

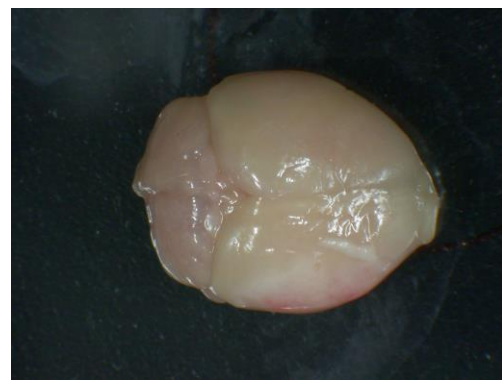
MCAo, Middle Cerebral Artery Occlusion

MCA occlusion is achieved using a siliconized filament introduced into the external carotid artery and advanced to the MCA to produce reduced blood flow. A laser Doppler ultrasound (Perimed-5000 series) is used to verify reduction of blood flow in the MCA territory during MCAo.

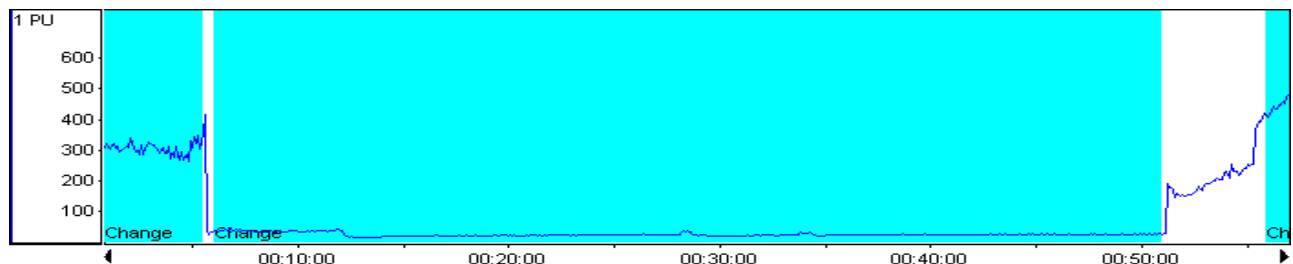
Core temperature is monitored continuously with a rectal probe and maintained at 37°C using a heating pad. To attach the Doppler probe (Perimed) to the skull table, a sagittal cut of the scalp is performed, with the probe placed 4mm lateral and 1mm posterior to Bregma, fixed in place with glue. With the mouse turned to the supine position, a neck incision is performed and the common carotid artery (CCA) exposed by blunt dissection. A loose suture is placed around the proximal CCA, the external (ECA) and internal carotid arteries (ICA) are exposed and the ECA is tightly ligated with suture. The proximal CCA is then ligated using the suture already in place. A second suture is loosely positioned around the distal CCA near the carotid bifurcation. Then a vascular clamp is placed on the ICA prior of CCA incision, distal to the proximal ligation to provide hemostasis. A siliconized filament is inserted into CCA, the ICA vascular clamp is removed, and the filament is advanced to the MCA until the blood flow Perimed records about 70% drop of the base line value. Subjects in which blood flow does not remain below 30% of baseline values for the entire period are excluded from the analysis. At the end of 45-55 min the filament is withdrawn and reperfusion is followed until it returned to 70-80% of the baseline. After removal of the filament, the ICA is briefly clamped to provide hemostasis while the distal CCA is ligated with a distal suture. The soft tissues are then allowed to fall back into place, and both surgical sites are closed using interrupted stitches of 4.0 Nylon monofilament. Mice are returned to a temperature controlled recovery chamber Thermocare with free access to food and water.



TTCstain-Evolution of infarct in mouse brain post 55-minute MCAo



Dorsal view of mouse brain post right MCAo



Model confirmation by LDF (Laser-Doppler flowmetry)

Blood flow measurements are recorded by a widely used Laser-Doppler flowmetry (PF 5010 Perimed, Inc) with a straight tip laser probe (model MT B500-0 L120, Perimed) glued to skull at 2 mm posterior to the bregma and 4-5 mm laterally from the midline. The model was confirmed if a $\geq 70\%$ reduction in cerebral blood flow was observed immediately after filament insertion.