

Oct 24, 2019

## Morris USF Lab protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.58vg9w6

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### Surgical Protocol

- 1 Methods were as previously described (Morris et al., 2010; Ott et al., 2012). Data were obtained from 13 adult cats of either sex. In some animals, a bilateral thoracotomy was performed. Prior to initiating the surgical protocol, atropine was injected to reduce mucus secretion in the airways. Dexamethasone was given to minimize brain stem swelling and prevent hypotension. Anesthesia induction and maintenance were with 5.0 and 1.0–3.0% isoflurane, respectively, mixed with medical grade air until decerebration. The trachea was intubated and catheters were placed in the femoral arteries and veins for intravenous administration of drugs and fluids and to monitor arterial blood pressure. Periodically, arterial blood was collected and analyzed for PO<sub>2</sub>, PCO<sub>2</sub>, pH, and HCO<sub>3</sub><sup>-</sup> concentrations; sodium bicarbonate solution (8.4%) was administered to correct metabolic acidosis as needed. Solutions of 6% Hetastarch or 5% Dextran in half-normal saline, 0.04–0.1% dopamine, and 0.075–0.3 mg/ml phenylephrine in lactated Ringer solution were administered intravenously as needed to maintain a mean blood pressure of at least 75 mmHg. To reduce bleeding during and following the decerebration process, both external carotid arteries were ligated caudal to the lingual artery branch. We performed an occipital craniotomy, a midcollicular transection and suction decerebration (Kirsten and St. John, 1978). The brainstem was exposed, and the pia mater removed for insertion of tungsten microelectrodes for measurement of neuron extracellular potentials. Immediately prior to the transection, an infusion of the neuromuscular blocker vecuronium bromide or pancuronium bromide was given and maintained to ensure that the subjects were paralyzed. Following decerebration, the isoflurane concentration was gradually reduced to zero. Following completion of all experimental protocols, an overdose of Euthasol was administered. Cardiac and respiratory activities were monitored until cessation.

### Nerve Isolation and Recording

- 2 The right hypoglossal (XII), left phrenic (Phr), left lumbar iliohypogastric (Lum), and right vagus (X) nerves were isolated from surrounding tissue and desheathed. In two subjects, the right recurrent laryngeal nerve (RLN) was used instead of the X nerve. To monitor and record efferent nerve activities, the XII nerve, Lum nerve, and X/RLN nerve were placed in coiled or hooked bipolar silver electrodes, covered with a combination of mineral oil and petroleum jelly, and wrapped in parafilm. The Phr nerve was floated in a pool of mineral oil in a neck pocket, sectioned and recorded in coiled or hooked bipolar silver electrodes. All nerve activity was amplified, full-wave rectified, low-pass filtered, and RC integrated ( $\tau = 200\text{--}500\text{ ms}$ ). Integrated nerve discharge activity was used to indicate phases of the respiratory cycle and stimulus effectiveness. Integrated nerve activity, tracheal pressure, end tidal CO<sub>2</sub>, and arterial blood pressure were monitored on a Grass polygraph and recorded digitally onto a hard disk drive for off-line analysis.

### Stimuli and Perturbations

- 3 Various stimuli and perturbations were applied.
- 3.1 Electrical stimulation of the superior laryngeal nerve(s) to elicit fictive swallows ( $n = 13$ ):

In all 13 subjects, the superior laryngeal nerves (SLNs) were isolated bilaterally; each was connected to a silver bipolar electrode and covered with a combination of mineral oil and petroleum jelly or a pledget soaked in mineral oil until the nerve was used for electrical stimulation. Fictive swallowing was evoked by electrical stimulation of the SLN (pulse duration, 0.1–0.25 ms; frequency, 5–22 Hz, Voltage 2.6–4.0 V, 33.3–51.5  $\mu\text{A}$ , train duration 2–120 s) and identified by changes in activities of the Phr, XII, and X/RLN nerves.

### 3.2 Water bolus administration to elicit fictive swallows (n = 13):

Water (5–25 mL) was rapidly injected (less than 5 s) through a polyethylene tube inserted into the mouth of each subject. A minimum of three water injection trials were performed with an inter-trial interval of at least 2 min.

### 3.3 Mechanical Stimulation to elicit fictive swallows (n = 5):

Insertion and deliberate movement of the water tube.

### 3.4 Stimulation of peripheral (n = 5) or central (n = 2) chemoreceptors:

Peripheral or central chemoreceptors were stimulated by 1 ml of CO<sub>2</sub>-saturated saline injected over a period of 30 s into the right carotid sinus or the vertebral artery, respectively.

### 3.5 Changes in mean arterial blood pressure (n = 7):

To produce a transient increase in blood pressure, the descending aorta was occluded with an embolectomy catheter for 30 s; mean arterial pressure was increased by at least 25 mmHg.

### 3.6 No- or delayed inflations (n = 10):

The ventilator was manipulated to delay or withhold lung inflation.

### 3.7 Esophageal balloon inflation to elicit fictive swallows (n = 1):

A balloon inserted into the esophagus was inflated.

### 3.8 Nebulized substances:


AITC was nebulized into the inspiratory line of the ventilator (n = 6). Compound 48/80 was also administered in 4 of the 6 subjects.

## Ventilation Mode Protocols

- Two modes of ventilation were used. In phrenic-triggered mode (PT), the integrated phrenic signal was used to trigger the ventilator to inflate the lungs and to allow passive deflation. There was a delay (mean duration:  $458 \pm 140$  ms) between the end of the inspiratory phase (Phr peak) and the end of lung inflation (TP peak). In free-run mode (FR), the ventilator rate was set to 30 breaths per minute with a gas flow rate adjusted to maintain arterial PCO<sub>2</sub> at  $30 \pm 0.5$  mmHg. In a protocol labeled "A mode," ventilator tidal volume was approximately doubled and the rate was approximately halved (adjustments to each were made to maintain a PCO<sub>2</sub> of 30–32 mmHg).

## Brainstem Neuron Recordings

- Extracellular neuronal activity was acquired from three or four multielectrode arrays with individually adjustable (submicrometer steps) high impedance tungsten microelectrodes. Electrode placement was guided by anatomical landmarks (obex, brain stem midline), appropriate stereotaxic coordinates derived from Berman (1968), and the results of previous studies (Connelly et al. 1992; Nuding et al. 2009b; Ott et al. 2012; Schwarzacher et al. 1995; Segers et al. 2008, 2015). Neurons in the vicinity of the dorsomedial medulla/nucleus of the solitary tract, the ventral respiratory column, the midline raphe nuclei and adjacent reticular formation, and the lateral tegmental field-parafacial region were recorded. Extracellular waveforms from single neurons were isolated using interactive spike sorting software (O'Connor et al. 2005).

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