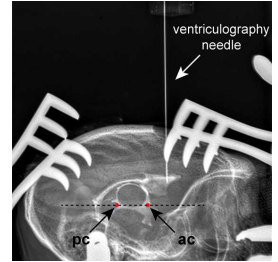


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Protocol for "ventriculography-assisted stereotaxic surgery in non-human primates

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We use this protocol and it's working

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Disclaimer

nothing to be disclosed

Abstract

An step-by-step procedure describing ventriculography-assisted stereotaxic surgery in non-human primates is provided here, including (i) animal pre-surgical preparation, (ii) stereotaxic surgery and (iii) post-surgical care. This protocol is intended to be used for intraparenchymal deliveries of viral suspensions.

Image Attribution

the author

Animal preparation

- 1 Pre-surgical anesthesia: to be induced with ketamine (5 mg/Kg) and midazolam (0.5 mg/Kg), administered intramuscularly.
- 2 Surgical shaving: comprising left and right superior and inferior limbs and thorax for the placement of adhesive EKG electrodes.
- 3 Induction of initial anesthesia: 2% isofluorane prior to intubation of the animal followed by intravenous injection of propofol (1.5 mg/Kg), fentanest (0.01 mg/Kg) and anectine (1 mg/Kg).
- 4 Intubation: a size 3 pediatrics endotracheal tube to be used. Keep oxygen volume between 10-15 ml/Kg under a respiratory rate of 15-20 rpm.
- 5 Catheter placement in the saphenous vein: to secure intravenous access to deliver a continuous infusion of saline solution with 5% glucose.
- 6 Pre-surgical anti-inflammatory treatment: comprising the subcutaneous delivery of carprofen (4 mg/Kg / 24h / 3 consecutive days), methylprednisolone (10mg/Kg; intravenous, dexamethasone (10 mg/Kg; intravenous) and enrofoxacin (10 mg/Kg / 24h / 7 days; intramuscular).
- 7 Animal monitoring: comprising rectal temperature, oxygen saturation, expired CO₂, EKG, respiration rate and blood pressure.
- 8 Surgical anesthesia: continuous intravenous infusion of propofol (6 mg/Kg/h)
- 9 Placement of the animal in the stereotaxic frame: macaque head fixed with elevated earbars, palate and orbital adaptors. Animal placed on top of an electric heat pad.

Stereotaxic surgical procedure

- 10 Animal head cleaned with an iodine solution. Skin treated with local anesthesia (lidocaine spray).
- 11 Preparation of the sterile surgical field and the sterile surgical instruments table.

- 12 Perform a surgical medial incision of the skin through the entire rostrocaudal extent of the head, followed by removal of the periosteum.
- 13 First craniotomy: drill a burr hole (4 mm in diameter) to expose the midsagittal venous sinus.
- 14 Secure access to the CSF space: a pediatric needle is inserted in the right lateral ventricle in the frontal lobe. Under the control of a stereotaxic micromanipulator, a orthogonal trajectory 1.2 mm to the midsagittal sinus is used to place a CSF pediatric needle into the ventricle. Adequate placement of the needle is confirmed upon CSF aspiration.
- 15 First ventriculography: Delivery of a bolus of 0.3 ml of X-ray contrast to properly delineate the ventricular system. The first ventriculography enables to calculate the location of the anterior and posterior commissures (ac and pc, respectively), as well as to measure the length of the ac-pc plane, required for proper calibration of the stereotaxic coordinates in keeping with the stereotaxic atlas of reference (see Figure 1, panels A & E)
- 16 Second craniotomy: another burr hole is drilled to expose the brain surface at the level of the calculated coordinates for the putamen nucleus
- 17 Fill the injection device (Hamilton syringe) with the viral suspension of AAV9-SynA53T.
- 18 Second ventriculography: Once the injection needle is located in the pre-calculated place of delivery within the putamen nucleus, a second X-ray plate is taken ensuring the right location of the tip of the needle (to be repeated for each AAV delivery; see Figure 1, panels B-D & F-G).
- 19 Delivery of the viral suspension: Pressure deliveries of AAV9-SynA53T were made in pulses of 5 ml/min for a total volume of 25 microliters each into three sites in the left putamen, each deposit spaced 1 mm in the rostrocaudal direction to obtain the highest possible transduction extent of the putamen. Once injections were completed, the needle was left in place for an additional time of 10 min before withdrawal to minimize AAV reflux through the injection tract.
- 20 Stereotaxic coordinates: 1mm rostral to ac, 1 mm dorsal to the ac-pc plane and 11 mm lateral to midline (rostral injection). 0 mm to ac, 1 mm dorsal to the ac-pc plane and 11.5 mm lateral to midline (mid injection). 1 mm caudal to ac, 1.5 mm dorsal to the ac-pc plane and 12.5 mm lateral to midline (caudal injection).



- 21 Once all AAVs were completed, the muscular plane was closed with absorbable suture, followed by surgical staples to close the skin and cleaned with an iodine solution.

Post-surgical care

- 22 Animals were kept under constant monitoring in individual cages with a libitum access to food and water. Once animals showed a complete post-surgical recovery (24 h), they were returned to the animal vivarium and housed in groups.
- 23 Post-surgical analgesia was achieved with a single intramuscular injection of flunixin meglumine (5 mg/Kg) delivered at the end of the surgical procedure and repeated 24 and 48 h post-surgery.
- 24 A similar schedule was conducted for antibiotic coverage (ampicillin, 0.5 ml/day).

Protocol references

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