

Surgical Model #: ICVC-R & THIRDVEN-R Intracerebroventricular (Lateral Ventricle) Cannulation (ICVC) and Third Ventricle Cannulation (TVC) Care and Use Document for Rats and Mice

Anesthetic: Isoflurane to effect

Analgesic: Buprenorphine (SQ): Rat and Mouse Dose 0.05 mg/kg

Basic Surgical Procedure Description:

An anesthetized and surgically prepared animal is immobilized in a stereotaxic frame. A 2-cm midsagittal skin incision is made on the scalp in order to visualize the skull landmarks: bregma and lambda. Blunt dissection is used to separate and retract the underlying fascia. A flat skull position is confirmed and cannula placement coordinates are calculated. Three holes are drilled to accept three anchoring screws, while a fourth hole is drilled to accept the cannula. A sterile guide cannula is stereotaxically placed. For an ICVC, the internalized tip is located in the lateral ventricle, while in TVC, the tip is located in the third ventricle. In rats, the guide cannula is anchored to the skull via cranioplastic cement and trephine screws, while in mice the guide cannula is anchored with cyanoacrylate glue. The guide cannula is sealed with a screw-on stylet or dummy cannula.

Cannula:

Guide cannulas consist of a length of 22-gauge (for rats) and 26-gauge (for mice) stainless steel hypodermic tubing encased in plastic. Plastics One, Roanoke, VA is our supplier (Phone: 1-540-772-7950). Injector cannula consist of a 28-gauge stainless steel hypodermic tubing for rats and a 33-gauge stainless steel tubing for mice.

Coordinates:

ICVC: Rats (>150g) AP= -0.8 mm, ML = +1.2 mm (left side) and DV= -4.8 mm

Mice (>16g) AP= -1.0 mm, ML = +1.0 mm (left side) and DV= -2.0–2.5 mm

TVC: Rats (>150g) AP= -0.8 mm, ML = 0.0 mm and DV= -8.0 mm

Coordinates listed are calculated in relation to bregma. Some variation occurs depending on the size and strain of the animal. Note: To date, TVC has not been validated in mice.

Quality Control:

Target coordinates and proper cannula placement are verified at the time of surgery by slowly injecting 5µl of methylene blue dye (2µl for mice) into the cannulas of the first two animals modified, while they are still under anesthesia. After waiting five minutes for the dye to circulate through the ventricles, the animals are euthanized. Decapitation is followed by brain removal and slicing at the point of cannula entry. Modifications only proceed if dye has diffused through the target ventricles into both hemispheres. Historically, Taconic has achieved 90-95% correct placement of cannulas in the target ventricle. Checking cannula placement after completion of studies is recommended. Head mount stability is also checked at the time of shipment. Angiotensin II testing is also available for confirmation of cannula placement prior to shipment.

Compound Administration:

Injector Cannulas will be provided with shipment. Injector cannulas are required to administer test articles via the guide cannula. Single injections should not exceed a rate of 1µl/min and a total volume of 5µl. Additional injections may be given after a washout period. Injector cannulas are typically attached to micro-syringes via a length of polyethylene tubing (PE 50). Injection is achieved by unscrewing and removing the dummy cannula and inserting an injector cannula until it locks in position. Connector assemblies from Plastics One can also be used for administration via infusion pumps. Note: Mice should be lightly sedated prior to cannula manipulation.

Housing:

Ideally, animals are individually housed in solid caging to minimize chances cannulas will be dislodged in wire bars of caging and lids. Group housing is adequate as long as cage mates refrain from chewing on one another's cannulas. Apply hot sauce to dummy cannula to prevent this occurrence. With appropriate care, cannulas are expected to last at least three weeks.