup>1</sup>, Song Liu<sup>1</sup>, Qiangjin Ruan<sup>1</sup>, Xunding Deng<sup>1</sup>, Dianchun Wang<sup>1</sup>, Quanshui Fan<sup>2</sup>, Xinghua Pan<sup>3</sup>

<sup>1</sup>Department of Neurosurgery, Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, Kunming 650032, Yunnan Province, China

<sup>2</sup>Disease Prevention & Control Centre, Chengdu Military Area Command of Chinese PLA, Kunming 650032, Yunnan Province, China

<sup>3</sup>Animal Experimental Centre, Department of Clinical Research, Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, Kunming 650032, Yunnan Province, China

## Abstract

The use of operating microscopes is limited by the focal length. Surgeons using these instruments cannot simultaneously view and access the surgical field and must choose one or the other. The longer focal length (more than 1 000 mm) of an operating telescope permits a position away from the operating field, above the surgeon and out of the field of view. This gives the telescope an advantage over an operating microscope. We developed a telescopic system using screen-imaging guidance and a modified portable video microscope constructed from a Computar MLH-10 × macro lens, a DFK-21AU04 USB CCD Camera and a Dell laptop computer as monitor screen. This system was used to establish a middle cerebral artery occlusion model in rats. Results showed that magnification of the modified portable video microscope was appropriate (5–20 ×) even though the Computar MLH-10 × macro lens was placed 800 mm away from the operating field rather than at the specified working distance of 152.4 mm with a zoom of 1–40 ×. The screen-imaging telescopic technique was clear, life-like, stereoscopic and matched the actual operation. Screen-imaging guidance led to an accurate, smooth, minimally invasive and comparatively easy surgical procedure. Success rate of the model establishment evaluated by neurological function using the modified neurological score system was 74.07%. There was no significant difference in model establishment time, sensorimotor deficit and infarct volume percentage. Our findings indicate that the telescopic lens is effective in the screen surgical operation mode referred to as “long distance observation and short distance operation” and that screen-imaging guidance using an modified portable video microscope can be utilized for the establishment of a middle cerebral artery occlusion model and micro-neurosurgery.

**Key Words:** portable video microscope; screen-imaging guidance; telescopic surgery; middle cerebral artery occlusion; cerebral infarction

**Abbreviations:** MCAO, middle cerebral artery occlusion; pvMa, portable video microscope

Xingbao Zhu<sup>☆</sup>, M.D., Ph.D., Associate chief physician, Associate professor, Department of Neurosurgery, Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, Kunming 650032, Yunnan Province, China

Corresponding author: Xingbao Zhu, Department of Neurosurgery, Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, Kunming 650032, Yunnan Province, China  
abcp66@gmail.com

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## INTRODUCTION

Middle cerebral artery occlusion (MCAO) in rats has been achieved using a number of methods<sup>[1-5]</sup>. Among them, the intraluminal suture model, first introduced by Koizumi *et al*<sup>[4]</sup> and later modified by Longa *et al*<sup>[5]</sup>, has become the most widely used model to study pathophysiology and therapeutic approaches in permanent and transient focal cerebral ischemia. In this model, a monofilament is advanced into the internal carotid artery until its tip is lodged in the anterior cerebral artery, blocking blood flow to the middle cerebral artery. This is a microsurgical technique, usually achieved with the assistance of an operating microscope. The focal length of the operating microscope is short (200–400 mm),

necessitating its placement near the operating field and in front of the surgeons.

This impedes observation and surgical manipulation. In contrast, the focal length of the telescope is longer (more than 1 000 mm) permitting a position away from the operating field, above the surgeons and out of the field of view.

The lens image is transformed onto the screen by digital video recording technology so there are two ways of observing anatomy: looking through the optic lens or at the monitor screen<sup>[6-21]</sup>. The two modes are “lens surgical operation mode” and “screen surgical operation mode”, respectively. In lens surgical operation mode, both observation and performance of the micrological surgery are “short distance work”, done solely via the microscope since observation through the optic lens is limited

by the focal length. Due to the short focal length, surgeons cannot view and access the surgical field simultaneously and must choose one or the other. In screen surgical operation mode, the micrological surgery is referred to as “long distance observation and short distance operation”. Therefore, any optical lens is effective since observation on the monitor screen is not limited by the focal length. The use of telescopic surgery should increase as digital video recording technology makes the screen surgical operation mode more available<sup>[6-21]</sup>.

The aim of this study was to develop and evaluate screen-based telescopic surgery for an intraluminal suture model of MCAO in rats. To do so, a portable video microscope (pvMa) was modified to a video near-viewing monocular telescope.

## RESULTS

### Quantitative analysis of experimental animals

A total of 27 rats were involved in the study. Intraluminal suture models of MCAO were created using screen-operative surgery with a modified pvMa. The MCAO model was successful in twenty rats which were included in the final analysis. In another seven rats the model failed and these were excluded from the final analysis.

### The modified pvMa increased the surgical convenience of MCAO surgery

#### Magnification efficacy of the modified pvMa

Magnification was appropriate (5–20 ×). A Computar MLH-10 × macro lens (CBC, Kyoto, Japan) was placed 800 mm away from the operating field although its specified working distance is 152.4 mm (zoom 1–40 ×).

#### Observation efficacy of the modified pvMa

Screen-imaging guidance using the modified pvMa led to an accurate, smooth, minimally invasive and comparatively easy surgical procedure during MCAO modeling. The screen imaging of the modified pvMa was clear, life-like, stereoscopic and matched the actual operation.

### The modified pvMa promoted the establishment of reproducible MCAO modeling

#### Model establishment time

The average time of MCAO model establishment was  $29.8 \pm 3.3$  minutes (Table 1).

#### Success rate of model establishment

Neurological function was evaluated using the modified neurological score system. High scores represented severe injury. A score  $\geq 7$  was considered successful injury of the MCAO<sup>[22]</sup>. Results showed an average score of  $13.5 \pm 1.2$  (12–15 scores) (Table 1). The success rate of MCAO modeling reached 74.07%.

#### Infarct volume

Normal brain tissue stained purple, while infarction foci remained white after staining with 2,3,5-triphenyl-2H-tetrazolium chloride (Figure 1). Quantitative analysis demonstrated that the infarct

volume percentage was  $22.8 \pm 0.8\%$  (21.4–24.6%, Table 1).

Table 1 Experimental data in the modified portable video microscope for middle cerebral artery occlusion

Rat	Model establishment time (min)	mNSS (scores)	Infarct volume percentage (%)
1	27	12	21.7
2	32	15	22.8
3	29	12	22.8
4	26	14	22.4
5	31	12	21.4
6	34	15	22.8
7	33	15	23.2
8	28	14	22.9
9	30	15	22.8
10	25	15	21.7
11	35	13	23.3
12	30	14	21.7
13	29	12	22.8
14	34	13	22.4
15	26	13	22.8
16	27	14	22.8
17	35	13	23.7
18	28	13	23.7
19	31	12	24.6
20	25	14	23.7
Mean	29.8	13.5	22.8
SEM	3.3	1.2	0.8

In the modified neurological score system (mNSS), high scores represent severe injury. Scores  $\geq 7$  were considered to indicate successful injury due to middle cerebral artery occlusion<sup>[22]</sup>.

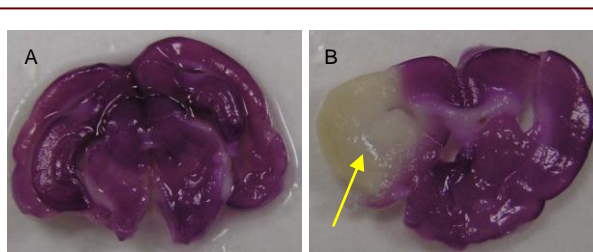


Figure 1 Brain blocks were stained with 2,3,5-triphenyl-2H-tetrazolium chloride. Normal brain is purple (A) and infarction loci is white/pale (B, arrow).

## DISCUSSION

Only a near-viewing telescope (the shortest viewing distance should not be greater than 1 000 mm) can be used in micrological surgery. With longer distances the surgeon is not able to adjust the focal length without changing his posture during the surgical procedure. There is only one Nicula 7–21 series monocular telescope still being produced with a shortest viewing distance of 500 mm. However, it has no standard mounts for the micro digital video camera or the fiber optic cable. To address this, we used the modified pvMa. Its key parts are a Computar MLH-10 × macro lens (CBC, Kyoto,

Japan) and a DFK-21AU04 USB CCD Camera (Imaging Source, Bremen, Germany). Magnification was appropriate (5–20 ×) and the macro lens was placed at a distance of 800 mm from the operating field even though its specified working distance is 152.4 mm (zoom 1–40 ×). The bifurcation of the common carotid artery in rats was observed using the modified pvMa. The screen imaging was clear, life-like and stereoscopic. Our results indicate that this device is suitable for micrological surgery. Intraluminal suture MCAO was performed under screen-imaging guidance using the modified pvMa. The screen image matched the actual operation and the micrological surgical procedure was accurate, smooth, minimally invasive and comparatively easy. Additionally, screen-based macroscopic surgery achieved a reproducible MCAO by intraluminal suture as evaluated by the modified neurological score system score and the infarct volume percentage. The data demonstrated that the modified pvMa is useful in micrological surgical procedures and screen-based macroscopic surgery is a practicable, simple, comfortable and cost-effective approach to establishing an intraluminal suture model of MCAO.

## MATERIALS AND METHODS

### Design

A randomized, controlled, animal experiment.

### Time and setting

The experiment was performed at the Animal Experimental Centre, Department of Clinical Research, Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, Yunnan Province, China from May to August in 2011.

### Materials

#### Animals

Twenty-seven clean, healthy, adult, male Sprague-Dawley rats, aged 45–90 days, weighing 180–250 g, were provided by the Animal Experimental Centre, Department of Clinical Research, Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, Yunnan Province, China (license No. SYXK (Dian) 2008-0005). Animals were housed in separate cages (temperature: 22–25°C; humidity: 50–60%) with free access to water and food in a 12-hour light-dark cycle. Experimental procedures were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China<sup>[23]</sup>.

#### Instruments

The modified pvMa and its components are as follows (Figure 2): (1) The micro-digital video camera: DFK-21AU04 USB CCD Camera (Imaging Source, Bremen, Germany). Technical parameters: product code: DFK 21AU04; type: color USB 2.0 camera with IR cut filter; connection: USB 2.0; manufacturer: Imaging Source; sensor: CCD; type: progressive scan; sensor specification: Sony ICX098BQ; format: 1/4"; resolution:

640 × 480 pixels; dynamic range: 8 bit; exposure time: 1/10 000 second to 60 minutes; gain: 0 dB to 36 dB; frames per second at maximum resolution: 60 frames/second; video formats at frame rate: 640 × 480, BY8 (RAW) at 60, 30, 15, 7.5, 3.75 frames/s 640 × 480, UYVY at 30, 15, 7.5, 3.75 frames/s; lens mount: C/CS mount; supply voltage: 8 V to 30 V via the USB 2.0 cable; current consumption: approximately 200 mA at 12 V DC; dimensions (height × width × length): 50.6 mm × 50.6 mm × 50 mm; weight: 265 g; maximum temperature (operation): –5°C to 45°C; maximum temperature (storage): –20°C to 60°C; maximum humidity (operation): 20–80%; maximum humidity (storage): 20–95%. (2) The optic lens: Computar MLH-10 × macro lens (CBC, Kyoto, Japan). The main technical parameters: product code: Computar MLH-10 ×; 1/2", C-mount; zoom: 0.084–0.84 × (zoom 1–40 ×); iris aperture (F): 5.6–32 c; horizontal visual angle: 3.60°–18.00°; nearest object image: 0.152 4 m; effective caliber: rear 6.40 mm, front 30.00 mm; front-lens thread (mm): 46.0 × 0.75; overall dimension (mm): 48 × 98.5; weight: 260 g. (3) The light source: custom made SPD-300-W LED spot optic source (CST, Dongguan, Guangdong Province, China). (4) The light source adapter: custom made COPL-40-W coaxial optic adapter (CST). (5) The monitor screen: Dell laptop computer.



Figure 2 Screen-based macroscopic surgery.

(A, B) The main part (macro lens (A) with micro digital video camera and light source adapter) of the portable video microscope system and shadowless lamp (B).

(C, D) Screen-based macroscopic surgery for middle cerebral artery occlusion.

### Methods

#### MCAO model establishment

The surgical procedure was performed using the screen-operative surgery method with the modified pvMa (Figure 3, supplementary Figure 1 online). Rats were anesthetized by intraperitoneal injection of chloral hydrate solution (3.6 g/kg; Sigma, St. Louis, MO, USA). A longitudinal midline incision was made in the ventral

cervical skin. Surgical procedures following the incision were performed and guided by screen imaging using the modified pvMa. The bifurcation of the left common carotid artery and the left internal carotid artery was carefully dissected and clearly exposed, and the distal portion of the external carotid artery and the proximal portion of the pterygopalatine artery were ligated with 5-0 silk. The common carotid artery and the internal carotid artery were temporarily clamped using microvascular clips. A 5-0 silk suture was then tied loosely around the origin of the external carotid artery. A small puncture was made in the wall of the external carotid artery with a pair of spring scissors and a 20 mm long, blunted and coated 4-0 monofilament nylon suture (Harvard Apparatus, Holliston, Massachusetts, USA) was inserted through the proximal external carotid artery into the internal carotid artery. This was advanced into the circle of Willis to effectively occlude the left middle cerebral artery. Prior to use, the suture tip was blunted by heating it near a flame and a 20 mm distal segment of the suture was then coated with poly-L-lysine solution (0.1% w/v, in deionized water; Sigma) and dried in a 60°C oven for 1 hour. The suture was inserted 18–20 mm from the bifurcation of the common carotid artery according to the animal's body mass<sup>[24-25]</sup>. Local cerebral blood flow was monitored in the cerebral cortex of each hemisphere in the region supplied by the middle cerebral artery using laser-Doppler flowmetry (Laserflo BPM2, Vasamedics, St. Paul, MN, USA) according to a previously described method<sup>[24]</sup>. Local cerebral blood flow was continuously measured (2-Hz sampling rate) 30 minutes before and after the suture was placed. The ipsilateral (left side) laser Doppler signal decreasing to 20% of baseline while the contralateral (right side) remained at baseline level represented the completion of MCAO<sup>[24]</sup>.

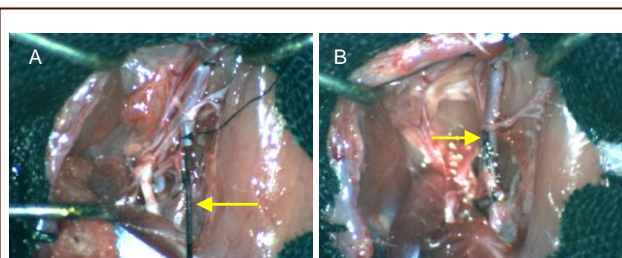


Figure 3 Middle cerebral artery occlusion guided by screen imaging using a modified portable video microscope. The middle cerebral artery was occluded by inserting suture via the proximal segment of the external carotid artery into the internal carotid artery (the arrow on A shows the inserted suture and the arrow on B shows the end of the inserted suture).

#### Identification of sensorimotor deficits

Sensorimotor deficits were measured using the modified neurological score system on day 3 before and after MCAO<sup>[26]</sup>. The score system was composed of motor, sensory, reflex, and balance tests and graded on a scale of 0–18. A normal score was 0, maximal deficit score was

18, and a score of 1 was awarded for the inability to perform the tasks or the lack of a tested reflex. Behavioral tests were conducted between 14:00 and 17:00 by two investigators blinded to the experiments, with a 5-minute rest period between each type of motor test. The testing sequence was randomized.

#### Calculation of infarction volume

Following the behavioral tests on day 3 after MCAO, rats were anesthetized with intraperitoneal administration of chloral hydrate (3.6 g/kg; Sigma) and sacrificed to calculate the infarct volume. First, the fresh brains were removed and stained with 2,3,5-triphenyl-2H-tetrazolium chloride (Sigma) to show the infarct foci<sup>[27]</sup>. The brains were quickly removed from solution and coronally sectioned into six 2 mm-thick slices. The flesh brain slices were incubated for 30 minutes in a 2% solution of 2,3,5-triphenyl-2H-tetrazolium chloride at 37°C and fixed by immersion in a 10% buffered formalin solution. The unstained area was defined as the ischemic lesion. Finally, the infarct volume was calculated according to a previously published method<sup>[28-33]</sup>. Brain sections were photographed with a charge-coupled device camera (EDC-1000HR Computer Camera, Electrim Corp.), and images were stored on a microcomputer. Using an image analysis program (BioScan Optimas, Edmonds, WA, USA), the volumes of the infarcted tissue and both hemispheres were calculated for each brain slice. The percentage of left hemisphere volume (%HLV) was calculated by the following equation, correcting for edema: %HLV = [total lesion volume – (right hemisphere volume – left hemisphere volume)]/left hemisphere volume × 100%.

#### Statistical analysis

The mean and standard error of mean of the modeling time, modified neurological score and volume of infarction were calculated using SPSS 12.0 software (SPSS, Chicago, IL, USA).

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**Author contributions:** Xingbao Zhu, Junli Luo and Song Liu participated in the screen-operative surgery. Guolong Chen, Qiangjin Ruan and Yun Liu participated in 2,3,5-triphenyl-2H-tetrazolium chloride staining and infarct volume calculation. Xunding Deng and Dianchun Wang participated in the behavioral tests. Quanshui Fan and Xinghua Pan participated in data collection, analysis and interpretation.

**Conflicts of interest:** None declared.

**Ethical approval:** This study received permission from the Animal Care and Research Committee of Kunming General Hospital, Chengdu Military Area Command of Chinese PLA, China.

**Supplementary information:** Supplementary data associated with this article can be found, in the online version, by visiting [www.nrronline.org](http://www.nrronline.org), and entering Vol. 7, No. 12, 2012 after selecting the "NRR Current Issue" button on the page.

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