PO2 DEPENDENT CHANGES IN INTRINSIC AND EXTRINSIC TONGUE MUSCLE ACTIVITIES IN THE RAT.

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ONLINE DATA SUPPLEMENT

Animals and surgical procedures.

No procedures were performed until animals were unresponsive to paw-pinch with a hemostat. Rectal temperature was maintained at 37 $^{\circ}$C. At the conclusion of all experiments animals were euthanized by an intravenous dose of sodium pentobarbital.

A constant flow of gas was directed across the inlet of the tracheotomy tube via a 't-tube' and gas mixtures were delivered to the animal by connecting the outflow port of the rotameter to the t-tube. Inspired gas concentrations were monitored with CO_2 (Applied Electrochemistry, model CD-3A) and O_2 analyzers (Beckman, model OM-11). Arterial pH (pH_a) and partial pressures of arterial O_2 (PaO_2) and CO_2 ($PaCO_2$) were determined with a blood gas analyzer (Instrumentation Laboratories 1640).

Electromyogram (EMG) recordings. EMG recordings of the intrinsic tongue retractor muscle (SL) was obtained by inserting two recording electrodes (~2 mm apart) into the dorsum of the tongue in the midline, anterior to the premolar eminence, ~0.8 cm from the tongue tip. The extrinsic tongue retractor muscles (HG) were exposed (E1) and two electrodes were inserted into the belly of this muscle. Correct electrode placement was confirmed by connecting the inserted electrodes to a stimulator (Grass, model S48) via a stimulus isolation unit (Grass, model SIU7C). Current (0.1-0.15 mA) was passed through the wires (60Hz, 500ms) and the direction of tongue movement was observed to ensure tongue retraction with stimulation (E2). Alternating current differential amplifiers (Grass, model 7WU16K) were used to amplify and filter (30-3000 Hz bandwidth) the

EMG. Tongue muscle EMG activities were sampled at 10KHz with an A-D converter (CED, Spike2). The amplified and filtered EMG signals were rectified and moving-time averaged with a time constant of 200 ms. The processed EMG signal is referred to as the integrated EMG (iEMG).

Denervation Protocol. To confirm that intrinsic tongue muscle EMG activities were not contaminated by volume conductance of electrical activity from adjacent muscles, a three-step denervation procedure was conducted at the conclusion of each experiment. This method has been described in detail elsewhere (E3). Briefly, EMG recordings were obtained with intact hypoglossal nerves. Subsequently, the medial XII branches were sectioned bilaterally at the point of bifurcation of lateral and medial XII branches thereby eliminating tongue protrudor muscle activities (i.e., transversus, verticalis and genioglossus muscles). If the EMG activities did not change as a result of the transection the lateral XII branches subsequently were sectioned bilaterally, approximately 8 mm distal to the point of bifurcation of the medial and lateral XII branches. This section eliminated only intrinsic tongue retractor activities (i.e., superior and inferior longitudinal muscles) leaving extrinsic retractor activities intact. In the final step of the protocol both XII nerves were transected proximal to the bifurcation, thereby eliminating all remaining extrinsic tongue retractor activities (i.e., hyoglossus and styloglossus muscles). In this way, elimination of activity following nerve branch section allowed us to confidently conclude that electrode placement was optimal. All denervation protocols were conducted under maximal drive conditions (E4) (i.e., 9% CO_2, 30% O_2, balance N_2) so that any

change of volume conductance would be amplified. The denervation protocol was based upon a previously published work in the rat (E5) and an example of the denervation protocol is shown in Figure E1.

Data Analysis. Average rate of rise was calculated by dividing peak iEMG activity by the time to peak, and the result expressed as a percentage of the maximal activity. Temporal differences in the burst onset of extrinsic and intrinsic activities were determined relative to the onset of the downward (negative) deflection of the esophageal pressure record associated with each inspiratory effort. Measures of average EMG, rate or rise of EMG and burst onset time were obtained from a series of 20 consecutive breaths collected immediately prior to blood gas sampling, pre and post vagotomy.

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FIGURE LEGEND

Figure E1. Representative raw extrinsic (HG) and intrinsic (SL) EMG recordings, esophageal pressure (Pes) and blood pressure (BP) during progressive denervation of the hypoglossal nerves. Panel A shows post vagotomy activities in the hypoglossal intact preparation. Panel B shows activities after bilateral section of the medial XII branches eliminating all tongue protrudor activities (i.e., genioglossus, transversus and verticalis muscles). Panel C shows the effect of sectioning the distal portions of the lateral XII branches removing intrinsic retractor activities (i.e., superior longitudinal muscles). Panel D shows the elimination of both extrinsic and intrinsic activities following bilateral section of the whole hypoglossal nerves.

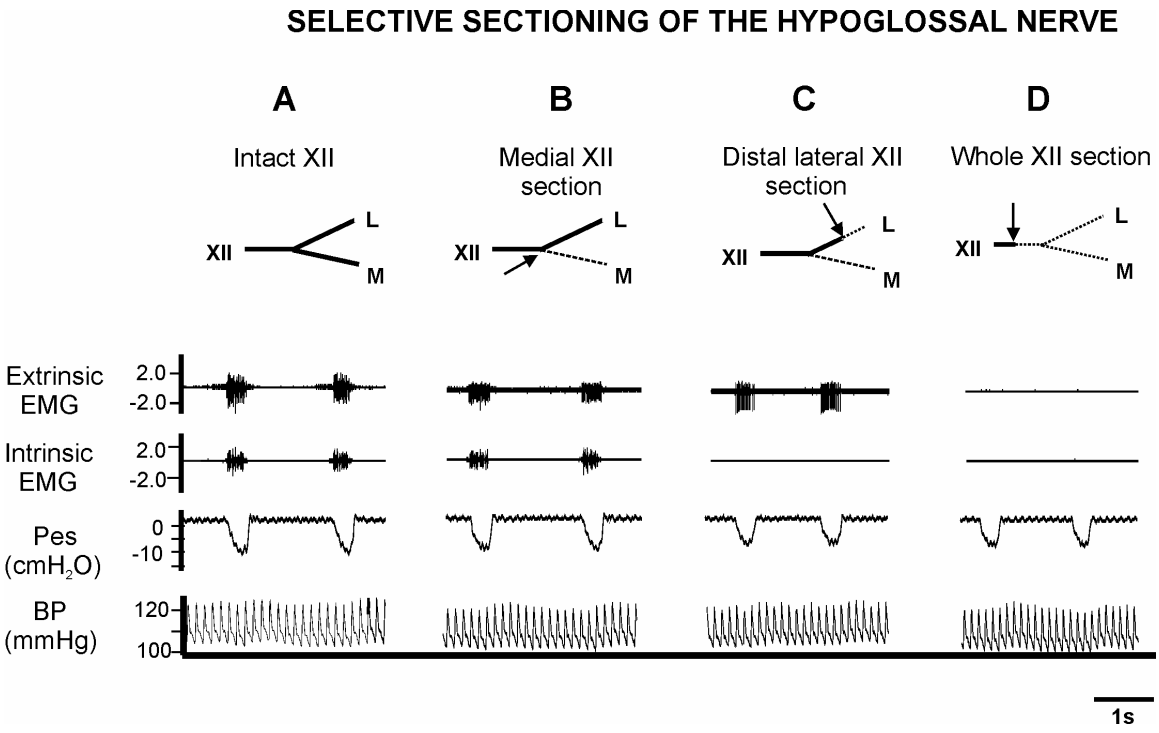
SELECTIVE SECTIONING OF THE HYPOGLOSSAL NERVE
A
B
C
D
Intact XII
Medial XII section
Distal lateral XII section
Whole XII section
XII
L
M
Extrinsic EMG
Intrinsic EMG
Pes (cmH2O)
BP (mmHg)
2.0
-2.0
0
-10
120
100
1s

Figure E1.