

Chronic recordings of hypoglossal nerve activity in a dog model of upper airway obstruction

MESUT SAHIN,¹ DOMINIQUE M. DURAND,¹ AND MUSA A. HAXHIU²⁻⁴

Departments of ¹Biomedical Engineering, ²Anatomy, ³Medicine,

and ⁴Pediatrics, Case Western Reserve University, Cleveland, Ohio 44106

Sahin, Mesut, Dominique M. Durand, and Musa A. Haxhiu. Chronic recordings of hypoglossal nerve activity in a dog model of upper airway obstruction. *J. Appl. Physiol.* 87(6): 2197–2206, 1999.—The activity of the hypoglossal nerve was recorded during pharyngeal loading in sleeping dogs with chronically implanted cuff electrodes. Three self-coiling spiral-cuff electrodes were implanted in two beagles for durations of 17, 7, and 6 mo. During quiet wakefulness and sleep, phasic hypoglossal activity was either very small or not observable above the baseline noise. Applying a perpendicular force on the submental region by using a mechanical device to narrow the pharyngeal airway passage increased the phasic hypoglossal activity, the phasic esophageal pressure, and the inspiratory time in the next breath during non-rapid-eye-movement sleep. The phasic hypoglossal activity sustained at the elevated level while the force was present and increased with increasing amounts of loading. The hypoglossal nerve was very active in rapid-eye-movement sleep, especially when the submental force was present. The data demonstrate the feasibility of chronic recordings of the hypoglossal nerve with cuff electrodes and show that hypoglossal activity has a fast and sustained response to the internal loading of the pharynx induced by applying a submental force during non-rapid-eye-movement sleep.

electroneurogram; cuff electrodes; upper airway loading; sleep; esophageal balloon

OBSTRUCTIVE SLEEP APNEA is characterized by occlusions of upper airways (UAWs) during sleep. The activity of the UAW dilator muscles plays an important role in the patency of the UAWs. Among those muscles, the genio-glossus (GG), which is innervated by the medial branch of the hypoglossal (HG) nerve, has been given particular attention, because the main function of the GG is to protrude the tongue. Anesthesia (13, 21) and sedation (4) can affect the HG activity level. Thus several chronic animal models have been developed to study the GG response to the loading of the UAWs in unsedated animals (13, 15, 19, 23). In these models, the airways are terminated with an elastic, resistive, or infinite load (total occlusion) to simulate the effects of the occlusions in patients with obstructive sleep apnea. In some other chronic animal models, the UAW is closed remotely with a computer-controlled valve (17) or an inflatable balloon placed in the trachea (22) to study the physiological consequences of the occlusions. In this study, we developed a new dog model of UAW obstruction whereby an external force is applied di-

rectly on the submental region to mechanically narrow the pharynx and, therefore, partially occlude the airways. Using this model, we studied the response of the HG nerve to loading of the pharynx during sleep.

Direct recordings of the HG nerve activity have not been reported in unsedated animals. Alternatively, in conscious animals and humans, electromyogram (EMG) recordings with wire electrodes have been utilized to study the activity of the muscles innervated by the HG nerve, especially the GG muscle. However, EMG recordings with wire electrodes have several problems: the signals favor the local activity (26) and contain movement artifacts, and the signal amplitudes are not reproducible from implant to implant. Another method of choice is to place a cuff electrode directly on the HG nerve. Although nerve cuff electrodes are not entirely free from the problems similar to those encountered with the EMG electrodes, the mechanical interface, and thus the reproducibility of recordings, is much more improved. The spiral-cuff-electrode design (20), which was previously shown to be able to record the HG and phrenic nerve discharges under different conditions in acute preparations (24, 25), was used. In this study, we also addressed the issues regarding chronic electrode implantations on the HG nerve, such as the nerve insult and the signal quality of the recordings.

METHODS

Two healthy beagles (young adult, 10–12 kg) with normal UAW anatomy were chronically implanted with cuff electrodes for recordings of HG activity and with electroencephalogram (EEG) and electrooculogram (EOG) electrodes for sleep staging. The cuff electrodes were implanted bilaterally in one animal (*beagle 1*, male) for durations of 17 mo (*cuff 1*) and 7 mo (*cuff 2*) and unilaterally in the other animal (*beagle 2*, female) for 6 mo (*cuff 3*). All the surgical and experimental procedures were designed according to the *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee of Case Western Reserve University, Cleveland, Ohio.

Surgical procedures. The animals were initially anesthetized with a short-acting barbiturate (thiopental, 25 mg/kg iv), intubated, and ventilated with a gaseous mixture of halothane, nitrous oxide, and oxygen. Incisions (5 cm long) were made on both sides of the upper neck area. Superficial muscles of the region were retracted, and an ~3-cm length of the HG nerve was separated from its surrounding tissue. A self-coiling spiral-cuff electrode (Fig. 1A) was implanted on the main trunk of the HG nerve near the bifurcation point of lateral and medial branches. A couple of loops were made in the electrode wires and sutured to a nearby muscle to avoid direct pulling on the electrode. Electrode wires were tunneled subcutaneously to an exit site between the scapulae. The incision was closed, and the animal was turned over for implantation of EEG and EOG electrodes.

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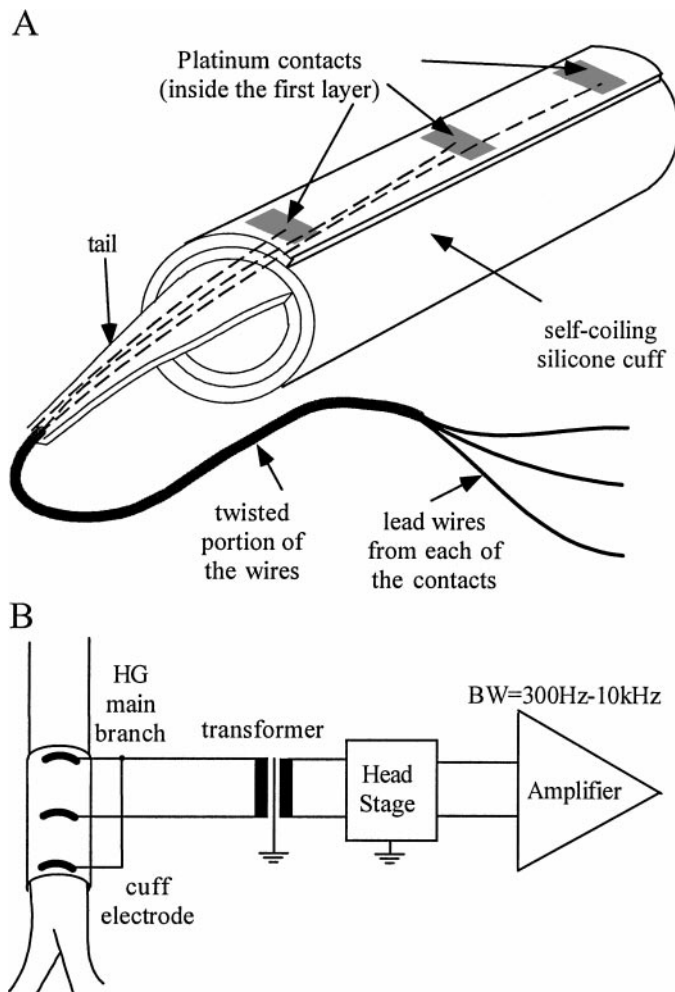


Fig. 1. *A*: self-coiling spiral-cuff electrode. Three platinum contacts were embedded in the innermost layer and exposed through windows cut out from silicone cuff on inside. *B*: tripolar connection of electrode contacts to amplifier with use of a transformer. HG, hypoglossal; BW, bandwidth.

Four stainless steel cortical screws (2 mm in diameter; Synthes), two on each side of the coronal suture symmetrically placed at the corners of a 12×12 -mm square, were screwed into the skull for recordings of EEG activity. Multistrand stainless steel Teflon-coated wires ($1 \times 7 \times 0.00135$ in.; Fort Wayne Metals, Fort Wayne, IN) were tied to the screws after the tips were deinsulated, and the area was covered with dental acrylic. Another set of three screws (separated by 6 mm on a line) were screwed into the sinus near the supraorbital ridge of the right eye for recordings of the eye movements (EOG). All the wires from the EEG and EOG electrodes were also tunneled subcutaneously to the exit site between the shoulders, and the incisions were closed. The electrode leads were attached to a connector that was kept inside a pocket on the dog's jacket.

Cuff electrodes. The self-coiling spiral design was chosen for the cuff electrodes, the ability of which to record the activity of the HG nerve has been demonstrated in anesthetized preparations (24, 25). A detailed description of the electrode fabrication can be found elsewhere (20, 25). The cuff electrodes used in this study were 20 mm in length and 2.5 mm in diameter (inner diameter of the first layer) and had 2.25–2.75 turns (Fig. 1*A*), snugly fitting the nerves in their resting position. Cuff electrodes had three contacts (each 9

mm apart, size of the exposed area = 2×1.25 mm) made from platinum foil (purity = 99.95%, thickness = 25 μ m; Goodfellow), which were spot welded to multistrand stainless steel (316 LVM) Teflon-coated wires ($1 \times 7 \times 0.00135$ in., Fort Wayne Metals) for connections. The electrode wires were twisted together before implantation to reduce the tension on the wires under bending forces. A longitudinal silicone piece from one end of the cuff to the other end was glued on the opposite side of the contacts on the first layer as a backbone to improve the longevity of the contacts. The electrode wires exited the cuff from one end on the first layer through a tapering tail that helped reduce the mechanical stress on the wires at the exit point. Nerve activity was recorded from the middle contact with respect to the end contacts that were shorted as shown in Fig. 1*B*.

Experimental setup. The dogs were trained to sleep lying on one side with their necks in a straight position in a one-side-open Faraday cage ($52 \times 70 \times 165$ cm) in the presence of the experimenter. The leads from the implanted electrodes were connected to the recording electronics before each session via a flat cable that was long enough to allow the animal to move freely inside the cage. A custom-designed apparatus with a pneumatic piston that could be advanced remotely (Fig. 2) was used to apply a perpendicular force on the submental region, ~ 2 cm rostral to the hyoid bone, thereby narrowing the pharyngeal portion of the UAWs (see the small dog head figure in Fig. 2*B*). The force applicator was held in place with the help of a thermoplastic mold that was worn around the animal's head. A small condenser microphone was mounted on the thermoplastic mold near the pharynx to record the snoring sounds. A custom-made cylindrical balloon was placed in the lower one-third of the thoracic portion of the esophagus (by having the animal swallow it with a bolus of soft food) before each sleep session for measurements of the esophageal pressure (Pes), as an estimate of the tracheal pressure. Respiratory abdominal movements were measured with RespiTrace (Ambulatory Monitoring) by using an inductive band transducer worn around the belly. All the raw signals were continuously digitized (Digital Data Recorder, model VR-10B, Instrutech) and recorded on videotapes for later analysis.

Experimental procedure. HG activity was recorded continuously in wakefulness (W) and sleep. Sleep experiments were held at night, usually before midnight. The animals were exercised 30–45 min before each session by being walked on a leash. The recording experiments (a total of 53 sessions) began at least 2 mo after surgery and were spread over time until animals were terminated. Each session lasted between 2 and 4 h and included multiple sleep cycles. A force was applied on the submental region to mechanically collapse and, therefore, load the UAWs internally during non-rapid-eye-movement (NREM) sleep. The submental force was increased in steps of 1 or 2 N, starting from zero up to a maximum value, waiting at least 10 breaths at each level. The maximum force was defined as the largest force value at which the animal was not aroused from sleep. The force transition from one level to the next took less than two breath cycles.

Sleep staging. EEG and EOG signals and the observed state of the animals were used to differentiate between W, NREM sleep, and rapid-eye-movement (REM) sleep stages. The NREM sleep stage was characterized by larger amplitudes and slower frequency components in the EEG signal relative to either the W or REM sleep stage. REM sleep was characterized by low amplitudes in EEG and often sharp edges in the EOG signal. The REM sleep stage was typically associated with twitches in the face and jerks in the legs.

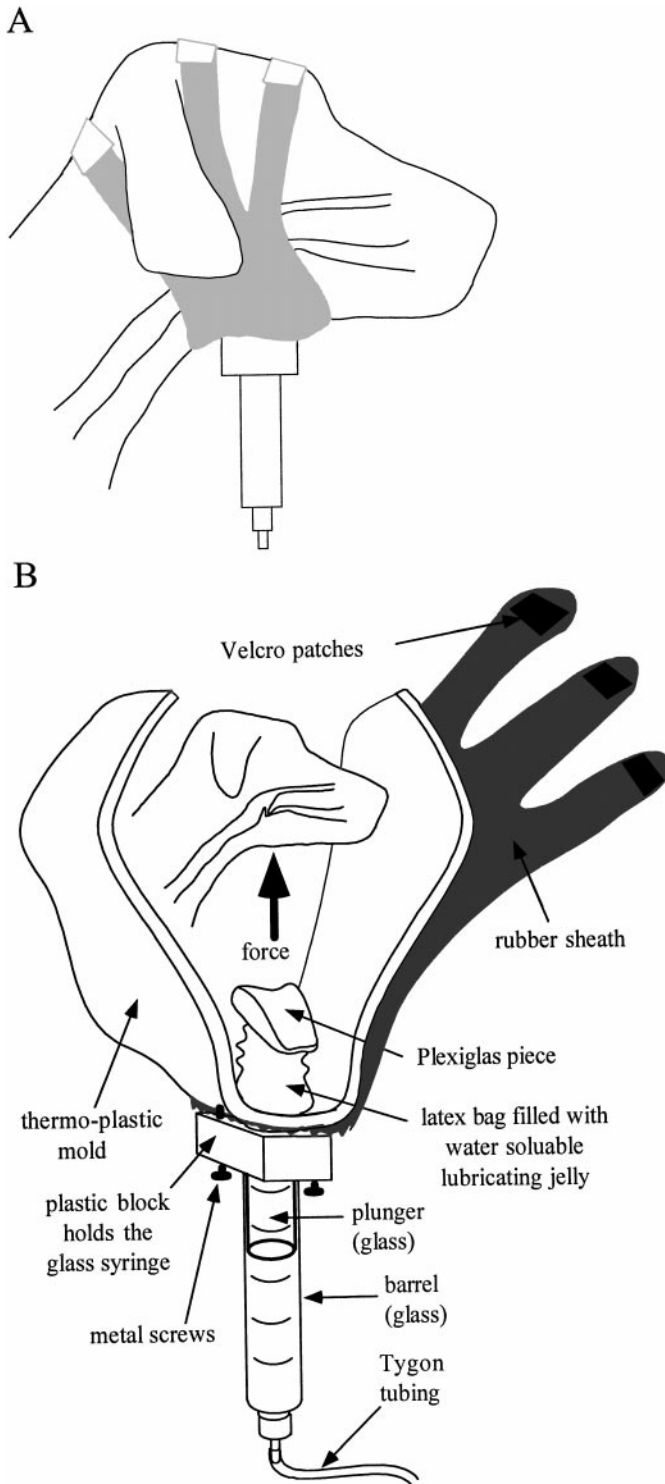


Fig. 2. Force applicator. A: the profile as worn by the animal. B: detailed presentation.

Force applicator. A 5-ml glass syringe was mounted on a thermoplastic mold that was shaped to fit comfortably around the dog's head (Fig. 2). The outside end of the plunger was cut off, and a Plexiglas piece with a relatively larger surface area (2.75 cm^2) was glued on the top by using fast-drying epoxy. The Plexiglas piece was shaped to conform to the anatomic structures in the submental area to minimize the disturbing effect of the force on the animal during sleep. A thin latex bag

was placed around the exposed end of the plunger, and the bag was filled with water-soluble lubricating jelly for smooth movement of the plunger. A piece of rubber sheath cut into an appropriate shape was wrapped around the mold, and the ends were held together over the head with the help of Velcro attachments to further stabilize the apparatus around the animal's head. A 40-cm-long flexible tubing (ID = 2.4 mm, OD = 4 mm; Tygon, Fisher Scientific) was attached to the syringe and continued with a longer and stiffer polyethylene tubing (ID = 3.05 mm, length = 2 m; TFE 9 Standard Wall, Zeus Industrial Products, Orangeburg, SC) to transmit the pressure to a remote transducer (Deltran, Utah Medical Products, Midvale, UT). The pressure measurements inside the system were scaled with the cross-sectional area of the syringe to determine the value of the submental force. Another syringe was included into the system for the remote control of the submental force by adding or removing air. The system had a volume of 18 cm^3 , excluding the syringes.

Pes measurements. A 5-cm-long cylindrical silicone tubing (2.5 mm diameter, Dow Corning) with very thin walls served as a sensor in the design of the esophageal balloon (Fig. 3A). The end was closed with a small ball made of silicone curing agent (MDX4-4210, Dow Corning). The open end of the cylindrical sensor was glued with the curing agent to a 14-cm-long silicone tube with a smaller diameter (ID = 0.64 mm, OD = 1.19 mm; Dow Corning), continued with another silicone tubing of 15-cm length (ID = 0.30 mm, OD = 0.64

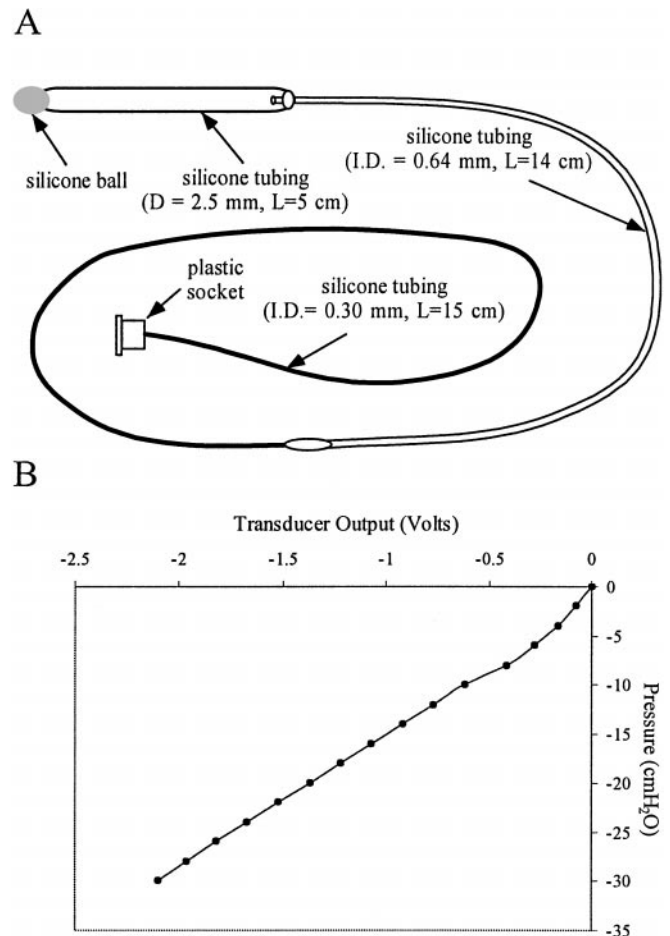


Fig. 3. A: esophageal balloon design. A 5-cm-long silicone tubing with very thin walls served as a pressure sensor. D, diameter; L, length, I.D., inner diameter. B: transfer function of esophageal balloon for steady negative pressures.

mm; SM-7755A Durometer, Sil-Med), and terminated with a plastic socket. The balloon was positioned inside the esophagus such that the pressure readings were maximum during the inspiratory phases. The first piece of tubing connected to the pressure sensor traveled along the esophagus to the mouth cavity. The thinner piece ran from behind the right teeth out through the corner of the mouth. The plastic socket was connected to a pressure transducer (Deltran, Utah Medical Products) during experiments. The balloon was deflated in the beginning of each session to avoid saturation in the mechanical transfer function of the balloon at large negative Pes values. The steady-state response of the balloon and the pressure transducer combination was nearly linear for the range of negative pressures measured in the esophagus (Fig. 3B). The response time (rise time from 10 to 90% of maximum) of the Pes measurement system was estimated as 170 ms by putting the balloon in a vacuum and quickly raising the pressure to the ambient pressure.

Signal conditioning. HG nerve recordings were first amplified with a step-up audio transformer (turn ratio = 1:5; part #24500, PICO Electronics) and then further amplified and filtered between 300 Hz and 10 kHz (P5 series, Grass Medical Instruments) as shown in Fig. 2. The HG signal was then digitized at a rate of 47.2 ksamples/s and converted to an appropriate format for storing the data on videotapes (Digital Data Recorder, model VR-10B, Instrutech). EEG and EOG signals were also amplified, band-pass filtered from 1 to 30 Hz, and digitized at 60 samples/s.

For frequency spectrum analysis, the raw electroenceurogram (ENG) signals were played from the videotapes off-line, resampled at a rate of 20,000 samples/s by using a data-acquisition board (NB-MIO-16P-5, National Instrument) and LabVIEW software tool, and stored on a personal computer. For breath-by-breath analysis and the temporal plots of the data, HG recordings were further filtered with a custom-designed band-pass filter (a third-order high-pass Butterworth filter at 900 Hz and a second-order low-pass Butterworth filter at 2400 Hz), rectified, and passed through a 100-ms time averager before they were sampled at a rate of 60 samples/s into the computer along with the other signals.

Data analysis. The MATLAB programming tool was used for breath-by-breath analysis. The inspiratory (Ti) and expiratory times and the breathing rate were measured from the Pes signal. The area under the phasic HG signal during the inspiratory period above the baseline was calculated as a measure of total HG output (AreaHG). The height of the phasic Pes (PeakPes) and the line integral during the inspiratory phase (AreaPes) were computed. A Student's *t*-test with an assumption of unequal variances was used for all measurements of statistical significance.

Histology. At the end of the study, animals were deeply anesthetized with pentobarbital (50 mg/kg iv) and perfused with saline followed by 4% paraformaldehyde. The main trunks and the branches of the HG nerves were dissected bilaterally from the surrounding tissue and cut 1–2 cm proximal and distal to the implanted cuff electrodes. The contralateral HG nerve that was not implanted in *beagle 2* was used as a control. Explanted nerves were placed in 3.5% glutaraldehyde. Histological sections were made at longitudinal locations of 5 and 10 mm away from each end of the cuff, at the edges of the cuff, and at locations corresponding to the center of all three contacts inside the cuff.

RESULTS

Dog model of UAW obstruction. During sleep sessions, the application of a small submental force ini-

tially produced a light snoring sound, and the sound level became louder with increasing force amplitude. At near-obstruction-force levels, the snoring sounds either became deeper and smoother or turned into louder intermittent vibratory sounds, the latter of which were usually associated with arousals. The near-obstruction state could be achieved only in deep-sleep stages. The two dogs used in this study were different in terms of the forces required to collapse their UAWs. It took larger submental forces to bring *beagle 1* to a near-obstruction state than *beagle 2* (6–8 vs. 3–4 N). Also, similar amounts of subobstructive levels of internal loading, judged by AreaPes parameter, could be achieved in *beagle 2* with one-half of the force level required in *beagle 1*. In general, the force levels that were needed to cause severe breathing difficulty in REM sleep were much lower than those required in NREM sleep in both dogs (0–3 vs. 6–8 N in *beagle 1* and 0–1 vs. 3–4 N in *beagle 2*). In some cases, the presence of the thermoplastic mold around the head was sufficient to cause the UAWs to collapse on a transition from NREM to REM sleep and an immediate arousal from sleep.

The effect of the submental force on various respiratory parameters was evaluated by varying the submental force from zero to the maximum level during NREM sleep in each dog (Fig. 4). The mean of 5–10 breaths preceding the onset of the force application was taken as control in each trial. The AreaPes and PeakPes parameters increased by 102 ± 55 and 45 ± 33 (SD)%, respectively, in *beagle 1* and 117 ± 87 and 79 ± 78 %, respectively, in *beagle 2*, and all changes were statistically significant ($P < 0.02$) when the post- and preloading mean values were paired from all the trials. The increase in the Ti (34 ± 20 and $21 \pm 15\%$ in *beagles 1* and *2*, respectively) and the decrease in the breathing rate (15 ± 6 and $12 \pm 4\%$ in *beagles 1* and *2*, respectively) were relatively smaller ($P < 0.02$). The changes in the abdominal movement (2 ± 19 and $0 \pm 31\%$ in *beagles 1* and *2*, respectively) were not statistically significant ($P = 0.47$ and $P = 0.13$, respectively). These data show that the submental force was able to reduce the size of the pharyngeal opening substantially and, therefore, load the UAWs internally. AreaPes is the

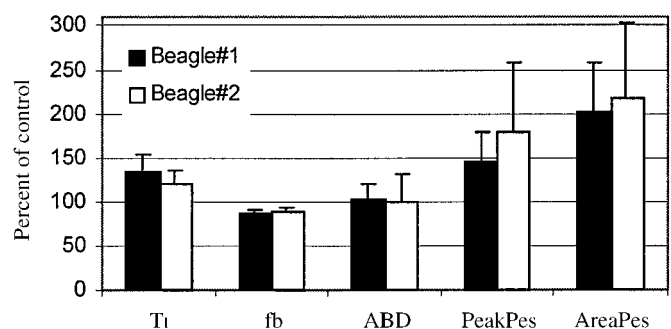


Fig. 4. Relative changes in various physiological parameters when submental force was increased from 0 (control) to a maximum level in non-rapid-eye-movement (NREM) sleep. Ti, inspiratory time; fb, breathing rate; ABD, abdominal movement; PeakPes, peak esophageal pressure (Pes); AreaPes, area of Pes during inspiration. Bar plots show mean + SD of relative increases in multiple trials (21 trials in *beagle 1* and 5 trials in *beagle 2*). Control values correspond to 100%.

parameter most sensitive to the internal loading of the UAWs. The narrowing in the UAWs due to the submental force is compensated for by an increase not only in PeakPes but also in T_i to allow the passage of a sufficient amount of air.

HG activity in NREM sleep. During NREM sleep without loading, phasic HG activity was low and sometimes not observable above the baseline level. The signal-to-noise ratio of the recordings, defined as the peak HG signal divided by the baseline level, had a value of 1.59 ± 0.43 ($n = 24$).

HG activity and the peak Pes increased simultaneously on application of the submental force (Fig. 5). In general, the phasic component did not have a reproducible shape during NREM sleep. It contained large spikes, especially while the dog was snoring. The rectified and averaged version of the HG activity was further filtered with a digital low-pass finite impulse response (120th order, frequency = 1.5 Hz) filter to remove the high-frequency components and, therefore, observe the envelope of the phasic component (not shown). The peak HG phasic activity at the maximum submental force values ranged between 0.40 and 0.81 μV for all the cuffs with a mean of 0.59 ± 0.13 μV ($n = 21$). These peak levels were much smaller than those observed during voluntary use of the tongue (see Fig. 9). The mean signal-to-noise ratio of 5–10 breaths measured at the maximum force level varied between 1.44 and 3.64 from trial to trial with a mean of 2.37 ± 0.74 ($n = 25$).

The relationship between the steady-state phasic HG response and the Pes was further investigated in the NREM sleep stage by plotting AreaHG against AreaPes in both animals (Fig. 6). The data show that the phasic component of HG is strongly correlated with the internal loadings of the UAWs ($R = 0.82$ and $R = 0.88$ in beagles 1 and 2, respectively). The HG nerve becomes active in each breath with increasing amounts of loading.

Temporal responses to UAW loading in NREM sleep. A typical force transition maneuver during NREM sleep is shown in Fig. 7A. The Pes swings and the

phasic HG activity are increased in the next breath after a step increase of 2 N in the submental force. Both responses persist as long as the submental force is held at the elevated level.

The temporal percent changes in AreaHG, AreaPes, and T_i are shown in Fig. 7, B, C, and D, respectively, during incremental force transitions of 2–6 N in NREM sleep. The first five breaths are taken as control. The transition begins within the sixth breath and ends within the seventh breath. Each bar in the plots represents the average of corresponding breaths from multiple trials ($n = 23$). All three parameters are increased in the next breath (seventh breath) after the loading of the UAWs, and all three parameters reach their steady state as soon as the force transition is complete before the eighth breath. All three parameters persist at their elevated levels as long as the force is applied.

HG activity in REM sleep. The HG nerve usually became more active after a transition from the NREM to REM sleep stage without applying the submental force while the force applicator was in place (Fig. 8A). The HG nerve was even more active when a small submental force was applied after the onset of REM sleep (Fig. 8B). Although the activity level was very variable in the REM sleep stage, the largest activity levels were usually associated with irregular abdominal movements and sometimes intermittent snoring sounds, which were suggestive of partial occlusions (Fig. 8B).

HG activity in W. The ENG signal was at the baseline level when the animals did not use their tongue, as shown during the first 10 s in Fig. 9A. There were no noticeable variations in the baseline level that could clearly be attributed to the position of the head or the posture of the animals when the dogs rested quietly. The small changes observed could result from physical deformation of the electrodes or other sources of noise. Small phasic variations were superimposed on the tonic activity during panting (Fig. 9A). The peak HG activity values were on the order of several microvolts

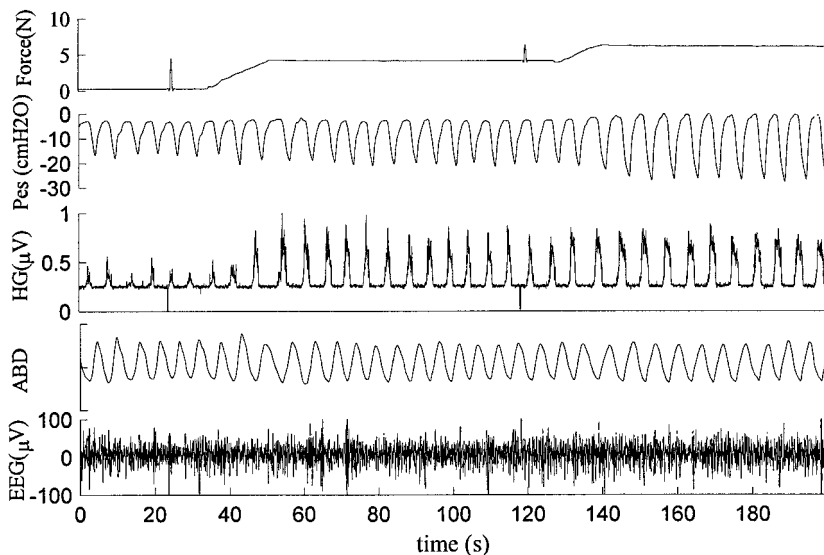


Fig. 5. Force transition maneuver in NREM sleep in beagle 1. Traces from top to bottom: submental force, Pes, rectified-integrated HG activity, ABD, and electroencephalogram (EEG) signal.

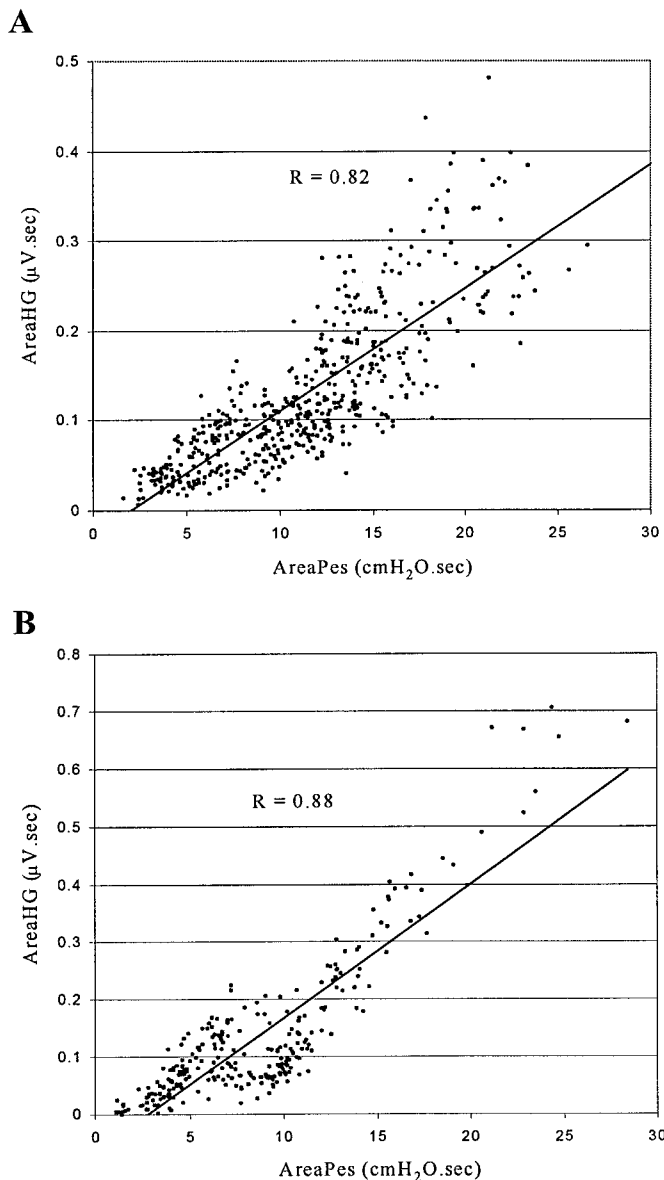


Fig. 6. Breath-by-breath measurements of area under phasic HG signal (AreaHG) vs. AreaPes in NREM sleep in *beagle 1* (A) and *beagle 2* (B). Data were obtained from 9 different force trials in each animal.

above a baseline level of ~ 0.25 μ V during various types of voluntary tongue movements, such as swallowing and licking water (Fig. 9, B and C).

ENG baseline signal. The combined impedance of nerve and cuff electrode were measured after the tissue encapsulation process was complete to estimate the

thermal noise generated in the signal source (nerve/cuff electrode) by using the Boltzmann equation (Fig. 10). Measurements were made by using the tripolar connection of the contacts (as in Fig. 1B) at various frequen-

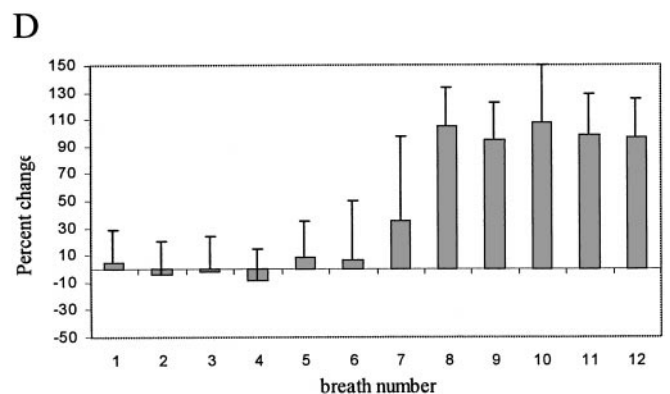
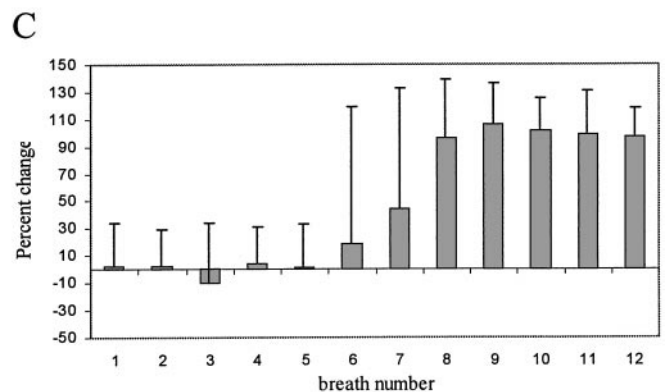
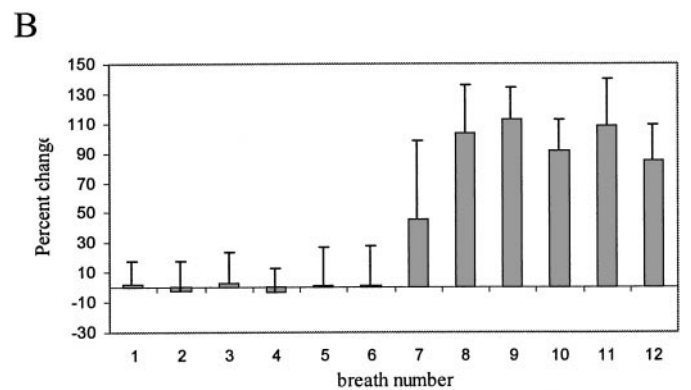
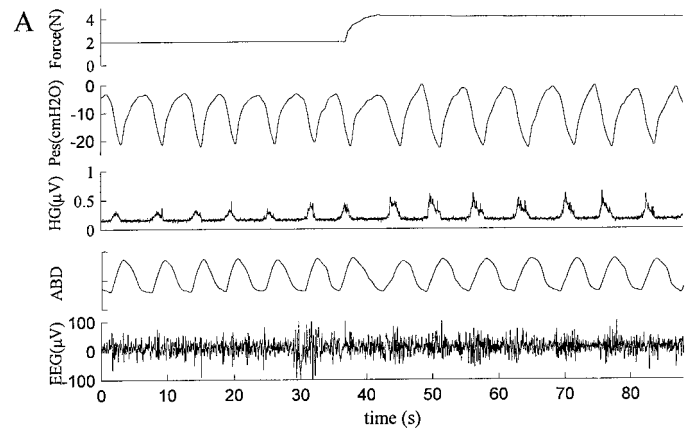


Fig. 7. Temporal responses to loading in NREM sleep. A: force transition maneuver in NREM sleep. Traces from top to bottom: submental force, Pes, rectified-averaged (100-ms) HG activity, ABD, and EEG signal. Relative increases in AreaHG (B), AreaPes (C), and T1 parameters (D) during force transition maneuvers. Force transition takes place within 6th and 7th breaths. Bars show average of corresponding breaths from 23 different trials. All measurements within a trial were normalized before corresponding breaths were averaged from all trials such that mean of the 5 breaths before force transition was 0% and mean of 5 breaths after force transition was 100%.

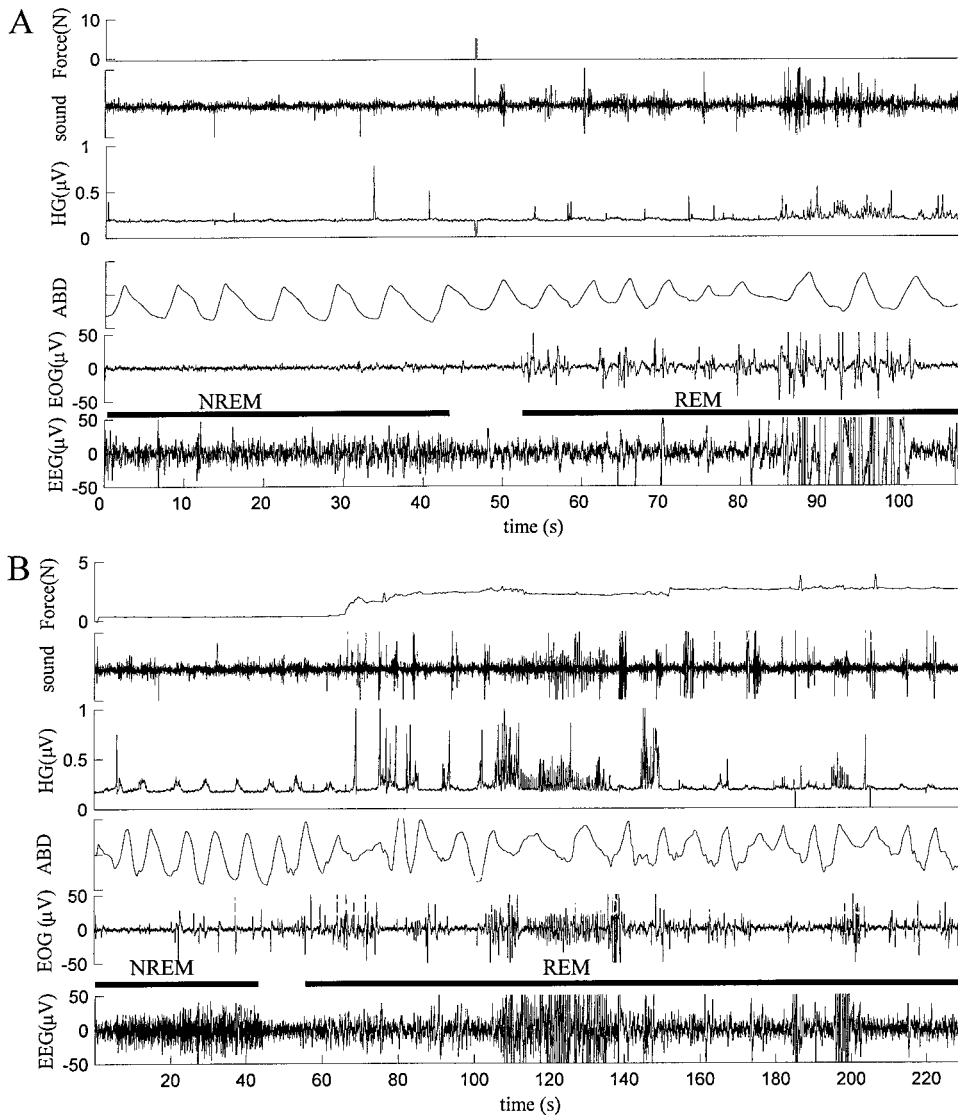


Fig. 8. HG activity recorded in *beagle 1*. *A*: during a transition from NREM to rapid-eye-movement (REM) sleep without application of submental force; *B*: while a submental force of 3 N is applied after onset of REM sleep. Note that HG nerve is very active during REM sleep, especially when there is a submental force. EOG, electrooculogram.

cies from 100 up to 10,000 Hz. The impedance values measured at 2 kHz were 3.3, 2.7, and 4.6 kΩ for cuffs 1, 2, and 3, respectively. The calculated thermal noise levels due to each one of these electrode impedances were in agreement with the measured baseline levels in the HG recordings, indicating that most of the baseline signal was due to thermal noise. There was never a noticeable increase in the baseline level because of the submental force in *beagle 1* (Fig. 5), with neither of the cuffs implanted and only a slight tonic response in *beagle 2* (not shown).

Nerve insult. In the study reported here, the instrumented dogs fully recovered and returned to their presurgical eating habits within 1 wk after surgery. Neither of the dogs had any observable functional loss in the use of their tongues for the duration of observation. There was no evidence in the behavioral pattern of the animals to suggest that the presence of the cuff electrodes on the HG nerves caused disturbance.

The histology sections from the explanted nerves showed an acceptable level of nerve insult (most of which could have been done during surgery) as indi-

cated by an increase in the interaxonal space and a decrease in the myelin thickness at locations corresponding to the midcuff levels compared with the control side (Fig. 11). These effects were expressed less at the level of the side contacts (not shown) and were completely absent in the proximal and distal sections from the implanted region (Fig. 11, *A* and *C*).

DISCUSSION

Chronic recordings of HG nerve. The results of this study demonstrated the feasibility of chronic recordings of the HG nerve with spiral-cuff electrodes. The cuff electrodes of this study were twice as long as the ones used for the initial demonstration of the HG recordings in the anesthetized animals (25). Longer cuffs were chosen because the amplitudes of ENG signals increase with increasing cuff lengths (27). The cuff-electrode implants in these dogs did not cause any observable functional impairment in the tongue function for implantation periods as long as 17 mo. The sample size is rather small in this study (3 electrodes).

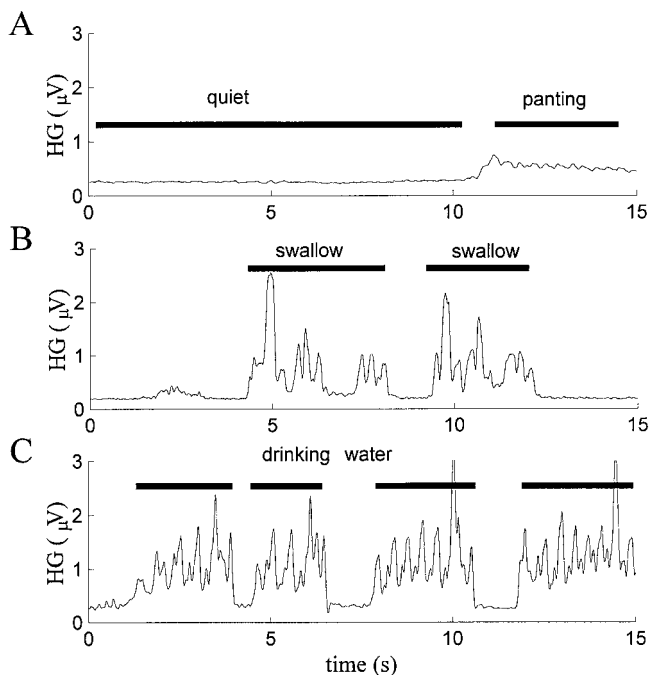


Fig. 9. Rectified-averaged HG nerve activity recorded during voluntary tongue movements: panting (A), swallowing (B), and drinking water (C).

However, these data provide valuable evidence for the feasibility of the cuff-electrode implants on the HG nerve when the implantation times and the size of the electrodes (20 mm long) are considered. In a recent study in which six adult dogs were implanted bilaterally with half-cuff electrodes (9 mm long) for 3 mo, no significant histological lesions were noted in the HG nerve, endoneurium, or perineurium (6).

Temporal HG response to loading. This study showed that phasic HG activity has a rapidly increasing and persistent response to the internal loadings of the

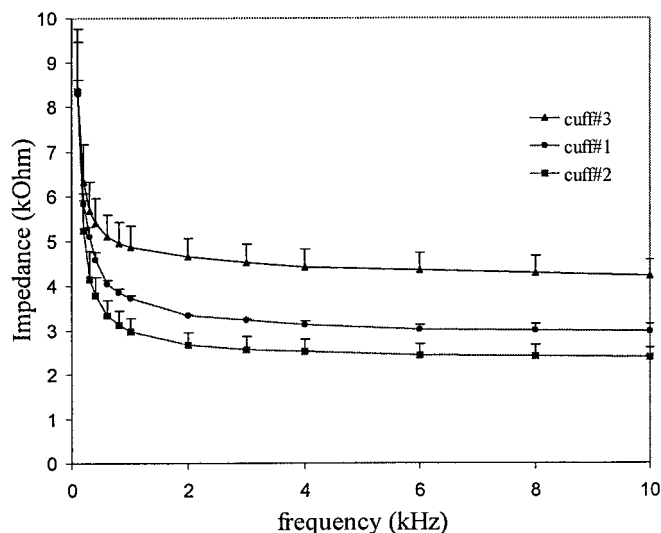


Fig. 10. Cuff/nerve impedances measured in tripolar connection (see Fig. 1B) at various frequencies. Plot for each cuff shows mean \pm SD of 3–4 sets of measurements taken every month after first 2 mo of surgery.

UAWs during NREM sleep. Contrary to some other reports, a progressive response spanning five or more breaths was not observable in our experiments (15, 16, 18). This progressive HG or GG response, which follows an increasing respiratory drive, is mediated through the chemoreflex mechanisms, whereas an immediate response probably involves the reflexes that are elicited by stimulation of the UAW mechanoreceptors. In sleeping dogs and humans, phasic GG activity increased on the first occluded breath and continued to increase progressively over the next two to three breaths (15, 16, 18). In another human study, the immediate response of the GG activity was completely absent despite a progressive response when a continuous negative airway pressure was applied to the UAWs during NREM sleep (1). However, a number of studies have shown the effectiveness of using oscillatory pressures as a mechanical stimulus in the UAWs to generate an immediate GG response. A study in dogs showed that high-frequency oscillations applied to the isolated UAWs at frequencies similar to those observed during snoring but at much less amplitude can induce immediate and sustained augmentation of GG activity in W and sleep (23). This finding was confirmed in normal subjects during sleep and in patients with sleep apnea (10). The immediate activation of the GG muscle by a brief stimulus (<1 s) of negative pressure was demonstrated in sleeping humans, although the response was much reduced and delayed compared with W (11, 30). These findings suggest that a changing pressure in the UAWs is a much more efficient stimulus than a continuous negative pressure in terms of eliciting the immediate GG response. This can explain the phasic HG response observed in this study, which increased rapidly after the force and reached its steady-state value as soon as the force transition was complete (the force was not applied abruptly so as not to arouse the animal from sleep). The external force was applied to the submental region such that it would narrow the pharyngeal passage (see the small head figure in Fig. 2B). Thus the submental force used in this study was more likely to produce oscillatory pressure changes (e.g., snoring) in the pharynx than a continuous airway pressure applied to the airways or the total occlusion of the airways. Moreover, the submental force is less likely to generate a progressive HG response because it should not alter the blood gases as much as would total occlusions.

The HG nerve signal recorded in this study might have both efferent and afferent components. The presence of afferent fibers in the HG nerve has been demonstrated, although they are only a few in number (28). The HG nerve signal recorded in this study should primarily contain efferent activity.

Temporal T_i response to UAW loading. The loading method used in this study also caused a fast increase in the T_i. During resistive and elastic loadings in NREM sleep, there is no discernible immediate first- or second-breath increase in the diaphragm activity and overall inspiratory drive in humans (2, 5, 12, 14, 31). Moreover, in dogs that were breathing through an endotracheal tube, the T_i did not increase significantly at the first

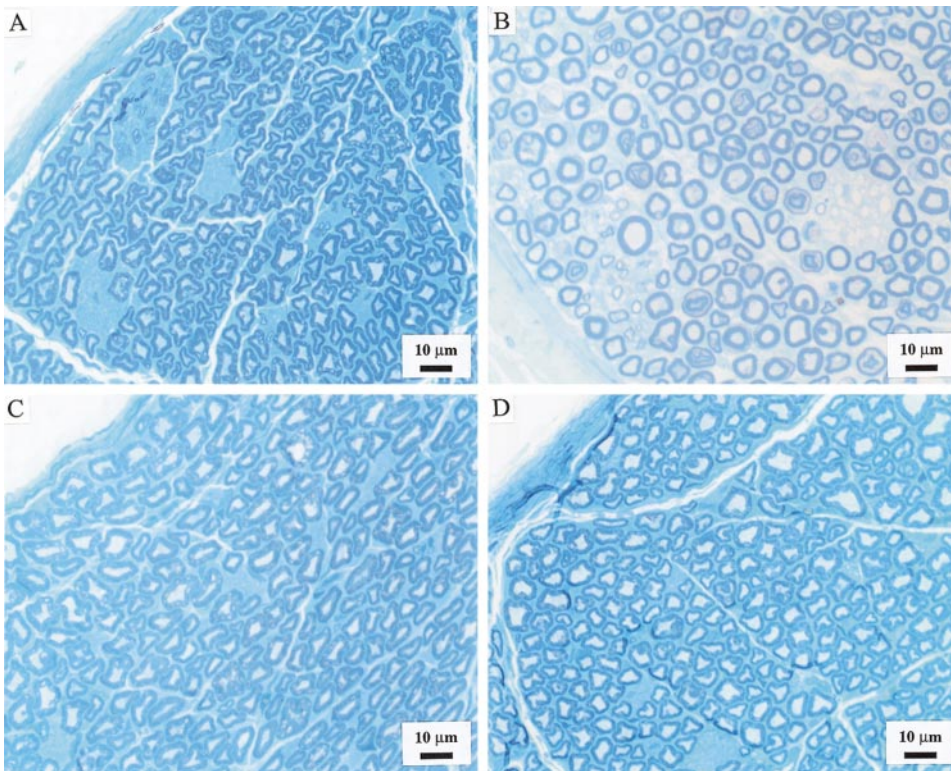


Fig. 11. Histology sections from both HG nerves in *beagle 2*. One side was implanted for 6 mo, and the other was the control side. Shown are sections proximal to (A), in the middle of (B), and distal to the cuff (C), and the control side (D).

breath as a response to resistive and elastic loadings during sleep (5). On the contrary, a negative airway pressure applied to either the nasopharynx or the larynx (but not the mouth) in anesthetized dogs produced a prolongation of the inspiratory period in the first breath, and it gradually returned to the control values within several breaths (29). Thus stimulation of airway mechanoreceptors may be essential for eliciting an immediate response in the respiratory drive. Unlike the quickly increasing and decaying or progressively increasing responses reported in these other studies, in this study the T_i increased when it loaded quickly and persisted as long as the submental force was applied. This discrepancy suggests a fundamental difference between the loading scheme used in this study and that used in the other studies in the way that they elicited the respiratory drive response.

HG activity in REM sleep. The irregular pattern of respiratory drive in REM sleep makes it difficult to compare the HG response to loading in REM sleep with that in NREM sleep. However, we observed that the HG nerve was more active in REM sleep, especially when submental force was applied, than in the preceding NREM sleep episodes. This observation seems to contradict some previous reports of GG recordings (15, 26). The discrepancy may be due to the nature of the loading scheme used in this study. A smaller force was able to collapse the UAWs in REM sleep compared with in NREM sleep. This could be attributed to the overall reduction in the tonic innervation of the UAW muscles in REM sleep. As a result, the submental force was probably able to stimulate the UAW mechanoreceptors much more efficiently in REM sleep than in NREM

sleep by being able to force the airways to a smaller size.

Tonic HG activity. The measured HG baseline signal levels were close to the estimated thermal noise levels and never increased as a response to the submental force with either of the cuffs in *beagle 1*. This suggests that the baseline signal mostly consisted of the thermal noise that was generated in the resistive component of the nerve/cuff impedance and that the tonic activity recorded with this implant was small compared with the thermal noise level. Although there was a small tonic response in *beagle 2*, the change in the HG baseline was much smaller than that of the phasic component and also that of the baseline variations between experiments. The variation in the HG baseline observed between experiments can be attributed to changes in the cuff-electrode impedance because of the reshaping of the cuff that is caused by the forces created by the surrounding muscles. In this study, a small size was chosen for the cuff-electrode contacts (2×1.25 mm) to increase the mechanical durability. This probably increased the contact impedances and thereby the thermal noise levels in the recordings. Otherwise, the presence of tonic HG activity has been demonstrated in human (26) and animal (8, 9, 15) studies with GG muscle recordings during sleep and W. Because the thermal noise level was higher than the HG tonic activity level, the decrease in the HG tonic activity at the onset of REM sleep, as reported by other groups (15, 26), was not observed in our recordings. This can also explain why the phasic component and the changes in the baseline signal in correlation with the head posi-

tion, which were demonstrated with GG recordings in cats (3), were not detectable during quiet W in our dogs.

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Address for reprint requests and other correspondence: D. M. Durand, Neural Engineering Center, Dept. of Biomedical Engineering, Case Western Reserve Univ., 10900 Euclid Ave., C. B. Bolton Bldg., Rm 3440, Cleveland, OH 44106 (E-mail: dxd6@po.cwru.edu).

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