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SPARC bilateral terminal phrenic neurophysiology preparation with moderate acute intermittent hypoxia

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Elisa Gonzalez-Rothi[1], Arash Tadjalli[1], Raphael Perim[1], Gordon Mitchell[1]

[1]University of Florida

SPARC
Tech. support email: **info@neuinfo.org**

Elisa Gonzalez-Rothi

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We use this protocol and it's working

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Abstract

Protocol for conducting bilateral phrenic neurograms in anesthetized, paralyzed, vagotomized and mechanically ventilated rats - moderate acute intermittent hypoxia-induced phrenic long-term facilitation protocol

Troubleshooting

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. Confirm adequate plane of anesthesia via toe pinch

3 Place intravenous line into tail vein for delivery of drugs/anesthesia/fluids (flush with heparinized saline)

4 Measure body temperature via rectal probe and maintain at 37.5 +/- 1 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 a hemostat, 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

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

8 once femoral line has been placed, begin conversion to urethane anesthesia

9 Perform conversion to urethane anesthesia:

9.1 prepare urethane solution: 2.1g in ddH2O to make up 10ml

9.2 deliver 10 ml/kg for a final dose of 2.1g/kg

9.3 deliver urethane solution via intravenous infusion at a rate of 6 ml/hr as isoflurane is reduced periodically over the course of 30 minutes

9.4 Throughout the influsion, closely monitor blood pressure responses, toe pinch and corneal blink reflex and adjust the withdrawl rate of isoflurane accordingly to maintain adequate plane of anesthesia

10 Isolate bilateral phrenic nerves using a dorsal approach:

10.1 Using a cautery, perform a ~ two inch dorsal midline incision from the base of the skull to the upper thoracic vertebrae.

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

10.3 Reflect the medial border of the scapulae and secure with an alligator clip or retractors.

10.4 Dissect the deep thoracic wall muscles and overlying fascia to expose the brachial plexus

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

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

10.7 Once the nerve is fully isolated, section the nerve distally and carefully desheath the proximal end of the nerve (~1 mm)

10.8 Fill the thoracic 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

10.9 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 urethane conversion, administer fluids as follows: 4:1 ratio of Lactated Ringers Solution + Sodium Bicarbonate at 1.5 ml/hr

12 Administer pancuronium bromide (paralytic) 10 minutes after urethane conversion complete: 1g in 1cc delivered via tail vein over a 3 minute period

13 Set baseline CO_2parameters (identify apneic/recruitment thresholds): Conducted no sooner than 20 minutes after pancuronium bromide delivery

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

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

13.3 Reduce inspired CO2 by 0.2% every 60 seconds until phrenic nerve burst activity ceases (apneic threshold)

13.4 Once nerve activity has ceased, it must remain absent for 1 minute

13.5 After 1 minute, increase inspired CO2 to 0.5% immediately and then increase by 0.2% every 30 seconds

13.6 Once phrenic nerve burst activity has resumed (recruitment threshold), set inspired CO2 at level which corresponds to an end-tidal CO2 reading of 2 to 3 mmHg above the recruitment threshold

13.7 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 two blood samples 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 Moderate Acute Intermittent Hypoxia Protocol:

15.1 Administer 3, 5-min hypoxic episodes (9-13% inspired O2)

15.2 Draw blood samples during each hypoxic episode (4 minutes after the start of the hypoxia) and titrate inspired gas concentrations to achieve the following criteria:PaO2 between 40 and 50-mmHg; PaCO2 within 2 mmHg of baseline

15.3 Adjust inspired O2 as needed during subsequent hypoxic episodes to reach target PaO2 values

16 Post Hypoxia Protocol:

16.1 Blood samples taken at 15, 30, 60 and 90 minutes post-hypoxia

16.2 PaO_2must remain above 150 mmHg at all subsequent blood draws

16.3 ·$PaCO_2$must remain +/- 1.5 mmHg relative to baseline (15 min and 30-min post episodic hypoxia are exceptions: CO_2only needs to be ± 2 mmHg of baseline value – **don’t take another blood sample if $CO_2$is outside this range, instead correct the $FICO_2$and/or pump rate and wait for next sample**)

16.4 If values are out of range, make adjustments as necessary to inspired CO_2 (or ventilation rate if needed), and repeat blood sample 5-min later

16.5 60 and 90 minutes are critical time points, take as many samples as necessary (5-min apart) to correct for the different variables

17 After obtaining a satisfactory 90 minute blood gas, perform a maximal chemoafferent reflex stimulation protocol: target = 90 mmHg end-tidal CO_2

18 At the end of the experiment, lidocaine the nerve trunk until phasic activity has ceased to assess any background electrical activity/noise

19 **Analysis:** Bilateral phrenic activity (amplitude and frequency), blood pressure (systolic, diastolic and mean arterial pressures) and heart rate are analuzed at baseline, H1, H2, H3, at 15, 30, 60 and 90 minutes post hypoxia, and during maximal chemoreceptor stimulation