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# Effects of targeted activation of tongue muscles on oropharyngeal patency in the rat

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ABSTRACT

Laboratory rats were acutely implanted with an electrode array composed of eight independently controllable contacts applied to ventral and dorsal aspects of the left and right hypoglossal nerves (HGNs) and their branches. Bipolar intramuscular electromyographic (EMG) electrodes were implanted into the left and right genioglossus, hyoglossus and styloglossus muscles to identify which muscles were activated during stimulation via the contacts. Elicited movements, including changes in the position of the tongue and in the size and the shape of the airway, were documented video-graphically through a surgery microscope and an endoscope. Constant current electrical stimulation activated various combinations of electrode contacts and the stimulation patterns were correlated with corresponding oral movements, airway sizes, and EMG activities. Results demonstrate that graded responses and differential activation of the various tongue muscles are achievable by stimulation of specific contacts in the electrode array. These effects are interpreted to result from the targeted activation of regions of the nerve lying under and between the electrodes. Further testing established that the muscle responses elicited by unilateral electrical stimulation with the present approach can be smoothly graded, that the muscle responses resulted in opening of the airway and could be reliably maintained for long durations.


## 1. Introduction

Understanding the response of peripheral nerves and their targets to electrical stimulation is foundational for evaluating the prospects for using this technology to treat diseases resulting from neurogenic causes. One such disease, obstructive sleep apnea (OSA), involves airway blockage consequent to reduced lingual muscle tone likely associated with inadequate neural activation normally provided by the hypoglossal nerve (HGN) [1]. Previous attempts to stimulate lingual muscles with intramuscular electrodes to increase tone [2–7] or to stimulate the HGN [8–12], resulted in promising but variable results. In particular, while stimulation of the HGN has been shown to be more effective than muscular stimulation [13], previous attempts to stimulate the entire HGN lacked selectivity in stimulating the portion(s) of the HGN to specifically target muscle movements that reliably enlarge the airway. Moreover, wholesale electrical stimulation of the HGN or one of its two major distal branches is problematic, generating muscle fatigue. Such fatigue has required intermittent stimulation, e.g., timed to respiration in order to achieve a minimally useful clinical result, i.e., opening of the airway [8,9,12,14–21]. Additionally, most OSA neurostimulation therapies to date utilized stimulation with a constant voltage [22], without direct control of the amount of the current delivered to the nerve. Since all portions of the HGN or its branches were activated due to the single channel cuff electrode design, both desirable and undesirable tongue movements (e.g., retraction) were generated and without any fine control, particularly of the retractor muscles [23].

The development and success of a neurostimulation approach to activating the tongue and, as a desired outcome, to open the airway, require a clear, scientific rationale in the design of the stimulating electrode and a full understanding of the precise effects of different forms of electrical activation on the HGN neuromuscular system. Any activation of the system must account for the differential innervation of lingual muscles by branches of the HGN, and the fact that while some lingual muscles function to open the airway, some do not. Indeed, based on the attachments of individual lingual muscles, some protrude the tongue, some flatten it within the oral cavity, and some pull it back into the airway [24]. The different muscles are differentially innervated by two main HGN branches. The medial branch innervates muscles generally regarded as protrusive, e.g., the genioglossus; the lateral branch innervate muscles thought to retrude the tongue, e.g., the styloglossus [25–27]. The HGN branches are represented centrally by a differential topographic arrangement of motor neurons in the hypoglossal nucleus in the medulla [28]. Correspondingly, therefore, the HGN is heterogeneous, containing axons that differ in their central origin and in their muscular targets. The present study in rats tests whether different portions of the HGN can be activated by an electrode array to affect

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different and distinct tongue movements and, by means of those stimulation-elicited movements, to reliably and reproducibly enlarge the airway.

The goal of the present study was to determine whether a multi-contact electrode array, each contact of which is driven by an independent current source, would produce distinct tongue movements that, in turn, act to improve airway patency as evidenced by the visual observation of tongue movements and oropharynx cross-sectional area (airway) changes. The experimental paradigm used for this proof-of-principle study was, therefore, to document whether specific electrode contacts within an array could selectively activate different regions of the HGN and elicit distinct tongue movements and corresponding changes in the size of the airway. The present study further evaluated whether the energy requirements to elicit such movements were within the range of a clinically applicable stimulation system, and whether the response to such stimulation could be maintained over a sufficient period of time to offer prospects for clinical benefit without muscle fatigue. Essentially, this animal study tests the hypothesis that selective activation of small subsets of the HGN with minimal electrical current pulses will result in distinct lingual movements that open the airway. The study, therefore, evaluates a potentially applicable OSA therapeutic approach.

## 2. Material & methods

### 2.1. Animal model

Thirty-seven male Sprague–Dawley rats (Charles River Laboratories International, Inc., Wilmington, MA), weighing from 300 to 450 g, aged 8–16 weeks, were used for the study. For the fatigue portion of the study, male Zucker rats weighing approximately 900 g (Charles River Laboratories International) were used. All laboratory procedures were approved by the University of California at San Diego's Laboratory Animal Care and Use Committee and followed the NIH Guide for the care and use of laboratory animals.

### 2.2. Acute surgery

Sprague–Dawley rats were calmed with Isoflurane gas before an IP injection of pentobarbital (Nembutal; 50 mg/kg) and allowed to reach a state of surgical anesthesia. The maxillae of the animals were secured into a non-traumatic head-holder while the animals were laid supine on a temperature controlled surgical platform. A ventral midline incision was made from the sternum to the mental region of the jaw. The digastric and mylohyoid muscles were reflected bilaterally (or unilaterally) and the HGNs were exposed [29]. Two micromanipulators were used to support stainless steel hooks to gently lift the left and right HGNs (proximal to their bifurcation below the hyoid bone) above the surrounding tissue. Additionally two more micromanipulators were used to position eight contact platinum–iridium electrode arrays (described below) against ventral aspects or dorsal aspects (cut, lifted and flipped over) of the left and right HGNs. Similarly the medial branches of HGNs (mHNs) were exposed. The surgical site was covered with mineral oil to protect it from drying and to prevent stray currents during stimulation (stimulus artifact control). Bipolar wire electrodes were inserted into the bellies of the left and right genioglossus (LGG & RGG), styloglossus (LSG & RSG), and hyoglossus (LHG & RHG) muscles. For video graphic (endoscopic or microscopic) recordings of the front and the back of the tongue, the lower mandible was lifted upward (ventrally, anatomically) exposing the oral cavity (using a rubber sling hooked onto the two lower incisors). The thin probe of an endoscope was gently lowered into the back of the throat and placed over the soft palate with its viewing-end (angled at 30°) facing upward and toward the laryngopharynx airway. The zoom lens of the video-scope was pointed toward the oral cavity capturing the anterior two-thirds of the tongue. The whole setup (Fig. 1A) was mounted on a vibration free surgical platform.

### 2.3. Stimulation electrodes

The stimulation electrodes consisted of 8 contact electrode arrays (Fig. 1B). The electrode tip was fabricated by securing two 4-wire platinum–iridium ribbon cables (Temp-Flex; 25 μm wire ribbon cables) with their eight metal tips (contacts) de-insulated and exposed. Care was taken to carve the exposed contact of the electrode tip into a semi-circular shape so that each array touched one complete half of the circumference of the nerve. In other words, two such electrode arrays sampled the entire nerve circumference by dividing HGN into 16 equal but independent stimulation sectors. The other end of the ribbon cables was attached to a small printed circuit board forming an edge connector. This edge connector in turn was plugged to an interface board, which subsequently was terminated with a DB-9 connector. This arrangement allowed the test animal to be located at a convenient distance from the stimulation pulse generator using standard shielded DB-9 cables (2–3 ft long).

### 2.4. Stimulation equipment

An eight channel laboratory stimulator (World Precision Instruments Model DS8000) was connected to the patch panels and allowed direct application of current controlled stimulation pulses to be applied to any combination of electrode contacts. The DB-9 connector from the interface board attached to a custom patch panel and allowed any of the eight stimulation contacts to be assigned to any of eight current sources. A second patch panel, interface board, and cabling system enabled a like arrangement for the second HGN electrode array (Fig. 1A).

### 2.5. Stimulation pulse

The stimulation pulse waveforms consisted of asymmetrical biphasic constant current pulses. A cathodic phase was applied first, followed by an equal area but opposite amplitude anodic phase, the cathodic phase lasting 200 μs and the anodic phase lasting 800 μs. Stimulation frequency was 3 Hz for determining thresholds and 50 to 100 Hz for monitoring the results of the various stimulation methods.

### 2.6. Intramuscular electromyogram (EMG)

Bipolar EMG electrodes were fabricated from a twisted pair of 25 micron ETFE insulated multiphase nickel alloy drawn-filled-tube silver core wires (35 N LT DFT 33 Ag/ETFE natural — Fort Wayne Metals). The proximal ends of two 10 inch long twisted wires were de-insulated and were crimped into gold connecting pins while the distal ends were fashioned into short parallel hooks after loading into the barrel of a 30G hypodermic needle. Using the needle as a guide, the electrode was inserted into various tongue muscles. The hypodermic needle was then slowly withdrawn leaving behind the wire hooked into muscle fibers. The distal ends of the paired wires inserted into the muscle resulted in a very small cross sectional area of exposed metal contacts in close proximity to each other and to the desired muscle, resulting in high electrode impedance, providing very good muscle selectivity and common mode rejection of distant signals.

The EMG electrodes were connected to interface boards nearby the animal preparation (Fig. 1). The interface boards were connected to a sixteen channel EMG amplifier (A-M Systems Model 3500). The amplifier channels were set to band pass signals from 50 to 3000 Hz with a gain of 50. The interconnecting cables were connected to independent differential input EMG amplifier channels. All of the sixteen amplifier outputs were available on a single DB-25 connector which was attached to a National Instruments SCB-68 interface box, which was then connected to a National Instruments NI-USB-6251 1.25 MS/s 16 bit 16