Development/Plasticity/Repair

Identification of a Novel Form of Noradrenergic-Dependent Respiratory Motor Plasticity Triggered by Vagal Feedback

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The respiratory control system is not just reflexive, it is smart, it learns, and, in fact, it has a memory. The respiratory system listens to and carefully remembers how previous stimuli affect breathing. Respiratory memory is laid down by adjusting synaptic strength between respiratory neurons. For example, repeated hypoxic bouts trigger a form of respiratory memory that functions to strengthen the ability of respiratory motoneurons to trigger contraction of breathing muscles. This type of respiratory plasticity is known as long-term facilitation (LTF). Although chemical feedback, such as hypoxia, initiates LTF, it is unknown whether natural modulation of mechanical feedback (from vagal inputs) also causes motor plasticity. Here, we used reverse microdialysis, electrophysiology, neuropharmacology, and histology to determine whether episodic modulation of vagally mediated mechanical feedback is able to induce respiratory LTF in anesthetized adult rats. We show that repeated obstructive apneas disrupt vagal feedback and trigger LTF of hypoglossal motoneuron activity and genioglossus muscle tone. This same stimulus does not cause LTF of diaphragm activity. Hypoxic episodes do not cause apnea-induced LTF; instead, LTF is triggered by modulation of vagal feedback. Unlike hypoxia-induced respiratory plasticity, vagus-induced LTF does not require 5-HT$_2$ receptors but instead relies on activation of α1-adrenergic receptors on hypoglossal motoneurons. In summary, we identify a novel form of hypoxia- and 5-HT-independent respiratory motor plasticity that is triggered by physiological modulation of vagal feedback and is mediated by α1-adrenergic receptor activation on (or near) hypoglossal motoneurons.

Introduction

Neural circuits that regulate motor behaviors such as breathing are not only designed to respond to reflexive feedback, but are also designed to learn so that motor control adapts to repeated experiences. For example, the mammalian respiratory system not only responds to feedback from chemical (e.g., O_2) and mechanical (e.g., lung volume) drives, it is also smart and learns to adapt to recurring stimuli by undergoing plasticity. One type of respiratory plasticity is long-term facilitation (LTF): it is triggered by repeated episodes of hypoxia, which strengthens respiratory motor outflow onto the motoneurons that drive breathing muscles, such as the diaphragm and genioglossus (tongue) (Baker and Mitchell, 2000; Feldman et al., 2003; Baker-Herman et al., 2004; Fuller, 2005).

Although chemical feedback, such as hypoxia, elicits LTF of respiratory motor activity, it is unknown whether physiological modulation of respiratory-related mechanical feedback can also trigger respiratory motor plasticity. This is biologically important because breathing is acutely sensitive to and regulated by mechanical feedback from both the lungs and upper airways (Hammouda and Wilson, 1932; Kuna, 1986; Kubin et al., 2006). It is also clinically relevant because intermittent modulation of mechanical feedback during obstructive sleep apnea (OSA), a disorder that afflicts 28 million North Americans (Guilleminault and Abad, 2004; Horner, 2008), could have neuroplastic effects on respiratory motor function.

Mechanical feedback from lung mechanoreceptors is relayed by vagus nerve afferents to the brainstem circuitry that controls breathing (van Lunteren et al., 1984; Kuna, 1986; Ezure et al., 2002; Kubin et al., 2006). We predict that repeated modulation of mechanical feedback will trigger respiratory motor plasticity, and we base this assertion on three lines of evidence. First, classic studies in *Aplysia* show that intermittent stimulation of respiratory-related mechanoreceptors causes LTF of respiratory gill-withdrawal reflex (Kandel, 2001). Second, high-intensity vagus nerve stimulation in rats has both short-term and long-term effects on breathing (Zhang et al., 2003). Third, electrical stimulation of vagal afferents influence other forms of brain plasticity, such as hippocampal long-term potentiation and memory retention (Clark et al., 1999; Ghacibeh et al., 2006; Zuo et al., 2007). Although experimental stimulation of vagal afferents can facilitate plasticity and modulate breathing, it is unknown whether physiologically relevant modulation of vagal feedback can also trigger respiratory plasticity.

We found that repeated modulation of vagal feedback caused by obstructive apneas triggers respiratory motor plasticity. Specifically, we show that repeated apneas not only cause hypoxia and affect vagal feedback, but they also induce LTF of genioglossus motor activity. LTF is triggered by vagal feedback and not by hypoxia because removal of vagal feedback even in the presence of episodic hypoxia prevents LTF. Importantly, modulation of vagal feedback itself is sufficient for triggering respiratory plasticity because episodic disruption of vagal feedback caused LTF. Last, we show that activation of α1-adrenergic receptors on (or

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near) hypoglossal motoneurons is required for vagus-mediated respiratory LTF.

Materials and Methods

Experiments were conducted on anesthetized, spontaneously breathing adult male Sprague Dawley rats ($n = 83$; 407 ± 21 g). All procedures and experimental protocols were approved by the Animal Care and Use Committee at the University of Toronto.

Surgical procedures

Rats were initially anesthetized with isoflurane in an induction chamber and then maintained under anesthesia via a nose cone (3–3.5% isoflurane in air). After abolition of the foot-pinch withdrawal reflex, they were placed on a servo-controlled heating pad (09585; FHC) and maintained at 36 ± 0.6°C. A midline ventral incision was made in the neck, and the trachea was sectioned just below the larynx; a tracheal tube was inserted for delivering anesthetic and oxygen (2–2.5% isoflurane in 50% O_2 and 50% N_2). The proximal end of the tracheal tube was connected to a custom-designed occluding device. The jugular vein was cannulated for administration of fluids to maintain fluid and acid-base balance (1:4 solution of 1 M sodium bicarbonate and lactated Ringer's solution; 1.5 ml/h). To record diaphragm electromyographic (EMG) activity, a 1–2 cm abdominal incision was made, and a custom-made bipolar electrode was fastened to the diaphragmatic fascia. To record upper airway respiratory activity, a pair of EMG needle electrodes (F-E2; Grass Technologies) were inserted into the genioglossus muscle. To record or modulate vagal afferent feedback, left and the right vagus nerves (cervical level) were carefully dissected and placed on either grounded bipolar recording electrodes or on custom-made cooling coils (see protocols below).

For microdialysis experiments, rats were placed in a stereotaxic frame (David Kopf Instruments), and their heads were secured with ear bars and a snout clamp. To position a microdialysis probe into the hypoglossal motor pool, a burr hole was made at the following coordinates (relative to bregma): 14.1 ± 0.08 mm posterior, 0.28 ± 0.02 mm lateral, and 9.1 ± 0.07 mm ventral.

Electrophysiological recordings. EMG and vagus nerve activity were amplified (NL104; Neurolog), filtered (bandpass, 1–3000 Hz for EMG and 500–5000 Hz for nerve activity respectively; NL126; Neurolog), integrated (time constant, 100 ms), digitized (1 kHz; Micro1401; Cambridge Electronic Design), and stored on a computer using Spike2 software (Cambridge Electronic Design). Specially written software (Labview; National Instruments) processed integrated EMG signals so that both the frequency and amplitude of the signals were automatically calculated and stored in 60 s bins.

Manipulation of vagal feedback. In some experiments, afferent vagal feedback was blocked by focally cooling both left and right vagus nerves (cervical level). To do this, a short length (~2 mm) of each nerve was laid on tiny cooling coils made from hollow copper tubing (Alloy 122; McMaster-Carr). Cooling coils were chilled to 3°C by constantly circulating ice-cold 95% ethanol through them using a peristaltic pump (502S; Watson-Marlow). A version of this technique has been shown previously to block vagal afferent feedback (Fishman et al., 1973; Phillipson et al., 1973).

Measuring CO_2 and O_2 levels. End-tidal CO_2 was monitored using a CO_2 analyzer (17630; VacuMed). Arterial O_2 saturation was measured using a pulse oximeter designed for rodents (MouseOx Pulse Oximeter; STARR Life Sciences Corp.). WINDAQ Waveform Browser software (Dataq Instruments) was used to digitize and analyze signals, which were then recorded using Spike2 software.

Microdialysis probes. Reverse dialysis was used to focally apply candidate receptor antagonists directly onto hypoglossal motoneurons by implanting a microdialysis probe (34 kDa cutoff membrane: 1 mm long × 250 μm wide; CMA/Microdialysis) into the hypoglossal nucleus. Probes were connected to FEP Teflon tubing (inside diameter, 0.12 mm; Eicom) and a 500 μl gas-tight syringe (MD-0050; Bioanalytical Systems). A syringe pump (MD-1001; Bioanalytical Systems) was used to continuously perfuse probes with 0.9% saline at a constant rate of 2 μl/min. A liquid-switch (MD-1508; Bioanalytical Systems) was used to switch between saline and candidate drugs without interfering with perfusion rate (Brooks and Peever, 2008; Burgess et al., 2008).

Drug preparation. All drugs were made on the day of experiments; they were dissolved in 0.9% saline, vortexed, and then filtered (0.2 μm nylon; Thermo Fisher Scientific). Ketanserin tartate [545.51 molecular weight (MW); Sigma Canada] and terazosin hydrochloride (423.89 MW; Sigma Canada) were used to block 5-HT_{2A} and α1-adrenergic receptors, respectively. The glutamate receptor agonist AMPA (267.08 MW; Tocris Bioscience) was used to activate AMPA receptors on hypoglossal motoneurons.

Experimental protocols

Rats were left to stabilize for 40–60 min before experiments began. Respiratory activity (i.e., genioglossus and diaphragm EMG), end-tidal CO_2, arterial O_2 saturation, and rectal temperature were continuously monitored and recorded for 60–90 min after experimental interventions. The following questions were addressed.

Study 1: do recurrent obstructive apneas induce LTF? Rats ($n = 12$) were exposed to 10 15-s obstructive apneas each separated by 1 min. Apneas were induced by inflating (15 s) a small, balloon-like device that was fitted into the tracheal tube. Obstructions were confirmed by monitoring CO_2 and O_2 levels as well as changes in vagus nerve activity (Fig. 1). Apneas were triggered during end expiration to mimic the obstruction pattern experienced in OSA patients (Sanders and Moore, 1983; Sanders et al., 1985). After repeated airway obstructions, respiratory activity was monitored for 60 min to determine whether this intervention triggered LTF.

Study 2: is apnea-induced LTF triggered by hypoxia or vagal feedback? Obstructive apneas not only cause hypoxemia (Guilleminault and Abad, 2004), they also affect mechanical vagal afferent feedback (van Lunteren et al., 1984; Strohl, 1985; Milsom, 1990; Bailey and Fregosi, 2006). The following set of experiments was therefore designed to determine how each of these variables contributes to apnea-induced LTF. To determine the role that vagal feedback plays in LTF activation, we induced repeated apneas with and without functional vagal afferents. We did this in two different ways. In one set of experiments ($n = 7$), we permanently removed vagal feedback by bilateral vagotomy before inducing repeated apneas. In another set ($n = 8$), we temporarily removed afferent inputs by focally cooling the left and right vagi (at the cervical level) during the repeated apnea protocol.

Study 3: can vagal afferent feedback itself trigger LTF? To determine whether modulation of vagal feedback alone (i.e., without hypoxia) causes LTF, we repeatedly suppressed afferent activity by cooling left and right vagi. It would be ideal to mimic the obstructive apnea protocol (i.e., 10 15-s episodes); however, it is not possible to cool and rewarm nerves in <1 min intervals. Therefore, nerves were cooled six times for 1 min each, with 1 min between each cooling episode, and respiratory activity was then monitored for another 60–90 min ($n = 6$).

Study 4: is apnea-induced LTF mediated by a serotonergic or noradrenergic mechanism? Previous studies show that blockade of either 5-HT_{2A} or α1-adrenergic receptors prevents hypoxia-induced LTF activation (McGuire et al., 2004; Golder and Mitchell, 2005; Neverova et al., 2007). Therefore, we aimed to determine whether 5-HT_{2A} and/or α1-adrenergic receptors contribute to vagus-mediated LTF. To do this, we antagonized either 5-HT_{2A} or α1-adrenergic receptors at the hypoglossal nucleus by perfusing 50 μM ($n = 6$) or 100 μM ($n = 4$) ketanserin or 1 μM terazosin ($n = 6$) for 20 min before repeated apneas. These concentrations of ketanserin and terazosin were used because previous microdialysis studies show that they effectively antagonize 5-HT_{2A} and α1-adrenergic receptors (Thorré et al., 1998; Rouquier et al., 1994; Yan, 2000; Yan et al., 2000; Kommalage and Höglund, 2005). Once the apnea protocol was complete, saline perfusion was resumed and respiratory activity was recorded for 60–90 min. A separate group of rats were exposed to repeated apneas under saline perfusion alone ($n = 11$). This group served as the control.

Control experiments. To show that breathing remained stable during the 60–90 min experimental period, we quantified respiratory activity for 90 min in a group of vagus-intact ($n = 8$) and vagotomized rats ($n = 6$). To demonstrate that vagus nerve cooling alone does not have long-

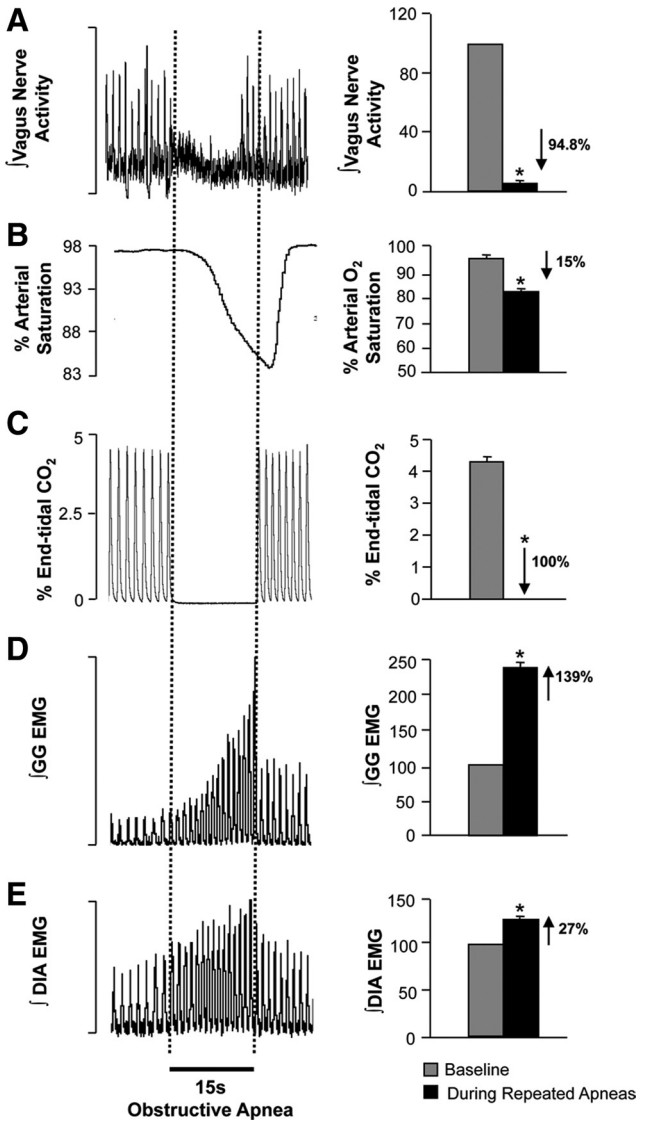

Figure 1. Obstructive apneas suppress both vagus nerve activity and arterial O_2 saturation. ***A***, An integrated trace of vagus nerve activity before, during, and after a 15 s apnea and group mean data showing that obstructive apneas suppress vagus activity. ***B***, A pulse oximeter trace showing arterial O_2 levels before, during, and after an apnea and group data showing that apneas reduce O_2 levels. ***C***, A trace showing end-tidal CO_2 levels before, during, and after an apnea and group data showing that CO_2 levels are undetectable during apneas. ***D***, ***E***, Integrated genioglossus (GG) and diaphragm (DIA) EMG activity before, during, and after an apnea and group data demonstrating that genioglossus and diaphragm activity reflexively increase during airway obstructions. Group data are expressed as percentage change from baseline ± SEM. *$p < 0.05$, significantly different from baseline.

term effects on respiratory activity, we cooled left and right vagus nerves for either a single, continuous 6 min ($n = 6$) or 13 min ($n = 7$) period; respiratory activity was then recorded for 60–90 min. The 6 and 13 min time periods are equivalent to either the sum of the six cooling periods (i.e., 6 min) or to the entire cooling procedure (i.e., 13 min).

Histology

Two procedures verified that microdialysis probes were located in the hypoglossal motor pool. (1) During the last 15 min of microdialysis experiments, 0.1 mM AMPA was perfused into the hypoglossal nucleus. This intervention should only effect hypoglossal motoneurons and hence genioglossus EMG activity; it should have no affect on either diaphragm EMG or respiratory frequency. (2) Postmortem histology confirmed the location of probes in the hypoglossal nucleus. Histological procedures were completed as follows. Rats were killed by an overdose of isoflurane anesthesia, and their brains removed and fixed in 4% paraformaldehyde (in 0.1 M PBS) for 48 h. Tissue was cryoprotected by soaking brains in 30% sucrose (in 0.1 M PBS) for 48 h. Brain tissue was sectioned into 30 μm slices using a freezing microtome (Leica); it was then mounted, dried, and stained with Neutral Red. Tissue sections were viewed using a light microscope (Olympus), and the location of probe lesion tracts were plotted on standardized rat brain maps (Paxinos and Watson, 1998).

Data analysis

Peak integrated inspiratory EMG amplitudes (i.e., genioglossus and diaphragm) as well as respiratory frequency were quantified on a breath-by-breath basis in 60 s intervals during all experimental protocols. Inspiratory amplitude and respiratory frequency are expressed as a percentage change from baseline ± SEM. Baseline values for inspiratory amplitude and respiratory frequency were acquired during the 60 s period before experimental interventions (e.g., repeated apneas). Data are quantified and expressed before (i.e., baseline) and at 15, 30, 45, and 60 min after experimental interventions. Peak integrated vagus nerve activities were averaged for 15–20 s before each airway occlusion. This value served as baseline vagus nerve activity. Average vagus nerve activities during airway occlusions were then expressed as a percentage change from baseline ± SEM. Arterial O_2 saturation and end-tidal CO_2 values are also expressed as a percentage change from baseline ± SEM.

Statistical analysis

The statistical tests used for analysis are included in Results. Comparisons between treatments (e.g., vagus intact vs vagotomy) for mean respiratory variables (e.g., amplitude or respiratory frequency) were made using a two-way repeated-measures (RM) ANOVA with *post hoc* Fisher's least significant difference tests to infer statistical significance. Paired *t* tests were used to determine whether respiratory variables were statistically different before and after apneas. Nonparametric Wilcoxon's signed-ranked tests were used when data were not normally distributed. A linear regression was used for correlation between the magnitude of LTF and the reflex response to repeated apneas at the end of each experiment. All statistical analyses used SigmaStat (SPSS) and applied a critical two-tailed value of $p < 0.05$. Data are presented as means ± SEM.

Results

Repeated obstructive apneas modulate vagal feedback and trigger LTF

Before determining whether repeated apneas trigger LTF, we wanted to verify that apneas actually modulate vagal feedback. We therefore quantified levels of vagus nerve activity before and during individual airway occlusions. Mean vagus nerve activity fell by 95 ± 0.3% (paired *t* test, $p = 0.01$) during individual obstructive apneas (Fig. 1*A*). We also showed that airway occlusion reflexively increased genioglossus and diaphragm inspiratory activity by 139 ± 6 and 27 ± 0.6% above baseline values, respectively ($n = 12$; paired *t* test, $p = 0.001$ for both muscles) (Fig. 1*D*,*E*). Occlusions also reduced end-expired CO_2 levels from 4.2 ± 0.2 to 0% (preocclusion baseline vs occlusion; Wilcoxon's signed-rank test, $p < 0.001$) (Fig. 1*C*), thus demonstrating that airway occlusions were complete. Last, we show that obstructive apneas induced hypoxemia, reducing arterial O_2 saturation by 14 ± 0.09% during occlusions (baseline, 95 ± 0.5% vs occlusion, 82 ± 0.09%; paired *t* test, $p = 0.037$) (Fig. 1*B*).

Recurrent airway occlusions triggered a potent and sustained increase in inspiratory genioglossus muscle activity that peaked at 60 ± 8% above baseline levels by 60 min (RM ANOVA, $p < 0.001$) (Fig. 2*A–C*). Apneas only amplified the magnitude of genioglossus inspiratory activity; they had no long-term effect on respiratory frequency (baseline, 58 ± 2 breaths/min vs 60 min post-apneas, 56 ± 3 breaths/min; RM ANOVA, $p = 0.43$) (Fig. 2*E*) or diaphragm muscle activity (RM ANOVA, $p = 0.25$) (Fig. 2*D*). LTF of inspiratory genioglossus tone was not attributable to changes in either O_2 or CO_2 levels because there were no long-

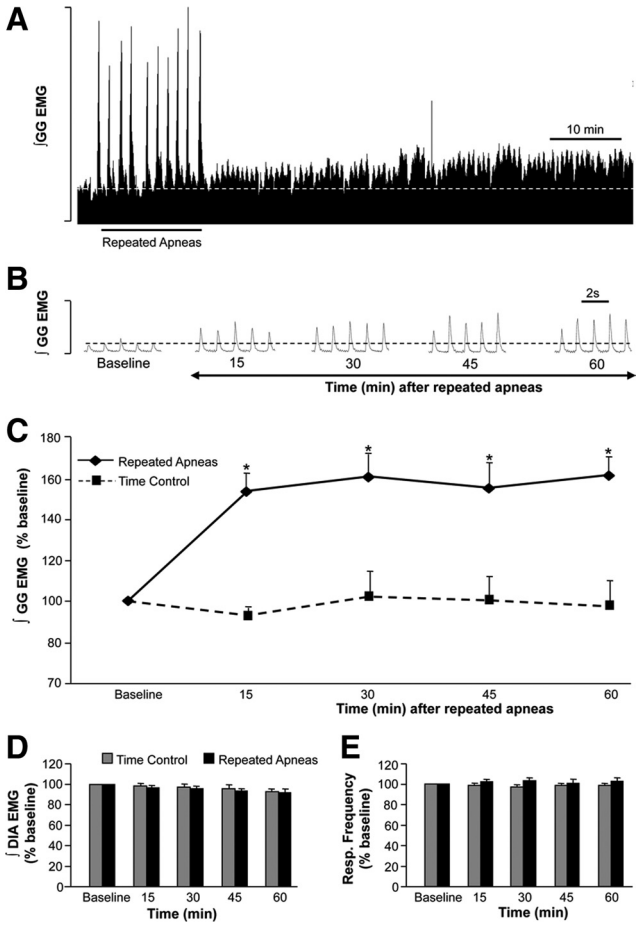

Figure 2. Repeated apneas trigger LTF of inspiratory genioglossus tone. ***A***, Genioglossus (GG) EMG activity before (baseline), during, and after repeated apneas. Note that inspiratory genioglossus tone increased above baseline levels (dotted line) after repeated apneas, i.e., LTF. ***B***, Integrated genioglossus EMG activity during baseline and at 15, 30, 45, and 60 min after repeated apneas. ***C***, Group mean data showing that repeated apneas trigger LTF of inspiratory genioglossus tone; at 15, 30, 45, and 60 min after repeated apneas, genioglossus activity increased above baseline levels. However, genioglossus activity remained stable across the 60 min recording period when repeated apneas were absent (i.e., time control). ***D***, ***E***, Group data demonstrating that diaphragm (DIA) EMG activity and respiratory frequency were unaffected by repeated apneas and remained stable in time-matched control recordings. Group data are expressed as percentage change from baseline ± SEM. $*p < 0.05$, significantly different from baseline.

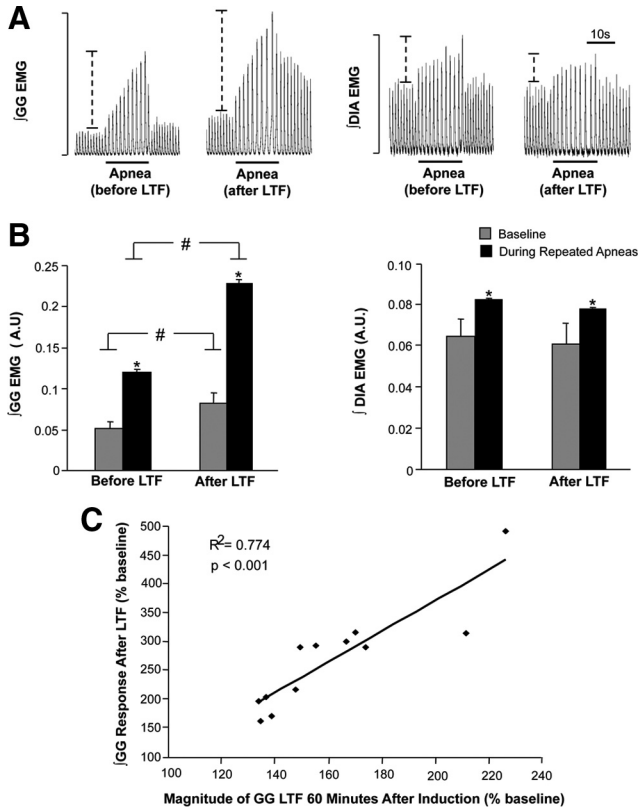

Figure 3. LTF strengthens genioglossus reflexes. ***A***, Apnea-induced changes in integrated genioglossus (GG) and diaphragm (DIA) EMG activity before and after LTF induction. ***B***, Group mean data demonstrating that the genioglossus response to apneas is significantly larger after LTF than before it. The diaphragm response to apneas is unaffected by LTF, with the response magnitude being equal before and after LTF induction. Note that gray bars represent the magnitude of genioglossus or diaphragm activity before apneas (i.e., baseline), whereas black bars represent the magnitude of the genioglossus or diaphragm response to apnea. ***C***, Group data showing a strong positive correlation between the magnitude of LTF and the subsequent magnitude of the genioglossus (GG) response to apneas. Group data are expressed as percentage change from baseline ± SEM. $*p < 0.05$ significantly different from baseline; $^{\#}p < 0.05$, significant difference before and after LTF. A.U., Arbitrary units.

term changes in either variable (RM ANOVA, $p > 0.05$ for both variables) (data not shown).

Last, we wanted to demonstrate that respiratory activity remained stable throughout the recording period. In a control group of rats, we recorded both genioglossus and diaphragm activity for at least 60 min without exposing them to repeated apneas. Both genioglossus and diaphragmatic activity as well as respiratory frequency remained constant throughout the 60 min recording period (RM ANOVA, $p > 0.05$ for all variables) (Fig. 2*C–E*).

LTF strengthens reflex pathways onto hypoglossal motoneurons

To determine whether apnea-induced LTF amplifies the responsiveness of hypoglossal motoneurons to respiratory reflexes, we quantified the magnitude of genioglossus responses to airway occlusions before and after LTF (i.e., at 60 min). During baseline, airway occlusions increased inspiratory genioglossus activity by 139 ± 6% ($n = 12$; paired t test, $p = 0.001$); however, this same intervention increased genioglossus activity by 181 ± 7% (paired t test, $p = 0.001$) after LTF induction (Fig. 3*A*,*B*). This enhancement was significantly larger than the baseline response (paired t test, $p = 0.023$). Importantly, there is a strong positive correlation between the magnitude of LTF and the magnitude of the reflex response ($r^2 = 0.774$; $p < 0.001$), suggesting that LTF strengthens hypoglossal motoneuron responsiveness in a dose-dependent manner (Fig. 3*C*). There was no difference between the magnitude of diaphragm responses to airway occlusions before and after LTF induction (baseline, 27 ± 0.6% increase; after LTF, 21 ± 0.5% increase; paired t test, $p = 0.148$), indicating that phrenic motoneuron responsiveness is unaffected by LTF mechanisms.

LTF is not triggered by repeated hypoxic episodes

Because obstructive apneas modulated vagal feedback and induced hypoxia, we aimed to dissociate their relative roles in eliciting LTF. To do this, we evoked apnea-induced hypoxia but prevented vagal feedback by cutting both left and right vagus nerves. Although repeated apneas caused LTF when vagal feedback was intact (Fig. 2*A–C*), bilateral vagotomy prevented LTF but hypoxic insults remained (baseline, 96 ± 0.1% vs occlusion,

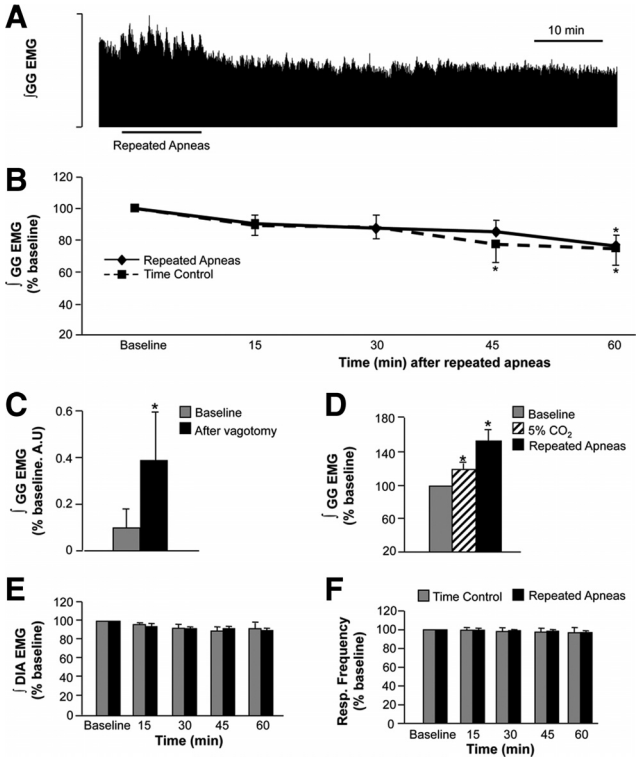

Figure 4. Vagotomy prevents apnea-induced LTF. ***A***, An EMG trace of genioglossus (GG) activity before, during, and after repeated apneas under vagotomy. Although apneas cause systemic hypoxia, they do not trigger LTF when vagal feedback is removed. ***B***, Group mean data showing that vagotomy prevents apnea-induced LTF; in fact, genioglossus activity decreases below baseline levels 60 min after apneas in vagotomized rats. In time control experiments (i.e., no apneas), genioglossus activity decreases below baseline levels at 45 and 60 min. ***C***, Group data showing that vagotomy causes a significant increase in inspiratory genioglossus EMG activity. ***D***, Even after vagotomy, inspiratory genioglossus activity can be reflexively increased by 5% CO_2 and obstructive apneas. ***E***, ***F***, Group data demonstrating that both inspiratory diaphragm (DIA) EMG activity and respiratory frequency are unaffected by repeated apneas and remained stable during the 60 min recording period in vagotomized rats. Group data are expressed as percentage change from baseline ± SEM. $^{*}p < 0.05$, significantly different from baseline. A.U., Arbitrary units.

$84 \pm 0.3\%$ O_2 saturation; paired t test, $p = 0.01$) (Fig. 4*A*). In fact, repeated apneas led to a gradual decline in genioglossus activity ($n = 8$; RM ANOVA, $p < 0.001$ at 60 min) (Fig. 4*B*), although respiratory frequency and diaphragm activity were unaffected (RM ANOVA, $p > 0.05$ for both variables) (Fig. 4*E*,*F*). There were no long-term changes in either arterial O_2 or CO_2 levels (RM ANOVA, $p > 0.05$) (data not shown).

Because vagotomy itself increased inspiratory genioglossus activity ($270 \pm 10\%$ above baseline; paired t test, $p = 0.01$) (Fig. 4*C*), we wanted to demonstrate that LTF was not masked by a ceiling effect and that genioglossus activity could indeed be elevated above post-vagotomy levels. Even after vagotomy, hypercapnia (5% CO_2) was able to increase genioglossus activity by $20 \pm 7\%$ ($n = 5$; paired t test, $p < 0.001$) (Fig. 4*D*). We also found that obstructive apneas increased genioglossus activity by $54 \pm 12\%$ after vagotomy (paired t test, $p < 0.001$) (Fig. 4*D*). Together, these findings suggest that lack of LTF after vagotomy is not attributable to the inability of genioglossus activity to respond to increased inspiratory drive.

Blockade of vagal feedback during repeated apneas prevents LTF

Because apnea-induced LTF was abolished by vagotomy, we wanted to further characterize the role that vagal feedback plays

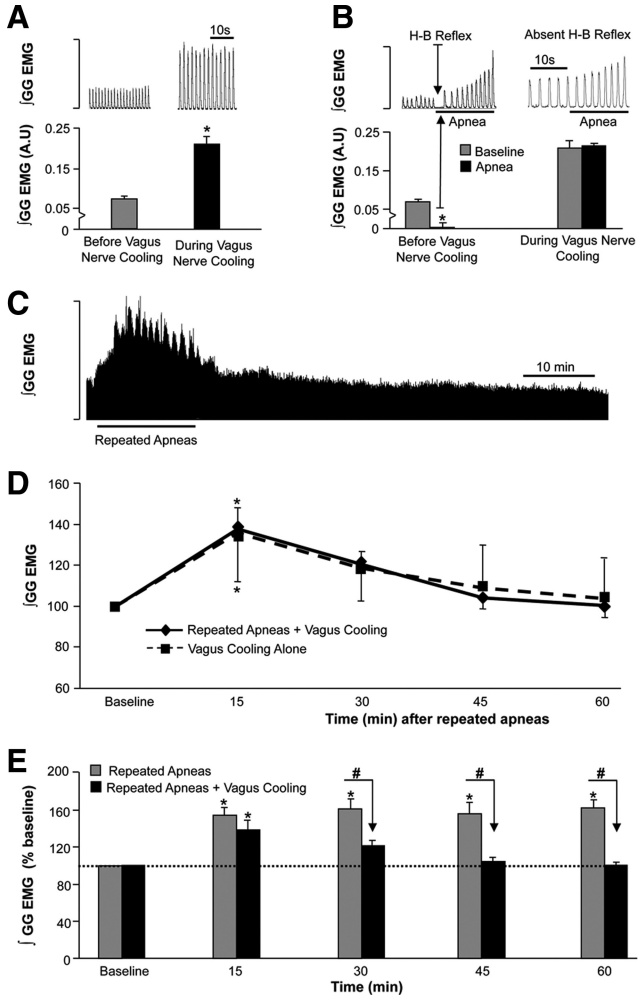

Figure 5. Vagal feedback is required for apnea-induced LTF. ***A***, An EMG trace showing inspiratory genioglossus (GG) activity before and during vagus nerve cooling (top traces). Group data showing that vagal cooling increases inspiratory genioglossus activity (bottom graph). ***B***, A genioglossus EMG trace showing that maintained lung inflation triggers the Hering–Breuer (H–B) reflex when vagal afferents are intact but that removal of afferent input by vagal cooling prevents the Hering–Breuer reflex (top traces). Group data showing that vagal cooling blocks the Hering–Breuer reflex (bottom graph). ***C***, An EMG trace of genioglossus activity before, during, and after repeated apneas and vagus nerve cooling. ***D***, Group data showing that (1) blockade of vagus afferent activity prevents apnea-induced LTF (solid line), and (2) a single 13 min period of vagal cooling (without apneas) has a transient effect on genioglossus activity (hatched line). ***E***, Group data showing that repeated apneas trigger LTF of genioglossus activity when vagal afferents are intact (gray bars) and that blockade of afferent feedback (by nerve cooling) prevents apnea-induced LTF (black bars). Group data are expressed as percentage change from baseline ± SEM. $^{*}p < 0.05$, significantly different from baseline; $^{\#}p < 0.05$, significant difference between the indicated data groups; A.U., Arbitrary units.

in initiating LTF. To do this, we reversibly blocked vagal afferent activity only during repeated obstructive apneas (i.e., a 13 min cooling period). Vagal feedback was switched off by focally cooling left and right vagus nerves and was switched on immediately after repeated apneas by rewarming the nerves.

Vagus nerve cooling caused a potent $217 \pm 11\%$ increase in inspiratory genioglossus activity ($n = 8$; cooling vs baseline, paired t test, $p = 0.03$) (Fig. 5*A*), suggesting that vagal feedback, which normally suppresses respiratory activity, was either reduced or absent (van Lunteren et al., 1984; Kubin et al., 2006). To further demonstrate that nerve cooling blocked vagal feedback, we activated the vagus-dependent Hering–Breuer lung-inflation reflex (Cohen, 1975; Mellen and Feldman, 2000) before and dur-

ing nerve cooling. Hering–Breuer reflex activation before nerve cooling caused an abrupt arrest in inspiratory genioglossus activity that lasted for 3.7 ± 0.4 s ($n = 8$; paired t test, $p < 0.001$) (Fig. 5*B*). However, during vagus nerve cooling, it did not trigger inspiratory arrest; in fact, motor output continued unperturbed (paired t test, $p = 0.915$) (Fig. 5*B*). These findings indicate that nerve cooling completely blocked vagal afferent feedback.

Next, we aimed to determine whether vagal feedback was required for apnea-induced LTF. Although repeated apneas caused systemic hypoxia (baseline, 95.3 ± 0.4 vs occlusion, 86.1 ± 0.08; paired t test, $p = 0.033$), they did not cause LTF of genioglossus activity when vagal afferents were blocked (RM ANOVA, *post hoc* tests, $p > 0.05$ at 30, 45, and 60 min) (Fig. 5*C–E*). Although genioglossus activity transiently increased after apneas (i.e., at 15 min), it returned to baseline within ~15-min and remained there throughout the recording period. This temporary increase at 15 min also occurred when nerves were cooled and apneas were not initiated (Fig. 5*D*). Therefore, continuous removal of vagal blockade itself did not cause long-lasting changes in upper airway activity, suggesting that repeated modulation of vagal feedback is required for eliciting LTF. Repeated apneas during vagal blockade had no long-term effect on either diaphragm activity or respiratory frequency (RM ANOVA, $p > 0.05$ for both variables). Neither arterial O_2 nor end-tidal CO_2 levels fluctuated during the 60 min experimental period (RM ANOVA, $p > 0.05$ for both variables) (data not shown).

Last, we wanted to show that effects of vagal cooling were transient and reversible. Although vagal blockade (i.e., without apneas) caused a robust increase in inspiratory genioglossus activity during the cooling procedure itself (Fig. 5*D*), this increase returned to baseline levels within 15 min after nerve rewarming (RM ANOVA, *post hoc* tests, $p > 0.05$ at 30, 45, and 60 min). We also show that cutting both left and right vagi at the end of experiments (i.e., 60 min after vagus nerve cooling) produced an immediate increase in inspiratory genioglossus activity ($n = 8$; 150 ± 12% increase; paired t test, $p = 0.02$) and decrease in respiratory frequency (40 ± 7% decrease; paired t test, $p = 0.01$). Together, these observations suggest that the cooling procedure itself did not damage vagal afferents or have long-term effects on respiratory output.

Repeated modulation of vagal feedback itself triggers LTF

Here, we aimed to determine whether modulation of vagal feedback alone could trigger LTF. We repeatedly blocked afferent feedback by cooling left and right vagi (six 1-min episodes each separated by 1 min) but without repeated apneas. Although repeated blockade of vagal feedback had no effect on arterial O_2 levels (paired t test, $p = 0.87$), it potently triggered LTF of genioglossus EMG activity (Fig. 6*A*). After repeated afferent blockade, inspiratory genioglossus activity increased above baseline levels and peaked at 58 ± 11% above baseline values by 60 min ($n = 6$; RM ANOVA, $p < 0.05$) (Fig. 6*B*). It is noteworthy that vagal blockade triggered genioglossus LTF that was identical in both magnitude and time course to that elicited by repeated apneas (RM ANOVA, $p > 0.05$) (Fig. 6*B*). Repeated vagal modulation had no lasting effect on either respiratory frequency or inspiratory diaphragm ($n = 6$; RM ANOVA, $p > 0.05$ for both variables) (data not shown).

To demonstrate that genioglossus LTF was not an artifact of nerve cooling per se, we continuously cooled the vagus nerves for 6 min, a time period equal to the sum of the six cooling episodes. Although we found that inspiratory genioglossus activity increased in response to cooling-induced vagal blockade, this effect

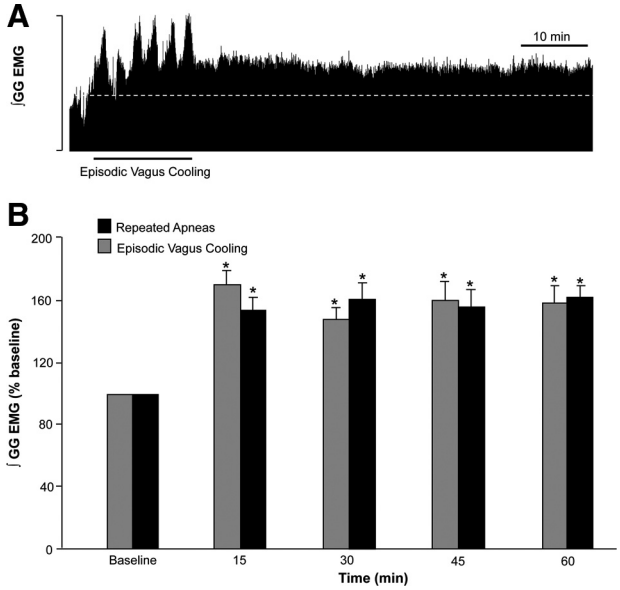

Figure 6. Repeated modulation of vagal feedback itself triggers LTF. ***A***, An EMG trace showing that repeated blockade of vagal feedback by vagal nerve cooling (6 1-min episodes, solid horizontal line) triggers LTF of genioglossus (GG) EMG activity. ***B***, Group data demonstrating that repeated modulation of vagal feedback induces LTF that is similar in both time course and magnitude to that triggered by repeated apneas alone. Group data are expressed as percentage change from baseline ± SEM. *$p < 0.05$, significantly different from baseline levels.

was only transient, with genioglossus activity returning to and remaining at baseline for the subsequent 60 min (RM ANOVA, $p > 0.05$ all time points). This finding demonstrates that LTF is not elicited by continuous removal of vagal feedback but instead requires repeated modulation of vagal activity for its activation.

LTF requires α1-adrenergic receptor activation on hypoglossal motoneurons

5-HT$_{2A}$ receptor blockade does not prevent LTF

The final goal of this study was to determine the neurochemical mechanisms that trigger apnea-induced LTF and in particular to determine which receptor mechanisms are activated on hypoglossal motoneurons, the presumed site of LTF. Because 5-HT$_{2A}$ receptors are abundantly expressed on hypoglossal motoneurons (Zhan et al., 2002) and because these receptors contribute to hypoxia-induced LTF (McGuire et al., 2004; Golder and Mitchell, 2005), we aimed to determine whether apnea-induced LTF also requires 5-HT$_{2A}$ receptor activation. Therefore, we antagonized 5-HT$_{2A}$ receptors onto hypoglossal motoneurons before LTF induction. However, before doing this, we wanted to demonstrate that saline perfusion onto motoneurons did not perturb LTF. We found that saline perfusion had no detectable effects on either basal genioglossus activity or apnea-induced LTF ($n = 11$; 73 ± 13% above baseline at 60 min; RM-ANOVA, $p < 0.001$) (Fig. 7*B*). However, we found that blocking 5-HT$_{2A}$ receptors by ketanserin perfusion (50 or 100 μM) onto hypoglossal motoneurons did not prevent LTF. In fact, repeated apneas still triggered robust genioglossus LTF, increasing it by 133 ± 24 and 73 ± 33% at a dose of 50 or 100 μM, respectively (RM ANOVA, $p < 0.001$ for 50 μM, $p = 0.006$ for 100 μM) (Fig. 7*A*). These findings suggest that 5-HT$_{2A}$ receptor activation is not required for LTF.

LTF is abolished by blocking α1-adrenergic receptors

Because episodic α1-adrenergic receptor activation induces LTF *in vitro* and blocking these receptors prevents hypoxia-induced LTF *in vivo* (Neverova et al., 2007), we hypothesized that α1-

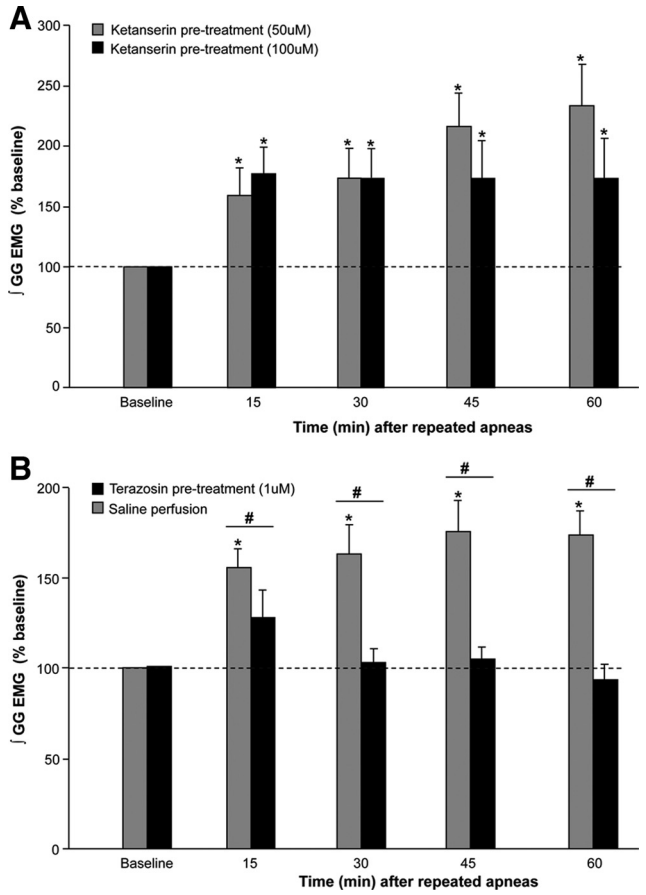

Figure 7. LTF requires α1-adrenergic receptor activation on hypoglossal motoneurons. ***A***, Group mean data showing that blockade of 5-HT_{2A} receptors by ketanserin perfusion at the hypoglossal motor pool has no measureable affect on LTF. ***B***, Group mean data demonstrating that saline perfusion at the hypoglossal motor pool does not interfere with apnea-induced LTF; however, blockade of α1-adrenergic receptors by terazosin perfusion prevents it. Group data are expressed as percentage change from baseline ± SEM. $^{*}p < 0.05$, significantly different from baseline; $^{\#}p < 0.05$, significant difference comparing groups at the indicated time point. GG, Genioglossus.

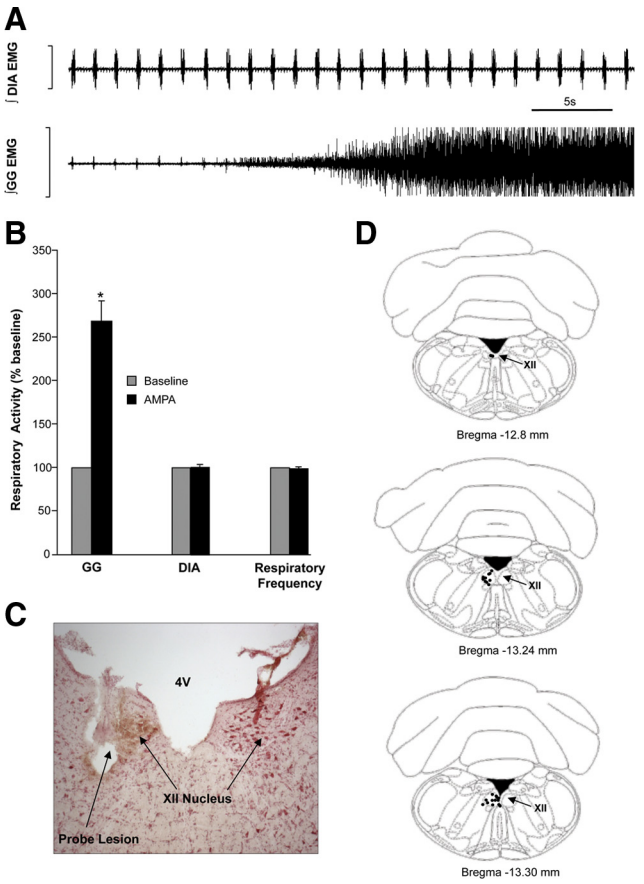

Figure 8. Microdialysis probes were located in the hypoglossal motor pool. ***A***, Genioglossus (GG) and diaphragm (DIA) EMG traces demonstrating that AMPA perfusion at the hypoglossal motor pool only affects genioglossus activity. ***B***, Group data showing that AMPA perfusion at the hypoglossal motor pool activates genioglossus alone but not diaphragm activity or respiratory frequency. ***C***, A photograph of a brainstem slice showing a microdialysis probe lesion in the hypoglossal motor pool. ***D***, Black filled circles represent the location of the lesions in the hypoglossal nucleus. Group data are expressed as percentage change from baseline ± SEM. $^{*}p < 0.05$, significantly different from baseline. 4V, Fourth ventricle; XII, hypoglossal nucleus.

adrenergic receptor activation may underlie apnea-induced LTF. Therefore, we blocked α1-adrenergic receptors on hypoglossal motoneurons before activating apnea-induced LTF. Antagonism of α1-adrenergic receptors completely abolished LTF of genioglossus activity. Specifically, we show that inspiratory genioglossus activity remained at baseline levels after repeated apneas ($n = 6$; RM ANOVA, $p = 0.10$) (Fig. 7*B*), indicating that apnea-induced LTF is α1-adrenergic receptor dependent.

Microdialysis probes are located in the hypoglossal nucleus

Postmortem histology performed in 25 rats demonstrates that microdialysis probes were located in the hypoglossal nucleus (Fig. 8*C*,*D*). Microdialysis of 0.1 mM AMPA into the hypoglossal nucleus at the end of each experiment caused a 269 ± 24% increase in genioglossus activity (paired *t* test, $p < 0.001$; $n = 25$); it had no effect on either inspiratory amplitude of diaphragm activity ($p = 0.676$) or respiratory frequency ($p = 0.504$) (Fig. 8*A*,*B*). This procedure verified that probes were located in the hypoglossal nucleus and that probes were functional; it also showed that motoneurons were viable and able to respond to glutamatergic excitation. Last, these results suggest that drug application did not influence respiratory rhythm-generating circuits because respiratory frequency was unaffected by dialysis procedures.

Discussion

Vagus-mediated LTF is a novel form of respiratory motor plasticity

Results show that repeated physiological modulation of vagal feedback triggers respiratory motor plasticity. Repeated apneas not only cause hypoxia and modulate vagal feedback, they also elicit LTF of genioglossus muscle tone; this same stimulus does not cause LTF of diaphragm activity. Apnea-induced LTF is not caused by repeated hypoxic episodes; instead, it is triggered by modulation of vagal feedback. In fact, repeated modulation of vagal feedback alone is sufficient for triggering LTF because intermittent disruption of vagal feedback itself causes LTF. Unlike hypoxia-induced plasticity that requires 5-HT_2 receptors (Fuller et al., 2001, McGuire et al., 2004; Golder and Mitchell, 2005), vagus-induced LTF requires α1-adrenergic receptor activation on hypoglossal motoneurons. We identify a novel form of hypoxia- and 5-HT-independent respiratory plasticity that is caused by physiological manipulation of vagal feedback and is mediated by α1-adrenergic receptor activation.

Apnea-induced LTF is triggered by vagal feedback not hypoxia

LTF is a type of CNS plasticity that enables the respiratory system to adapt to repeated experiences (e.g., recurrent apneas). Mechanisms mediating LTF have typically been studied by episodic modulation of chemical feedback caused by hypoxic exposure. Previous studies show that intermittent hypoxia elicits LTF of both phrenic and hypoglossal motoneuron activity. Although hypoxia is a powerful stimulus that causes plasticity, our results show that apnea-induced LTF is not induced by hypoxia. Perhaps the degree of hypoxic intensity was insufficient to recruit the neurochemical pathways (e.g., serotonin) necessary for hypoxia-induced LTF. Indeed, a previous study in rats shows that hypoxic intensity predicts LTF magnitude (Tadjalli et al., 2007). However, lack of hypoxia-induced LTF is nonetheless surprising because brief and mild hypoxic episodes (15 s) trigger LTF in anesthetized rats (Peng and Prabhakar, 2003). One possibility is that hypoxia-induced LTF, like the negative pressure airway reflex, is sensitive to and suppressed by different types and levels of anesthesia (Eikermann et al., 2008).

Episodic modulation of vagal feedback is the primary mechanism triggering apnea-induced LTF. This result is significant because it shows that respiratory plasticity can be triggered by a hypoxia-independent mechanism. Importantly, it demonstrates that repeated modulation of mechanical feedback is able to evoke respiratory plasticity.

Vagus-mediated and hypoxia-induced LTF share common mechanisms, for example, both are elicited by pattern-dependent but activity-independent processes. For example, intermittent hypoxia (three 5-min episodes) evokes LTF of phrenic and hypoglossal motor output, whereas a single continuous exposure does not (Baker and Mitchell, 2000). Repeated modulation of vagal feedback induced by either obstructive apneas or vagal nerve cooling also triggers LTF; continuous modulation does not cause plasticity.

Vagus-mediated LTF is not driven by an activity-dependent mechanism. Although repeated apneas and vagal inactivation potently increase inspiratory drive onto hypoglossal motoneurons (Figs. 2, 6), this activation itself does not induce LTF, suggesting that it is activity independent. This point is strengthened by the fact that phrenic motor output is repeatedly increased during apneas, yet this activation does not cause LTF of diaphragm activity. Furthermore, episodic hypercapnia that potently excites both phrenic and hypoglossal nerve outflow does not cause LTF (Baker et al., 2001). *In vitro* studies also show that repeated K^+-mediated excitation of hypoglossal motoneurons does not induce LTF (Bocchiaro and Feldman, 2004).

Mechanical feedback from lung stretch receptors is relayed by vagus nerve afferents to the neural circuits that drive breathing (van Lunteren et al., 1984; Kuna, 1986). Lung mechanoreceptors function to couple lung mechanics with respiratory motor drive. Therefore, mismatch between vagal afferent feedback and efferent inspiratory drive (e.g., airway obstruction) activates respiratory reflexes such as the Breuer–Hering reflex (Cohen, 1975; Kubin et al., 2006; Widdicombe, 2009). Although vagal feedback has been shown to influence LTF expression (Golder and Martinez, 2008), this is the first documentation that physiologically relevant disruption of vagal feedback actually triggers respiratory motor plasticity. Previous studies also show, albeit using high-intensity electrical stimulation, that vagal afferents can cause both short- and long-term changes in respiratory control (Zhang et al., 2003). However, these studies show that vagal stimulation causes LTF of phrenic activity, whereas we show that vagal modulation only triggers LTF of genioglossus inspiratory tone. Such differences probably reflect different stimulation parameters (e.g., apnea-induced vagal modulation vs electrical stimulation) and thus activation of different brain circuits.

Vagus-mediated LTF only strengthens genioglossus muscle tone; it does not have long-term effects on diaphragm activity. This is specific to this type of plasticity because hypoxia-induced LTF increases inspiratory drive at both phrenic and hypoglossal motor pools. LTF of genioglossus muscle tone is biologically significant because it indicates that vagus-induced plasticity only boosts hypoglossal motoneuron function, suggesting pathway specificity.

A noradrenergic mechanism underlies vagus-mediated LTF

Hypoxia-induced LTF is mediated by a 5-HT_{2A} receptor-dependent process because blockade of such receptors prevents LTF (Fuller et al., 2001; Golder and Mitchell, 2005). In contrast, vagus-mediated LTF does not require 5-HT_{2A} receptor activation. Blockade of 5-HT_{2A} receptors on (or near) hypoglossal motoneurons does not abolish apnea-induced LTF, whereas antagonism of α1-adrenergic receptors does. This finding suggests that vagus-mediated LTF is driven by a noradrenergic mechanism on hypoglossal motoneurons themselves. Indeed, *in vitro* studies show that repeated α1-adrenegic receptor activation elicits LTF of hypoglossal motoneuron activity (Neverova et al., 2007). However, noradrenergic activation of interneurons within and around the hypoglossal motor pool (Peever et al., 2002; Chamberlin et al., 2007) may also play a role in triggering vagus-mediated LTF.

Episodic vagus modulation may trigger LTF by causing repeated noradrenaline release from the locus ceruleus (LC). Anatomical tracing studies demonstrate that vagal afferents project to the nucleus of the solitary tract (NTS), which in turn innervates LC neurons (Cedarbaum and Aghajanian, 1978; Clavier, 1978; Kalia and Sullivan, 1982; Berthoud and Neuhuber, 2000). Importantly, vagal nerve modulation increases c-Fos activation of noradrenergic LC cells (Naritoku et al., 1995; Cunningham et al., 2008), which in turn project directly to hypoglossal motoneurons (Aldes et al., 1992). We hypothesize that obstructive apneas modulate vagal afferents, which indirectly (i.e., via NTS) activate LC neurons to increase noradrenaline release onto hypoglossal motoneurons. Based on *in vitro* studies (Neverova et al., 2007), we assert that repeated α1-adrenergic receptor activation subsequently amplifies AMPA-driven currents by inducing PKC-dependent mechanisms; this in turn heightens hypoglossal motoneuron activity and causes LTF of genioglossus motor tone.

Why does vagus-mediated LTF only amplify genioglossus motor tone? Although manipulation of vagal afferents directly affects both phrenic and hypoglossal motor outflow, repeated modulation only triggers plasticity of hypoglossal function; it never affects phrenic activity. Anatomical tracing data show that noradrenergic LC neurons densely innervate hypoglossal motoneurons (Aldes et al., 1992), but they do not directly innervate phrenic motoneurons (Dobbins and Feldman, 1994). In fact, findings suggest that phrenic motoneurons do not receive direct noradrenergic inputs. Thus, apnea-induced vagal modulation only triggers noradrenergic-dependent LTF of hypoglossal motor outflow; it does not directly affect phrenic motoneuron activity and hence plasticity. This is in sharp contrast to hypoxia-induced plasticity, which evokes LTF of both phrenic and hypoglossal motor outflow via serotonergic-dependent mechanisms (Bach and Mitchell, 1996; Fuller et al., 2001).

Although a noradrenergic mechanism underlies vagus-mediated LTF, both serotonergic and noradrenergic processes

have been shown to underlie respiratory LTF. For example, systemic administration of either 5-HT_{2A} (Fuller et al., 2001; McGuire et al., 2004; Golder and Mitchell, 2005) and α1-adrenergic (Neverova et al., 2007) receptor antagonists prevents hypoxia-induced LTF *in vivo*. Immunohistochemical studies demonstrate that hypoxia stimulates multiple types of neurons in numerous brain regions including both the noradrenergic LC and serotonergic raphe nuclei (Erickson and Millhorn, 1994; Teppema et al., 1997; Bodineau and Larnicol, 2001). In contrast, vagal modulation predominately activates LC cells that project to hypoglossal motoneurons (Naritoku et al., 1995; Cunningham et al., 2008). Therefore, we hypothesize that intermittent hypoxia recruits both serotonergic and noradrenergic mechanisms that cause both phrenic and hypoglossal LTF, whereas vagus-mediated LTF specifically activates a noradrenergic pathway that only causes plasticity in hypoglossal motoneurons.

Scientific importance and clinical relevance

Direct electrical stimulation of vagal afferents enhances hippocampal long-term potentiation in rodents (Zuo et al., 2007), significantly improves learning tasks in humans (Clark et al., 1999), and is associated with LTF of phrenic motor activity in rats (Zhang et al., 2003). Current results indicate that physiologically relevant modulation of vagal feedback also triggers plasticity, illustrating that natural as well as artificial vagal manipulation induces CNS plasticity.

During sleep, breathing is frequently punctuated by transient obstructive apneas, which manifest clinically as OSA (Remmers et al., 1978; Badr, 2002). Reduced upper airway motoneuron activity and hence muscle tone during sleep is an underlying cause of OSA (Horner, 2008). Diminished airway motor tone results in airway narrowing or complete airway obstruction, which leads to hypoventilation and asphyxia. Obstructive apneas also activate vagus-mediated airway reflexes that function to heighten pharyngeal muscle tone and reopen the airway. Apnea-induced LTF may be clinically relevant because it (1) may be triggered by obstructive apneas themselves and (2) may serve to boost hypoglossal motoneuron activity and thus heighten genioglossus motor tone. Apnea-induced LTF may therefore function to prevent or minimize airway obstructions during sleep (Mateika and Narwani, 2009; Richerson, 2010). Genioglossus motor tone is elevated in OSA patients (Mezzanotte et al., 1992; Fogel et al., 2001); however, it is unknown whether apnea-induced LTF is the underlying cause.

Our results also show that LTF acts to strengthen the genioglossus response to individual obstructive apneas; diaphragmatic responses to apneas are unaffected after LTF (Fig. 3). This observation suggests that LTF only increases hypoglossal motoneuron responsiveness to respiratory stimuli and that phrenic motoneuron excitability is unaffected. LTF of genioglossus responses to airway obstruction may therefore function to mitigate persistent obstructive apneas in OSA.

In summary, we propose that recurrent obstructive apneas, as experienced in OSA, cause repeated modulation of vagal feedback, which in turn triggers LTF of hypoglossal motoneuron function by a noradrenergic-dependent mechanism. LTF of genioglossus muscle activity could potentially function to strengthen upper airway motor tone and hence improve effective lung ventilation in OSA. Understanding the intracellular pathways underlying vagus-mediated LTF could therefore aid with development of effective pharmacological treatments for OSA.

References

Aldes LD, Chapman ME, Chronister RB, Haycock JW (1992) Sources of noradrenergic afferents to the hypoglossal nucleus in the rat. Brain Res Bull 29:931–942.

Bach KB, Mitchell GS (1996) Hypoxia-induced long-term facilitation of respiratory activity is serotonin dependent. Respir Physiol 104:251–260.

Badr MS (2002) Pathophysiology of obstructive sleep apnea. Oral Maxillofac Surg Clin North Am 14:285–292.

Bailey EF, Fregosi RF (2006) Modulation of upper airway muscle activities by bronchopulmonary afferents. J Appl Physiol 101:609–617.

Baker TL, Mitchell GS (2000) Episodic but not continuous hypoxia elicits long-term facilitation of phrenic motor output in rats. J Physiol 529:215–219.

Baker TL, Fuller DD, Zabka AG, Mitchell GS (2001) Respiratory plasticity: differential actions of continuous and episodic hypoxia and hypercapnia. Respir Physiol 129:25–35.

Baker-Herman TL, Fuller DD, Bavis RW, Zabka AG, Golder FJ, Doperalski NJ, Johnson RA, Watters JJ, Mitchell GS (2004) BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia. Nat Neurosci 7:48–55.

Berthoud HR, Neuhuber WL (2000) Functional and chemical anatomy of the afferent vagal system. Auton Neurosci 85:1–17.

Bocchiaro CM, Feldman JL (2004) Synaptic activity-independent persistent plasticity in endogenously active mammalian motoneurons. Proc Natl Acad Sci U S A 101:4292–4295.

Bodineau L, Larnicol N (2001) Brainstem and hypothalamic areas activated by tissue hypoxia: Fos-like immunoreactivity induced by carbon monoxide inhalation in the rat. Neuroscience 108:643–653.

Brooks PL, Peever JH (2008) Glycinergic and $GABA_A$-mediated inhibition of somatic motoneurons does not mediate rapid eye movement sleep motor atonia. J Neurosci 28:3535–3545.

Burgess C, Lai D, Siegel J, Peever J (2008) An endogenous glutamatergic drive onto somatic motoneurons contributes to the stereotypical pattern of muscle tone across the sleep–wake cycle. J Neurosci 28:4649–4660.

Cedarbaum JM, Aghajanian GK (1978) Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. J Comp Neurol 178:1–16.

Chamberlin NL, Eikermann M, Fassbender P, White DP, Malhotra A (2007) Genioglossus premotoneurons and the negative pressure reflex in the rat. J Physiol 579:515–526.

Clark KB, Naritoku DK, Smith DC, Browning RA, Jensen RA (1999) Enhanced recognition memory following vagus nerve stimulation in human subjects. Nat Neurosci 2:94–98.

Clavier R (1978) Afferent projections to the locus coeruleus of the rat as demonstrated by horseradish peroxidase technique. Anat Rec 190:365.

Cohen MI (1975) Phrenic and recurrent laryngeal discharge patterns and the Hering-Breuer reflex. Am J Physiol 228:1489–1496.

Cunningham JT, Mifflin SW, Gould GG, Frazer A (2008) Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by vagal nerve stimulation. Neuropsychopharmacology 33:1884–1895.

Dobbins EG, Feldman JL (1994) Brainstem network controlling descending drive to phrenic motoneurons in rat. J Comp Neurol 347:64–86.

Eikermann M, Malhotra A, Fassbender P, Zaremba S, Jordan AS, Gautam S, White DP, Chamberlin NL (2008) Differential effects of isoflurane and propofol on upper airway dilator activity muscle activity and breathing. Anesthesiology 108:897–906.

Erickson JT, Millhorn DE (1994) Hypoxia and electrical stimulation of the carotid sinus nerve induce Fos-like immunoreactivity within catecholaminergic and serotoninergic neurons of the rat brainstem. J Comp Neurol 348:161–182.

Ezure K, Tanaka I, Saito Y, Otake K (2002) Axonal projections of pulmonary slowly adapting receptor relay neurons in the rat. J Comp Neurol 446:81–94.

Feldman JL, Mitchell GS, Nattie EE (2003) Breathing: rhythmicity, plasticity, chemosensitivity. Annu Rev Neurosci 26:239–266.

Fishman NH, Phillipson EA, Nadel JA (1973) Effect of differential vagal cold blockade on breathing pattern in conscious dogs. J Appl Physiol 34:754–758.

Fogel RB, Malhotra A, Pillar G, Edwards JK, Beauregard J, Shea SA, White DP (2001) Genioglossal activation in patients with obstructive sleep apnea versus control subjects. Mechanisms of muscle control. Am J Respir Crit Care Med 164:2025–2030.

Fuller DD (2005) Episodic hypoxia induces long-term facilitation of neural drive to tongue protrudor and retractor muscles. J Appl Physiol 98:1761–1767.

Fuller DD, Zabka AG, Baker TL, Mitchell GS (2001) Phrenic long-term facilitation requires 5-HT receptor activation during but not following episodic hypoxia. J Appl Physiol 90:2001–2006; discussion 2000.

Ghacibeh GA, Shenker JI, Shenal B, Uthman BM, Heilman KM (2006) The influence of vagus nerve stimulation on memory. Cogn Behav Neurol 19:119–122.

Golder FJ, Martinez SD (2008) Bilateral vagotomy differentially alters the magnitude of hypoglossal and phrenic long-term facilitation in anesthetized mechanically ventilated rats. Neurosci Lett 442:213–218.

Golder FJ, Mitchell GS (2005) Spinal synaptic enhancement with acute intermittent hypoxia improves respiratory function after chronic cervical spinal cord injury. J Neurosci 25:2925–2932.

Guilleminault C, Abad VC (2004) Obstructive sleep apnea syndromes. Med Clin North Am 88:611–630, viii.

Hammouda M, Wilson WH (1932) The vagus influences giving rise to the phenomena accompanying expansion and collapse of the lungs. J Physiol 74:81–114.

Horner RL (2008) Pathophysiology of obstructive sleep apnea. J Cardiopulm Rehabil Prev 28:289–298.

Kalia M, Sullivan JM (1982) Brainstem projections of sensory and motor components of the vagus nerve in the rat. J Comp Neurol 211:248–265.

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038.

Kommalage M, Höglund AU (2005) Involvement of spinal serotonin receptors in the regulation of intraspinal acetylcholine release. Eur J Pharmacol 509:127–134.

Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR (2006) Central pathways of pulmonary and lower airway vagal afferents. J Appl Physiol 101:618–627.

Kuna ST (1986) Inhibition of inspiratory upper airway motoneuron activity by phasic volume feedback. J Appl Physiol 60:1373–1379.

Mateika JH, Narwani G (2009) Intermittent hypoxia and respiratory plasticity in humans and other animals: does exposure to intermittent hypoxia promote or mitigate sleep apnoea? Exp Physiol 94:279–296.

McGuire M, Zhang Y, White DP, Ling L (2004) Serotonin receptor subtypes required for ventilatory long-term facilitation and its enhancement after chronic intermittent hypoxia in awake rats. Am J Physiol Regul Integr Comp Physiol 286:R334–R341.

Mellen NM, Feldman JL (2000) Phasic lung inflation shortens inspiration and respiratory period in the lung-attached neonate rat brain stem spinal cord. J Neurophysiol 83:3165–3168.

Mezzanotte WS, Tangel DJ, White DP (1992) Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism). J Clin Invest 89:1571–1579.

Milsom WK (1990) Mechanoreceptor modulation of endogenous respiratory rhythms in vertebrates. Am J Physiol 259:R898–R910.

Naritoku DK, Terry WJ, Helfert RH (1995) Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. Epilepsy Res 22:53–62.

Neverova NV, Saywell SA, Nashold LJ, Mitchell GS, Feldman JL (2007) Episodic stimulation of α1-adrenoreceptors induces protein kinase C-dependent persistent changes in motoneuronal excitability. J Neurosci 27:4435–4442.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. New York: Academic.

Peever JH, Shen L, Duffin J (2002) Respiratory pre-motor control of hypoglossal motoneurons in the rat. Neuroscience 110:711–722.

Peng YJ, Prabhakar NR (2003) Reactive oxygen species in the plasticity of respiratory behavior elicited by chronic intermittent hypoxia. J Appl Physiol 94:2342–2349.

Phillipson EA, Fishman NH, Hickey RF, Nadel JA (1973) Effect of differential vagal blockade on ventilatory response to CO2 in awake dogs. J Appl Physiol 34:759–763.

Remmers JE, deGroot WJ, Sauerland EK, Anch AM (1978) Pathogenesis of upper airway occlusion during sleep. J Appl Physiol 44:931–938.

Richerson GB (2010) Respiratory plasticity in sleep apnoea: should it be harnessed or restrained? J Physiol 588:3–4.

Rouquier L, Claustre Y, Benavides J (1994) Alpha 1-adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: a microdialysis study. Eur J Pharmacol 261:59–64.

Sanders MH, Moore SE (1983) Inspiratory and expiratory partitioning of airway resistance during sleep in patients with sleep apnea. Am Rev Respir Dis 127:554–558.

Sanders MH, Rogers RM, Pennock BE (1985) Prolonged expiratory phase in sleep apnea. A unifying hypothesis. Am Rev Respir Dis 131:401–408.

Strohl KP (1985) Respiratory activation of the facial nerve and alar muscles in anaesthetized dogs. J Physiol 363:351–362.

Tadjalli A, Duffin J, Li YM, Hong H, Peever J (2007) Inspiratory activation is not required for episodic hypoxia-induced respiratory long-term facilitation in postnatal rats. J Physiol 585:593–606.

Teppema LJ, Veening JG, Kranenburg A, Dahan A, Berkenbosch A, Olievier C (1997) Expression of c-fos in the rat brainstem after exposure to hypoxia and to normoxic and hyperoxic hypercapnia. J Comp Neurol 388:169–190.

Thorré K, Ebinger G, Michotte Y (1998) 5-HT4 receptor involvement in the serotonin-enhanced dopamine efflux from the substantia nigra of the freely moving rat: a microdialysis study. Brain Res 796:117–124.

van Lunteren E, Strohl KP, Parker DM, Bruce EN, Van de Graaff WB, Cherniack NS (1984) Phasic volume-related feedback on upper airway muscle activity. J Appl Physiol 56:730–736.

Widdicombe J (2009) Lung afferent activity: implications for respiratory sensation. Respir Physiol Neurobiol 167:2–8.

Yan QS (2000) Activation of 5-HT2A/2C receptors within the nucleus accumbens increases local dopaminergic transmission. Brain Res Bull 51:75–81.

Yan Q, Reith ME, Yan S (2000) Enhanced accumbal dopamine release following 5-HT(2A) receptor stimulation in rats pretreated with intermittent cocaine. Brain Res 863:254–258.

Zhan G, Shaheen F, Mackiewicz M, Fenik P, Veasey SC (2002) Single cell laser dissection with molecular beacon polymerase chain reaction identifies 2A as the predominant serotonin receptor subtype in hypoglossal motoneurons. Neuroscience 113:145–154.

Zhang Y, McGuire M, White DP, Ling L (2003) Episodic phrenic-inhibitory vagus nerve stimulation paradoxically induces phrenic long-term facilitation in rats. J Physiol 551:981–991.

Zuo Y, Smith DC, Jensen RA (2007) Vagus nerve stimulation potentiates hippocampal LTP in freely-moving rats. Physiol Behav 90:583–589.