

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.

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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 innervates muscles thought to retract 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 μ m 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 μ s and the anodic phase lasting 800 μ s. 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

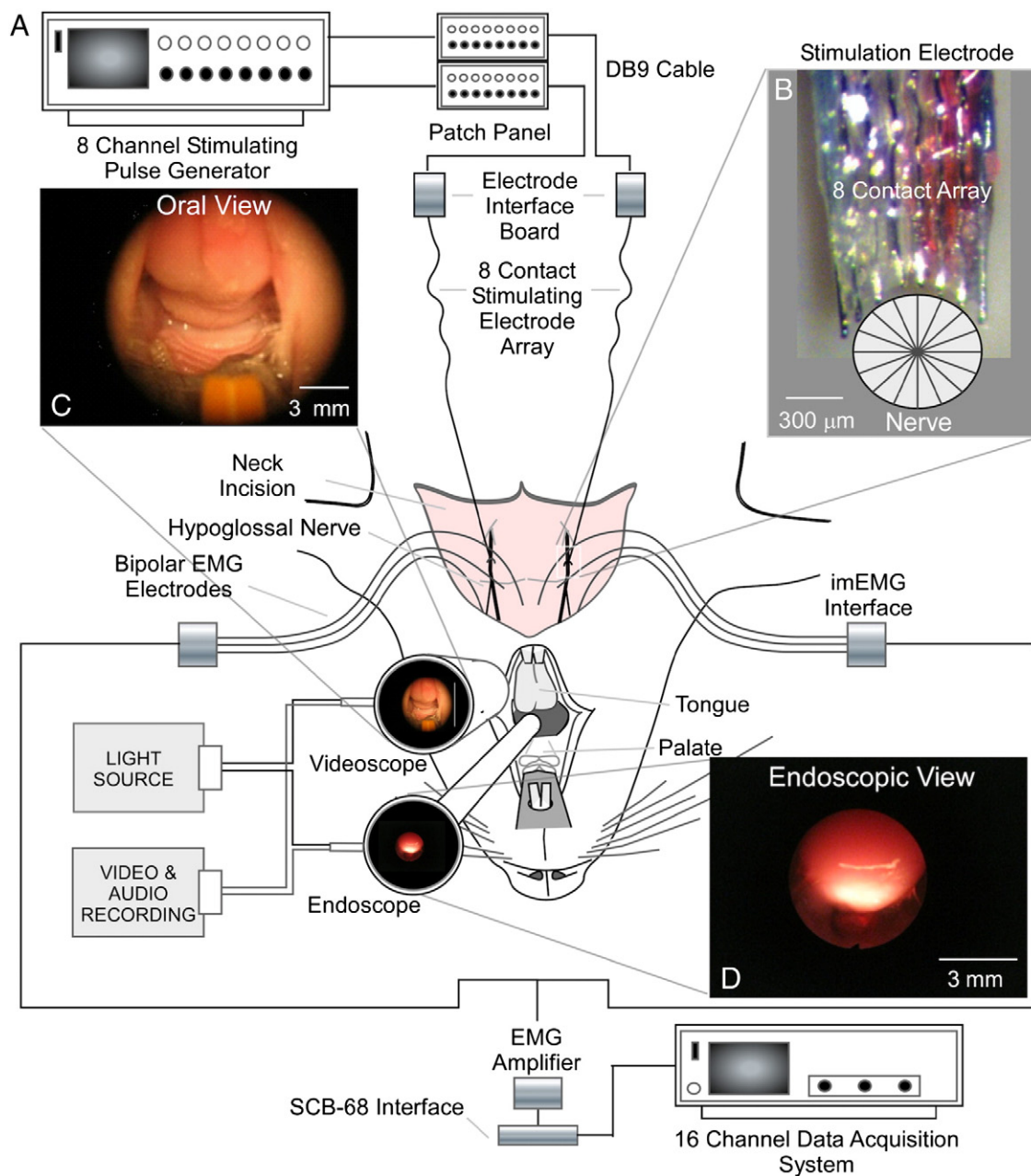


Fig. 1. Schematic illustration of the experimental setup: The anesthetized rat was positioned supine with its mouth opened using a non-traumatic head holder and elastic ties, and its HGNs exposed surgically. An 8-channel stimulator (A) was connected to a patch panel and eight contact electrode array (B) via an interface board and was used to stimulate the HGN. EMG signals from three pairs of extrinsic muscles were amplified and captured with a 16-channel data acquisition system. Magnified images and movies of the oral (C) and oropharyngeal (D) airways were captured and recorded using an endoscope and video scope attached to a light source. Same setup was used for all experiments (see Materials & methods (Section 2) for more details).

channel data acquisition system (DAQ). The EMG signals were sampled at 25 kHz on each channel by the DAQ. Samples were triggered by the stimulator pulse leading edge and 500-point digital records were collected per channel. Signal Express laboratory signal capture software (National Instruments) running on Windows XP Pro on a Mac Book Pro computer was used to capture and display the EMG signals.

2.7. Data collection

Tongue movements and changes in the airway opening were observed using a surgical microscope (Zeiss Model OPMI-1-FC) and a rigid arm endoscope (Karl Storz Model 7217BA and Olympus Model A70942A) attached to the flexible arm of the fiber optic light source

from FOSTEC (Schott-Fostec, LLC Model LR92240). Still and moving images were recorded using a digital camera with 10× zoom (Canon Power Shot SD600; Fig. 1C, D). Video graphic (endoscopic or microscopic) images in all cases were collected over three trials of stimulation ($n = 3$) (see Data analysis, below). Characteristic frames (corresponding to each motion and resultant airway size) were imported into KM Player image management software, the area of the left and right airway openings (between the soft palate & the tongue base, also referred as “oral” or between the dorsal pharyngeal wall and the rear tongue, also referred as “oropharyngeal”) were outlined using Macromedia Fireworks, and areas calculated using ImageJ, then averaged and normalized (relative to their respective unstimulated values) to obtain net changes.

2.8. Data analysis

In all cases (Figs. 2–10), except for the EMG experiment (Fig. 11), the data collected for statistical analysis were still images of the airway captured 5 s before and 5 s after the onset of stimulation of each contact on the HGN. The stimulus duration at each contact was 10 s with 10 s rest between stimulus pulses. Each figure in the Results section presents data from a different experiment, i.e., where the stimulus paradigm or the method of video capture, microscopic or endoscopic, was varied (see legends, Figs. 2–10). Each figure depicts the results from a single, representative rat; 1–3 rats were assessed per experiment. Sizes of the airway were measured during stimulation of each contact and at rest (unstimulated). The quantitative data presented as histograms in Figs. 2–7, 9, and 10 are means of the airway size during stimulation of each contact averaged over 3 trials in a single case, allowing 1–2 min of complete recovery between trials. The SDs of the average sizes of the airway during stimulation across the 3 trials indicate that the means and histogram profiles are reproducible and had minimal dispersion.

3. Results

3.1. Targeted stimulation of the HGN — effects on the oral and oropharyngeal airways

Differential activation of the HGN, unilaterally, by selective stimulation of individual contacts in the multi-contact electrode assembly resulted in distinct tongue movements and changes in the size of the airway. Thresholds for neuromuscular activation were determined by

observing the start of the noticeable movements (twitches) in the tongue while the HGN was initially stimulated at 3 Hz. Current amplitude was slowly increased from the sub-threshold levels until twitching motions of the tongue could be readily observed. Stimulation thresholds ranged from 10 to 180 μ A. Maximal stimulation levels were determined by gradually increasing the current amplitude beyond the threshold levels at 50 to 100 Hz until the tongue motion changed into another movement, indicative of a secondary muscle group joining the response, or when no further tongue movement was noted. Stimulation frequency ranged from 50 to 100 Hz. Maximal stimulation levels were typically 3 to 4 times of threshold levels, but could be as high as 2.4 mA. These threshold current levels were noted for each of the eight stimulating contacts, one after the other.

The electrode array, applied to dorsal and ventral halves of the nerve allowed activation of sixteen contact points, each contacting 1/16th of the surface of the HGN (Fig. 1B) and the elicitation of corresponding motor effects. A representative case is shown in Fig. 2. Stimulating each of 8 contacts covering the ventral half of the nerve resulted in tongue movements evident as distinct, instantaneous changes in tongue shape, variable in degree depending on the activated contact, and most pronounced ipsilateral to the stimulated nerve. The stimulation-elicited lingual movement affected the size of the oral and oropharyngeal airways as evaluated through a surgery microscope and endoscope (Fig. 1C, D), respectively. These changes were minimal for many of the contacts but large for some. The surgery microscope evaluated the oral airway, defined as the space between the soft palate and the dorsal surface of the posterior tongue. The endoscope evaluated the oropharyngeal airway, defined as the area between the postero-dorsal oropharyngeal wall and the dorsum of the posterior tongue base, i.e., the more

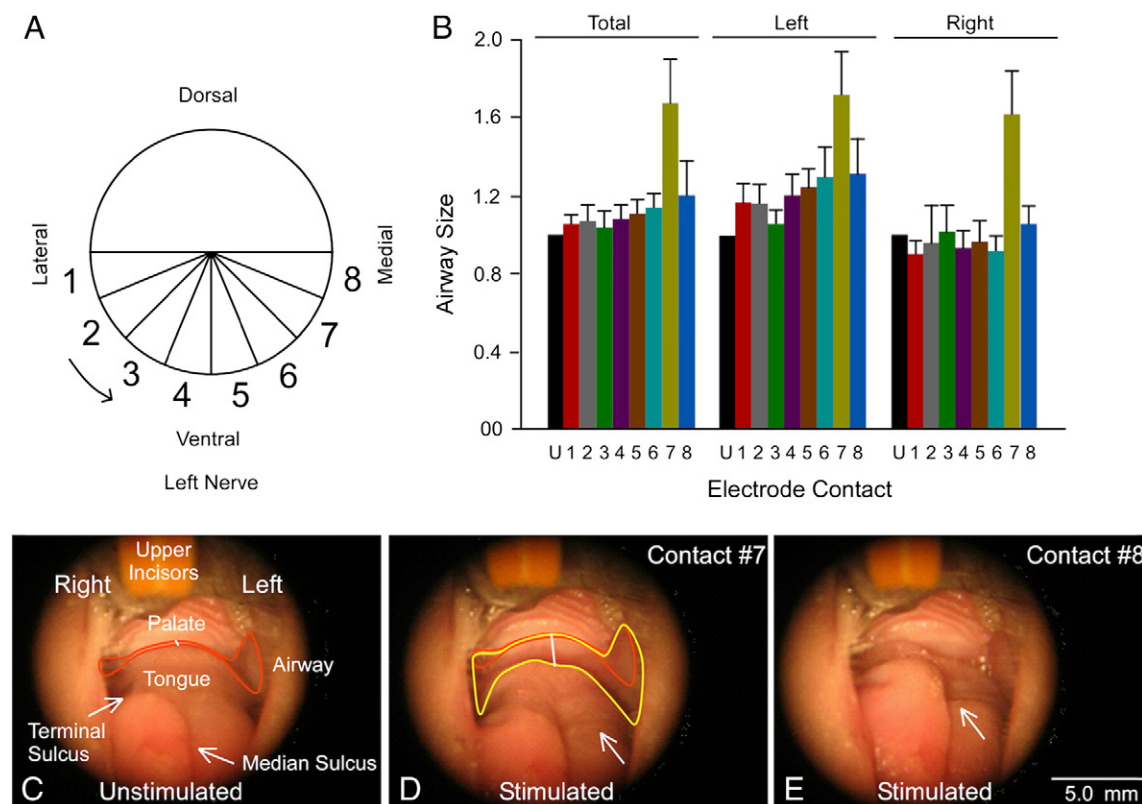


Fig. 2. Selective stimulation of the HGN: Effects on the oral airway. A: Separate populations of HGN motor axons (1/16th HGN) were selectively stimulated with the present multi-contact electrode, in this case applied to the ventral half of the left nerve. B: Sequential activation of contacts 1–8 resulted in opening of the airway, bilaterally, that was most evident for contact 7 (olive bar in histogram; black bars (U) = no stimulation). The opening of the airway, resulting from tongue movement (C–E), was recorded and quantified (B). The area of the left and the right oral airway opening, between the hard palate and the posterior tongue, were marked (yellow in D), summed, averaged, and normalized relative to their unstimulated values (red in C, D) to document net changes. Also observed were topographic changes in the surface of the anterior tongue ipsilateral to activation indicated by arrows in D, E compared the tongue surface (in C). Such surface changes were recorded but not quantified. Error bars in B represent standard deviations (SD) for each experimental trial ($n = 3$). Arrows indicate terminal sulcus and median sulcus. The white vertical lines in C & D indicate the airway midline and the reference point for calculating airway increases on either side.

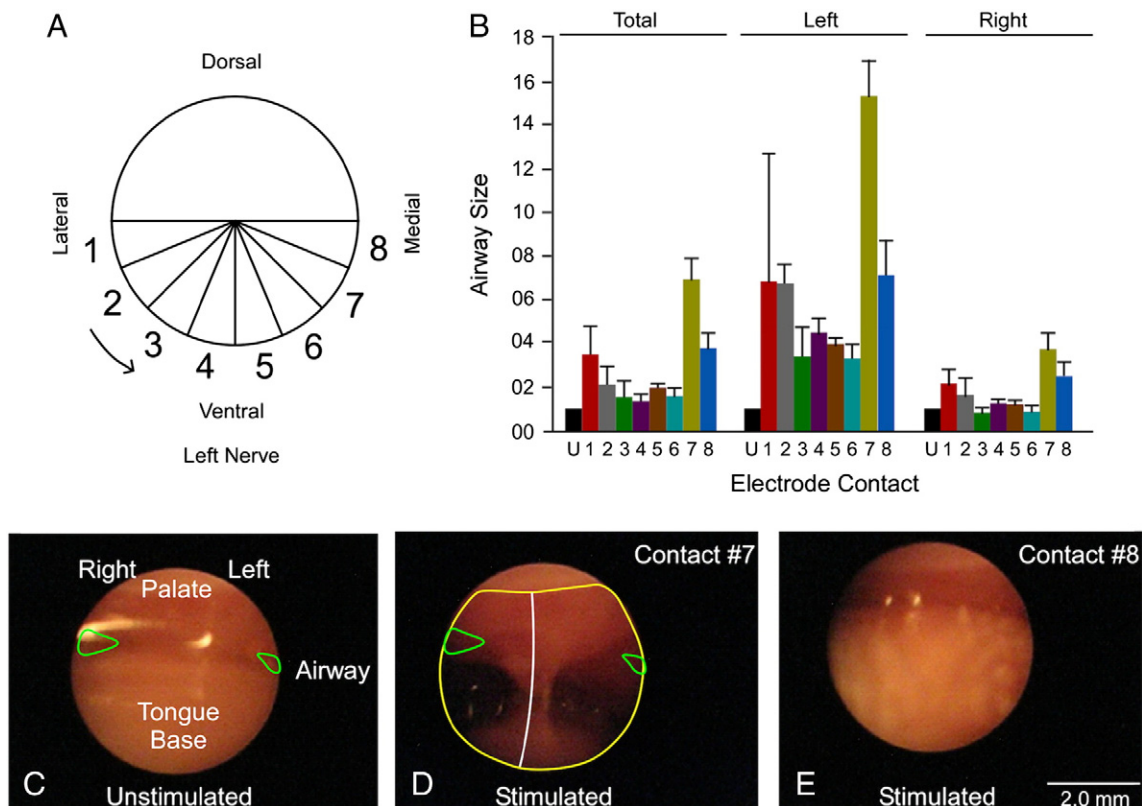


Fig. 3. Selective stimulation of the HGN: Effects on the oropharyngeal airway: Separate populations of HGN motor axons (1/16th HGN) were selectively stimulated with the present multi-contact electrode applied to the ventral surface of the left nerve (A). This is the same case as that in Fig. 2B. Sequential activation of contacts 1–8 resulted in opening of the oropharyngeal airway, bilaterally, that was most pronounced ipsilaterally for most contacts, especially contact 7 (olive in B), as for the oral airway (Fig. 2) and, to a lesser degree, contacts 1, 2 and 8. The area of the left and the right oropharyngeal airway opening (C–E), between the soft palate and the tongue base, were marked (yellow in D), summed, averaged, and normalized relative to their unstimulated values (green in C, D) to document net changes. White vertical line in D, midline of opened airway. Error bars in B represent standard deviations (SD) for each experimental trial ($n = 3$). Black bars (U) = no stimulation.

proximal, deeper space than the oral airway. Thus, for example, differential activation of electrode contact 7 resulted in a 1.7-fold increase in oral airway opening compared to the unstimulated oral airway (Fig. 2). Remarkably, a similar increase (1.6-fold) was observed on the side of the airway contralateral to the stimulated nerve. Moreover, in the same preparation, activation of the same contacts (7) resulted in a dramatic increase (15-fold) in the size of the ipsilateral oropharyngeal airway, a 4-fold increase in the contralateral airway and a total of 7-fold increase bilaterally, as documented with an endoscope (Fig. 3). Thus, while contacts 1, 2, 7 and 8 were, in contrast to the other contacts, effective in opening the airway ipsilateral to the stimulated nerve, a significant effect was also observed contralaterally in both oral and oropharyngeal airways (Figs. 2B, 3B).

In a second case (Figs. 4, 5), more numerous contacts were tested for their effectiveness in opening the airway compared with the previous case. The effect was dominant ipsilaterally to a greater extent (maximum 6-fold in the oral and 11-fold in the oropharynx). Contacts 1, 2, 3, 5, 6 and 8 were effective in opening the oral airway (Fig. 4B; >3-fold) as well as the oropharyngeal airway (Fig. 5B; >10-fold). Also observed were unique topographic changes in the surface of the tongue (mid-line deviation, depression, elevation, ripples, etc.; arrow in Fig. 4F). In this case, the segments of the HGN that, when activated, were most effective in the opening of both oral and oropharyngeal airways, were at contacts 1, 2, and 3.

3.2. Unilateral/bilateral stimulation of the HGN and its medial branch – effects on the oral and oropharyngeal airways

Stimulating a single effective contact, i.e., activating 1/16th of the HGN (or its mHN), resulted in bilateral opening of the airway despite

the fact that the activation was applied to a single nerve, unilaterally (Fig. 6). Activating the most effective single contacts on both nerves resulted in opening of the airway that was generally greater than the same activation restricted to a single nerve. The oropharyngeal airway, compared to the oral airway, showed a greater increase in diameter, which was, in some instances, 10 fold (Fig. 6G) that in the unstimulated condition.

3.3. Co-activation of adjacent contacts – titration of differential effects on tongue movements

To test how lingual movement is affected by simultaneous, graded activation of two closely spaced HGN segments, adjacent contacts were stimulated with varying current strengths (Fig. 7). For example, two adjacent (1/16th portion of the HGN) contacts (A and B) with similar threshold values were simultaneously stimulated either by increasing the current in a stepwise fashion (12 steps; 10 μ A interval) from a sub-threshold level (30 μ A) to a supra-threshold level (140 μ A), or by decreasing current from a supra-threshold level to a sub-threshold level (Fig. 7B, C). Activating these two contacts above threshold elicited distinctly different movements. Contact A activation resulted in “depression and flattening of the rear tongue” (Fig. 7, compare D and E, blue line). Contact B activation generated “ripples in the anterior 2/3 of the tongue” (Fig. 7G, black arrows inside the box). In order to characterize any differential effects on tongue movement elicited by activation of the two nerve segments they were simultaneously stimulated in a graded fashion, but while an increasing current gradient was applied to one of the contacts, a decreasing gradient was applied to the other contact. Visual inspection and photographic

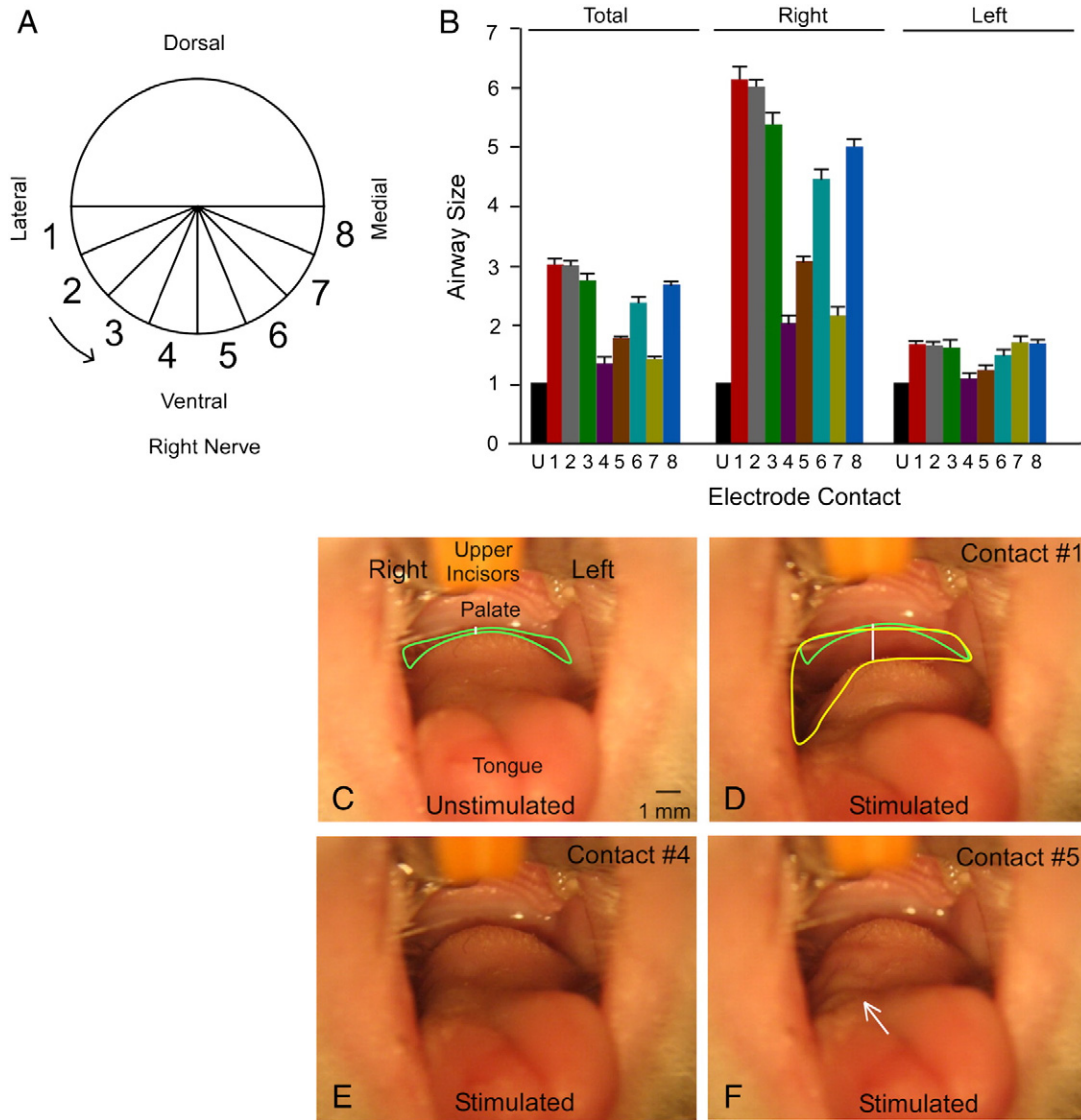


Fig. 4. Selective stimulation of the HGN enlarges the oral airway: Small portions (1/16th) of the HGN stimulated with the multi-contact electrode assembly elicited opening of the airway that was greater for some contacts than others. In this example, an eight contact array was applied to the ventral half of the right HGN for successive activation of contacts 1 to 8, from lateral to medial (A). B: Increases in the airway, measured as the area bordered by the soft palate, rear tongue, and pharyngeal walls, were normalized relative to their unstimulated values (U, black bars). Error bars in B represent standard deviations (SD) for each experimental trial ($n = 3$). Airway changes were different from contact to contact. Contacts 1, 2, 3, 5, 6 and 8 were the most effective in opening the entire (total) airway (>3 fold vs. unstimulated). Increases were not only greatest in the ipsilateral to the stimulated nerve (right, >6 fold in contacts 1 and 2), but also evident on the contralateral airway side (left). C: Video-microscopic image of the airway in the unstimulated condition (green line). D: Dramatically enlarged airway during activation of contact 1 (yellow outline). E: Slightly enlarged airway during activation of contact 4. Also observed were topographic changes in the surface of the anterior tongue ipsilateral to activation indicated by white arrow in F compared the smooth tongue surface (in C).

documentation determined the corresponding onset and plateau or cessation of the two movements.

The contact B-elicited movement, “ripples in the anterior 2/3 of the tongue”, with increasing current, starts to appear at current step 7 (90 μ A) and plateaus at step 11 (130 μ A) (Fig. 7B, black curve). At the same time, the contact A-elicited movement “depression and flattening of the rear tongue”, with decreasing current from an initial supra-threshold strength, disappears at step 10 (50 μ A) (Fig. 7B, orange curve).

Sequential photography during this experiment documented that the initial maximum “depression and flattening of the rear tongue” movement at supra-threshold activation of contact A (Fig. 7E, blue line), subsequently, with decreasing activation, declines, shrinking the oral airway (Fig. 7F, red line), eventually reaching a minimum comparable to the unstimulated condition (Fig. 7G, green line). Simultaneously, increasing the activation of contact B elicited the onset of “ripples in the anterior 2/3 of the tongue” that became most evident as the declining

movement “depression and flattening of the rear tongue” ceased (Fig. 7G, gray arrows and box). In the reverse experiment, “ripples in anterior 2/3 of the tongue” disappear at step 8 (70 μ A) while “depression and flattening of the rear tongue” increase and plateau at step 11 (130 μ A).

3.4. Fatigability test: chronic stimulation effects of the HGN in the Zucker rat

The Zucker rat, typically weighing more than twice the weight of a wild type rat, a genetic model for research on obesity [30] was used to test the time course of lingual muscle fatigue with continuous stimulation of the HGN. The rat was placed in the supine position to mimic the sleeping position that, in humans, most threatens airway closure, i.e., when the tongue loses tone and “falls” by gravity into the oropharyngeal airway. Constant supra-threshold stimulation (350 μ A at 100 Hz) of one contact on both the right and left HGNs resulted in a

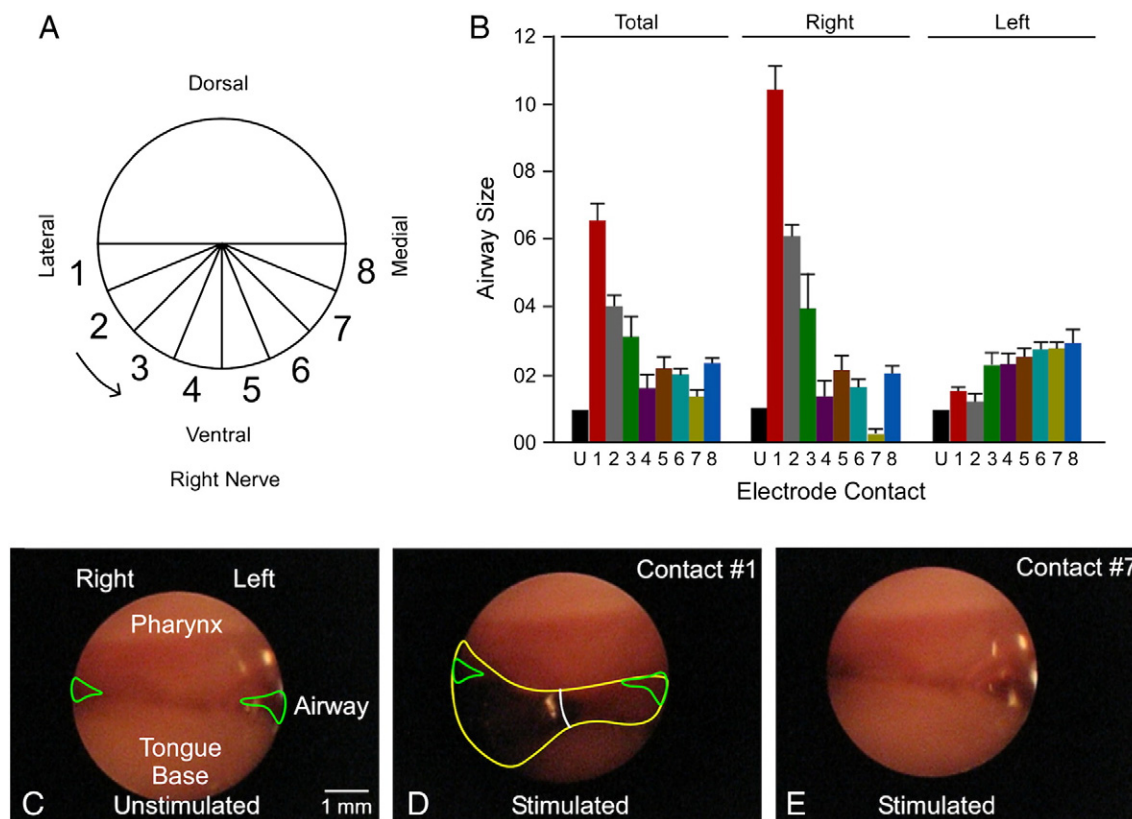


Fig. 5. Selective stimulation of the HGN enlarges the oropharyngeal airway: Eight contacts (1–8) applied to the ventral half of the right HGN were successively activated (A). B: Increases in the oropharyngeal airway diameter, measured from endoscopic images as the area bordered by the pharynx, tongue base, and pharyngeal walls, were normalized relative to their unstimulated values (U, black bars). Error bars in B represent standard deviations (SD) for each experimental trial ($n = 3$). Contacts 1, 2, and 3 were the most effective in opening the entire (total) airway (>6 fold in contact 1 vs. unstimulated) and its right hand side (RHS; >10 fold in contact 1). Increases were also evident, but of lesser magnitude, on the contralateral airway side (LHS, left hand side); contacts 3 thru 8. C: Endoscopic image of the airway in the unstimulated condition (green outlines). D: Dramatically enlarged airway during activation of contact 1 (yellow outline). E: Minimally enlarged airway during activation of contact 7. The white vertical line in D indicates the airway midline.

gradual but slow decrease in the size of the oral airway (Fig. 8). Shrinkage of the airway was documented photographically, occurring over a time course of 155 min (Fig. 8B–D). Measurements of the right and left average airway diameters quantify the slow decline to the small airway of the resting, unstimulated tongue ($T_{1/2} = 65$ to 70 min) (Fig. 8F).

3.5. Selective stimulation of the medial branch of the HGN vs. the entire HGN – effects on the oral and oropharyngeal airways

The multi-contact electrode was applied more distally than in the previous experiments and localized unilaterally to a major subset (approximately half) of the HGN, i.e., its medial branch (mHN), known to innervate the genioglossus muscle, intrinsic muscles and geniohyoid muscle of the tongue [25–27]. Each of the 8 contacts was activated separately, and all elicited a tongue movement that opened the oropharyngeal airway (Fig. 9). The degree of airway opening differed from contact to contact, but each of them at least doubled the airway size. While all segments of the nerve elicited lingual movements that widened the airway, contacts 2, 3 and 4 were most effective. Moreover, while the effect was most pronounced in the airway ipsilateral to the stimulated HGN branch where the airway opening reached a four-fold increase (Fig. 9B, D) over the resting, unstimulated condition, the airway contralateral to stimulation also opened the airway but to a lesser extent (two-fold increase). The same contacts that maximally opened the ipsilateral airway were the ones maximally effective in opening the contralateral airway.

Since activating each contact, with the electrode applied to one-half of the mHN branch, was effective in opening the airway, albeit to greater or lesser degrees, simultaneous activation of all 8 contacts evaluated the effect of topographically maximal stimulation of the entire electrode assembly on airway size. The rationale for this test is that wholesale stimulation of the medial nerve branch would include activation of the nerve subsets targeted by each contact of the multi-contact electrode in the previous experiment. The corresponding hypothesis is that bulk nerve branch stimulation would be at least as effective in opening the airway as the most effective single contact. Indeed, as with single contact stimulation, stimulating the entire half of the nerve resulted in tongue movement and airway opening that was most pronounced on the ipsilateral side, but was also evident contralaterally (Fig. 10). In general, the magnitude of the airway increases effected by stimulating the entire 8-contact electrode was comparable to, and only slightly greater than, those effected by stimulating the single most effective contact (compare Figs. 9, 10).

3.6. Identification of extrinsic muscles of the tongue via EMG recordings

The differential effect on tongue movements elicited by stimulating portions of the HGN presumably resulted from selective activation of some but not all of the various muscles that comprise the tongue. To demonstrate directly the selective activation of specific muscles by single contact stimulation of 1/16th of the HGN, EMG recordings were obtained from three extrinsic muscles i.e. the genioglossus, hyoglossus,

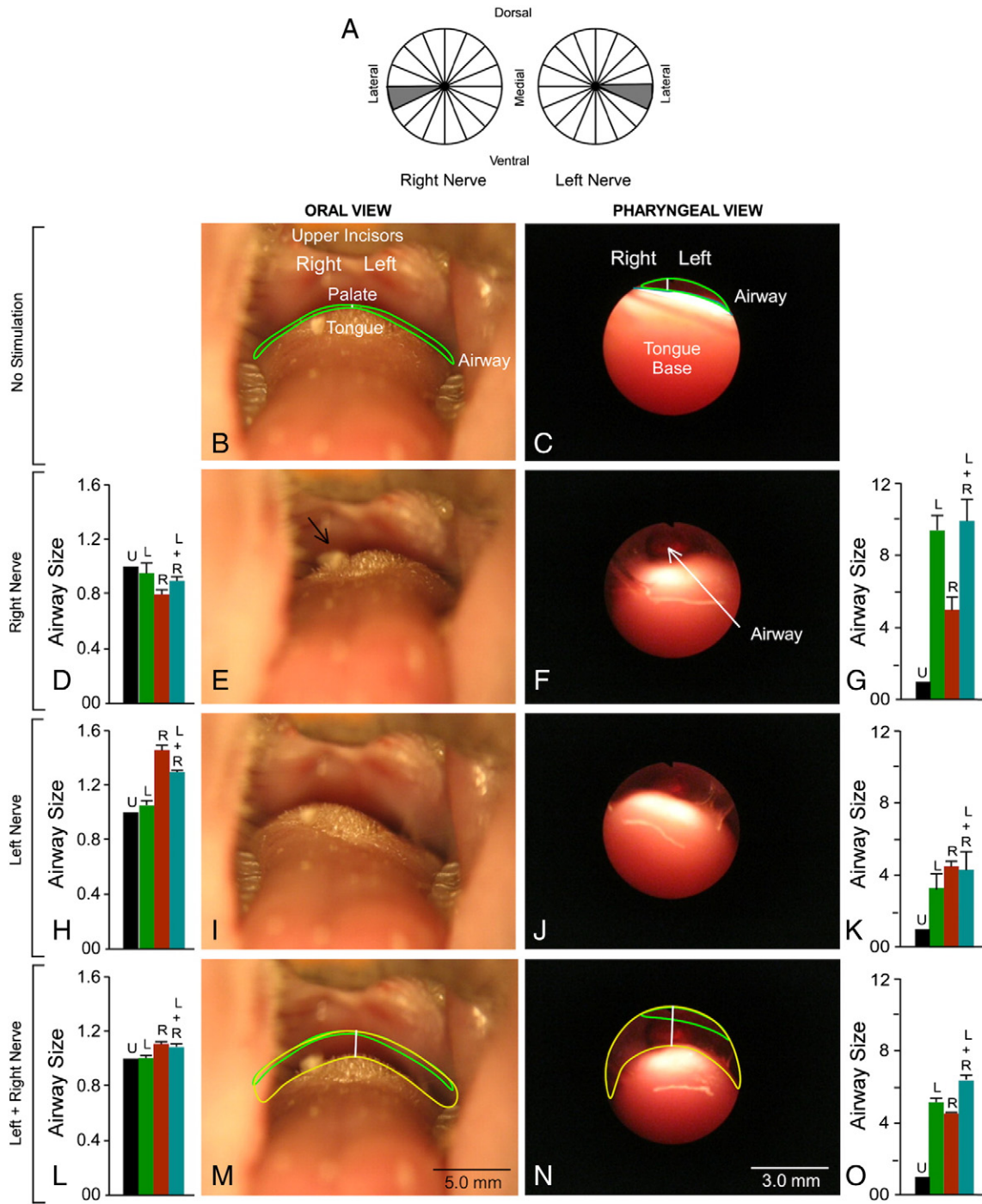


Fig. 6. Airway increases during unilateral vs. bilateral partial stimulation of the HGN: Single contacts on the right and left nerves were activated individually or simultaneously to effect tongue movements that enlarged the oral and, to a greater extent, the oropharyngeal airway. A: Diagram of the portions (1/16th) of the HGNs stimulated via the present eight contact electrode assembly applied to the ventral half of each nerve (right – contact 1; left – contact 8). The diameter of the airway was recorded microscopically (B, E, I, M) and endoscopically (C, F, J, N) and quantified (D, H, L, and G, K, O, respectively). Unilateral nerve stimulation, resulted in the tongue movement evident as increasing only slightly, the oral airway (H, I) but, to a much greater extent, the oropharyngeal airway both ipsilaterally and contralaterally (F, G, J, K). Simultaneous activation of the contacts on both nerves elicited enlargement of the airway that was bilaterally symmetrical (L, M, N, O). The white vertical lines indicate the airway midline. Green outline, unstimulated and yellow, stimulated. White arrow in F, larynx. Error bars in B represent standard deviations (SD) for each experimental trial (n = 3). Black bars (U) = no stimulation.

and styloglossus muscles, bilaterally (Fig. 1). A representative case is shown in Fig. 11. Unilateral activation of a single contact on the dorsal aspect of the left HGN elicited a single pronounced EMG response restricted to the ipsilateral genioglossus muscle (Fig. 11A & B). By contrast, activation of a single contact on the lateral right HGN elicited EMG responses restricted to the right side of the tongue, in this instance involving both the styloglossus and hyoglossus muscles (Fig. 11C & D).

Thus, in terms of specificity and laterality of the nerve-elicited muscle activation stimulating a single contact activates one or two specific muscles; the activated muscles are restricted to the same side of the tongue as the stimulated nerve segment. Stimulating both the right and left HGN contacts simultaneously, predictably, activated all three aforementioned muscles: the left genioglossus, the right styloglossus, and the right hyoglossus (Fig. 11G–I).

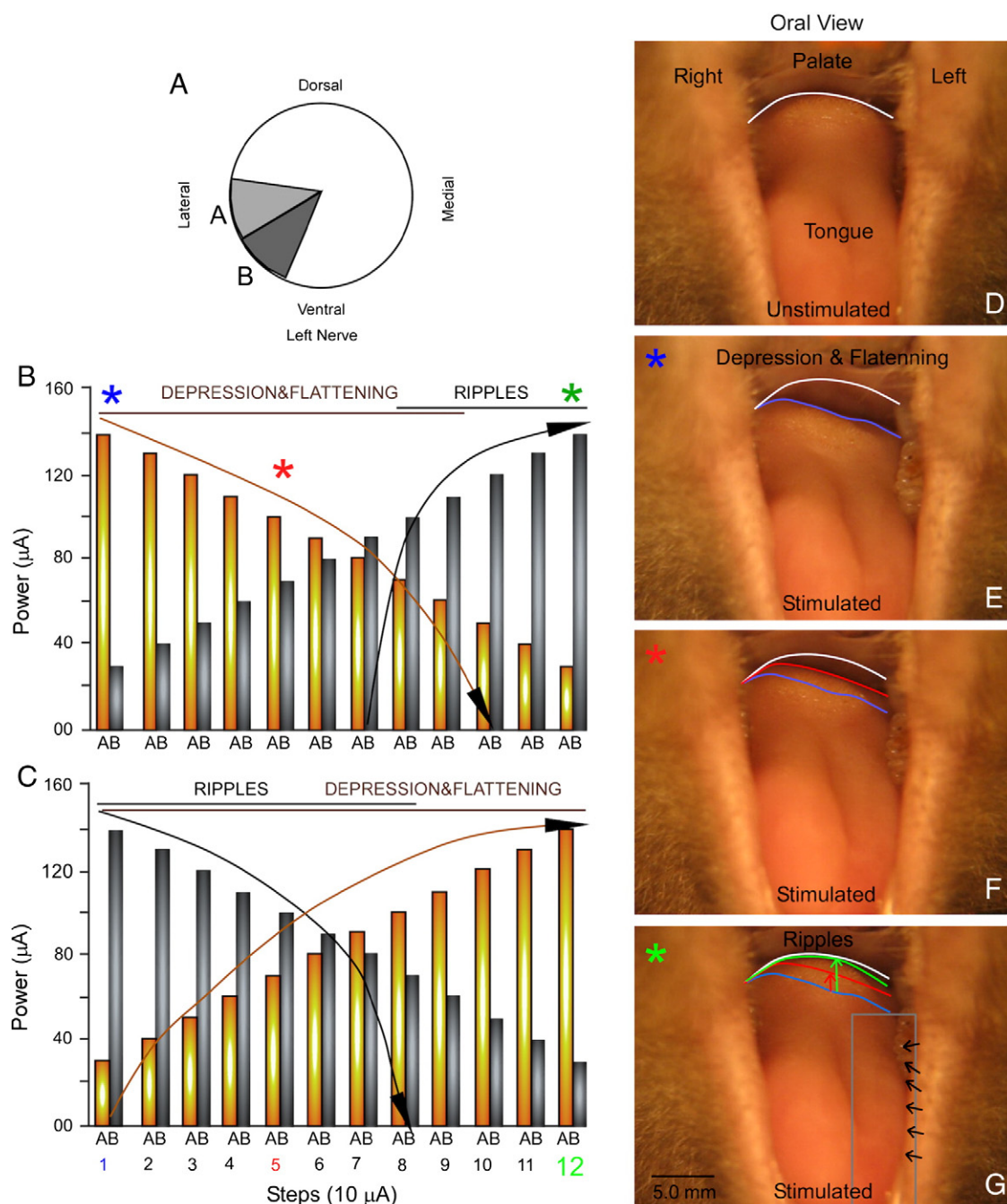


Fig. 7. Current steering: Ramping the current strength applied to two adjacent electrode contacts (A) differentially up and down (see Sections 3.3, 4.2) elicited systematic modulation of two distinct tongue movements. Stimulating contacts A and B with comparable current strengths effected different tongue movements; “depression and flattening of the rear tongue” in the case of contact A (blue line in E and red/green lines in F and G); “ripples in the anterior 2/3 of the tongue” (black arrows and gray box in G) in the case of contact B (compare the unstimulated condition, D). B: Simultaneously stimulating contact A by decreasing current in 12 steps (10 µA interval) from a supra-threshold level (140 µA) ending in a sub-threshold level (40 µA), while stimulating B in a corresponding ramp up fashion (contact A—orange histogram; contact B—gray histogram) resulted in disappearance of tongue flattening commensurate with the appearance of rippling. C: Reverse, confirmatory experiment to that depicted in B. Colored asterisks in B are based on the visual inspection of the appearance of the above movements and correspond to the microscope images in E, F, and G. Black and red arrows in B and C indicate current strengths eliciting the onset and increase of tongue rippling, and presence and disappearance of tongue depression, respectively (duration of motor events indicated by the horizontal lines at top).

4. Discussion

4.1. Targeted activation of the HGN

The present results demonstrate that by systematically activating individual contacts of a multi-contact electrode array, portions of the HGN can be selectively targeted, independently controlled and partially stimulated to differentially activate specific lingual muscles and to effect

changes in the size of the oropharyngeal airway. Activation of certain single contacts elicited some tongue movements that were more effective in opening the airway than others. It is likely, therefore, that each contact stimulates a different set of HGN fibers, and those subsets of the HGN differentially innervate lingual muscles some of which optimize the opening of the upper airway.

Anatomical studies have demonstrated for the rat and other vertebrates a topographic arrangement of motor neurons in the hypoglossal

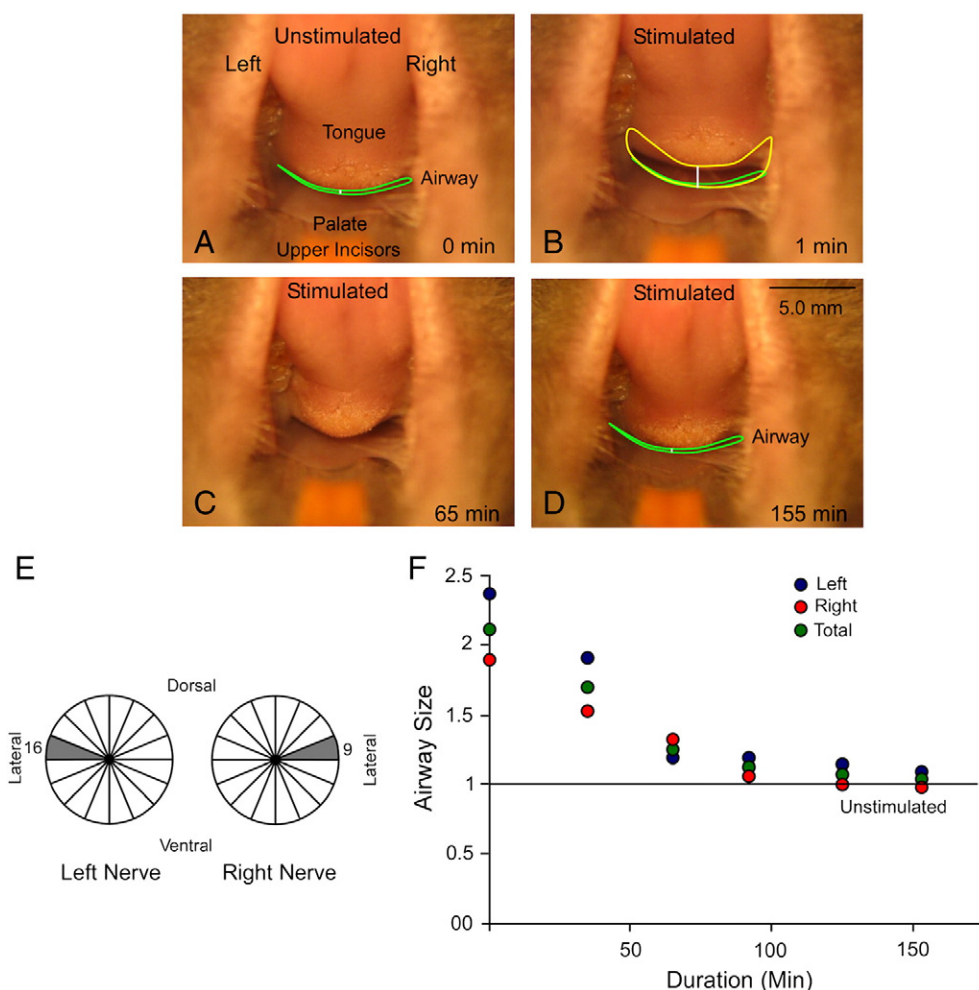


Fig. 8. Fatigability test: Chronic, selective HGN stimulation effects in a Zucker rat. The obese rat, in a supine position, adds the effect of gravity from the heavy tongue. Thus, opening the airway requires contraction of tongue muscles that elevate the tongue's weight. Two contacts that effected elevation of the posterior tongue (tongue base, E), were continuously stimulated simultaneously at supra-threshold values (350 μ A at 100 Hz) for a period of 155 min and photographed intermittently ((A–D) at 0, 1, 65 and 155 min elapsed time). The area of the left, right and both (A and B; green—unstimulated and yellow—stimulated) airway openings (between the palate and the posterior tongue) were marked (A, B, D), summed, averaged ($n = 3$) and normalized (to their respective unstimulated values, A) to obtain the left or right or total change (at 1, 34, 65, 92, 125 and 155 min elapsed time, F). A decline in the airway opening indicated fatigue of the stimulated tongue. Steady but a slow decline (F; left/blue, right/red and total/green airway change) attaining a level of resting/unstimulated tongue in 150 min ($T_{1/2} = 60$ to 70 min, approximately).

nucleus. Generally, motor neurons innervating tongue retrusor muscles are located dorsally in the nucleus, while neurons innervating protrusor muscles are located ventrally. More specifically, within the dorsal hypoglossal nucleus division the motor neurons innervating the hyoglossus and the styloglossus appear to sequester toward the caudal and rostral portions of the division, respectively [26,31–33]. The dorsal division also innervates two intrinsic muscles, the superior and inferior longitudinalis muscles; however, intrinsic motor neuronal pools are differentially located rostrocaudally [33]. The ventral division is similarly divisible into separate motor neuronal pools with different muscular targets; it consists of a larger medial subdivision that innervates the transverse and vertical intrinsic muscles, and a smaller lateral subdivision innervating the genioglossus [26,34]. This central topographic arrangement of motor neurons innervating different muscles implies that, in the periphery, the HGN nerve itself may be similarly organized. In other words, axons of the different motor neuronal pools likely track toward the tongue in particular segments of the nerve. The present results, showing differential effects of stimulating different topographic components of the nerve, are consistent with activation of the axons of different motor neuronal pools [35].

At present there is no published anatomical evidence for the various motor neuronal pools distributing their axons as distinct fascicles within the HGN. A fascicular organization is clear histologically for some

nerves, e.g., the axillary and radial nerves [36–38], but such organization is not evident in the main trunk of the HGN [39,40]. Nevertheless, based on the present results and the established topography of motor neurons in the medulla, we predict that the HGN, too, has a topographic organization of axons with clustering of similar functional groups representing particular motoneuronal pools, albeit without any histologically separate fascicles. Tracing of the peripheral course of genioglossus motor axons with a neuroanatomical marker suggests an organized grouping of fibers within the HGN en route to the musculature [41]. In the present study, selective activation of different hypoglossal motor neurons, effected by different contacts stimulating different portions of the nerve, is also supported by the present EMG recordings (Fig. 11). Activation of a segment of the HGN, in turn, activates one or two muscles, but not all, indicating specificity in the relationship between parts of the nerve and individual neuromuscular groups.

4.2. Multi-contact stimulation and the titration of response

Current steering allows the current distribution between any two independent contacts to be controlled such that regions of activation within the nerve bundle may be selectively activated in a graded fashion. Practically, by doing so, a nerve is divided into an unlimited number

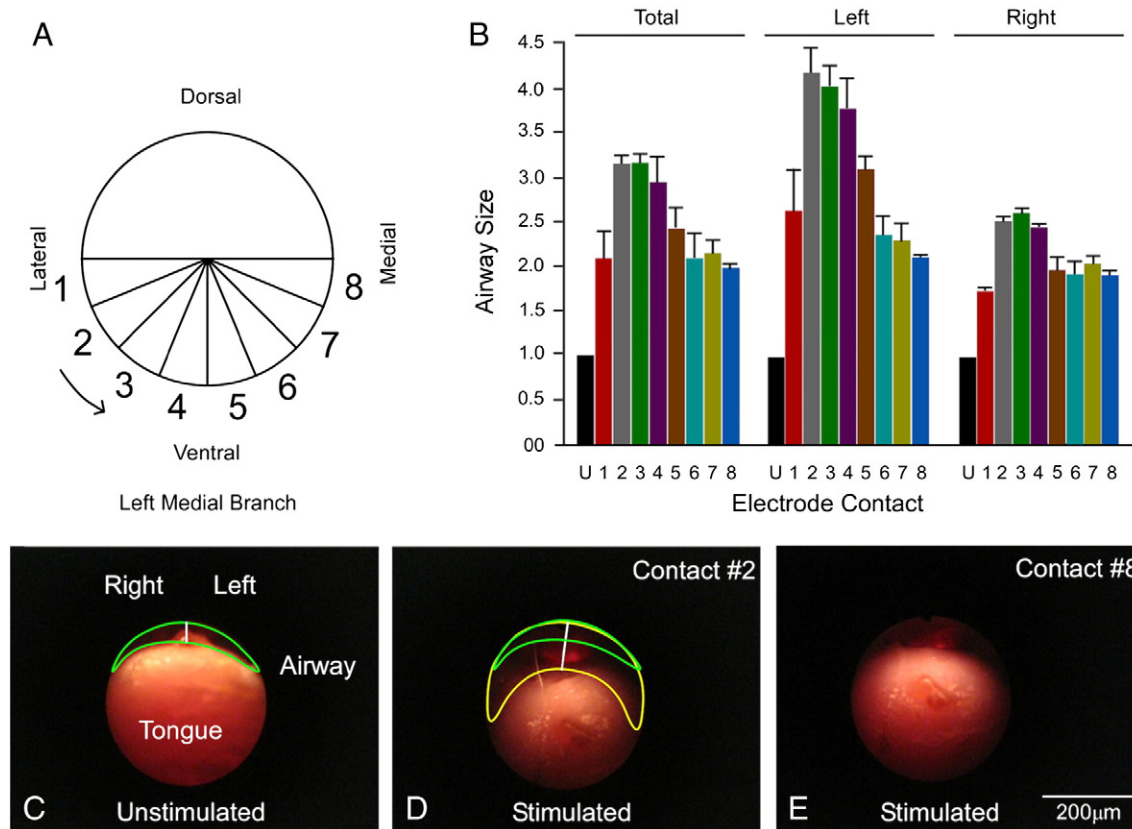


Fig. 9. Selective stimulation of the medial branch of the HGN: Effects on the oropharyngeal airway. A: Small populations of motor axons were selectively stimulated with the multi-contact electrode applied to the ventral surface of the medial branch of the left HGN. Opening of the airway, primarily resulting from genioglossus muscle contraction (see text) were recorded via endoscopic photographs C–E and quantified (B). Every contact resulted in some tongue movement. Airway enlargement was elicited bilaterally (B, D), but was especially pronounced on the left, ipsilateral to the stimulated nerve; contacts 2, 3, 4, and 5 were the most effective (B). Error bars in B represent standard deviations (SD) for each experimental trial ($n = 3$). The white vertical lines indicate the airway midline. Green outline, unstimulated and yellow, stimulated. Black bars (U) = no stimulation.

of hypothetical sectors that can be stimulated independently from each other. Thus, for example, in a previous study of sciatic nerve, stimulation of different motor nerve fascicles by selective activation of contacts in a multi-contact electrode permitted “steering” of excitation to portions of the nerve [42]. In our experimental use of current steering, we selected two adjacent (1/16th portion of the HGN) contacts (A and B in Fig. 7) with similar threshold values but mediating distinct tongue movements. When these two contacts were simultaneously stimulated by gradually varying current strengths, they not only activated two distinct motor groups but also did so in a smooth and graded manner. In other words, the regions of the nerve lying between electrode contacts were selectively recruited and activated. The implication of such current steering is that a multi-contact electrode can be tuned, directing excitation to nerve fibers that can effect a desired motor response, activating only those motor groups that move the tongue to open the airway while avoiding responses that do not. Moreover, the present test, stimulating two closely adjacent electrode contacts, demonstrates the precision by which specific neuro-muscular units can be controlled with the multi-contact electrode technology. Finally, the finding that activation can be applied in a graded and cyclical manner with consistent, repetitive responses, allows for mitigation of muscle fatigue. Thus, with any potential therapeutic application of a multi-contact electrode to a human nerve, the effective contacts could be activated sequentially and intermittently to effect desirable responses from several motor groups while mitigating against wholesale neuromuscular fatigue that would be caused by whole nerve or entire major nerve branch stimulation (see 4.8, below).

4.3. Selective activation of the targeted tongue musculature; effects on the airway

Selective activation of all contacts, one by one, either opened the oropharyngeal airway, or showed no change, i.e., compared with the unstimulated condition. By contrast, we did observe more varied kinds of motions, i.e. forward, backward, ripples, depression, deviation etc., in the more freely movable anterior tongue. The former response would be expected to improve airway patency of the retrolingual airway; the latter would improve patency in the velopharynx. Both effects, in different combinations, affect the net airway patency. These effects of stimulation, together with bilateral changes in the tongue's position during unilateral HGN stimulation (see Figs. 2, 4 & 6), relate to the arrangement of the different lingual muscle groups and the biomechanics of the organ as a muscular hydrostat [40]. This is consistent with a reported role for tongue retrusors in protecting the shape and size of the human upper airway [43].

Previous studies of the mechanism by which co-activation of the various muscle groups effect lingual movements have generated data consistent with the muscular-hydrostat model [44]. The hydrostat model proposes that the mammalian tongue is a cylindrical structure with a constant volume that adjusts its shape and size by co-activating both its protrusor and retrusor muscles. Thus, protrusion of the tongue not simply results by the action of the genioglossus, a protrusor, alone but also reflects the combined activities of the intrinsic verticalis and transversus muscles [45]. Similarly, retrusive movements involve the styloglossus and hyoglossus muscles along with superior and inferior

longitudinal muscles [45,46]. Coactivation of antagonistic muscle groups prevents the occlusive effect of the retractor, thereby improving oropharyngeal patency. Indeed airway flow increased more during coactivation of a retrusor muscle together with a genioglossus, protrusor, muscle stimulation than during genioglossus muscle stimulation alone [10,25,47].

Very recently, Kairaitis proposed that pharynx, like the tongue, also works as muscular hydrostat. The pharyngeal luminal shape and

patency are therefore controlled, in part, by the complex interplay of the muscles of the pharyngeal wall [48]. The posterior tongue musculature merges with the musculature of the pharynx [49,50], and the lingual/pharyngeal complex also attaches to both the hyoid bone inferiorly and the cranial base superiorly [51–53]. As a consequence of these attachments, the true hydrostatic behavior of the posterior tongue is expected to deviate from and act differently from that of the anterior free end of the tongue. Hence, the position of the posterior tongue is most relevant to the maintenance of oropharyngeal patency while the anterior tongue is more involved in non-respiratory activities. Not surprisingly, therefore, our results showing differential effects of HGN stimulation on the anterior (two-thirds) versus posterior (one-third) tongue suggest the existence of antero-posterior physio-anatomical dichotomy within the tongue body (Figs. 2B, 3B, 4B, 5B). With HGN stimulation, the posterior of the tongue at the level of retroglottal airway experienced either an incremental increase in airway size or no change irrespective of the motion in the anterior of the tongue. This may explain why a retrusion effect is minimally expressed in oropharyngeal airway (Figs. 3B, 5B).

4.4. Co-stimulation of agonist and antagonist muscles in optimum proportion improves airway patency

The tongue's musculature is comprised of four pairs of intrinsic (verticalis, transversalis, superior, and inferior longitudinalis) and extrinsic (genioglossus, hyoglossus, styloglossus, and palatoglossus) muscle fibers [54]. Extrinsic tongue muscles change the position of the tongue, whereas the intrinsic tongue muscles alter its shape [55,56]. Amongst the extrinsics the genioglossus muscle is considered to be the tongue protrusor (opening effect on the airway) while styloglossus and hyoglossus muscles are considered retrusors (closing effect on the airway). The geniohyoid, a non-lingual muscle, innervated by HGN, also affects the tongue by altering the position of the hyoid complex [5,52,53]. Recent data suggest that the airway opening or closing effect is a result of a complex interplay of the two groups of muscles that are traditionally regarded as simple antagonists i.e. retrusors and protrusors. These two muscle groups, when contracting simultaneously, control both the tongue's position and its tone and stiffness. This stiffness improves oropharyngeal patency by preventing the occlusive effect of the retractor muscles [47,57]. Fuller et al. showed that the muscles that protrude and retract the tongue are co-activated during the respiratory drive and that the inspiration phase is always associated with tongue retraction force [57].

Consistent with the aforementioned studies, this suggests that the retrusive effect of the retrusor muscles at the rear of the tongue is rendered insignificant, i.e., non-retrusive, in the presence of protrusive forces. Fregosi et al. reported that the stimulation of the genioglossus muscle alone dilates the airway, but does not change the oropharyngeal stiffness [rats, 25]. Likewise, Eisele et al. also showed that co-activating the tongue muscles by whole HGN stimulation (i.e. co-stimulation of both protrusor and retrusor muscles), even though effecting an apparent retraction of the anterior tongue, resulted in the net increase in the inspiratory flow (assisted by the aforementioned oropharyngeal

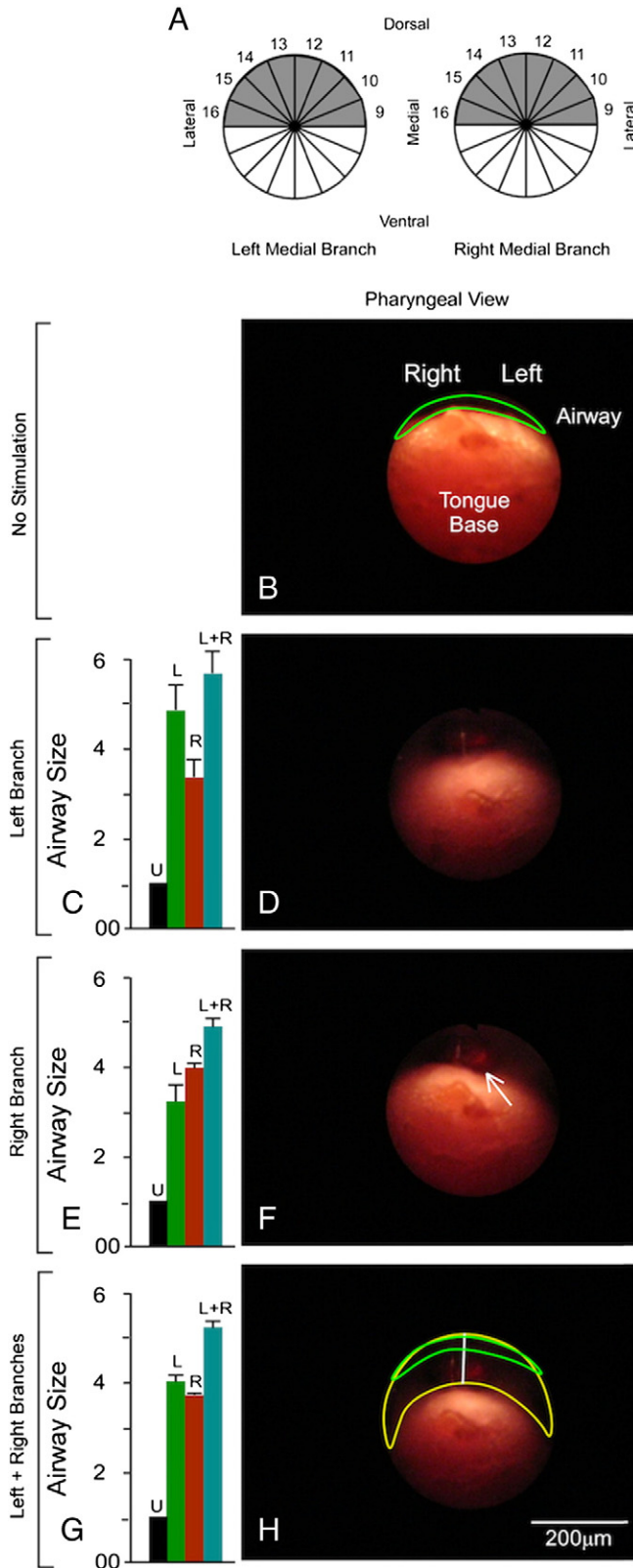


Fig. 10. Stimulation of the right and left medial branches of the HGNs – Effects on the oropharyngeal airway: All eight contacts of the electrode applied to the dorsal half of the medial HGN branches on the right and left sides were simultaneously stimulated unilaterally or bilaterally (A), thus activating the genioglossus muscle and, to some extent, the intrinsic muscles of the tongue (see Section 3.2). Distinct effects (on the opening of the airway) resulting from tongue movements were recorded (endoscopic photographs B, D, F and H) and quantified (C, E and G). Areas of the left or right (unilateral) or both (bilateral) airways were outlined (B, H; green and yellow lines), summed, averaged ($n = 3$) and normalized to obtain net changes in the airway. Unilateral nerve stimulation resulted in the tongue movement and airway opening both ipsilaterally and contralaterally (C–F). Airway changes were more symmetrical when the mHNS were stimulated bilaterally (G, H). Arrow in F, larynx. Error bars in B represent standard deviations (SD) for each experimental trial ($n = 3$). The white vertical lines indicate the airway midline. Black bars (U) = no stimulation.

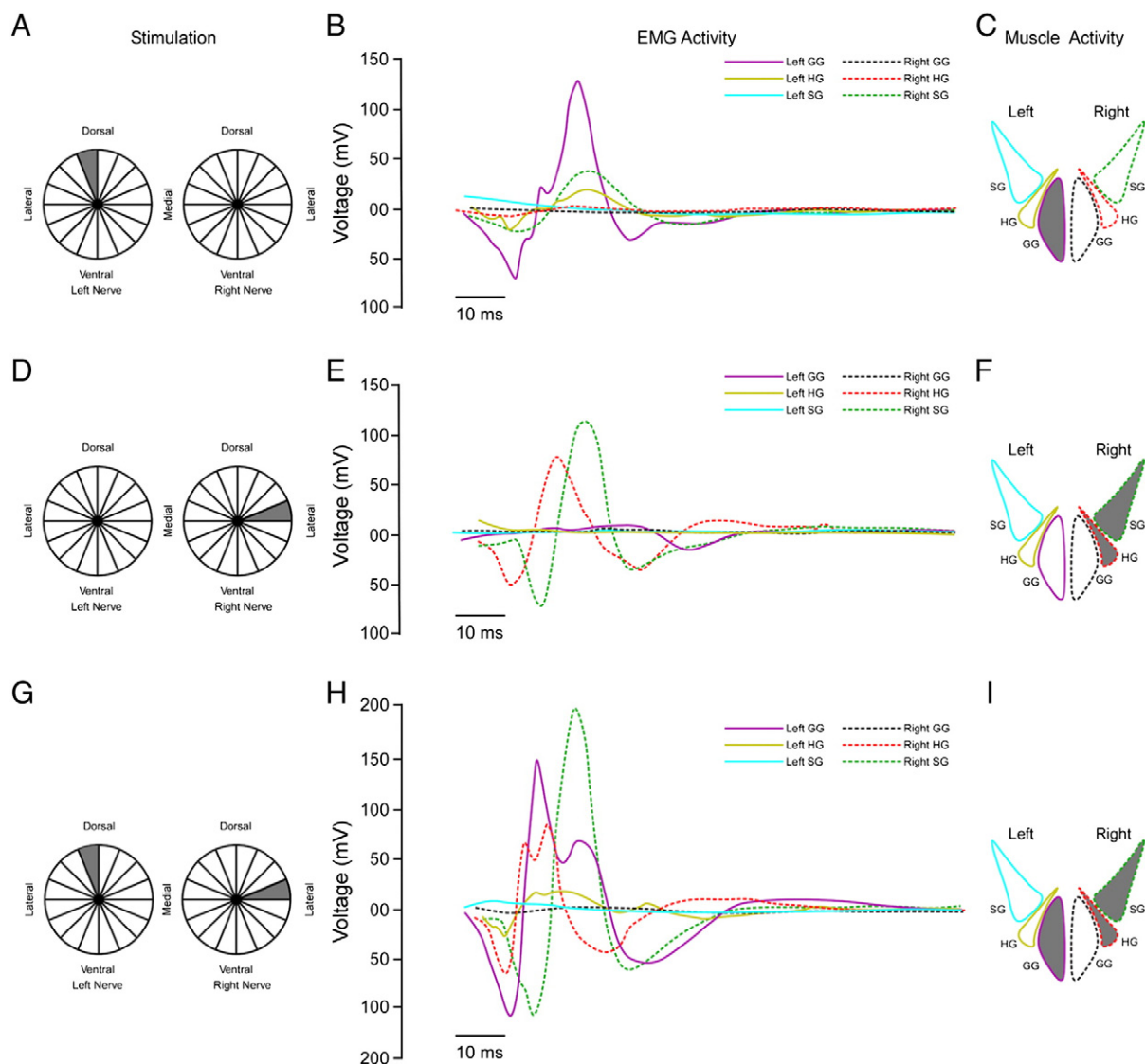


Fig. 11. Selective stimulation of single HGN contacts differentially activates specific tongue muscles identified via intra-muscular EMG recordings. One contact on each of the right (D) and left (A) nerves was stimulated either independently or both at the same time (G). EMG activity from the styloglossus (SG), hyoglossus (HG) and genioglossus (GG) muscles, bilaterally, was recorded. Unilateral partial nerve stimulation of the left nerve selectively elicited a response from the left GG muscle (B—purple, C—shaded). Unilateral stimulation of the right nerve elicited a response from the right SG & HG muscles (E—red, green, F—shaded). All three muscles responded when both nerves were selectively stimulated simultaneously (H, I—shaded).

stability) in awake and anesthetized human subjects [12]. In the present study we measured increases in the size of the airway during HGN stimulation at atmospheric pressure. Whether the stiffness of the airway walls is maintained as in the Fregosi study [25] is not known. Certainly, airway patency depends upon both its remaining open and not subject to collapse during pressure changes in breathing.

4.5. Effects of lingual movements on oropharyngeal patency

Reduced oropharyngeal muscle tone is the major cause of the oropharyngeal collapse in patients with obstructive sleep apnea [58,59]. The anterior wall of the oropharynx is formed by the posterior surface of the tongue. Hence, the oropharyngeal wall is anchored by both its own muscles, i.e. the overlapping superior and middle pharyngeal constrictor muscles, as well as by posterior lingual muscles. Based on the observation that all six pairs of tongue muscles interact anatomically and physiologically with the superior pharyngeal constrictor muscles at the inferior, posterior human tongue [49,50], Niimi et al. postulated that the stability of the pharynx is dependent on both the stiffness of the constrictor muscles and the posterior tongue, including, notably, some

retrusor muscles. Hence, it is also likely that any movement of the retroglossal muscles that stretch or stiffen, irrespective of whether from a protrusor or a retrusor or both (retrusor and protrusor fiber working against each other) imparts indirect drag to the superior pharyngeal constrictor muscles of the oropharyngeal wall, perhaps making the soft wall of pharynx less prone to collapse (particularly during a low pressure event, i.e., during inspiration). Tongue stiffness, in turn, increases the inertia (stability) and thus prevents the tongue from falling into the retroglossal airway [25]. Interestingly, in our study, the persistent absence of the active closure of the airway no matter which contact was stimulated points to the fact that movement in the tongue musculature (retrusion or protrusion or both) leads to primarily one kind of effect, i.e., the stabilization of the retroglossal airway, i.e., of the oropharynx.

Therefore, the controlled co-activation of a small fraction of the retrusor (antagonist) musculature together with the activation of protrusor (agonist) muscles can act to improve oropharyngeal patency while not significantly leading to tongue retrusion. Similarly, activation of any tongue muscle, irrespective of its role in isolation as a retrusor or protrusor, can possibly achieve beneficial lingual motions relative to the

oropharyngeal patency and can thus be a potentially effective outcome of selective methods of electrical stimulation.

4.6. Duration of the muscle response elicited by HGN stimulation

Vital functions performed by the mammalian tongue can be grouped into phasic activities, e.g., quick motions involved in speech, chewing, swallowing and suckling—mostly performed by the anterior two-thirds of the tongue, or tonic activities, e.g., maintaining tongue's posture and preventing its collapse into the retroglossal airway—mostly performed by the posterior one-third of the tongue [60,61]. Because the tonic activities are constant, certain muscles of the tongue, like the heart and diaphragm, are categorized as fatigue-resistant [62–64]. Consistent with the above notion, the posterior third of the human tongue has an unusually high capillary density of fatigue resistant muscle fibers [40,65]. More recently, muscular twitch tension studies in rats also suggested that motor units of the hypoglossal nucleus and its muscles (styloglossal and genioglossal muscles) are fatigue resistant [66,67].

We tested fatigue resistance using our stimulation paradigm. The animal tested (Zucker strain; Jackson Labs) was an un-trained animal with no prior exposure to electrical stimulation. The Zucker rat is bred specifically to mimic the morbidly obese, older human, and, except for activities associated with eating, this animal rarely engages in physical exertion. The animal's fat-laden tongue undoubtedly adds undue load to the musculature that controls the airway, although we cannot rule out, in addition, a possible influence of fat infiltration on muscle contractility. Nevertheless, the fatigue response at ten fold higher current and five fold higher frequency (to what is generally expected for use in therapy), using only one of the sixteen contacts, stimulating only 1/16th of the total HGN fiber population, resulted in a very slow and only a modest decline that lasted for close to 2 h. This suggests that in any clinical application, with training, stimulating at closer to the expected values, and with the opportunity to use multiple contacts to load-share the stimulation response, a response might be sustained for a much longer period, e.g., for the duration of the sleeping period. In human neurostimulation it is common to employ duty-cycling to the stimulation regime to avoid fatigue of the stimulated muscle i.e. by giving the muscle an opportunity to rest and recover from its bout of operation before resuming activity. Thus, in any application of the present technology, as the tongue musculature becomes adapted to the stimulation and becomes more fit with use, it is expected that even better fatigue resistance and hence sustained and improved clinical outcomes could be achieved.

4.7. Stimulation energies are low and within an acceptable range for clinical application

To gain insight into potential application of selective HGN activation to the clinical condition, the present study evaluated the intensity of neural stimulation required to effect tongue movement and airway opening. The stimulation energies that activated the HGN in the present study were clearly within the safety range of typical implanted stimulator design [68,69]. Thresholds of 10 μ A to 180 μ A at 200 μ s phase durations are easily achieved and effective, as were the supra-maximal levels of 2 to 3 mA. Moreover, with the present technological approach it was possible to achieve only muscle movements that result in airway opening. The rate of stimulation of the motor nerve needs only to be of a sufficient frequency to partially activate the nerve and to achieve, as a result, an increase in the oropharyngeal opening. Presumably, in any human application, the neurostimulation induced lingual movement, whatever its onset characteristics, involve muscle contraction that will not be perceived by the sleeping patient. Conceivably, for clinical application involving potential implantation of a multi-contact electrode with characteristics similar to that used in the present animal study, the implanted system could function at frequencies that are closer to

15 to 30 Hz, lower than the 50 to 100 Hz utilized in portions of this study [68,69]. Such a stimulation paradigm would have implications for the energy consumption consideration, size of the battery driving any such implant, and how often and how long a pulse generator driving the electrode would have to be re-charged or eventually replaced.

4.8. Implications of targeted HGN stimulation for OSA therapy

Obstructive sleep apnea (OSA) is commonly characterized by repetitive episodes of obstructions of the retroglossal airway, blood oxygen desaturation, and subsequent arousal from sleep [70,71]. Airway blockage associated with a reduction of lingual muscle tone is presumably associated with inadequate neural activation normally provided by the HGN [72,73]. Physical management of the airway in humans is the standard approach for addressing OSA. Current treatment, predominantly, employs positive airway pressure (PAP) to open the oropharynx, or to prevent it from collapsing during sleep-related muscle relaxation. Since 30–60% OSA patients do not tolerate PAP therapy [74], a number of investigations have explored the benefits of electrical stimulation of the tongue or its innervation on airway patency [11,75–77]. Previous neurostimulation efforts have activated the entire HGN, or all fibers of one of its major branches. This approach necessitates applications of a “closed loop system” wherein the HGN activation is timed to the respiratory cycle to avoid the problem of electrical stimulation-induced muscle fatigue [12,14,15,17–20,78]. Such efforts required respiration sensing and control methods to deliver well-timed delivery of stimulation in order to achieve a useful clinical result, i.e., some opening of the airway. Alternatively, an “open-loop system”, without a respiratory feedback, would activate selected muscle groups. In this approach, by switching from contact to contact, different motor units belonging to multiple muscle groups are activated each in turn, thus mitigating fatigue and restoring the tone and tongue position present during wakeful rest [79]. In the present animal study, due to technical limitations in fabricating a multi-contact nerve cuff electrode for the small HGN of the rat, one-half of the nerve was tested in each experiment. In a potential therapeutic application to the human HGN, a larger multi-contact cuff electrode could encircle the entire nerve [40] to target effective neuro-muscular subsets throughout the whole nerve.

In the present study we have demonstrated that stimulating a small sub-region of the HGN, merely 1/16th of the nerve, albeit the right region, can lead to desirable tongue movements with significant increases in the opening of the airway in an open-loop manner, i.e., not requiring respiration feedback and associated sensor technology. We also showed that this opening effect is not limited to the ipsilateral side of the oral cavity but also involves its contralateral side. Similar results were obtained from the activation of the mHN alone, except that the maximum increment in the opening of the oropharynx was less than one-third compared to the whole nerve. Thus, based on our results, stimulating the mHN alone does not impart any additional benefit compared to partial nerve stimulation; in fact, the efficacy of the airway opening decreases. Possibly, the geniohyoid and genioglossus muscles, both innervated by the mHN, have counter-productive effects on the airway opening.

5. Summary

In summary, the results of this study suggest that tongue muscles in the rat can respond to selective activation of different portions of the HGN by producing unique and desirable motions that increase the airway opening. Undesirable movements, i.e., airway closure, can be avoided. The results also suggest that graded responses can be achieved, that the regions of the nerve lying between electrode contacts may be selectively activated, that relatively low excitation energies are required, and that simultaneous activation of appropriate muscle groups can generate a coordinated opening of the oropharyngeal airway. We further show that partial stimulation of the whole HGN is

more productive than partial stimulation of its medial branch despite a reportedly dominant role of the medial branch in tongue protrusion. Finally we show that the unilateral nerve stimulation is sufficient for the bilateral effect on airway patency, and that the muscle response elicited by electrical stimulation can be expected to be maintained for a long duration. By switching activation between contacts, different motor units can be activated cyclically, mitigating fatigue, and mimicking the natural motor activity of the tongue. These results have implications for the design and the future success of a neurostimulation device for OSA patients.

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References

- [1] Horner RL. Neural control of the upper airway: integrative physiological mechanisms and relevance for sleep disordered breathing. *Compr Physiol* Jan 2012;2(1): 479–535.
- [2] Guilleminault C, Powell N, Bowman B, Stoohs R. The effects of electrical stimulation on obstructive sleep apnea syndrome. *Chest* 1995;107:67–73.
- [3] Edmonds LC, Daniels BK, Stanson AW. The effects of transcutaneous electrical stimulation during wakefulness and sleep in patients with obstructive sleep apnea. *Am Rev Respir Dis* 1992;146:1030–6.
- [4] Schnall RP, Pillar G, Kelsen SG, Oliven A. Dilatory effects of upper airway muscle contraction induced by electrical stimulation in awake humans. *J Appl Physiol* 1995;78: 1950–6.
- [5] Bishara H, Odeh M, Schnall RP, Gavriely N, Oliven A. Electrically-activated dilator muscles reduce pharyngeal resistance in anesthetized dogs with upper airway obstruction. *Eur Respir J* Sep 1995;8(9):1537–42.
- [6] Oliven A, Schnall RP, Pillar G, Gavriely N, Odeh M. Sublingual electrical stimulation of the tongue during wakefulness and sleep. *Respir Physiol* 2001;127:217–26.
- [7] Mann EA, Burnett T, Cornell S, Ludlow CL. The effect of neuromuscular stimulation of the genioglossus on the hypopharyngeal airway. *Laryngoscope* Feb 2002;112(2): 351–6.
- [8] Fairbanks David W. Feasibility and performance of the electrical stimulation of the HGN. *ENT J* 1993;72(1):52–7.
- [9] Decker MJ, Haaga J, Arnold JL, Atzberger D, Strohl KP. Functional electrical stimulation and respiration during sleep. *J Appl Physiol* 1993;75:1053–61.
- [10] Yoo PB, Durand DM. Effects of selective HGN stimulation on canine upper airway mechanics. *J Appl Physiol* 2005;99:937–43.
- [11] Schwartz AR, Smith PL, Liven A. Electrical stimulation of the hypoglossal nerve: a potential therapy. *J Appl Physiol* 2014;116:337–44.
- [12] Eisele DW, Smith PL, Alam DS, Schwartz AR. Direct HGN stimulation in obstructive sleep apnea. *Arch Otolaryngol Head Neck Surg* Jan 1997;123(1):57–61.
- [13] Hadley AJ, Kolb I, Tyler DJ. Laryngeal elevation by selective stimulation of the hypoglossal nerve. *J Neural Eng* 2013;10:1–8.
- [14] Eisele DW, Schwartz AR, Smith PL. Tongue neuromuscular and direct HGN stimulation for obstructive sleep apnea. *Otolaryngol Clin North Am* 2003;36(3):501–10.
- [15] Kezirian EJ, Boudewyns A, Eisele DW, Schwartz AR, Smith PL, Van de Heyning PH, et al. Electrical stimulation of the hypoglossal nerve in the treatment of obstructive sleep apnea. *Sleep Med Rev* 2010;14(5):299–305.
- [16] Van de Heyning PH, Badr MS, Baskin JZ, Cramer Bornemann MA, De Backer WA, Dotan Y, et al. Implanted upper airway stimulation device for obstructive sleep apnea. *Laryngoscope* Jul 2012;122(7):1626–33.
- [17] Strollo Jr PJ, Soose RJ, Maurer JT, de Vries N, Cornelius J, Froyovich O, et al. Upper-airway stimulation for obstructive sleep apnea. *N Engl J Med* Jan 9 2014;370(2): 139–49.
- [18] Eastwood PR, Barnes M, Walsh JH, Maddison KJ, Hee G, Schwartz AR, et al. Treating obstructive sleep apnea with hypoglossal nerve stimulation. *Sleep* Nov 1 2011; 34(11):1479–86.
- [19] Schwartz AR, Bennett ML, Smith PL, De Backer W, Hedner J, Boudewyns A, et al. Therapeutic electrical stimulation of the hypoglossal nerve in obstructive sleep apnea. *Arch Otolaryngol Head Neck Surg* 2001;127(10):1216–23.
- [20] Schwartz AR, Barnes M, Hillman D, Malhotra A, Kezirian E, Smith PL, et al. Acute upper airway responses to hypoglossal nerve stimulation during sleep in obstructive sleep apnea. *Am J Respir Crit Care Med* Feb 15 2012;185(4):420–6.
- [21] Hu L, Xu X, Gong Y, Fan X, Wang L, Zhang J, et al. Percutaneous biphasic electrical stimulation for treatment of obstructive sleep apnea syndrome. *IEEE Trans Biomed Eng* Jan 2008;55(1):181–7.
- [22] Fairbanks DW, Fairbanks DNF. Neurostimulation for obstructive sleep apnea: investigations. *Ear Nose Throat J* 1993;72:52–7.
- [23] Oliven A. Treating obstructive sleep apnea with hypoglossal nerve stimulation. *Curr Opin Pulm Med* Nov 2011;17(6):419–24.
- [24] Hiemae KM, Palmer JB. Tongue movements in feeding and speech. *Crit Rev Oral Biol Med* 2003;14(6):413–29.
- [25] Fuller DD, Williams JS, Janssen PL, Fregosi RF. Effect of co-activation of tongue protruder and retractor muscles on tongue movements and pharyngeal airflow mechanics in the rat. *J Physiol* Sep 1 1999;519(Pt 2):601–13.
- [26] Uemura-Sumi M, Itoh M, Mizuno N. The distribution of hypoglossal motoneurons in the dog, rabbit and rat. *Anat Embryol (Berl)* 1988;177(5):389–94.
- [27] Altschuler SM, Bao X, Misalis RR. Dendritic architecture of hypoglossal motoneurons projecting to extrinsic tongue musculature in the rat. *J Comp Neurol* 1994;342: 538–50.
- [28] Sawczuk A, Mosier KM. Neural control of tongue movement with respect to respiration and swallowing. *Crit Rev Oral Biol Med* 2001;12(1):18–37.
- [29] Gilliam EE, Goldberg SJ. Contractile properties of the tongue muscles: effects of hypoglossal nerve and extracellular motoneuron stimulation in rat. *J Neurophysiol* 1995;74(2):547–55.
- [30] Argilés JM. The obese Zucker rat: a choice for fat metabolism 1968–1988: twenty years of research on the insights of the Zucker mutation. *Prog Lipid Res* 1989; 28(1):53–66.
- [31] Krammer EB, Rath T, Lischka MF. Somatotopic organization of the hypoglossal nucleus: a HRP study in the rat. *Brain Res* 1979;170(3):533–7.
- [32] Chibuzo GA, Cummings JF. An enzyme tracer study of the organization of the somatic motor center for the innervation of different muscles of the tongue: evidence for two sources. *J Comp Neurol* 1982;205(3):273–81.
- [33] McClung JR, Goldberg SJ. Organization of motoneurons in the dorsal hypoglossal nucleus that innervate the retrusor muscles of the tongue in the rat. *Anat Rec* 1999;254(2):222–30.
- [34] Aldes LD. Subcompartmental organization of the ventral (protruder) compartment in the hypoglossal nucleus of the rat. *J Comp Neurol* 1995;353:89–108.
- [35] Huang J, Sahin M, Noormahamand C, Durand D. Activation patterns of the tongue muscles with selective stimulation of the HGN. *Conf Proc IEEE Eng Med Biol Soc* 2004;6:4275–8.
- [36] Aszmann OC, Dellon AL. The internal topography of the axillary nerve: an anatomic and histologic study as it relates to microsurgery. *J Reconstr Microsurg* 1996;12(6): 359–63.
- [37] Stewart JD. Peripheral nerve fascicles: anatomy and clinical relevance. *Muscle Nerve* 2003;28(5):525–41.
- [38] Campero M, Serra J, Ochoa JL. Peripheral projections of sensory fascicles in the human superficial radial nerve. *Brain* 2005;128(Pt 4):892–5.
- [39] Captier G, Canovas F, Bonnel F, Seignarbieux F. Organization and microscopic anatomy of the adult human facial nerve: anatomical and histological basis for surgery. *Plast Reconstr Surg* 2005;115:1457–65.
- [40] Zaidi FN, Meradows P, Jacobowitz O, Davidson TM. Tongue anatomy and physiology, the scientific basis for a novel targeted neurostimulation system designed for the treatment of obstructive sleep apnea. *Neuromodulation* 2012;10:1525–35.
- [41] Lee S, Eisele DW, Schwartz AR, Ryugo DK. Peripheral course of genioglossal motor axons within the HGN of the rat. *Laryngoscope* 1996;106(10):1274–9.
- [42] Tarter MD, Mortimer JT. Selective and independent activation of four motor fascicles using a four contact nerve-cuff electrode. *IEEE Trans Neural Syst Rehabil Eng* 2004; 12(2):251–7.
- [43] Mateika JH, Millroad DL, Kim J, Rodriguez HP, Samara GJ. Response of human tongue protruder and retractors to hypoxia and hypercapnia. *Am J Respir Crit Care Med* 1999;160:1976–82.
- [44] Kier WM, Smith KK. Tongues, tentacles and trunks: the biomechanics and movement of muscular hydrostats. *Zool J Linn Soc* 1985;83:207–324.
- [45] Smith KK, Kier WM. Trunks, tongues and tentacles: moving with skeletons of muscle. *Am Sci* 1989;77:22–35.
- [46] Gilbert RJ, Napadow VJ, Gaige TA, Wedeen VJ. Anatomical basis of lingual hydrostatic deformation. *J Exp Biol* 2007;210(Pt 23):4069–82.
- [47] Oliven A, Odeh M, Geitini L, Oliven R, Steinfeld U, Schwartz AR, et al. Effect of coactivation of tongue protruder and retractor muscles on pharyngeal lumen and airflow in sleep apnea patients. *J Appl Physiol* 2007;103(5):1662–8.
- [48] Kairaitis K. Is the pharynx a muscular hydrostat? *Med Hypotheses* 2010;74(3): 590–5.
- [49] Kokawa T, Saigusa H, Aino I, Matsuoka C, Nakamura T, Tanuma K, et al. Physiological studies of retractive movements of the human tongue. *J Voice* 2006;20(3):414–22.
- [50] Saigusa H, Yamashita K, Tanuma K, Saigusa M, Niimi S. Morphological studies for retractive movement of the human adult tongue. *Clin Anat* 2004;17(2):93–8.
- [51] Takahashi S, Ono T, Ishiwata Y, Kuroda T. Breathing modes, body positions, and suprahyoid muscle activity. *J Orthod* 2002;29(4):307–13.
- [52] Strohl KP, Wolin AD, van Lunteren E, Fouke JM. Assessment of muscle action on upper airway stability in anesthetized dogs. *J Lab Clin Med* Aug 1987;110(2):221–30.
- [53] Wiegand DA, Latz B, Zwillich CW, Wiegand L. Geniohyoid muscle activity in normal men during wakefulness and sleep. *J Appl Physiol* 1990;69:1262–9.
- [54] McClung JR, Goldberg SJ. Functional anatomy of the hypoglossal innervated muscles of the rat tongue: a model for elongation and protrusion of the mammalian tongue. *Anat Rec* 2000;260(4):378–86.
- [55] Dobbins EG, Feldman JL. Differential innervation of protruder and retractor muscles of the tongue in rat. *J Comp Neurol* 1995;357(3):376–94.
- [56] Bailey EF, Huang YH, Fregosi RF. Anatomic consequences of intrinsic tongue muscle activation. *J Appl Physiol* 2006;101(5):1377–85.
- [57] Fuller D, Mateika JF, Fregosi RF. Co-activation of tongue protruder and retractor muscles during chemoreceptor stimulation in the rat. *J Physiol* 1998;507(1):265–76.
- [58] Smith PL, Wise RA, Gold AR, Schwartz AR, Permutt S. Upper airway pressure–flow relationships in obstructive sleep apnea. *J Appl Physiol* 1988;64:789–95.

- [59] Lowe AA. The tongue and airway. *Otolaryngol Clin North Am* 1990;23(4):677–98.
- [60] Abd-El-Malek S. Observations on the morphology of the human tongue. *J Anat* 1938; 73:201–10.
- [61] Abd-El-Malek S. The part played by the tongue in mastication and deglutition. *J Anat* 1955;89(2):250–4.
- [62] Sokoloff AJ, Yang B, Li H, Burkholder TJ. Immunohistochemical characterization of slow and fast myosin heavy chain compositions of muscle fibers in the styloglossus muscle of the human and macaque. *Arch Oral Biol* 2007;52:533–43.
- [63] Sokoloff AJ, Daugherty M, Li H. Myosin heavy-chain composition of the human hyoglossus muscle. *Dysphagia* Jun 2010;25(2):81–93.
- [64] Smith JC, Goldberg SJ, Shall MS. Phenotype and contractile properties of mammalian tongue muscles innervated by the hypoglossal nerve. *Respir Physiol Neurobiol* Jul 28 2005;147(2–3):253–62.
- [65] Granberg I, Lindell B, Eriksson PO, Pedrosa-Domellöf F, Stål P. Capillary supply in relation to myosin heavy chain fiber composition of human intrinsic tongue muscles I. *Cells Tissues Organs* 2010;192:303–31.
- [66] Sutlive TG, McClung JR, Goldberg SJ. Whole-muscle and motor-unit contractile properties of the styloglossus muscle in rat. *J Neurophysiol* 1999;82(2):584–92.
- [67] Sutlive TG, Shall MS, McClung JR, Goldberg SJ. Contractile properties of the tongue's genioglossus muscle and motor units in the rat. *Muscle Nerve* 2000;23(3):416–25.
- [68] Agnew William F, McCreery DB. Considerations for safety with chronically implanted nerve electrodes. *Epilepsia* 1990;31(Suppl. 2):S27–32.
- [69] Agnew WF, McCreery DB, Yuen TG, Bullara LA. Evolution and resolution of stimulation-induced axonal injury in peripheral nerve. *Muscle Nerve* 1999;22(10): 1393–402.
- [70] Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndromes. *Annu Rev Med* 1976;27:465–84.
- [71] Badr MS. Pathogenesis of obstructive sleep apnea. *Prog Cardiovasc Dis* 1999;41(5): 323–30.
- [72] Jordan AS, White DP. Pharyngeal motor control and the pathogenesis of obstructive sleep apnea. *Respir Physiol Neurobiol* Jan 1 2008;160(1):1–7.
- [73] White DP. Pathogenesis of obstructive and central sleep apnea. *Am J Respir Crit Care Med* 2005;172:1363–70.
- [74] Weaver TE, Sawyer AM. Adherence to continuous positive airway pressure treatment for obstructive sleep apnoea: implications for future interventions. *Indian J Med Res* 2010;131:245–58.
- [75] Huang J, Sahin M, Durand DM. Dilation of the oropharynx via selective stimulation of the HGN. *J Neural Eng* 2005;2:73–80.
- [76] Van Zutphen C, Janssen P, Hassan M, Cabrera R, Bailey EF, Fregosi RF. Regional velopharyngeal compliance in the rat: influence of tongue muscle contraction. *NMR Biomed* 2007;20(7):682–91.
- [77] Hu Lianggang, Xu Xiaomei, Gong* Yongsheng, Fan Xiaofang, Wang Liangxing, Zhang Jianhua, et al. Percutaneous biphasic electrical stimulation for treatment of obstructive sleep apnea syndrome. *IEEE Trans Biomed Eng* 2008;55(1):181–7.
- [78] Kezirian EJ, Goding Jr GS, Malhotra A, O'Donoghue FJ, Zammit G, Wheatley JR, et al. Hypoglossal nerve stimulation improves obstructive sleep apnea: 12-month outcomes. *J Sleep Res* Feb 2014;23(1):77–83.
- [79] Mwenge GB, Rombaux P, Dury M, Lengelé B, Rodenstein D. Targeted hypoglossal neurostimulation for obstructive sleep apnoea: a 1-year pilot study. *Eur Respir J* Feb 2013;41(2):360–7.