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SPARC bilateral phrenic neurophysiology preparation with phrenic afferent stimulation - spinal intact study V.1

Forked from a private protocol

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ABSTRACT

Protocol for conducting bilateral phrenic neurograms in anesthetized, paralyzed, vagotomized and mechanically ventilated rats to test the cardiorespiratory effect of phrenic afferent stimulation in spinal intact animals.

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FORK NOTE

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1 Isoflurane induction: 2.5-3% in 100% O2 in a closed chamber for 3-5 minutes

2 Transfer to nose cone and maintain at 3% in 100% 02.

3 Place intravenous line into tail vein for delivery of drugs/anesthesia/fluids (flush with heparinized saline). Reduce isoflurane anesthesia to 1%. Begin urethane infusion (1.8g/kg in ddH2O at 6ml/hr) while slowly decreasing isoflurane.Confirm adequate plane of anesthesia via toe pinch and transfer to surgical station.

4 Measure body temperature via rectal probe and maintain at 37.0 +/- 0.5 C via heated surgical table

5 Perform tracheostomy and initiate mechanical ventilation with the following parameters: rate = 70 breaths/minute; tidal volume = (0.7 x body weight (in grams)) mL; inspired gases: 50% O2 + a small percentage of CO2 added to gas mixture reach a target end-tidal CO2 to be maintained at ~ 45mmHg throughout the surgical procedure + balance N2

6 Perform bilateral vagotomy

7 Place femoral arterial line to monitor arterial blood pressure and withdraw periodic blood samples (pre-fill the catheter with heparinized saline and ensure no bubbles are present in the line)

7.1 make a 1/2 inch ventral incision just distal to the groin on the inner thigh to expose the femoral artery, vein and nerve

7.2 gently dissect the surrounding fascia to isolate the femoral artery

7.3 tie off the distal end of the femoral artery and place a loose suture at the proximal end. Using an alligator clamp, pull the suture to temporarily prevent blood flow at the proximal end of the femoral artery

7.4 make a small partial incision into the wall of the femoral artery and insert the arterial catheter into the proximal portion of the artery, carefully loosening the proximal suture to enable advancement of the catheter into the vessle (~1 cm into the proximal portion of the vessel.

7.5 tie the catheter into the vessel using the proximal suture and secure in place with additional sutures as needed

7.6 ensure no kinks in the vessel and open the stop cock at the end of the catheter to ensure unresisted flow into the catheter

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7.7 flush the catheter with heparinized saline and connect to the pressure transducer/amplifier to ensure adequate blood pressure readings

8 Isolate bilateral phrenic nerves using a dorsal approach:

8.1 Using a cautery, perform a ~ two inch incision just lateral to midline and medial to shoulder blade.

8.2 Using a cautery, dissect the dorsal musculature connecting the medial border of the scapulae to the posterior trunk on both sides.

8.3 Reflect the medial border of the scapulae and secure with an alligator clip.

8.4 Dissect the deep thoracic wall muscles and overlying fascia to expose the brachial plexus. Using suture, visulaize the tendon running horizontal above brachial plexus, pass 4.0 suture under tendon twice and place basket stich around tendon. Pull suture toward midline of animal and secure to open phrenic pocket.

8.5 Cut the brachial plexus distally and reflect the nerve bundle to expose the phrenic nerve

8.6 Using fine forceps, carefully isolate the phrenic nerve from the surrounding fascia without touching or pulling on the nerve

8.7 Once the nerve is fully isolated, section the nerve distally and carefully desheath the proximal end of the nerve (~1 mm). Add 0.9% saline to phrenic pocket.

8.8 Repeat to isolate right phrenic nerve.

9 In groups recieving a dorsal rhizotomy. Cut left C3, C4, C5, C6 dorsal rootlets

9.1 Using cautery, make a ~1inch medial incision from base of skull.

9.2 cut through underlying muscles to expose verterbral processes. Using cautery, seperate musculature from C2 vertebrae. Using microcurette remove muscles from C3-C6 vertebrae.

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9.3 Using ronguers, remove the left portion of C3-C6 vertebrae.

9.4 Using microscissors and fine forceps, cut and remove dura over C3-C6.

9.5 visuallize and cut all dorsal rootlets from C3-C6 with microscissors.

9.6 Add saline soaked cotton above spinal cord

10 Set up phrenic nerve recordings.

10.1 Place magentic base with L-shaped rod so that the rod is running lenth wise with the rat and above the midline.

10.2 Pull and wrap suture around both tendons around the rod.

10.3 Fill the phrenic cavity with 0.9% saline and suck the distal end of the proximal nerve stump into the suction electrode. The proximal end of the nerve sheath that was retracted in the prior step will form a tight seal with the tip of the suction electrode, preventing any leakage or loss of suction. The tip of the nerve should be visible within the tip of the electrode and both the inner wire and the desheathed portion of the nerve should be fully submerged in saline within the electrode

10.4 Carefully place the uninsulated tip of the outer wire against the proximal stump of the nerve proximal to the tip of the electrode and check recording for signal quality

11 Following completion of surgical procedure, administer fluids as follows: 4:1 ratio of Lactated Ringers Solution + Sodium Bicarbonate at 1.0 ml/hr

12 Administer pancuronium bromide (paralytic): 1g in 1cc delivered via tail vein over a 3 minute period

13 Set baseline CO_2parameters : Conducted no sooner than 20 minutes after pancuronium bromide delivery

13.1 Draw an initial blood sample to ensure base excess of +/- 3

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13.2 Hyperinflate the lungs over 2 respiratory cycles by briefly occluding the expiratory line

13.3 Wait at least 20 minutes before starting the experimental protocol - must have “stable” baseline activity for at least 15 minutes, defined as no trend increases/decreases in nerve activity greater than 5%

14 Take a blood sample during baseline separated by at least 5 minutes to establish baseline blood gas values: Criteria = PaO2 above 150 mmHg; SBE within +/- 3. Wait at least 5-minutes after blood sample before starting experimental protocol

15 Begin phrenic afferent stimulation protocol

15.1 Set inspiratory-triggered stimulation threshold: using contralateral integrated phrenic amplitude, set horizontal cursor in the middle of the phrenic amplitude.

15.2 Attach left phrenic electrode to stimulator. Right phrenic electrode is recorded.

15.3 Deliver first current. All stimulation is biphasic, inspiratory triggered stimulation using a wide pulse (each phase 1.0ms) or narrow pulse (each phase 0.1ms). Stimulation is delivered for 20seconds at pre-determined current. Stimulation is turned off and output is recorded for 5 minutes.

15.4 Repeat until all 12 currents have been delivered. All currents are randomly presented to each animal so that each current is delivered first in one animal.

15.5 Draw blood a sample after 4 currents and adjust inspired gas concentrations to keep PaCO2 within 2 mmHg of baseline and PaO_2must remain above 150 mmHg at all blood draws.

15.6 PaO_2must remain above 150 mmHg at all subsequent blood draws

16 At the end of the experiment, deliver hypercapnia 12% inspired CO2 for max response.

17 **Analysis:** Bilateral phrenic activity (amplitude and frequency), blood pressure (systolic, diastolic and mean arterial pressures) and heart rate are analyzed at baseline, and during and after each current and during hypercapnia.

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