Behavioral/Systems/Cognitive

Endogenous Glutamatergic Control of Rhythmically Active Mammalian Respiratory Motoneurons *In Vivo*

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The transmission of rhythmic drive to respiratory motoneurons *in vitro* is critically dependent on glutamate acting primarily on non-NMDA receptors. We determined whether both non-NMDA and NMDA receptors contribute to respiratory drive transmission at respiratory motoneurons in the intact organism, both in the state of anesthesia and in the same animals during natural behaviors. Twenty-seven rats were implanted with electroencephalogram and neck electrodes to record sleep–wake states and genioglossus and diaphragm electrodes for respiratory muscle recordings. Microdialysis probes were inserted into the hypoglossal motor nucleus (HMN). Under anesthesia, non-NMDA or NMDA receptor antagonism significantly decreased respiratory-related genioglossus activity, indicating a contribution of each receptor to respiratory drive transmission at the HMN. However, despite the presence of respiratory-related genioglossus activity in the same rats across sleep–wake states, neither non-NMDA receptor antagonism at the HMN nor glutamate uptake inhibition had any effect on respiratory-related genioglossus activity. These results showed that, compared with anesthesia, respiratory drive transmission through the non-NMDA receptor is low in the behaving organism. In contrast, glutamate uptake inhibition increased tonic genioglossus activity in wakefulness and non-rapid-eye-movement sleep, indicating a functional endogenous glutamatergic modulation of tonic, but not respiratory, motor tone. Such an effect on tonic drive may contribute to the suppression of both tonic and respiratory-related genioglossus activity in wakefulness and sleep with NMDA receptor antagonism at the HMN. These data do not refute previous identification of a glutamatergic (mostly non-NMDA receptor activating) respiratory drive to hypoglossal motoneurons, but this mechanism is more prominent in anesthetized or *in vitro* preparations.

Key words: **respiration; motoneuron; glutamate; hypoglossal motor nucleus; genioglossus muscle; sleep; NMDA; non-NMDA**

Introduction

The rhythmic activation of respiratory motoneurons is an essential motor act to maintain ventilatory homeostasis. *In vitro* studies have shown that the main component of respiratory drive transmission at the phrenic and hypoglossal motor nuclei is via glutamate acting primarily on non-NMDA receptors (McCrimmon et al., 1989; Liu et al., 1990; Greer et al., 1991; Funk et al., 1993; Berger, 2000; Rekling et al., 2000; Wang et al., 2002). Although both non-NMDA and NMDA receptors colocalize on individual hypoglossal motoneurons (O'Brien et al., 1997), the lesser of involvement of NMDA receptors in modulating respiratory drive transmission *in vitro* may be because deafferentation and removal of tonic drives blocks some voltage-dependent NMDA receptors or because of developmental changes in glutamate signaling from the neonate to the adult organism (Mayer and Westbrook, 1985; Rekling et al., 2000). Both non-NMDA and NMDA receptors significantly modulate respiratory drive transmission at the hypoglossal motor nucleus (HMN) in anesthetized or decerebrate adult animals (Steenland et al., 2006; Zuperku et al., 2008). We developed a chronic animal model for manipulation of neurotransmission at the HMN (Chan et al., 2006) and so test the hypothesis that both non-NMDA and NMDA receptors modulate respiratory-related genioglossus activity in intact freely behaving adult rats *in vivo*. Because motoneurons are relatively hyperpolarized in sleep compared with wakefulness (Chase et al., 1980), we also hypothesized that the NMDA receptor contribution to genioglossus activity would be less in sleep and most apparent in wakefulness.

Previous studies characterizing the non-NMDA and NMDA receptor contributions to hypoglossal motor activity have only been performed *in vitro* (Greer et al., 1991; Funk et al., 1993; Rekling et al., 2000; Wang et al., 2002) or in anesthetized or decerebrate preparations (Steenland et al., 2006; Zuperku et al., 2008). The magnitude of respiratory-related hypoglossal nerve or genioglossus activity in those reduced preparations, however, is large compared with the conscious animal (Roda et al., 2004; Sood et al., 2005). Thus, the magnitude and exclusive role of glutamate as the mediator of respiratory drive transmission at the HMN may be lessened or overridden in the intact freely behaving organism as the mechanisms modulating the respiratory network become more complex. For example, a variety of sleep-state-dependent neural systems express respiratory-related activity *in vivo* (Lindsey et al., 1992; Guyenet et al., 1993; Orem and Kubin, 2000), project to respiratory motoneurons (Travers and Nor-

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gren, 1983; Dobbins and Feldman, 1995), and influence respiratory motor activity (Kubin et al., 1998; Chan et al., 2006). A second aim of this study then was to characterize the non-NMDA and NMDA receptor contributions to respiratory drive transmission at the HMN in intact freely behaving rats across sleep–wake states and in the same rats studied under anesthesia in which the non-NMDA and NMDA receptor contributions are expected to be significant (Steenland et al., 2006). We also determined for the first time whether non-NMDA and NMDA receptors contribute to tonic genioglossus activity across sleep–wake states. Such tonic activity is also relevant to the respiratory function of the upper airway by contributing to airway patency (Horner, 1996).

Materials and Methods

Experiments were performed on 27 male Wistar rats (mean body weight, 269 g; range, 245–290 g). Procedures conformed to the recommendations of the Canadian Council on Animal Care, and the University of Toronto Animal Care Committee approved the protocols. Rats were housed individually, maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.), and had access to food and water *ad libitum*.

Surgical procedures

Instrumentation for studies in freely behaving rats. Sterile surgery was performed with the rats anesthetized with intraperitoneal ketamine (85 mg/kg) and xylazine (15 mg/kg). In addition, the rats were intraperitoneally injected with saline (3 ml, 0.9%) for fluid loading, atropine sulfate (1 mg/kg) to minimize airway secretions, and buprenorphine (0.03 mg/kg) to reduce potential postoperative pain. An anesthesia mask was placed over the snout, and the rats spontaneously breathed a 50:50 mixture of room air and oxygen. Any additional anesthesia during surgery was administered, as necessary, by inhalation (isoflurane, typically 0.2–2%). Effective anesthesia was judged by abolition of the pedal withdrawal and corneal blink reflexes. The rats were then implanted with electroencephalogram (EEG) and neck electromyogram (EMG) electrodes for determination of sleep–wake states and with genioglossus and diaphragm electrodes for respiratory muscle recordings (Morrison et al., 2003; Sood et al., 2005; Chan et al., 2006). Tests for the accurate placement of the genioglossus electrodes and their function throughout the experiments included EMG recordings during surgery and observing tongue movements in response to electrical stimulation of the genioglossal wires at both the beginning and end of the experiments (Morrison et al., 2003; Sood et al., 2005; Chan et al., 2006). Removal of the tongue, by dissection, at the completion of all experiments confirmed the physical location of the electrodes to the genioglossus muscle. The size, configuration, and placement of the genioglossus and diaphragm electrodes were consistent across experiments, and each animal served as its own control.

Using a stereotaxic frame (model 962; David Kopf Instruments), microdialysis guides (CMA/11; Chromatography Sciences Company) were targeted 3 mm above the HMN during surgery (Morrison et al., 2003; Sood et al., 2005; Chan et al., 2006). The guides were placed 13.89 ± 0.11 mm posterior to bregma, 0.12 ± 0.012 mm lateral to the midline, and 6.99 ± 0.071 mm ventral to bregma. A dummy cannula was placed inside the microdialysis guide to keep it patent until the day of the experiment. Electrodes were connected to pins and inserted into a miniature plug (model STC-89PI-220ABS; Carleton University). The plug and microdialysis guides were affixed to the skull with dental acrylic and anchor screws.

On completion of surgery, the rats were transferred to a clean cage and kept warm under a heating lamp until full recovery as judged by normal locomotor activity, grooming, drinking, and eating. The rats were given soft food for the first day after surgery. The rats recovered for an average of 8.4 d (range, 6–14 d) before the experiments were performed.

Follow-up studies under anesthesia. After completion of the protocol in the freely behaving rats (see below), the same animals were then acutely anesthetized with intraperitoneal injection of urethane (1 g/kg). The microdialysis probe was left in the hypoglossal motor nucleus, but the inlet and outlet tubing were connected together to prevent loss of the internal solution. After the onset of surgical anesthesia, the rats were tracheotomized and spontaneously breathed a 50:50 mixture of room air and oxygen; the rats were also given atropine (1 mg/kg) to minimize airway secretions. Subsequent anesthesia was administered as necessary by inhalation (halothane, typically 0.2–0.9%). When halothane was initiated within an animal, typically no, or only minor, adjustments were necessary across the experiment. We routinely use this anesthetic regimen because it provides highly reliable preparations with stable respiratory and EEG activities over the course of the experiments (Liu et al., 2005; Steenland et al., 2006) that is not possible, for example, with periodic bolus doses of supplemental urethane, which can transiently suppress genioglossus activity. The femoral artery and vein were cannulated for blood pressure measurement and intravenous infusions, respectively. The rats received continuous intravenous fluid (0.4 ml/h) containing 7.6 ml of saline, 2 ml of 5% dextrose, and 0.4 ml of 1 M $NaHCO_3$. Core body temperature was monitored with a rectal probe and maintained between 36 and 38°C with a water pump and heating pad (T/Pump-Heat Therapy System; Gaymar).

Protocol and recording procedures

Freely behaving rats. For recordings, a lightweight shielded cable was connected to the plug on the rat's head. The cable was attached to a counterbalanced swivel that permitted free movement. All rats were studied in a noise-attenuated, electrically shielded cubicle (EPC-010; BRS/LVE) and supplied with fresh bedding, food, and water. A video camera inside the cubicle allowed for continuous monitoring without disturbing the animal. For habituation, the rats were connected to the cable and swivel apparatus the day before the experiments.

Experiments started between 7:00 A.M. and 8:00 A.M. and were performed during the day, i.e., the normal sleep period of rats. At the start of the experiment, the internal cannula was removed from the guide, and the microdialysis probe (240 μm diameter, 1 mm membrane; CMA/11 14/01; Chromatography Sciences Company) was inserted. The probes projected 3 mm from the tip of the guide and were therefore targeted to the HMN. The ease of insertion of the probe ensured that the rat was minimally disturbed by the procedure. The probes were perfused with artificial CSF (ACSF) at a flow rate of 2.1 μl/min, with the ACSF being made fresh on the day of each experiment. The composition of the ACSF was as follows (in mM): 125 NaCl, 3 KCl, 1 KH_2PO_4, 2 $CaCl_2$, 1 $MgSO_4$, 25 $NaHCO_3$, and 30 glucose. The ACSF was warmed to 37°C and bubbled with CO_2 to a pH of 7.40 ± 0.003 (SEM). The $CaCl_2$ was added after the ACSF was warmed to 37°C.

After insertion of the microdialysis probe, at least 45 min were allowed to elapse before sleep–wake states and respiratory muscle activities were analyzed (Morrison et al., 2003; Sood et al., 2005; Chan et al., 2006). At least two full cycles containing periods of active and quiet wakefulness, and non-rapid-eye-movement (NREM) and REM sleep, were recorded in each animal for each applied drug (see below). At the end of the experiment, the microdialysis probes were washed out with ACSF for at least 1 h.

Follow-up studies under anesthesia. After completion of the protocol in the freely behaving rats, the same animals were then prepared for acute, i.e., nonrecovery, studies under anesthesia (see above). To ensure consistent positioning between rats, they were placed in a stereotaxic apparatus (model 962; David Kopf Instruments), and the head was secured with blunt ear bars. The same EEG, genioglossus, and diaphragm EMG signals were recorded by reconnecting the amplifiers to the appropriate electrical contacts on the head plug. The microdialysis tubing was also reconnected to the perfusion pump, and ACSF was perfused through the microdialysis probe for at least 1 h before any interventions were performed. After perfusion with ACSF, the same drugs that were applied in the same rat during wakefulness and sleep were then reapplied under anesthesia. The time for drugs to be applied under anesthesia was at least 3 h after the last applied drugs in the conscious experiments. The studies under anesthesia were performed as positive controls to confirm our previous observations in the same anesthetized preparation (Steenland et al., 2006).

Interventions

Study 1: NMDA receptor antagonism at the HMN. Experiments were performed in seven rats to determine whether NMDA receptors at the HMN

modulate genioglossus activity (1) across natural sleep–wake states and (2) during anesthesia. In the same rats studied under both conditions, genioglossus activity was recorded during control perfusion of ACSF into the HMN, followed by D-2-amino-5-phosphonovaleric acid (D-APV) dissolved in ACSF (1 mM, pH 7.21 ± 0.034; Sigma). D-APV is a competitive NMDA receptor antagonist (Olverman et al., 1984; Rekling, 1992; O'Brien et al., 1997; Wang et al., 2002). In a previous study, we showed that this dose of D-APV delivered by microdialysis perfusion into the HMN produced appropriate antagonism of the NMDA receptor in response to application of exogenous agonist but did not affect responses to non-NMDA receptor stimulation (Steenland et al., 2006). The protocol for delivery of drugs in the freely behaving rats was described above. For the experiments under anesthesia, D-APV was applied for 15 min and washed out for at least 30 min. At the end of the anesthesia experiments, serotonin (10 mM, pH 6.36 ± 0.026; Sigma) dissolved in ACSF was perfused as a positive control to activate the genioglossus and confirm that the HMN was intact and able to respond to manipulation of neurotransmission (Jelev et al., 2001; Sood et al., 2005).

Study 2: non-NMDA receptor antagonism at the HMN. Experiments were performed in seven separate rats to determine whether non-NMDA receptors at the HMN modulate genioglossus activity (1) across natural sleep–wake states and (2) during anesthesia. In the same rats studied under both conditions, genioglossus activity was recorded during control perfusion of ACSF into the HMN, followed by 6-cyano-7-nitroquinoxaline-2, 3-dione disodium salt (CNQX) dissolved in ACSF (0.1 mM, pH 7.39 ± 0.006; Sigma). CNQX is a competitive non-NMDA receptor antagonist (Honore et al., 1988; Funk et al., 1993; Wang et al., 2002). In a previous study, we showed that microdialysis perfusion of this dose of CNQX into the HMN produced appropriate antagonism of the non-NMDA receptor in response to application of exogenous agonist but did not affect responses to NMDA receptor stimulation (Steenland et al., 2006). The protocol for delivery of drugs in the freely behaving rats was described above. For the anesthetized experiments, CNQX was applied for 15 min and washed out for at least 30 min. At the end of all the experiments, serotonin (10 mM, pH 6.35 ± 0.041) was also applied as a positive control to activate genioglossus muscle (Jelev et al., 2001; Sood et al., 2005). In a separate group of six rats, additional experiments were also performed across sleep–wake states during microdialysis perfusion of ACSF and 1 and 5 mM CNQX into the HMN using the identical protocol for the 0.1 mM CNQX and the 1 mM D-APV experiments. In anesthetized rats, 1 mM CNQX is sufficient to fully abolish genioglossus activity, even in vagotomized animals with high respiratory-related genioglossus activity (Steenland et al., 2006).

Study 3: endogenous stimulation of glutamate receptors at the HMN. Experiments were performed in seven rats to determine the effect on genioglossus activity of elevating endogenous glutamate across sleep-wake states. Genioglossus activity was recorded during perfusion of ACSF into the HMN, followed by the glutamate uptake inhibitor dihydrokainate (DHK) dissolved in ACSF (2 mM, pH 7.32 ± 0.060; Sigma) (Funk et al., 1993; Fallgren and Paulsen, 1996). At the end of the experiments in three rats, DHK was also coadministered with 0.1 mM of the non-NMDA receptor antagonist CNQX. At the end of the experiments in another three rats, DHK was coadministered with 1 mM of the NMDA receptor antagonist D-APV. At the conclusion of each study, drugs were washed out for at least 1 h, and then serotonin (10 mM, pH 6.36 ± 0.026) was also applied as a positive control as in studies 1 and 2 to activate genioglossus muscle.

Electrophysiological recordings

The electrical signals were amplified and filtered (Super-Z head-stage amplifiers and BMA-400 amplifiers/filters; CWE). The EEG was filtered between 1 and 100 Hz and the EMG signals between 100 and 1000 Hz. The electrocardiogram was removed from the diaphragm signal using an oscilloscope and an electronic blanker (model SB-1; CWE). The moving-time averages of the genioglossus and diaphragm signals were also obtained (time constant, 200 ms). Each signal, along with blood pressure for the anesthesia experiments [DT-XX transducer (Ohmeda) and PM-1000 Amplifier (CWE)] were recorded on computer (Spike 2 software, 1401 interface; Cambridge Electronic Design).

Data analysis

Analysis of EMG and EEG signals. The EMGs were analyzed every 5 s from the respective moving-time average signals (above electrical zero). The genioglossus and diaphragm signals were analyzed breath-by-breath, which corresponded to ~7–10 breaths for each 5 s epoch. For each breath in each 5 s epoch, the analysis of the genioglossus EMG was time locked to breathing as defined by the peak and trough of the diaphragm signal. Visual inspection of the computer-identified peaks and troughs of the diaphragm and genioglossus signals was performed during the automated analysis, and any artifacts were discarded and not used in the analysis. Peak inspiratory genioglossus activity was defined as the peak moving-time average of the genioglossus EMG in the 100 ms spanning the end of diaphragm inspiration, and tonic activity was defined as the minimal activity in expiration. For each 5 s epoch, genioglossus activity was then quantified as mean tonic activity (i.e., the mean minimal activity in expiration from the number of breaths in that epoch) and the mean amplitude of respiratory-related activity (i.e., mean peak inspiratory activity − tonic activity). Tonic genioglossus activity will include both basal tone and any background spontaneous activity that may occur during certain behaviors, e.g., twitches in REM sleep or behaviors in active wakefulness. Likewise, although clear phasic variations in genioglossus activity can occasionally occur in behaviors such as REM sleep or active wakefulness, whether the activity that occurs in synchrony with the diaphragm is truly "respiratory" or random activation attributable to behavioral or REM sleep processes cannot be determined with this or other methods, but such activations do not affect measurements in quiet wakefulness or NREM sleep. Mean neck muscle activity, diaphragm amplitude, and respiratory rate were also calculated in consecutive 5 s bins for all the periods of sleep and wakefulness in each rat. The EEG was sampled by computer at 500 Hz and then analyzed on overlapping segments of 1024 samples, windowed using a raised cosine (Hamming) function, and subjected to a fast Fourier transform to yield the power spectrum. The window was advanced in steps of 512 samples, and the mean power spectrum of the EEG signal over each 5 s epoch was calculated. The power contained within six frequency bands was recorded as absolute power and also as a percentage of the total power of the signal. The band limits were δ_2 (0.5–2 Hz), δ_1 (2–4 Hz), θ (4–7.5 Hz), α (7.5–13.5 Hz), β_1 (13.5–20 Hz), and β_2 (20–30 Hz); the ratio of high (20–30 Hz) to low (0.5–2 Hz) frequency activity was also calculated as an index of relative EEG activation (Hamrahi et al., 2001).

Measurements. The moving-time averages of the genioglossus, neck, and diaphragm EMGs were averaged for each 5 s period across sleep–wake states in each rat and subsequently sorted into periods of active and quiet wakefulness, NREM sleep, and REM sleep as identified by standard EEG and EMG criteria (Morrison et al., 2003; Sood et al., 2005; Chan et al., 2006). The EEG and neck EMG signals were scored visually for identification of sleep–wake states but without reference to the genioglossus or diaphragm signals to minimize bias when selecting periods for analysis. Active wakefulness was identified as low-voltage EEG activity with high levels of neck muscle activity associated with behaviors such as grooming, eating, and drinking. Quiet wakefulness was associated with an absence of such overt behaviors. For the studies in the freely behaving rats, data were analyzed at least 30 min after a switch between drugs to allow for the lag time of the tubing connected to the microdialysis probe. For the anesthetized rats, measurements were taken over 1 min periods at the end of each period of drug perfusion as described previously (Steenland et al., 2006).

Histology

At the completion of each experiment, a microdialysis probe with the membrane cut at the tip was inserted into the guide cannula, and a 1% solution of potassium permanganate was microinjected at 2.1 μl/min for 5 min to mark the sites of microdialysis (Morrison et al., 2003; Sood et al., 2005; Chan et al., 2006). After electrical stimulation of the genioglossal wires to recheck tongue movement, the rats were then overdosed with intraperitoneal urethane (6 g/kg) and perfused intracardially with 0.9% saline and 10% Formalin. The brain was then removed and fixed in 10% Formalin. The medullary regions were blocked, transferred to 30% sucrose, and cut in 50 μm coronal sections with a cryostat (CM 1850;

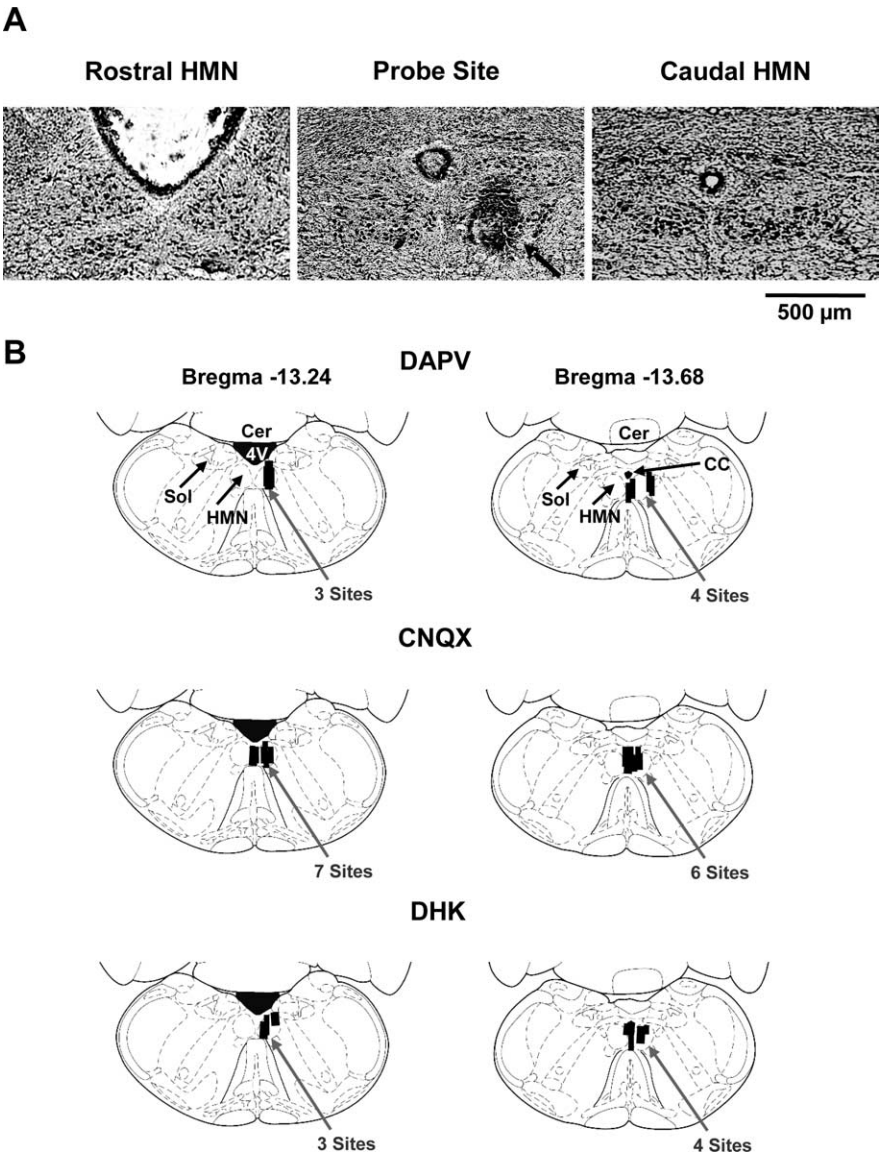

Figure 1. Example and group data showing location of the microdialysis probes. ***A***, Histological section showing an example of a lesion site made by the microdialysis probe in one rat. ***B***, Also shown are the distribution of individual microdialysis sites from all rats administered the NMDA receptor antagonist D-APV ($n = 7$), the non-NMDA receptor antagonist CNQX ($n = 13$), and the glutamate reuptake inhibitor DHK ($n = 7$). The sizes of the bars represent the apparent size of the lesions from the histological sections. CC, Central canal; Cer, cerebellum; Sol, nucleus of the tractus solitarius; 4V, fourth ventricle.

Leica). Sections were mounted and stained with Neutral Red, and the lesion site left by the microdialysis probes were marked on a corresponding standard cross-section using a stereotaxic atlas of the rat brain (Paxinos and Watson, 1998).

Statistical analysis

The analyses performed for each statistical test are included in the text when appropriate. Differences were considered significant if the null hypothesis was rejected at $p < 0.05$ using a two-tailed test. Where *post hoc* comparisons were performed after ANOVA with repeated measures (RM), the Bonferroni's corrected p value was used to infer statistical significance. Analyses were performed using SigmaStat (SPSS).

Results

Sites of microdialysis

Figure 1 shows an example of a lesion site made by the microdialysis probe in the HMN. This figure also shows the sites of microdialysis from the rats contributing to each of studies 1–3. The microdialysis probes were successfully implanted into, or immediately adjacent to, the HMN in all rats.

Study 1: NMDA receptor antagonism at the HMN in freely behaving rats

Respiratory-related genioglossus activity

Figure 2*A* shows a representative trace of genioglossus muscle activity across sleep–wake states with and without application of the NMDA receptor antagonist D-APV to the HMN. The group data in Figure 2*B* show that NMDA receptor antagonism at the HMN significantly modulated the amplitude of respiratory-related genioglossus activity ($F_{(1,6)} = 7.61$; $p < 0.03$, two-way ANOVA-RM). The amplitude of respiratory-related genioglossus activity was decreased by D-APV in both active wakefulness ($t = 2.76$; $p = 0.033$) and NREM sleep ($t = 2.37$; $p = 0.030$) but not during quiet wakefulness ($t = 0.48$; $p = 0.638$) or REM sleep ($t = 1.06$; $p = 0.305$) (Bonferroni's *post hoc* paired t tests) (Fig. 2*B*).

Tonic genioglossus activity

Figure 2*C* shows that NMDA receptor antagonism at the HMN significantly modulated tonic genioglossus muscle activity ($F_{(1,6)} = 8.28$; $p < 0.028$, two-way ANOVA-RM). Compared with ACSF controls, tonic genioglossus activity was significantly decreased by D-APV at the HMN during active wakefulness ($t = 5.74$; $p < 0.001$, Bonferroni's *post hoc* paired t test) but not during quiet wakefulness, NREM sleep, or REM sleep (Fig. 2*C*). This result demonstrates that glutamate contributes to tonic motor activity via an NMDA receptor mechanism during behaviors engaged during active wakefulness and is consistent with the voltage-gated nature of this receptor (Mayer and Westbrook, 1985).

Specificity of responses

The effects of D-APV at the HMN were specific to genioglossus muscle because there were no significant effects on diaphragm activation ($F_{(1,6)} = 0.58$; $p = 0.475$, two-way ANOVA-RM) or neck muscle activity ($F_{(1,6)} = 5.50$; $p = 0.058$).

Study 2: non-NMDA receptor antagonism at the HMN in freely behaving rats

Respiratory-related genioglossus activity

Non-NMDA receptor antagonism with 0.1 mM CNQX at the HMN had no effect on the amplitude of respiratory-related genioglossus muscle activity in freely behaving rats ($F_{(1,6)} = 0.42$; $p = 0.542$, two-way ANOVA-RM) (Fig. 3*A*). This lack of effect is noteworthy because it is in contrast to the significant modulation observed in the same rats when studied subsequently under anesthesia (see below) and the expectations from *in vitro* experiments (Greer et al., 1991; Funk et al., 1993; Rekling et al., 2000).

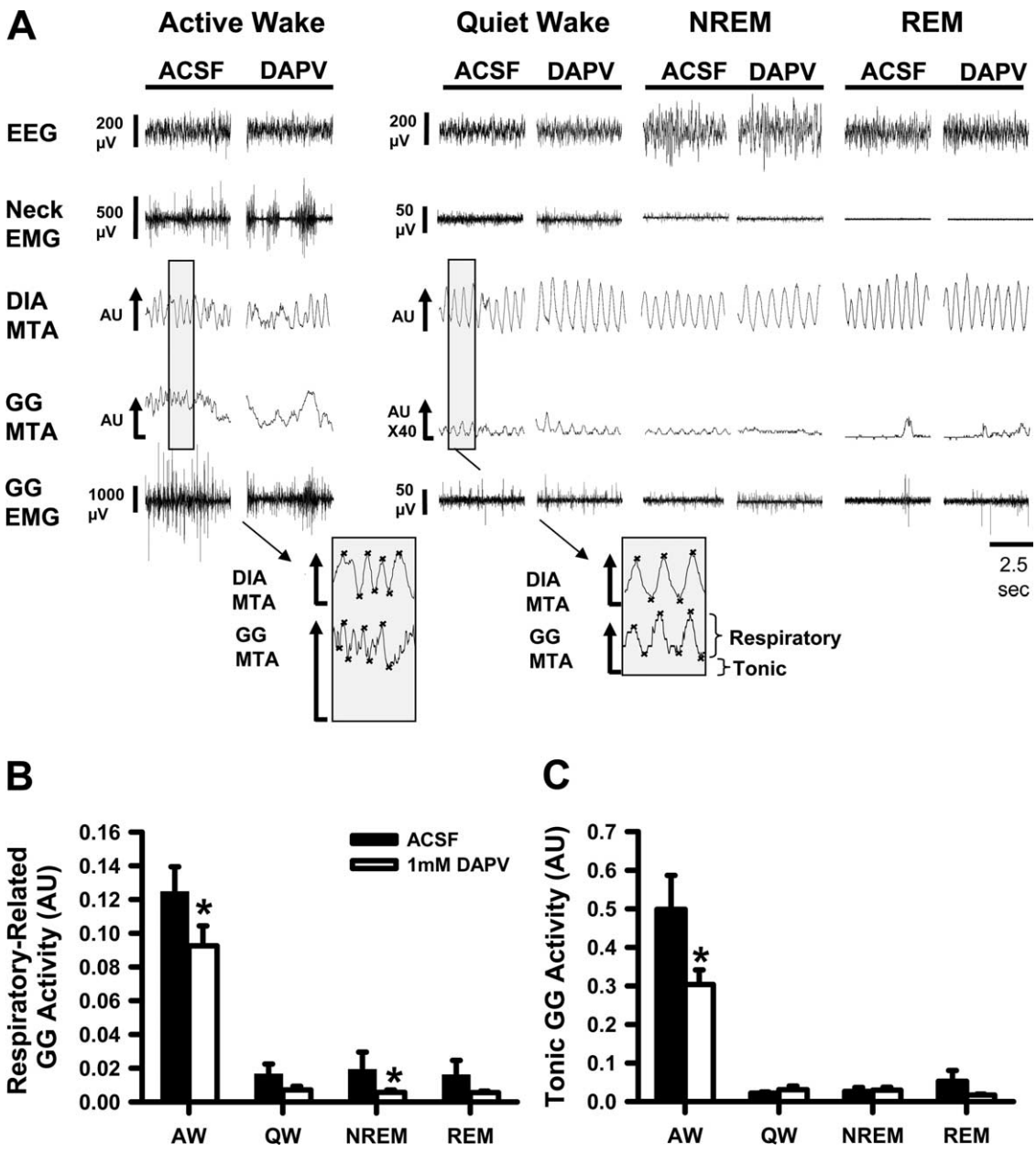

Figure 2. NMDA receptor-mediated modulation of hypoglossal motor output to genioglossus (GG) muscle in freely behaving rats. Example (***A***) and group data (***B***, ***C***) showing the effect of microdialysis perfusion of D-APV into the HMN compared with ACSF controls. D-APV reduced tonic genioglossus muscle tone during active wakefulness (AW) and reduced the amplitude of respiratory-related genioglossus activity during active wakefulness and NREM sleep. In the example trace, the genioglossus and diaphragm (DIA) signals are displayed as their moving-time averages (MTA) in arbitrary units (AU). The baseline of the integrator (i.e., electrical zero) is shown for the genioglossus moving-time averages signal, and the arrows denote an increase in EMG activity. Note the magnified scale for quiet wakefulness (QW), NREM sleep, and REM sleep compared with active wakefulness. The inset shows magnified genioglossus and diaphragm signals at the same scale, and the peaks and troughs of both signals from the moving-time averages are identified for periods of active and quiet wakefulness (for details of the detection and analysis of the genioglossus signal, see Materials and Methods). All values in the group data are displayed as mean + SEM from seven rats; $*p < 0.05$ indicates a significant difference from the respective ACSF control.

Likewise, there were no statistically significant effects of 1 or 5 mM CNQX at the HMN on the amplitude of respiratory-related genioglossus activity ($F_{(2,8)} = 0.25$; $p = 0.140$, two-way ANOVA-RM), with genioglossus activity even tending to increase with 1 mM CNQX rather than any hypothesized decrease with either 1 or 5 mM CNQX (Fig. 3*C*).

Tonic genioglossus activity

Non-NMDA receptor antagonism at the HMN with 0.1 mM CNQX also had no effect on tonic genioglossus activity compared with ACSF controls ($F_{(1,6)} = 0.22$; $p = 0.656$, two-way ANOVA-RM) (Fig. 3*B*). Likewise, there were no significant effects of 1 or 5 mM CNQX at the HMN on tonic genioglossus activity ($F_{(2,8)} = 2.66$; $p = 0.123$, two-way ANOVA-RM) (Fig. 3*D*).

Specificity of responses

With these interventions at the HMN, there was also no nonspecific effect of 0.1 mM CNQX on diaphragm activation ($F_{(1,6)} = 0.22$; $p = 0.658$, two-way ANOVA-RM) or neck muscle activity ($F_{(1,6)} = 0.42$; $p = 0.541$). Likewise, for 1 and 5 mM CNQX at the HMN, there were also no significant effects on diaphragm ($F_{(2,8)} = 3.22$; $p = 0.091$, two-way ANOVA-RM) or neck muscle activity ($F_{(2,8)} = 0.18$; $p = 0.842$).

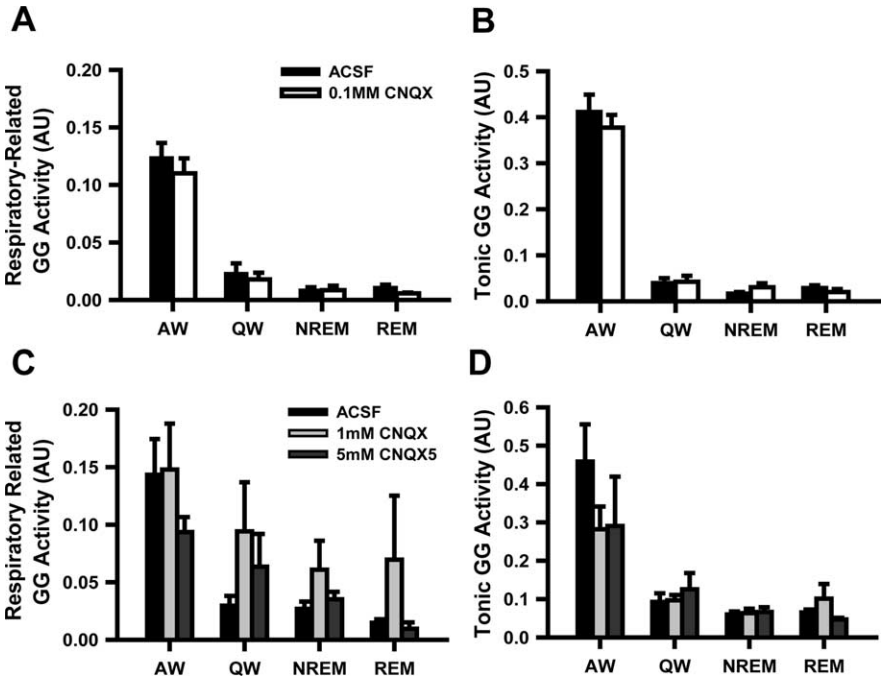

Figure 3. Non-NMDA receptor-mediated modulation of hypoglossal motor output to genioglossus (GG) muscle in freely behaving rats. ***A***, Group data from seven rats showing that 0.1 mM CNQX at the HMN had no effect on the amplitude of respiratory-related genioglossus activity across any sleep–wake state. ***B***, There was also no effect of CNQX on tonic genioglossus activity. Likewise, in a separate set of experiments in six rats, application of 1 and 5 mM CNQX to the HMN had no statistically significant effects on respiratory-related or tonic genioglossus activities (***C***, ***D***). For additional details, see Results. All values are displayed as mean + SEM. Abbreviations are as for Figure 2.

Study 3: endogenous stimulation of glutamate receptors at the HMN

The above results show that the endogenous level of non-NMDA receptor activation at the HMN is low and does not contribute significantly to tonic or respiratory-related genioglossus muscle activity across sleep–wake states, whereas NMDA receptor activation contributes to both measures of genioglossus activity in active wakefulness. In a third study, the glutamate reuptake inhibitor DHK was perfused into the HMN to determine whether increased endogenous glutamate at the HMN modulates genioglossus muscle activity.

Respiratory-related genioglossus activity

Figure 4*A* shows a representative trace of genioglossus muscle activity across sleep–wake states with and without application of the glutamate reuptake inhibitor DHK to the HMN. The group data in Figure 4*B* showed that DHK at the HMN did not augment the amplitude of respiratory-related genioglossus muscle activity in any sleep–wake state ($F_{(1,6)} = 0.14$; $p = 0.721$, two-way ANOVA-RM). This result reinforces the concept from study 2 that rhythmic glutamatergic drives to the HMN are low in freely behaving rats, despite the presence of rhythmic respiratory-related genioglossus activity (Figs. 2–4).

Tonic genioglossus activity

There was a significant effect of DHK at the HMN on tonic genioglossus muscle activity ($F_{(1,6)} = 10.91$; $p < 0.016$, two-way ANOVA-RM). The representative trace of genioglossus activity from one rat (Fig. 4*A*) and the group data from all rats (Fig. 4*B*) show that tonic genioglossus activity was elevated by DHK at the HMN during both quiet wakefulness ($t = 4.02$; $p < 0.001$) and NREM sleep ($t = 3.27$; $p = 0.005$) but not during active wakefulness ($t = 2.02$; $p = 0.059$) or REM sleep ($t = 2.94$; $p = 0.772$) (all Bonferroni's *post hoc* paired *t* tests after ANOVA-RM). These state-specific effects of glutamate reuptake inhibition indicate that there were no significant genioglossus responses when tonic motor tone was either very high (i.e., active wakefulness) or low (i.e., periods of atonia in REM sleep). The effects of DHK at the HMN were specific to genioglossus muscle because there were no significant effects on diaphragm activation ($F_{(1,6)} = 0.08$; $p = 0.785$, two-way ANOVA-RM) or neck muscle activity ($F_{(1,6)} = 0.80$; $p = 0.406$).

In a subset of animals at the end of the DHK experiments, we determined whether the excitatory responses to glutamate reuptake inhibition were attenuated by coapplication of the NMDA or non-NMDA receptor antagonists. Figure 4*D* shows that the increased genioglossus activity produced by DHK at the HMN was decreased by the NMDA receptor antagonist D-APV in three of three rats in both quiet wakefulness and NREM sleep. Figure 4*E* shows that the increased genioglossus activity produced by DHK was decreased by the non-NMDA receptor antagonist CNQX in two of three rats in quiet wakefulness and three of three rats in NREM sleep.

Overall, these results suggest that glutamate reuptake inhibition with DHK increased tonic genioglossus activity via both non-NMDA and NMDA receptor mechanisms. This result further suggests that the lack of effect of the NMDA and non-NMDA receptor antagonists on tonic genioglossus activity without glutamate reuptake inhibition in quiet wakefulness and NREM sleep (Figs. 2*C*, 3*B*,*D*) were attributable to normally low levels of endogenous glutamate modulating the HMN.

Follow-up studies under anesthesia

The representative trace of genioglossus activity from one rat and the group data from all rats in Figure 5*A* illustrate that halothane-maintained anesthesia caused a large increase in the amplitude of respiratory-related genioglossus activity when compared with the levels observed in the same rats during NREM sleep ($t = 9.27$; $p < 0.001$, paired *t* test).

The example from one rat and the group data in Figure 5*B* show that NMDA receptor antagonism at the HMN with D-APV caused a statistically significant suppression of respiratory-related genioglossus activity ($F_{(3,18)} = 52.92$; $p < 0.001$, one-way ANOVA-RM; $t = 2.68$; $p = 0.046$ from Bonferroni's *post hoc* paired *t* test). This result confirms our previous observations in similarly anesthetized rats that NMDA receptors contribute to respiratory drive transmission at the HMN (Steenland et al., 2006). Genioglossus activity increased slightly after washout of D-APV such that levels were similar to the initial ACSF condition ($t = 1.68$; $p = 0.330$). At the end of the experiment, application of 10 mM serotonin caused a clear increase in genioglossus activity confirming an intact HMN ($t = 8.59$; $p < 0.001$) (Fig. 5*B*).

The example from one rat and the group data in Figure 5*C*

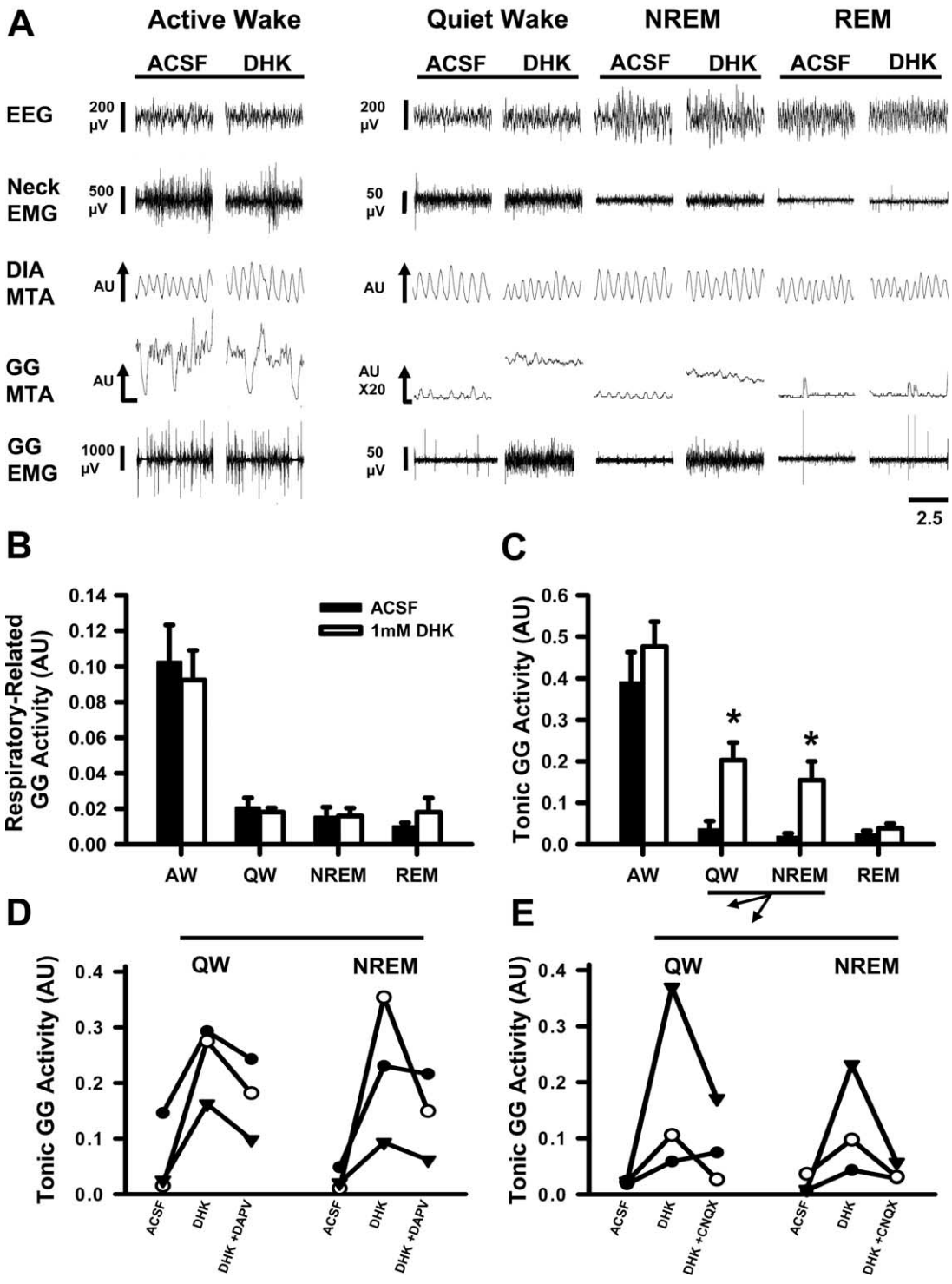

Figure 4. Increased tonic genioglossus (GG) activity with glutamate reuptake inhibition at the HMN in freely behaving rats. ***A–C***, Example (***A***) and group data (***B***, ***C***) showing that DHK at the HMN elevated tonic genioglossus activity during quiet wakefulness (QW) and NREM sleep but was unable to reverse the periods of genioglossus suppression during REM sleep. There was no effect of DHK on the amplitude of respiratory-related genioglossus activity. All values are displayed as mean + SEM from seven rats; $*p < 0.05$ indicates a significant difference from the respective ACSF control. Note the magnified scale for quiet wakefulness, NREM sleep, and REM sleep compared with active wakefulness (AW). ***D***, The increased genioglossus activity produced by DHK at the HMN was decreased by the NMDA receptor antagonist D-APV in three of three rats in both quiet wakefulness and NREM sleep. ***E***, The increased genioglossus activity produced by DHK was decreased by the non-NMDA receptor antagonist CNQX in two of three rats in quiet wakefulness and three of three rats in NREM sleep. The individual responses in each rat can be identified by the individual data points and corresponding lines in ***D*** and ***E***. DIA, Diaphragm; MTA, moving-time averages; AU, arbitrary units.

show that non-NMDA receptor antagonism at the HMN with CNQX also caused a significant suppression of respiratory-related genioglossus activity ($F_{(3,18)} = 19.22$; $p < 0.001$, one-way ANOVA-RM; $t = 2.70$; $p = 0.044$ from Bonferroni's *post hoc* paired *t* test). This result also confirms our previous observations in similarly anesthetized rats that non-NMDA receptors contribute to respiratory drive transmission at the HMN (Steenland et al., 2006). After washout of CNQX, respiratory-related

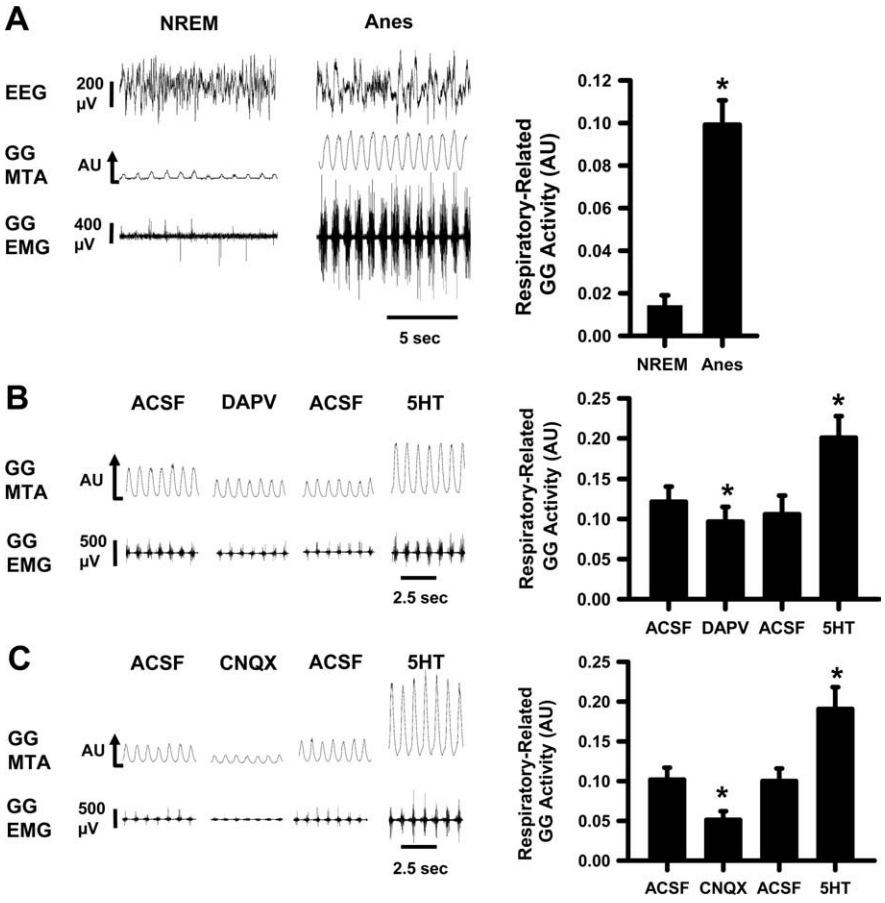

Figure 5. NMDA and non-NMDA receptor activation at the HMN contributes to respiratory-related genioglossus (GG) activity in anesthetized rats. ***A***, Example and group data ($n = 14$ rats) showing that anesthesia (Anes) increased respiratory-related genioglossus activity compared with the levels observed in the same rats in NREM sleep. ***B***, ***C***, Examples and group data ($n = 7$ rats for each) showing that NMDA and non-NMDA receptor antagonism at the HMN with D-APV and CNQX, respectively, decreased genioglossus activity, indicating the contribution of each receptor type to the manifestation of respiratory-related genioglossus activity under anesthesia. Application of serotonin [5-hydroxytryptamine (5HT)] at the end of all the experiments confirmed an intact HMN capable of responding to manipulation of neurotransmission. The representative traces in ***A–C*** come from different animals, but the group data show similar baseline genioglossus activities under anesthesia. All values are displayed as mean + SEM; $*p < 0.05$ indicates a significant difference from the respective ACSF control. DIA, Diaphragm; MTA, moving-time averages; AU, arbitrary units.

genioglossus activity returned to levels similar to the initial ACSF condition ($t = 0.09$; $p = 1.00$). At the end of the experiments, application of 10 mM serotonin caused a robust activation of genioglossus activity ($t = 4.75$; $p < 0.001$), again confirming an intact HMN. Overall, these results show that respiratory-related glutamatergic drive to the HMN is relatively low in freely behaving rats across natural sleep–wake states but is present under anesthesia when both NMDA and non-NMDA receptors contribute to the manifestation of respiratory-related genioglossus activity.

Discussion

Respiratory-related activity

This study is the first to identify the endogenous non-NMDA and NMDA receptor modulation of hypoglossal motor activity in an intact freely behaving organism *in vivo*. Using the same methodology in the same anesthetized and freely behaving animals, we show that both non-NMDA and NMDA receptor mechanisms at the HMN contribute significantly to respiratory-related genioglossus activity in anesthetized adult rats. This result obtained under anesthesia is significant because it shows that, in addition to the fundamental role of non-NMDA receptor activation in the expression of respiratory-related hypoglossal motor activity, originally identified as the principal mechanism operating *in vitro* (Greer et al., 1991; Funk et al., 1993, 1997; Rekling et al., 2000), NMDA receptor activation is also involved *in vivo*. The lesser involvement of NMDA receptors in respiratory drive transmission at the HMN in neonatal preparations may be influenced by deafferentation and removal of tonic excitatory inputs that are normally present *in vivo* and/or a function of an immature respiratory network (Rekling et al., 2000). As an example of the latter, neonatal hypoglossal motoneurons from NMDA receptor knockout mice have inspiratory synaptic currents that are indistinguishable from wild-type animals, which questions the importance of NMDA receptors in inspiratory drive transmission in these animals at birth (Funk et al., 1997). At the phrenic motor nucleus, both non-NMDA and NMDA receptors are involved in respiratory drive transmission in anesthetized adult animals (Bohmer et al., 1991; Pierrefiche et al., 1994; Chitravanshi and Sapru, 1996), whereas NMDA receptors again play a lesser role in neonatal *in vitro* and *in vivo* preparations (McCrimmon et al., 1989; Liu et al., 1990; Greer et al., 1991; Funk et al., 1997) and in juvenile anesthetized rats *in vivo* (McCrimmon et al., 1989). Overall, the results suggest that both non-NMDA and NMDA receptors function to modulate transmission of rhythmic inspiratory drive at respiratory motoneurons and that the conditions for the significant involvement of both receptors are met in the anesthetized adult *in vivo* preparation with an intact neuraxis.

Anesthetized versus conscious rats

The magnitude of respiratory-related hypoglossal motor activity is high *in vitro* (Smith et al., 1991; Rekling et al., 2000), and certain anesthetics increase hypoglossal premotor neuronal activity and augment the magnitude of respiratory-related hypoglossal motor tone compared with the same animals studied when awake without anesthetic (Roda et al., 2004). For example, halothane anesthesia increases hypoglossal nerve activity and induces strong c-Fos protein expression in the Kölliker-Fuse nucleus (Roda et al., 2004), which has excitatory projections to the HMN (Kuna and Remmers, 1999). In the present experiments, anesthesia was maintained with halothane, and there was a large increase in respiratory-related genioglossus activity compared with the same animals studied in sleep (Fig. 5), despite the hyperpolarizing effect of volatile anesthetics on hypoglossal motoneurons (Sirois et al., 2000).

Non-NMDA receptors mediate the major component of

respiratory-related hypoglossal motor activity *in vitro* (Greer et al., 1991; Funk et al., 1993, 1997; Rekling et al., 2000). Despite the significant contribution of this non-NMDA receptor mechanism in anesthetized adult rats *in vivo* (Fig. 5) (Steenland et al., 2006), there was no effect of non-NMDA receptor antagonism on respiratory-related genioglossus activity when the same animal was studied with the same interventions across sleep–wake states (Fig. 3). Because respiratory-related genioglossus activity was present in the same animal under both conditions, this result shows that the expression of this respiratory-related activity is not significantly dependent on non-NMDA receptor activation in the intact behaving organism while it was under anesthesia.

Although there was no evidence for a decrease in respiratory-related genioglossus activity after non-NMDA receptor antagonism in the freely behaving rats (Fig. 3), it may be of some concern that the lesser activity compared with anesthetized rats may have made it more difficult to detect a change. However, α1 receptor antagonism at the HMN significantly suppresses respiratory-related genioglossus activity in both wakefulness and sleep (Chan et al., 2006), i.e., unlike the responses to CNQX. One potential explanation for these results is that, in the intact conscious organism, there are a number of neuronal groups expressing rhythmic respiratory-related activity (Lindsey et al., 1992; Veasey et al., 1995; Oyamada et al., 1998; Nattie, 1999; Orem and Kubin, 2000), with these groups having direct and indirect projections to respiratory motoneurons (Travers and Norgren, 1983; Manaker and Tischler, 1993; Dobbins and Feldman, 1995; Kuna and Remmers, 1999; Rukhadze and Kubin, 2007). Accordingly, nonglutamatergic and sleep-state dependent premotor inputs are positioned to influence respiratory motor activity either directly or indirectly via modulation of rhythmic glutamatergic inputs. Overall, the current data show that, in contrast to anesthetized or *in vitro* preparations, respiratory drive transmission through the non-NMDA receptor at the HMN is low in rats across all sleep–wake states. Reinforcing this lack of endogenous glutamatergic respiratory drive onto non-NMDA receptors is that glutamate reuptake inhibition increased tonic but not respiratory-related genioglossus activity in quiet wakefulness and NREM sleep, i.e., behavioral states in which the respiratory control system and respiratory-related genioglossus activity are at their most stable. Nevertheless, there is the potential concern that some of the transient genioglossal motor activations that characterize active wakefulness and REM sleep may not be truly "respiratory," although they are associated with diaphragm activation, i.e., this activity may be a product of both inspiratory and non-inspiratory drives of which the non-NMDA receptor inspiratory component may be small and undetectable relative to the overall input. This concern, however, further suggests that non-NMDA receptors contribute minimally to respiratory drive transmission at the HMN, even in these states with more variable genioglossus activity.

In this study, we identified a contribution of NMDA receptors to respiratory drive transmission at the HMN because respiratory-related genioglossus activity decreased significantly in active wakefulness and NREM sleep with NMDA receptor antagonism (Fig. 2*B*). This effect is consistent with suppression of medullary inspiratory neuronal discharge after NMDA receptor antagonism in decerebrate dogs, i.e., with this latter response caused by NMDA receptors contributing a tonic drive that modulates the expression of inspiratory burst amplitude (Krolo et al., 2000). In the present study, a suppressant effect of NMDA receptor antagonism on respiratory-related genioglossus activity may be mediated by a similar mechanism. There are a number of other tonically active depolarizing inputs to the HMN in the intact organism, including serotonergic and noradrenergic neurons (Kubin et al., 1998; Fenik et al., 2005; Chan et al., 2006), that could depolarize hypoglossal motoneurons and enable the magnesium block of the NMDA receptor to be removed. Our studies in conscious rats suggest a significant tonic excitatory noradrenergic input to the HMN but not so for a serotonergic drive (Sood et al., 2005, 2006; Chan et al., 2006).

Tonic motor activity

This study is also the first to determine the endogenous glutamatergic influences on tonic hypoglossal motor activity *in vivo*. The results show that endogenous glutamate at the HMN contributes to the expression of tonic genioglossus activity via an NMDA receptor mechanism but only at times when the motoneurons were most active, i.e., during behaviors engaged during active wakefulness (Fig. 2*C*). This result is consistent with the voltage-gated nature of this receptor (Mayer and Westbrook, 1985). In contrast to this significant effect of NMDA receptor antagonism on tonic genioglossus activity during active wakefulness, there was no effect of non-NMDA receptor antagonism on tonic activity across sleep–wake states (Fig. 3). We showed previously that the dose of CNQX delivered to the HMN antagonizes the non-NMDA receptor response to application of exogenous agonist but does not affect NMDA receptor responses and likewise for D-APV and NMDA receptor antagonism (Steenland et al., 2006). Moreover, these doses of antagonist were effective in the same animals when studied under anesthesia (Fig. 5), and even the higher doses of CNQX did not significantly affect tonic activity. Overall, the results showed that endogenous activation of non-NMDA receptors at the HMN contributes minimally to resting tonic motor tone in this freely behaving preparation *in vivo* and that NMDA receptors contributed to the expression of tonic motor activity but only during behaviors engaged during active wakefulness.

Glutamate uptake inhibition, however, caused clear increases in tonic genioglossus activity, with this response confined to quiet wakefulness and NREM sleep (Fig. 4*A*,*C*). In contrast, glutamate uptake inhibition had no significant effects on tonic genioglossus activity in active wakefulness, i.e., at times when tonic activity was already high and may have been masked by ongoing behavioral activation. The lack of response of tonic genioglossus activity to glutamate uptake inhibition in REM sleep suggests that glutamate levels were already minimal. The lack of response to DHK in REM sleep also fits with the inability of exogenously applied glutamate to excite trigeminal motoneurons in REM sleep (Burgess et al., 2008), implicating the recruitment of a strong inhibitory REM mechanism that attenuates glutamate receptor-mediated excitation. Nevertheless, it is not known whether the tonic genioglossus response to DHK is attributable to increased glutamate concentrations at the synapse and/or the extracellular space. The response of the motoneuron to increased endogenous glutamate may be affected by regional variations in glutamate receptor subunit composition and/or desensitization kinetics (Funk et al., 1995; Rekling et al., 2000).

References

Berger AJ (2000) Determinants of respiratory motoneuron output. Respir Physiol 122:259–269.

Bohmer G, Schmid K, Schauer W (1991) Evidence for an involvement of NMDA and non-NMDA receptors in synaptic excitation of phrenic motoneurons in the rabbit. Neurosci Lett 130:271–274.

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.
Chan E, Steenland HW, Liu H, Horner RL (2006) Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. Am J Respir Crit Care Med 174:1264–1273.
Chase MH, Chandler SH, Nakamura Y (1980) Intracellular determination of membrane potential of trigeminal motoneurons during sleep and wakefulness. J Neurophysiol 44:349–358.
Chitravanshi VC, Sapru HN (1996) NMDA as well as non-NMDA receptors mediate the neurotransmission of inspiratory drive to phrenic motoneurons in the adult rat. Brain Res 715:104–112.
Dobbins EG, Feldman JL (1995) Differential innervation of protruder and retractor muscles of the tongue in rat. J Comp Neurol 357:376–394.
Fallgren AB, Paulsen RE (1996) A microdialysis study in rat brain of dihydrokainate, a glutamate uptake inhibitor. Neurochem Res 21:19–25.
Fenik VB, Davies RO, Kubin L (2005) REM sleep-like atonia of hypoglossal (XII) motoneurons is caused by loss of noradrenergic and serotonergic inputs. Am J Respir Crit Care Med 172:1322–1330.
Funk GD, Smith JC, Feldman JL (1993) Generation and transmission of respiratory oscillations in medullary slices: role of excitatory amino acids. J Neurophysiol 70:1497–1515.
Funk GD, Smith JC, Feldman JL (1995) Modulation of neural network activity in vitro by cyclothiazide, a drug that blocks desensitization of AMPA receptors. J Neurosci 15:4046–4056.
Funk GD, Johnson SM, Smith JC, Dong XW, Lai J, Feldman JL (1997) Functional respiratory rhythm generating networks in neonatal mice lacking NMDAR1 gene. J Neurophysiol 78:1414–1420.
Greer JJ, Smith JC, Feldman JL (1991) Role of excitatory amino acids in the generation and transmission of respiratory drive in neonatal rat. J Physiol 437:727–749.
Guyenet PG, Koshiya N, Huangfu D, Verberne AJ, Riley TA (1993) Central respiratory control of A5 and A6 pontine noradrenergic neurons. Am J Physiol 264:R1035–R1044.
Hamrahi H, Chan B, Horner RL (2001) On-line detection of sleep-wake states and application to produce intermittent hypoxia only in sleep in rats. J Appl Physiol 90:2130–2140.
Honore T, Davies SN, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, Nielsen FE (1988) Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 241:701–703.
Horner RL (1996) Motor control of the pharyngeal musculature and implications for the pathogenesis of obstructive sleep apnea. Sleep 19:827–853.
Jelev A, Sood S, Liu H, Nolan P, Horner RL (2001) Microdialysis perfusion of 5-HT into hypoglossal motor nucleus differentially modulates genioglossus activity across natural sleep-wake states in rats. J Physiol 532:467–481.
Krolo M, Stuth EA, Tonkovic-Capin M, Hopp FA, McCrimmon DR, Zuperku EJ (2000) Relative magnitude of tonic and phasic synaptic excitation of medullary inspiratory neurons in dogs. Am J Physiol Regul Integr Comp Physiol 279:R639–R649.
Kubin L, Davies RO, Pack L (1998) Control of upper airway motoneurons during REM sleep. News Physiol Sci 13:637–656.
Kuna ST, Remmers JE (1999) Premotor input to hypoglossal motoneurons from Kolliker-Fuse neurons in decerebrate cats. Respir Physiol 117:85–95.
Lindsey BG, Hernandez YM, Morris KF, Shannon R (1992) Functional connectivity between brain stem midline neurons with respiratory-modulated firing rates. J Neurophysiol 67:890–904.
Liu G, Feldman JL, Smith JC (1990) Excitatory amino acid-mediated transmission of inspiratory drive to phrenic motoneurons. J Neurophysiol 64:423–436.
Liu X, Sood S, Liu H, Horner RL (2005) Opposing muscarinic and nicotinic modulation of hypoglossal motor output to genioglossus muscle in rats in vivo. J Physiol 565:965–980.
Manaker S, Tischler LJ (1993) Origin of serotoninergic afferents to the hypoglossal nucleus in the rat. J Comp Neurol 334:466–476.
Mayer ML, Westbrook GL (1985) The action of *N*-methyl-D-aspartic acid on mouse spinal neurones in culture. J Physiol 361:65–90.
McCrimmon DR, Smith JC, Feldman JL (1989) Involvement of excitatory amino acids in neurotransmission of inspiratory drive to spinal respiratory motoneurons. J Neurosci 9:1910–1921.
Morrison JL, Sood S, Liu H, Park E, Nolan P, Horner RL (2003) Role of inhibitory amino acids in control of hypoglossal motor outflow to genioglossus muscle in naturally sleeping rats. J Physiol 552:975–991.
Nattie E (1999) CO2, brainstem chemoreceptors and breathing. Prog Neurobiol 59:299–331.
O'Brien JA, Isaacson JS, Berger AJ (1997) NMDA and non-NMDA receptors are co-localized at excitatory synapses of rat hypoglossal motoneurons. Neurosci Lett 227:5–8.
Olverman HJ, Jones AW, Watkins JC (1984) L-glutamate has higher affinity than other amino acids for [^{3}H]-D-AP5 binding sites in rat brain membranes. Nature 307:460–462.
Orem J, Kubin L (2000) Respiratory physiology: central neural control. In: Principles and practice of sleep medicine, Ed 3 (Kryger MH, Roth T, Dement WC, eds), pp 205–220. Philadelphia: Saunders.
Oyamada Y, Ballantyne D, Muckenhoff K, Scheid P (1998) Respiration-modulated membrane potential and chemosensitivity of locus coeruleus neurones in the in vitro brainstem-spinal cord of the neonatal rat. J Physiol 513:381–398.
Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, Ed 4. San Diego: Academic.
Pierrefiche O, Foutz AS, Champagnat J, Denavit-Saubie M (1994) NMDA and non-NMDA receptors may play distinct roles in timing mechanisms and transmission in the feline respiratory network. J Physiol 474:509–523.
Rekling JC (1992) Interaction between thyrotropin-releasing hormone (TRH) and NMDA-receptor-mediated responses in hypoglossal motoneurones. Brain Res 578:289–296.
Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL (2000) Synaptic control of motoneuronal excitability. Physiol Rev 80:767–852.
Roda F, Pio J, Bianchi AL, Gestreau C (2004) Effects of anesthetics on hypoglossal nerve discharge and c-Fos expression in brainstem hypoglossal premotor neurons. J Comp Neurol 468:571–586.
Rukhadze I, Kubin L (2007) Differential pontomedullary catecholaminergic projections to hypoglossal motor nucleus and viscerosensory nucleus of the solitary tract. J Chem Neuroanat 33:23–33.
Sirois JE, Lei Q, Talley EM, Lynch III C, Bayliss DA (2000) The TASK-1 two-pore domain K^+ channel is a molecular substrate for neuronal effects of inhalation anesthetics. J Neurosci 20:6347–6354.
Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL (1991) Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254:726–729.
Sood S, Morrison JL, Liu H, Horner RL (2005) Role of endogenous serotonin in modulating genioglossus muscle activity in awake and sleeping rats. Am J Respir Crit Care Med 172:1338–1347.
Sood S, Raddatz E, Liu X, Liu H, Horner RL (2006) Inhibition of serotonergic medullary raphe obscurus neurons suppresses genioglossus and diaphragm activities in anesthetized but not conscious rats. J Appl Physiol 100:1807–1821.
Steenland HW, Liu H, Sood S, Liu X, Horner RL (2006) Respiratory activation of the genioglossus muscle involves both non-NMDA and NMDA glutamate receptors at the hypoglossal motor nucleus in vivo. Neuroscience 138:1407–1424.
Travers JB, Norgren R (1983) Afferent projections to the oral motor nuclei in the rat. J Comp Neurol 220:280–298.
Veasey SC, Fornal CA, Metzler CW, Jacobs BL (1995) Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. J Neurosci 15:5346–5359.
Wang J, Irnaten M, Venkatesan P, Evans C, Baxi S, Mendelowitz D (2002) Synaptic activation of hypoglossal respiratory motoneurons during inspiration in rats. Neurosci Lett 332:195–199.
Zuperku EJ, Brandes IF, Stucke AG, Sanchez A, Hopp FA, Stuth EA (2008) Major components of endogenous neurotransmission underlying the discharge activity of hypoglossal motoneurons in vivo. Adv Exp Med Biol 605:279–284.