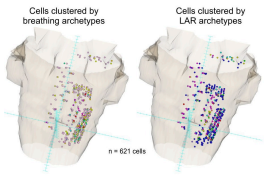
Feb 13, 2020 Version 2

# Morris USF Lab protocol V.2

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**We use this protocol and it's working**

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## Troubleshooting

## Surgical Protocol

1 Methods were as previously described (**Morris et al., 2010**; **Ott et al., 2012**). Data were obtained from 13 decerebrated, paralyzed, and artificially ventilated adult cats of either sex. 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 $PO_2$, $PCO_2$, pH, and $HCO_3$- 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 and 0.04–0.1% dopamine 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**). Immediately prior to the transection, an infusion of the neuromuscular blocker vecuronium bromide was given and maintained throughout the remainder of the experiment to ensure that subjects were paralyzed. Following decerebration, the isoflurane concentration was gradually reduced to zero. The brainstem was exposed, and the pia mater removed for insertion of tungsten microelectrodes for measurement of neuron extracellular potentials. 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 ($\tau$ = 200–500 ms). Integrated nerve discharge activity was used to indicate phases of the respiratory cycle and stimulus effectiveness. Integrated nerve activity, tracheal pressure, end tidal CO2, 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 µA, train duration 2–120 s) and identified by changes in activities of the Phr, XII, and X/RLN nerves.

3.2 Water bolus administation 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 CO2-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 adminstered in 4 of the 6 subjects.

## Ventilation Mode Protocols

4 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 ± 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 PCO2 at 30 ± 0.5 mmHg. In a protocol labeled "A mode," ventilator tidal volume was approximately doubled and the rate was aproximately halved (adjustments to each were made to maintain a PCO2 of 30-32 mmHg).

## Brainstem Neuron Recordings

5 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).