

BLF22 Surgical Protocol

Cerebral Perfusion (MCA Occlusion Model) in Mice

APPLICATION BASICS

Stroke Model:	Middle Cerebral Artery Occlusion
Cerebral Cortex:	Ischemia & Reperfusion
Species:	Mice (C57Bl/6)
Body Weight:	22 - 30 grams
Duration:	Acute
PROBE TYPE:	EZ Fine or JF OmegaWave, Japan
	M: Monofiber (Transonic®, USA)

Middle Cerebral Artery Occlusion Model

Transgenic manipulations make the mouse an ideal model for stroke. Experimental data is easily compared with control data in dose response studies where researchers can assess the effects of various pharmacological agents and dosages on cerebral blood flow.

Laser Doppler technology is used to assess cerebral perfusion during middle cerebral artery occlusion by confirming a sudden drop in cerebral blood flow, and subsequent reperfusion of the ischemic area. Laser Doppler Monitors give high resolution perfusion measurement in approximately 1 mm³ of tissue at a depth of 1 mm just below the surface of the Probe. Therefore, when using Laser Doppler Monitors for ischemia/reperfusion or dose response studies, it is critical that the measurement site is the same throughout the entire measurement sequence. If the site changes during a treatment, reliable data cannot be obtained and compared.

This becomes a challenge in Middle Cerebral Artery (MCA) occlusion studies where the animal must be positioned on its back with the neck extended for insertion and advancement of the occluding filament into the carotid artery to produce the stroke. The fiber cannot be easily advanced into the internal carotid via standard protocols with the mouse fixed in an upright position; however, turning the animal over can displace the position of the Laser Probe. This is resolved in the mouse with a flexible fiber Probe.

Because the skull of the mouse is very thin, the laser light will penetrate through the skull layer without effect. Therefore, cerebral tissue perfusion can be measured over the skull without performing a craniotomy which also requires that the animal be in an upright position as described in a previous protocol (LD-105-sp). A Laser Doppler Probe using bare fiber (Type EZ Fine or Type JF, Omegawave, Inc., Japan), can be affixed to the skull using super glue, thus ensuring that the measurement site will not change during the experiment. The mouse can be repositioned on its back to advance the filament in the carotid after gluing the Laser Doppler Probe fibers in place.

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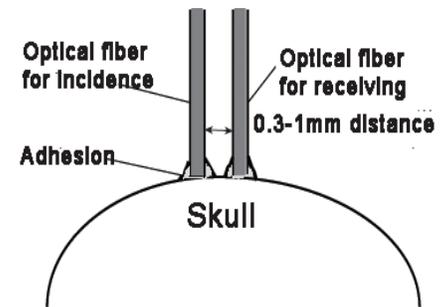


Fig. 1: Type EZ Fine Laser Doppler Probe (Omegawave, Inc.) affixed with superglue to the skull of a mouse.

ACKNOWLEDGEMENT

Protocol courtesy of S. Kashima, OmegaWave, Inc.

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Cerebral Perfusion (MCA Occlusion Model) in Mice cont.

The EZ Fine Probe uses an acrylic resin coated silica glass fiber that is 0.25 mm diameter. It can be cut with a special cutting tool set, the glued portion removed after each experiment and the remainder of the fiber reused. Alternatively, a Monofiber Probe (Type M, Transonic Systems Inc.®) may be used similarly, though the received light signal is stronger from a two fiber Probe.

Surgical Procedure

- Anesthetize the animal as required.
- With the animal in the prone position, expose the skull by a midline scalp incision.
- Position the two optical fibers (Incidence fiber and Receiving fiber) on the skull separated 0.3 mm – 1 mm apart. Glue in place approximately 2 mm posterior to the bregma.
- Turn the animal to a supine position and expose the carotid complex through a ventral midline incision. Ligate the common carotid artery and the external carotid artery and its branches with 5-0 silk suture.
- Make a transverse incision in the artery and introduce the nylon filament into the external carotid artery. Gently advance it into the internal carotid until there is a slight resistance (approximately 10 mm) indicating the origin of the middle cerebral artery at the Circle of Willis.
- Observe a sudden drop in cerebral blood flow recorded by the laser Doppler confirming occlusion of the MCA.
- Occlusion may be observed for 1 to several hours.
- For reperfusion, withdraw the filament and remove the sutures on the common carotid artery and internal carotid artery, keeping the external carotid permanently occluded.

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