Transection of the superior sagittal sinus enables bilateral access to the rodent midline brain structures

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ABSTRACT:

Stereotaxic access to brain areas underneath the superior sagittal sinus (SSS) is notoriously challenging. As a major drainage vessel, covering the whole extension of the sagittal fissure, the SSS impedes direct bilateral access to underlying regions for recording and stimulation probes, drug-delivery cannulas, and injection devices. We now describe a new method for transection and retraction of the SSS in rats, that allows the accurate placement of microinjection devices, or chronic electrode probes, while avoiding hemorrhage and the ensuing deleterious consequences for local structures, animal health, and behavior. To demonstrate the feasibility of this approach we evaluated its consequences acutely during surgery, and thereafter during surgical survival, recovery, behavioral testing, as well as postmortem analysis of histological impact in the related brain structures. This method provides a new approach enabling direct access for manipulation and recording of activity in brain areas previously obstructed by the SSS.

DETAILED PROTOCOL:

MATERIALS

Animals

Current protocol was validated with Rats of 2 to 3 months old. Although not tested, it seems feasible that this method is applicable to animals of different age or species, with minimal adaptations.

10% (vol/vol) iodopovidone

Ketamine (Imalgene 1000 100 mg/ ml)

Medetomidine (DOMTOR 1 mg/ml)

Isoflurane (IsoVet 1000mg/g)
Lubricating ophthalmic gel (Lubrithal)
Atipamizole (Antisedan 5 mg/ml)
Lidocaine gel (Lidonostrum Gele 2% 20 mg/g gel))
Saline solution (NaCl 0.9%)
Ringer's Lactate Solution (Braun)

Digital Stereotaxic Frame (Stoelting, cat. no. 51900)
Stereo Microscope (A.KRÜSS Optronic, cat. no. MSL4000-10/30-S)
Fiber Optic Illuminator (World Precision Instruments, cat. no. Z-LITE-Z)
Micromotor High Speed Drill (Stoelting, cat. no. 51449)
Germinator 500 (Braintree Scientific, cat. no. GER 5287-120V)
Anesthesia System (VetTech, cat. no. AN070)
Oxygen Concentrator 5L (VetTech, cat. no. AN037/5)
Heating Pad (FOCHEA)
Cauterizer (Fine Science Tools, cat. no. 18010-00)

Hemostats (Fine Science Tools, cat. no. 91309-12)
Splinter Forceps (Zepf Medical Instruments, cat. no. 10-8002-09)
Straight Spring Scissors (Fine Science Tools, cat. no. 91500-09)
Dumont #7 Forceps – for Bone Flap (Fine Science Tools, cat. no. 11271-30)
Jeweler #5 Forceps – for Durotomy (World Precision Instruments, cat. no. 555229F)
Curved Forceps – for Sinus' manipulation (Fine Science Tools, cat. no. 91117-10)
Scalpel Handle #4 (Fine Science Tools, cat. no. 10004-13)
Ligation Aid (Fine Science Tools, cat. no. 18062-12)
0.5mm diameter burrs for micro drill (Fine Science Tools, cat. no. 19007-05)

Scalpel Blades #4 (RazorMed)
Gelatin sponge hemostats (Cutanplast, cat. no. 0561010)
Cotton tipped applicators (Henry Schein, cat. no. 100-9175)
Non-woven swabs (Hartmann, cat. no. 411831)
1ml Syringes (Henry Schein, cat. no. 9003310)
10ml Syringes (Henry Schein, cat. no. 9003304)
Hypodermic 23G needles (Henry Schein, cat. no. 9003337)

Absorbable Surgical Suture (Surgicryl, cat. no. 11151516)
Silicone Grease (Sigma-Aldrich, cat. no. 769711-1EA)
Agarose (Nzytech, cat. no. MB14402)

SURGICAL PROCEDURE (TIMING ~ 2 hours)

1. Prepare the surgical procedure by setting up the operating area, stereotaxic apparatus and sterilized equipment**.** Turn on the anesthesia system (1% isoflurane, 99% air, 250 CC/min).

CRITICAL STEP: The surgery should be conducted under sterile conditions to avoid contamination of the surgical wound and to minimize the risk of post-operative infection complications. The sterility of surgical instruments must be maintained throughout the procedure.

2. Weight the rat and anesthetize the animal with intraperitoneal injection of Ketamine (37,5 mg/kg) and Medetomidine (0,5 mg/kg).

2. Shave rat's head and place the animal on the heating pad.

3. Place the rat in the stereotaxic apparatus using blunt ear bars. Hold one ear bar in the stereotaxic frame, guide it towards the ear canal and tighten. Holding the animal, place the second ear bar in the other ear canal and tighten. Adjust until animal's head cannot be moved from side to side. Align the head with the center of the apparatus. Secure animal's tooth in the mouth holder and fix the air mask. Adjust the screws until the animal's head is properly fixed and in a leveled position.

CRITICAL STEP: Ensure proper insertion of earbars into the ear canals to avoid pressure damage to the nerves and skull.

4. Following careful fixation, apply ophthalmic gel to prevent corneal dehydration. Scrub the surgical site with 10% iodopovidone and 70% ethanol to avoid contamination. Check the

animal's reflexes by toe-pinch reflex to ensure adequate anesthesia. If reflexes are shown, wait until deeper anesthesia. Supplementation of anesthetics might be necessary (Fig. 1a).

CRITICAL STEP: A volatile mixture of 0.5 to 1% isoflurane in oxygen should be delivered throughout the procedure to maintain anesthesia.

5. Make a sagittal incision along the sagittal suture, starting rostrally between the eyes and extending caudally until the back of the ears. Retract skin and muscle using hemostatic clamps. Expose the skull by scrapping periosteum off the bone. Manage occasional bone or muscle hemorrhages with sterile saline and by applying gentle pressure (Fig. 1b).

6. Identify the stereotaxic landmarks - Bregma and Lambda, using the sutures on the skull surface. Validate the horizontality of the skull by confirming if the dorsoventral coordinates of Bregma and Lambda have a difference of less than 0.1mm. Adjust nose bar to reach "flat-skull" position (Fig. 1c)

- CRANIOTOMY:

7. Mark the outline of the craniotomy on the skull surface (e.g.: AP +/-2; ML +/-2).

We did not test the procedures at more posterior coordinates where the SSS is thicker, namely in the confluence of the SSS and the transverse sinuses. However, when carefully performed to cause minimal damage and preserve the surrounding vasculature, the procedure might be applicable in other sections of the SSS.

CRITICAL STEP: The craniotomy should extend +/- 2mm bilaterally from the midline to allow enough space for the sinus removal procedure.

8. The craniotomy should be opened by removing a single bone flap. Using a hand-held high speed drill with a small round tip bit, start drilling carefully the outlines of the craniotomy, taking special care when drilling over the sagittal sinus to avoid tears (Fig. 1d).

9. Lift the single bone piece using a double hand technique (Fig. 1e-f).

CRITICAL STEP: Due to particularly frequent dural attachments under the coronal suture, scrape the dura from the bone flap to preserve the integrity of the underlying vasculature.

Use forceps (Dumont #7 Forceps) on one hand to slowly lift the bone flap and a cauterizer with the smallest tip attached on the other hand to scrape the dura of the bone as it is gradually lifted.

TIP: Continuous irrigation of the craniotomy with sterile saline facilitates the pealing of the dura. When needed, apply gentle pressure to control small bleeding events.

- DUROTOMY:

10. Excise the dura mater at both sides of the sagittal sinus using precision-tipped forceps (Jeweler #5 Forceps) (Fig. 1g).
Open each durotomy at the lateral limit of the craniotomy, next to the bone, and extend it carefully along the anterior and posterior boundaries of the craniotomy until the border of the sagittal sinus. Control small bleeding events with saline and gentle pressure. In the rare event of a dural tear or damage to a cerebral vein, achieve hemostasis using gelatin sponge soaked in saline.

- Ligation and sectioning of the sinus:

11. Slowly lift and fold the bilateral dura flaps over the sinus with curved forceps. Elevate the attached sinus and thread a suture beneath it, using a vascular ligation instrument (Ligation Aid) threaded with approximately 10cm of 1.5 absorbable suture (Fig. 1h-i).
Use one hand to lift the sinus and the other to slide the tip of the instrument under the sinus, carefully avoiding cerebral veins. Once the instrument's tip is visible on the opposite site, lower the sinus and carefully thread the suture under it without removing the instrument, stabilizing it very carefully.

CRITICAL STEP: In order to promote a smooth sliding of the suture and avoid damaging the underside of the sinus, the thread should be covered in sterile silicone grease.

CRITICAL STEP: To prevent mechanical damage to the cortical surface, place pieces of gelatin sponge soaked in saline over the brain on each side of the sinus. Gently push the foam under the sinus.

CRITICAL STEP: Take particular care to cover the whole extent of the exposed cortex and to keep the craniotomy well irrigated.

12. Once a 2-3cm loop of suture is threaded, carefully retract the instrument while firmly holding the suture in place at the opposite side of the sinus (Fig. 1j).

13. Cut the loop in the middle leaving the suture in place, crossing underneath the sinus, with two strands of thread perpendicular to the sinus. Gently pull one strand towards the anterior limit of the craniotomy and the second towards the posterior limit (Fig. 1k-l).

14. Double ligate the one at the posterior border, followed shortly by the anterior one (Fig. 1m).

15. Lift the sinus, as described before, with care and gelatin sponge slid under the sinus. Gently lifting the sinus with one hand, cauterize the mid-point between sutures and extend the severed borders of the sinus posteriorly and anteriorly, thus exposing the longitudinal fissure (Fig. 1n).

CRITICAL STEP: It is noteworthy to state that at any stage of this procedure bleeding is likely. In some cases, extensive bleeding might even be expected, depending on the local vasculature of the animal. At such times, copious amounts of saline and applying gentle pressure over a piece of gelatin sponge resolve bleeding events.

16. After reestablishing hemostasis, clean the craniotomy from gelatin sponge pieces and remove excess moisture with a cotton-swab. Wipe dry the bone surrounding the craniotomy.

17. Cover the whole extension of the craniotomy with a single drop of 1.5% agarose at body temperature (~37ºC) (Fig. 1o).

CRITICAL STEP: After solidifying, the agarose lid will mechanically stabilize the remaining sinus edges and the suture knots left on them, while avoiding intracranial pressure increase and ventricular expansion.
Furthermore, it will prevent periosteum or fascia to grow over the brain surface.

18. Suture the skin incision, sterilize the wound and apply lidocaine analgesic ointment. Set isoflurane perfusion to 0% to exclusively deliver oxygen to the animal. Inject Atipamezole (1 mg/kg) intraperitoneally to revert anesthesia Inject 5 ml of Ringer Solution subcutaneously. When motion reflexes are regained, place the animal in a clean heated cage until fully recovered (Fig. 1p).