Materials

Microbalance (Mettler Toledo AG245)
Cryostat (Microm HM500)
Tissue Tek (Fisher Scientific)
Microscope slides (Fisher Scientific)
Cresyl violet (Fluka, Sigma): three components are made and then mixed: 0.3 g of cresyl violet in 50 ml of H_2O; 3.48 ml of glacial acetic acid in 300 ml of H_2O; and 5.44 g of sodium acetate in 200 ml of H_2O
Cover glass (Corning, Fisher Scientific)
Mounting medium (Permount, Fisher Scientific)
Scanner

Acknowledgments

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[28] Proximal Middle Cerebral Artery Occlusion Surgery for the Study of Ischemia–Reoxygenation Injury in the Brain

By ARTURO J. CARDOUNEL, WILLIAM A. WALLACE, and CHANDAN K. SEN

Introduction

A variety of experimental models are used in studying ischemic brain injury. The three main classes of *in vivo* rodent models involve global ischemia through multiple vessel occlusion, hypoxia/hypovolemia in order to produce global alterations in cerebral blood flow, or focal ischemia through common carotid artery (CCA) occlusion or craniectomy followed by middle cerebral artery (MCA) occlusion.[1,2] A newer and now widely used approach involves MCA occlusion through the use of an intravascular nylon occluder. This method was developed by Koizumi *et al.*[3] with a more recent modification by Longa *et al.*[2] This has become the most widely used

[1] P. Lipton, *Physiol. Rev.* **79**(4), 1431 (1999).
[2] E. Z. Longa *et al.*, *Stroke* **20**(1), 84 (1989).
[3] Y. Y. Koizumi, T. Nakazawa, and G. Ooneda, *Jpn. J. Stroke* **8,** 1 (1986).

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model to study the pathophysiology of neuronal injury and to test therapeutic interventions following temporary focal ischemia. The model is relatively easy to perform, minimally invasive, and does not require craniectomy, which may influence blood–brain permeability, intracranial pressure, and brain temperature.[4] However, several inherent complications have been reported using this model. These limitations include (i) insufficient middle cerebral artery occlusion (MCAO) and premature reperfusion,[5] (ii) coagulation and thrombus formation,[3,6] (iii) inadvertent subarachnoid hemorrhage,[2,3,7,8] and (iv) hypothermia during and postsurgery.[9,10] These concerns must be addressed and a standardized model must be developed in order to limit variability and optimize reproducibility. This article describes a MCAO model in which the previous concerns are addressed through laser Doppler flowmetry (LDF) measurements, pre- and postsurgical heparinization, and telemetric intracranial temperature monitoring.

Middle Cerebral Artery Occlusion

Male Wistar rats weighing between 300 and 350 g are anesthetized in an acrylic induction chamber (Harvard Apparatus, NP 60-5246) through administration of halothane or isofluorane until unconscious. The rat continues to receive 1–1.5% halothane delivered via a tabletop anesthesia machine and vaporizer (Harvard Apparatus, NP 72-3011; NP-72-3038) via spontaneous respiration through a nose cone. Halothane is mixed with oxygen-enriched air in order to maintain normal arterial pO_2 and pCO_2 values.

A femoral venous line is established for drug delivery. Heparin (150 IU/kg) is infused at the onset of surgery. A femoral arterial line is inserted for continuous blood pressure measurement. Arterial blood samples are taken prior to and during the surgical and recovery periods for the determination of blood gases, pH, and glucose levels. Body temperature is monitored and maintained at 37° using a homoeothermic blanket with a rectal probe. The brain temperature is monitored throughout the surgical and recovery periods using a minithermister (Minimitter)

[4] W. R. Hudgins and J. H. Garcia, *Stroke* **1**(5), 375 (1970).
[5] L. Belayev *et al*., *Stroke* **27**(9), 1616 (1996).
[6] C. H. Rabb, *Stroke* **27**(1), 151 (1996).
[7] J. B. Bederson, I. M. Germano, and L. Guarino, *Stroke* **26**(6), 1086 (1995).
[8] Y. Kuge *et al*., *Stroke* **26**(9), 1655 (1995).
[9] Q. Zhao *et al*., *Brain Res*. **649**(1–2), 253 (1994).
[10] D. Corbett, M. Hamilton, and F. Colbourne, *Exp. Neurol*. **163**(1), 200 (2000).

implanted in the skull superior to the dura. A probe holder is glued to the skull surface over the cortical area supplied by the middle cerebral artery for LDF. The middle cerebral artery occlusion is performed as described by Longa *et al.*[2] with some minor modifications as described later.

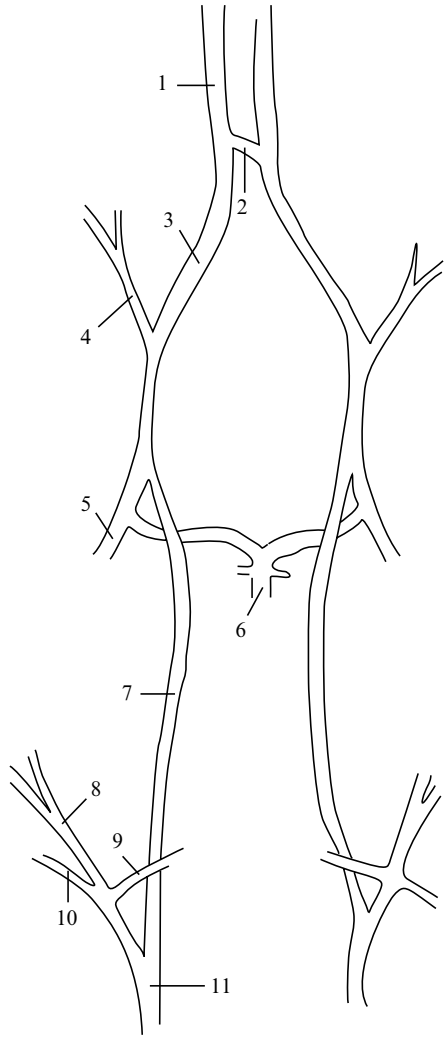
The dorsal skull and ventral neck are shaved with clippers (Harvard Apparatus, NP 52-5204) using a #40 blade (Harvard Apparatus, NP-52-5212), taking care to clean away any excess fur. The rat is placed in the prone position on the operating table. The scalp is cleaned with a providone/iodine solution. Using medium-weight scissors, a midline incision is made on the rat's scalp. The fascia is then cut away and the bregma identified. Just medial to this suture line, the soft tissue and periosteum are removed with a currette, exposing the skull.

A bench-top motor drill (AH 59-7455, AH 59-9860) is then used to thin the bone of the skull at 4 mm lateral and 1.5 mm posterior to the bregma bilaterally to allow for cortical blood flow measurements using a laser Doppler (Moor Instruments). A 1-cm section of PE 50 tubing is secured to the skull with superglue or dental cement in order to hold the laser Doppler in place for the remainder of the procedure. Following placement of the laser Doppler probe, the rat is placed in the supine position.

The midline incision site on the neck is prepared aseptically with a providone/iodine solution. Using medium-weight scissors, a midline incision is made over the neck of the rat. Dissection is performed lateral to the right sternomastoid muscle, and the omohyoid muscle is then identified and divided. Under an operating microscope the right common carotid artery is exposed and dissected free of surrounding connective tissue (Fig. 1).

The external branch of the right common carotid artery is then isolated and the branches of the occipital and superior thyroid arteries are coagulated. The external carotid artery is ligated with a 6-0 braided silk suture at the bifurcation of the lingual and maxillary arteries. A microclip (NP 61-0186) is placed across the external carotid artery at the bifurcation with the common carotid, thereby maintaining blood flow through the internal carotid. Next, a 6-0 silk suture is tied loosely around the mobilized ECA stump. A previously prepared 5-cm length of poly-L-lysine-coated 4-0 monofilament nylon suture, with its tip blunted by heating near a flame, is inserted into the external carotid artery (ECA) lumen through a puncture proximal to the distal ECA ligation.[5] The silk suture around the ECA is then tightened around the intraluminal nylon suture to prevent bleeding and the microvascular clip is removed. The ECA stump is then dissected free and traction is applied in order to advance the suture tip into the internal carotid artery (ICA).

Care must be taken while advancing the sutures as not to advance the suture into the extracranial pterygopalatine artery. This can be avoided



1. Internal ethmoidal artery
2. Anterior communicating artery
3. Anterior cerebral artery
4. Middle cerebral artery
5. Posterior cerebral artery
6. Basilar artery
7. Internal carotid artery
8. External carotid artery
9. Occipital artery
10. Cranial thyroid artery
11. Common carotid artery

FIG. 1. Anatomy of head and neck vasculature in the adult rat.

through visualization of the filament as it is advanced. The pterygopalatine artery runs on a ventral axis, whereas the ICA is located more dorsal; proper illumination of the surgical site will allow visualization of the filament.

The suture is advanced gently through the internal carotid artery a total distance of approximately 18 mm to the origin of the middle cerebral artery. Slight resistance will be felt as the suture reaches the origin of the MCA; proper placement of the filament is verified by a drop in LDF greater than 70%. The occurrence of subarachnoid hemorrhage (SAH) can be determined by measuring LDF on the ischemic and contralateral cortex. A significant drop in LDF on the nonischemic hemisphere suggests SAH, which can be verified later on necropsy.

LDF is monitored prior to and during the ischemic period as well as at reflow. Any animals that may have incomplete ischemia or early reperfusion can thus be disqualified. Following 90 min of ischemia, the rat is reanesthetized in the induction chamber and transferred back to the operating table in the supine position.

The neck wound is reopened, and the suture is withdrawn back into the external carotid artery, reestablishing blood flow to the internal carotid artery and middle cerebral artery. This should be verified by LDF. The remaining suture is then trimmed to 1–2 mm outside of the vessel.

The wounds are then closed and infiltrated with 0.5% xylocaine. Analgesia is provided as needed with acetaminophen (1 g/kg) by oral gavage. Behavioral assessments are performed at this time and daily thereafter.[11] The animals are then returned to their cages and monitored until fully recovered.

At a time point of choice (usually 24–72 h) postreperfusion, the animals are killed under deep halothane anesthesia for the harvest of brain tissue. The brain temperature is monitored continuously and is maintained at 37° using a heat lamp. This is vital to the reproducibility of the model, as only slight changes in cranial temperature can have a large impact on the severity of ischemic injury.[9,10]

Behavioral Assessment

Ten-Point Neuro Scale

This 10-point scale may be used to evaluate general neurological function. Four points are awarded for a reduction in resistance to contralateral push; three points are awarded if contralateral circling is evident; two

[11] A. C. DeVries *et al.*, *Neurosci. Biobehav. Rev.* **25**(4), 325 (2001).

points are awarded for the appearance of contralateral shoulder adduction, and one point is awarded for contralateral forelimb flexion when suspended vertically by the tail.

Visual Placing

A test of visual acuity and reflexes is performed by lowering the animal slowly toward a table. A positive score is recorded if the animal extends its forepaws before touching the table two out of three times.

Forelimb Strength

The animal is suspended by its forelimbs on a rope suspended between two posts. The time until the rodent falls is measured up to 90 s. A score of 0 is given if the animal falls immediately. A score of 90 is given if the animal does not fall. The exercise is repeated and the best of three trials is recorded.

Bridge Walking

This tests the balance and coordination of the animal. The rodent is placed at the center of a bridge 60 cm long. The bridge is suspended above a foam egg crate. The time required for the rodent to reach a platform on

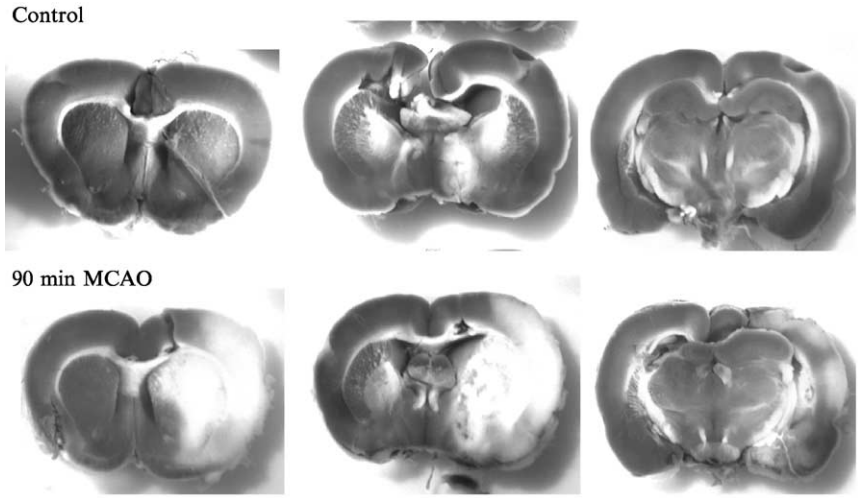


FIG. 2. Infarct size determination by TTC staining. (Top) Normal noninfarcted brain slices. Viable tissue stains red, whereas nonviable infracted tissue is white. (Bottom) A large infarct occupying the cortex and striatum. The infarct is located mainly in the frontal and midcortical regions.

either side within 2 min is recorded. If the animal falls, the fall time is recorded.

Histology

One group of experimental animals is euthanized at 72 h postreperfusion. The brains are removed and frozen rapidly (-20°) in 2-methylbutane and are stored at -80°. The brains are then sectioned with a cryostat, 20 μm thick from anterior to posterior, and stained with cresyl violet.

TTC Stain

A second set of experimental animals is euthanized at 72 h postreperfusion. The brains are removed rapidly and 2-mm sections are cut using a rodent brain matrix. The sections are placed in a 2% TTC solution and incubated for 30 min at 37°. The serial sections are then removed and washed in phosphate-buffered saline. Images are captured by video microscopy using the SNAPPY image capture software, and infarct areas are determined using IT software (Fig. 2).

Acknowledgment

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[29] Carotid Chemodenervation Approach to Study Oxygen Sensing in Brain Stem Catecholaminergic Cells

By Olivier Pascual, Jean-Christophe Roux, Christophe Soulage, Marie-Pierre Morin-Surun, Monique Denavit-Saubié, and Jean-Marc Pequignot

In mammals, an oxygen deficit in the air induces an immediate and sustained increase in ventilation in order to improve the delivery of oxygen from pulmonary airways to the blood circulation and then to the tissues. The neuronal network, which generates and controls the ventilation, is located in the brain stem. This network is vital and still able to generate a respiratory motor output when isolated in a brain stem preparation. Furthermore, lesioning this network suppresses the ventilation of the whole animal. Neuronal respiratory groups include the dorsal group within

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