Postsynaptic Inhibition of Hypoglossal Motoneurons Produces Atonia of the Genioglossal Muscle During Rapid Eye Movement Sleep

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Study Objectives: Hypoglossal motoneurons were recorded intracellularly to determine whether postsynaptic inhibition or disfacilitation was responsible for atonia of the lingual muscles during rapid eye movement (REM) sleep.

Design: Intracellular records were obtained of the action potentials and subthreshold membrane potential activity of antidromically identified hypoglossal motoneurons in cats during wakefulness, nonrapid eye movement (NREM) sleep, and REM sleep. A cuff electrode was placed around the hypoglossal nerve to antidromically activate hypoglossal motoneurons. The state-dependent changes in membrane potential, spontaneous discharge, postsynaptic potentials, and rheobase of hypoglossal motoneurons were determined.

Analyses and Results: During quiet wakefulness and NREM sleep, hypoglossal motoneurons exhibited spontaneous repetitive discharge. In the transition from NREM sleep to REM sleep, repetitive discharge ceased and the membrane potential began to hyperpolarize; maximal hyperpolarization (10.5 mV) persisted throughout REM sleep. During REM sleep there was a significant increase in rheobase, which was accompanied by barrages of large-amplitude inhibitory postsynaptic potentials (IPSPs), which were reversed following the intracellular injection of chloride ions. The latter result indicates that they were mediated by glycine; IPSPs were not present during wakefulness or NREM sleep.

Conclusions: We conclude that hypoglossal motoneurons are postsynaptically inhibited during naturally occurring REM sleep; no evidence of disfacilitation was observed. The data also indicate that glycine receptor-mediated postsynaptic inhibition of hypoglossal motoneurons is crucial in promoting atonia of the lingual muscles during REM sleep.

Keywords: atonia, hypoglossal, IPSP, motoneuron, OSA, postsynaptic inhibition, REM sleep

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INTRODUCTION

Patency of the upper airway, which is essential to maintain ventilatory processes during wakefulness as well as nonrapid eye movement (NREM) and rapid eye movement (REM) sleep, is dependent on the degree of contraction, i.e., tone, of the genioglossus muscle. In turn, the genioglossal muscle is activated by hypoglossal motoneurons.[1–4] In pathological conditions such as obstructive sleep apnea (OSA), episodic periods of apnea/hypopnea occur during sleep, most frequently during REM sleep. Patients with OSA are therefore subjected to recurrent episodes of hypoxia that can occur hundreds of time each night.[4] In an attempt to elucidate the synaptic mechanisms that control the activity of hypoglossal motoneurons during naturally occurring REM sleep, we obtained intracellular records from hypoglossal motoneurons to directly measure their changes in membrane excitability. Knowledge of the state-dependent changes in excitability of hypoglossal motoneurons will not only to advance our understanding of the normal state-dependent control of hypoglossal motor processes but also serve as a foundation for determining and manipulating those processes that result in pathological conditions such as OSA.

Previously, human[5] and animal studies[6–8] of the state-dependent activity of hypoglossal motoneurons have used extracellular techniques to record the activity of the hypoglossal nerve or genioglossal muscle in conjunction with the ejection of neurotransmitter agonists and antagonists into the brainstem in the region of the hypoglossal nucleus. These studies were not designed to examine the underlying subthreshold and suprathreshold processes that determine the intrinsic excitability of hypoglossal (and other) motoneurons. Therefore, to obtain direct measures of the factors that are responsible for changes in hypoglossal motoneuron excitability during naturally occurring states of sleep and wakefulness, and specifically REM sleep, we used intracellular recording techniques in intact, unanesthetized cats. We monitored directly the crucial membrane potential events (both suprathreshold and subthreshold) that are responsible for regulating the state-dependent discharge of hypoglossal motoneurons and the tone of the genioglossal muscles.

METHODS

Five adult male cats (3.5–4.5 kg) were used in a previous study of field potential activity,[9] in addition to being used for intracellular recordings that were the subject of the current report. All animals were in good health and approved for research by veterinarians of the UCLA Department of Laboratory Animal Medicine. Experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011). Appropriate procedures were conducted to minimize pain, discomfort, or stress of the animals and only the minimum number of animals was used that was necessary to yield reliable scientific data.

Surgical Procedures

Details of the surgical procedures that were used to obtain electrophysiological data from hypoglossal motoneurons in the

intact, unanesthetized cat and for monitoring electroencephalographic (EEG), electro-oculographic (EOG), pontogeniculooccipital (PGO) and electromyographic (EMG) activity have been previously reported.[9–11] Briefly, during isoflurane anesthesia, screw electrodes were placed in the calvarium to monitor EEG activity. A small hole (1 mm in diameter) was drilled in the caudal parietal bone to provide access for a microelectrode to the hypoglossal motor nucleus. A pair of screw electrodes was placed in the infraorbital region of the frontal bone to record EOG activity; flexible wire electrodes were inserted into the neck muscles to monitor EMG activity. A silicone-based stimulating cuff electrode[9,12] was implanted (via a submandibular approach) around the trunk of the hypoglossal nerve, proximal to the bifurcation into its medial and lateral nerve branches to antidromically identify hypoglossal motoneurons. The leads from the cuff electrode were tunneled subcutaneously to the dorsal occipital bone, where they were externalized and bonded with acrylic resin to a head implant. All electrode leads were connected to a female Winchester plug, which was also bonded to the calvarium with acrylic resin. A head-restraining device was cemented to the acrylic mound for maintaining, without pain or discomfort, stereotaxic alignment of the head of the animal during recording sessions. Following a recovery period of 10 to 14 days, the animals were habituated daily (4 to 9 days; 2 to 4 h per day) to a stereotaxic apparatus (Kopf 880 head-holder, David Kopf Instruments, Tujunga, CA). Experiments (recording) were conducted when the animals exhibited normal amounts of and transitions between wakefulness and NREM and REM sleep states.[13]

Stimulation and Recording Procedures

Field potential recordings were obtained from each animal in order to localize the optimal site to record antidromically activated motoneurons.[9] Subsequently, microelectrode penetrations were aligned subtentorially at 10–12° (posterior-to-anterior) to pass through the cerebellum to access the hypoglossal nucleus (stereotaxic coordinates: P12 to 14, L0.5 to 1.0, H-6.5).[14] Intracellular recordings from hypoglossal motoneurons were obtained using glass microelectrodes filled with a solution of either 3 M KCl (tip resistance = 10–20 MΩ) or 2 M K-citrate (tip resistance = 20–35 MΩ). A reference (ground) silver wire electrode was placed, acutely, in contact with tissue surrounding the access hole during recording sessions. Intracellular current injections were carried out via recording microelectrodes using an Axoclamp 2A preamplifier (Axon Instruments, Inc., Union City, CA) operating in current clamp mode. Polysomnographic and intracellular signals (10x low-gain and 100x high-gain) were simultaneously monitored on a chart recorder (Grass Instruments, Middleton, WI) and oscilloscope, respectively, and were stored on a video cassette recorder using a pulse code modulation (PCM) multiplex adapter.

Data Analysis

Intracellular recordings were digitized off-line at 10 μs and 50 μs bin widths to examine action potentials and other motoneuron membrane properties, respectively. Axograph software was used for measuring the magnitude of the digitized intracellular data (action potentials, membrane potentials, inhibitory postsynaptic potentials [IPSPs]) as described previously.[9,12,15]

Hypoglossal motoneurons were identified by antidromic activation by the ipsilateral hypoglossal nerve via the cuff electrode (single rectangular pulses, 0.05 ms pulse-width, 0.25 Hz frequency, 2–7 V intensity). The antidromic spike amplitude was measured from the baseline membrane potential to the peak of the spike.[16] The membrane potential was obtained by subtracting the steady DC voltage drop of the cell from the extracellular voltage registered upon withdrawal of the electrode from the cell.[17] For the analysis of the waveform and frequency of IPSPs, only cells recorded with citrate-filled microelectrodes were used because of the chloride-dependent nature of these potentials.[18] To examine the ion dependency of the IPSP, chloride-filled microelectrodes were used. Accordingly, chloride ions were injected into the impaled cell by passive diffusion from the recording electrode or by passing steady hyperpolarizing currents (up to 5 nA). The rheobase of the cell was assessed by determining the threshold current necessary to produce an action potential in at least 90% of the trials with long (20–50 msec) depolarizing current pulses at a repetition rate of 1/sec.[16]

A paired two-tailed Student t-test was used for statistical analysis of changes in the membrane potential during NREM compared with REM sleep. Because rheobase data were collected from individual motoneurons during either NREM or REM sleep, the difference in these values was analyzed using the unpaired two-tailed Student t-test. Statistical significance was set at $P < 0.05$; data were expressed as means ± standard error of the mean.

RESULTS

Intracellular data were obtained from motoneurons (n = 28) that were antidromically identified following stimulation of the ipsilateral trunk of the hypoglossal nerve. Only data from cells that were healthy and undamaged from electrode placement were included in this study. All cells had antidromic spike amplitudes ranging from 60–91 mV (mean = 72.4 ± 1.4 mV) and stable potentials free from signs of membrane deterioration and/ or injury discharge. The mean duration for which recordings were obtained was: 4.0 ± 0.6 min (range = 0.5–10.2 min; n = 23 cells) and 2.1 ± 0.4 min (range = 0.6–6.3 min; n = 15 cells) for NREM and REM sleep, respectively. Cells with membrane potentials of -60.8 mV and -55.4 mV were recorded only during wakefulness for approximately 3 min. For the entire pool of motoneurons that were recorded during NREM or REM sleep states (n = 26), the mean membrane potential during NREM sleep was -58 ± 0.7 mV, compared to -68.6 ± 1.1 mV during REM sleep.

Figure 1 is a recording that was obtained during wakefulness from a hypoglossal motoneuron that was positively identified by antidromic activation. Following antidromic invasion, a representative full-sized (somadendritic) action potential with an overshoot spike potential was observed (spike size = 91 mV, antidromic latency = 1.1 msec, membrane potential = -60.8 mV; Figure 1A and 1B). In addition, this and other antidromic spikes were typically followed by a prolonged after-hyperpolarizing potential (AHP; duration = 63.6 msec, amplitude = 6.4 mV; Figure 1B).

All intracellular data were collected during multiple recording sessions over a period of 2–4 w. Of the 28 motoneurons that were recorded, a subgroup of 12 hypoglossal motoneurons (spike size = 70.8 ± 2.3 mV, range = 60–82 mV) were selected

for analysis of the membrane potential during naturally occurring NREM sleep, the transition to REM sleep, and during the subsequent period of REM sleep. The remaining cells were excluded from the analysis of hyperpolarization because they were only recorded during a single state (wakefulness, n = 2; NREM sleep, n = 11; REM sleep, n = 3).

For those cells exhibiting state changes (n = 12), recordings within and across animals revealed that the membrane potential of hypoglossal motoneurons exhibited a consistent pattern of state-dependent activity, as shown in Figure 2. The transition from NREM to REM sleep began with the presence of PGO waves, which were correlated with a decrease in the amplitude of EMG activity, the cessation of slow EEG waves, and the occurrence of low voltage/fast EEG activity. The spontaneous discharge of hypoglossal motoneurons terminated at the onset of this transition period. The end of the transition phase was determined by the cessation of EMG activity, as shown in Figure 2. The membrane potential of the representative motoneuron (-52 mV during NREM sleep) as shown in Figure 2 began to hyperpolarize at the beginning of the transition phase (that lasted for approximately 30 sec). Hyperpolarization continued until the membrane potential reached a maximum of -71 mV, which occurred 50 sec after the onset of REM sleep (Figure 2). This cell exhibited a maximal hyperpolarization of 19 mV during REM sleep.

For the pool of 12 motoneurons with stable records during NREM and REM sleep, similar to that shown in Figure 2, the mean value of the membrane potential was -57.5 ± 0.9 mV during NREM sleep (range = -52 to -64 mV), compared to -68 ± 1.3 mV (range = -60 to -74 mV) during REM sleep. Quantitatively, there was maximal hyperpolarization of 10.5 ± 1.4 mV (range = 4–19 mV) during REM sleep. The REM sleep related membrane hyperpolarization, compared with the level

of polarization during NREM sleep, was statistically significant (P < 0.0001, degrees of freedom = 11, *t*-value = 7.383, n = 12).
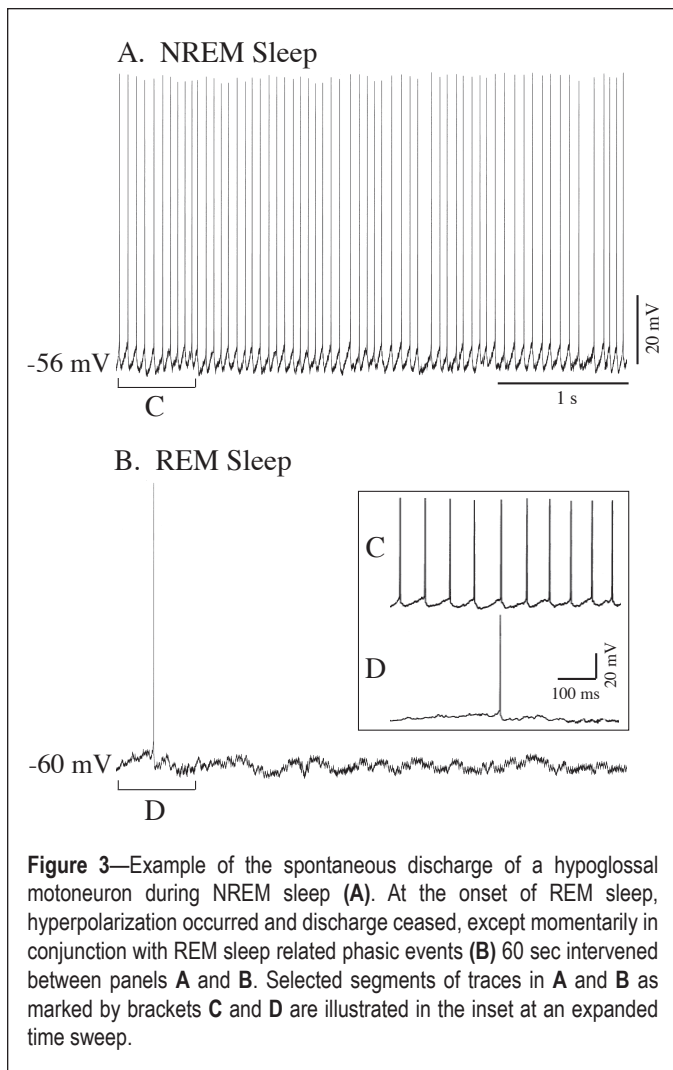
Spontaneous action potentials were observed in seven of the 12 hypoglossal motoneurons (58%) that were recorded during NREM sleep: these cells either ceased firing completely (n = 4)
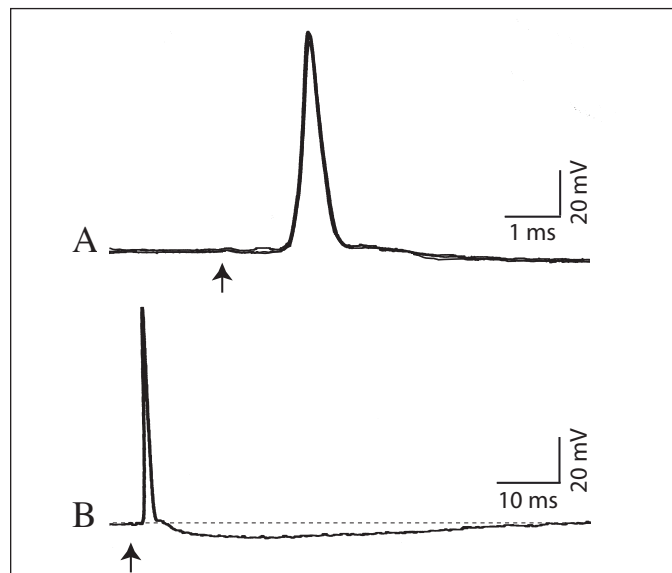
Figure 1—Positive identification of an intracellularly recorded hypoglossal motoneuron. **(A)** Superimposed traces of three consecutive trials of antidromic activation of the motoneruron. This representative hypoglossal motoneuron exhibited antidromically evoked action potentials with a constant latency following stimulation of the ipsilateral hypoglossal nerve (arrows) during naturally occurring wakefulness. **(B)** An example of a postspike after-hyperpolarizing potential is presented with an expanded time base. Hypoglossal nerve stimulus: 3.4 V (arrowheads) **(A,B)**.
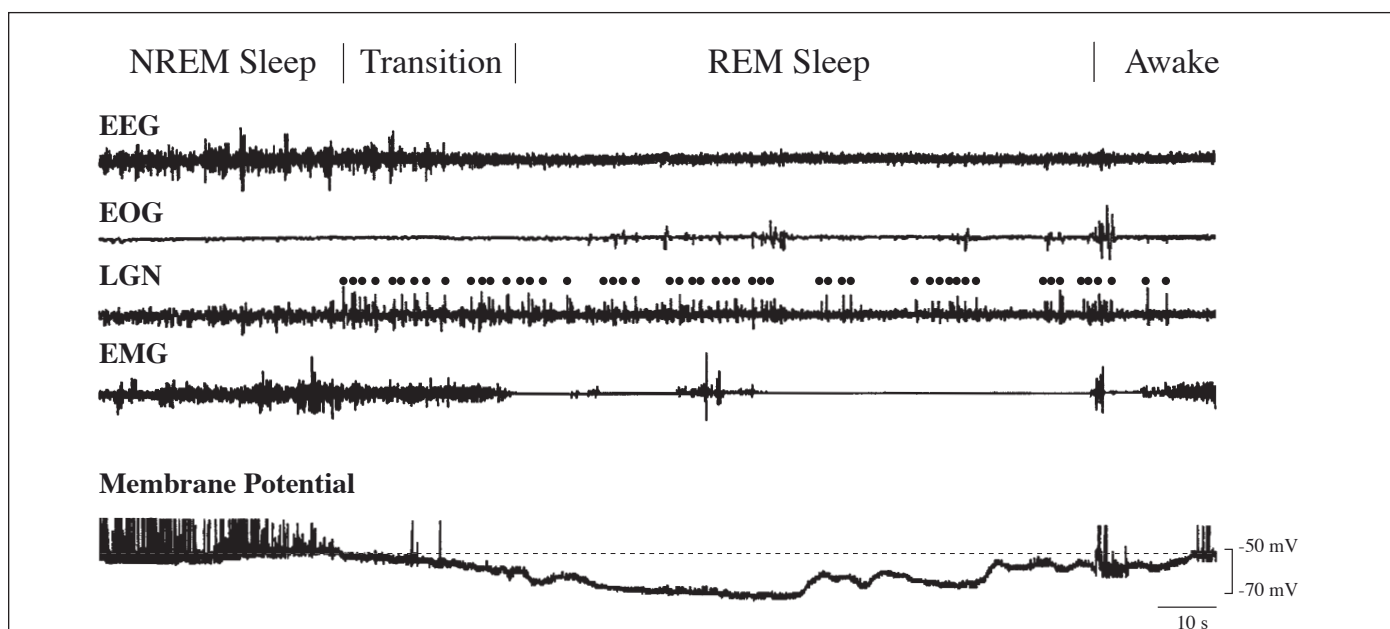
Figure 2—Hypoglossal motoneuron hyperpolarization during the transition from nonrapid eye movement (NREM) to rapid eye movement (REM) sleep and throughout REM sleep. During the transition phase, only sporadic firing occurred. In contrast, during REM sleep, discharge ceased. The membrane potential (dashed line) returned to the same level as during NREM sleep when the animal awoke. Action potentials are truncated. PGO waves are indicated by dots above the LGN trace. EEG, electroencephalogram; EMG, electromyogram; EOG, electro-oculogram; LGN, lateral geniculate nucleus; PGO, pontogeniculo-occipital.

A. NREM Sleep

-56 mV

C

20 mV

1 s

B. REM Sleep

C

D

100 ms

20 mV

-60 mV

D

Figure 3—Example of the spontaneous discharge of a hypoglossal motoneuron during NREM sleep **(A)**. At the onset of REM sleep, hyperpolarization occurred and discharge ceased, except momentarily in conjunction with REM sleep related phasic events **(B)** 60 sec intervened between panels **A** and **B**. Selected segments of traces in **A** and **B** as marked by brackets **C** and **D** are illustrated in the inset at an expanded time sweep.

or exhibited sporadic discharge during the succeeding period of REM sleep (n = 3; Figure 3). Phasic discharge consisted of one or several spikes that occurred in conjunction with episodes of threshold depolarization that were superimposed on the tonic hyperpolarization that was present throughout REM sleep. Other phasic events (such as myoclonic twitches and eye movements) occurred in conjunction with the phasic periods of hypoglossal motoneuron depolarization. Of the seven cells that exhibited spontaneous discharges during NREM sleep, the mean firing frequency was 10.9 Hz (range = 7.5 to 15.6 Hz); three of these cells discharged intermittently during REM sleep at a mean frequency of 0.17 Hz (range = 0.02 to 0.26 Hz). The remaining five motoneurons (42%) did not exhibit spontaneous activity (spiking) throughout the entire period of NREM and REM sleep.

Prominent subthreshold membrane activity arose at the onset of the transition from NREM to REM sleep. Specifically, large-amplitude IPSPs (up to 2.5 mV), which began to appear during the transition phase, persisted throughout REM sleep (Figure 4B). The frequency of these IPSPs was 38.9 Hz and 46.5 Hz for the representative motoneurons shown in Figure 4B and 4C, respectively. REM sleep-specific IPSPs were present in eight motoneurons with membrane potentials that ranged from -60 to -69 mV (Figure 4B). The four additional motoneurons
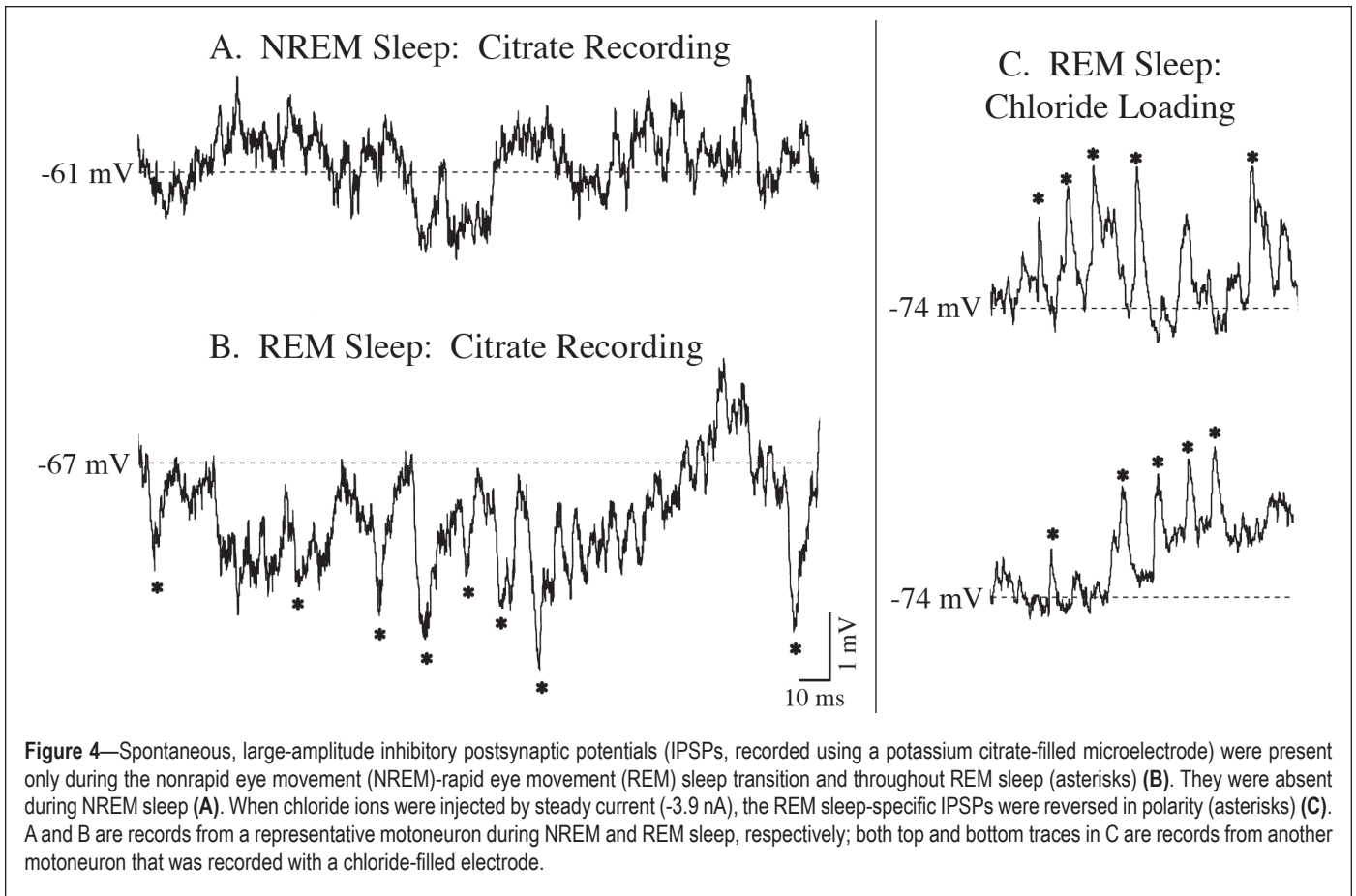
(whose membrane potentials ranged from -73 to -74 mV) that were recorded during REM sleep were subjected to chloride ion loading; in these cells polarity of the REM sleep-specific IPSPs was reversed (Figure 4C). The three motoneurons that did not exhibit IPSPs had membrane potentials that ranged from -71 to -72 mV, which is near or at the reversal potential of chloride ions. In contrast to REM sleep, IPSPs did not occur during NREM (Figure 4A) or wakefulness (not shown) in spite of the relatively depolarized level of the membrane potential during these states (see Discussion).

The rheobase current of six motoneurons during NREM sleep and another three during REM sleep was quantified to determine this direct measure of the intrinsic membrane excitability of hypoglossal motoneuron during these states. Figure 5 presents examples of the marked increase in rheobase during REM sleep (2 nA, Figure 5B) compared to that recorded during NREM sleep (0.3 nA, Figure 5A). The mean rheobase was 0.65 ± 0.20 nA (range = 0.3–1.5 nA, n = 6) during NREM sleep, compared to 2.40 ± 0.89 nA (range = 1.1–4.1 nA, n = 3) recorded during REM sleep. This difference in rheobase between NREM and REM sleep was statistically significant (P = 0.03, degree of freedom = 7, t-value = 2.68, n = 9).
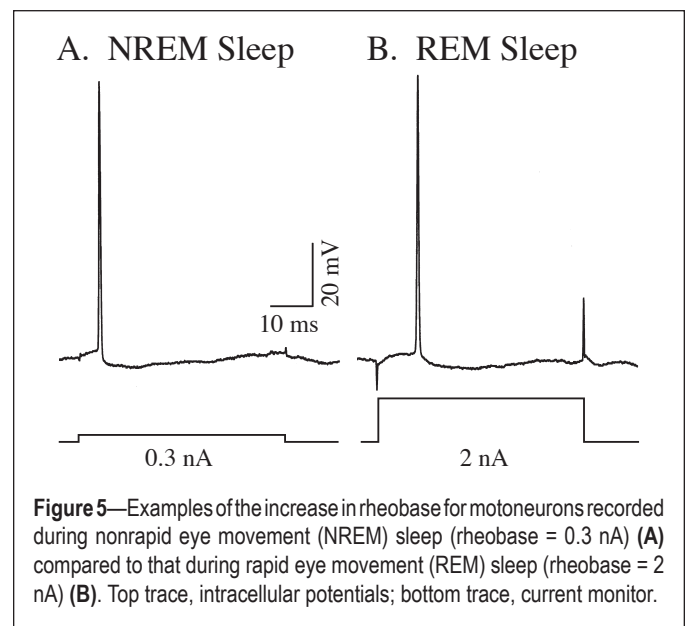
DISCUSSION

This is the first description of the spontaneous changes in the membrane potential activity of hypoglossal motoneurons, which were documented by recording intracellularly during naturally occurring states of sleep and wakefulness. In addition, a coherent set of intracellularly documented state-dependent changes in subthreshold and suprathreshold patterns of hypoglossal motor control is presented. The data reveal that the normal state-dependent mechanisms that control hypoglossal motor activity during NREM and REM sleep are comparable to those that modulate the membrane potential of other brainstem as well as spinal cord motoneurons.[10,19,20] Specifically, we postulate that during REM sleep, hypoglossal and other motoneurons are subjected to glycinergic postsynaptic inhibition, which constitutes the crucial mechanism that is responsible for atonia of the muscles that are innervated by these motoneurons (discussed in the next paragraphs). We are not aware of any previous intracellular studies (in any species) that have examined the synaptic control of hypoglossal motoneurons during sleep and wakefulness. All other intracellular experiments dealing with hypoglossal motoneurons have used current-clamp or whole-cell voltage-clamp techniques under acute conditions, i.e., either in anesthetized or decerebrate animals[12,21–28] or have been conducted in slice preparations.[2,29,30]

In the current experiment, we examined the following intracellularly recorded properties of the membrane potential of hypoglossal neurons: membrane polarization, frequency of discharge, rheobase, and postsynaptic potentials. Determinations were made of the changes in these membrane potential parameters that were temporally and functionally correlated with variations in the animals' state of sleep and wakefulness. We determined that hypoglossal motoneurons exhibit a significant increase in hyperpolarization during REM sleep (10.5 mV) based on their membrane potential level during NREM. The degree of hyperpolarization during REM sleep compared to that present during NREM sleep was similar to that reported

A. NREM Sleep: Citrate Recording

-61 mV

B. REM Sleep: Citrate Recording

-67 mV

C. REM Sleep: Chloride Loading

-74 mV

-74 mV

1 mV

10 ms

Figure 4—Spontaneous, large-amplitude inhibitory postsynaptic potentials (IPSPs, recorded using a potassium citrate-filled microelectrode) were present only during the nonrapid eye movement (NREM)-rapid eye movement (REM) sleep transition and throughout REM sleep (asterisks) **(B)**. They were absent during NREM sleep **(A)**. When chloride ions were injected by steady current (-3.9 nA), the REM sleep-specific IPSPs were reversed in polarity (asterisks) **(C)**. A and B are records from a representative motoneuron during NREM and REM sleep, respectively; both top and bottom traces in C are records from another motoneuron that was recorded with a chloride-filled electrode.

for digastric (11 mV),[19] trigeminal (approximately 10 mV),[10,31] and spinal motoneurons (8–9 mV)[20,32,33] in comparable experimental paradigms during naturally occurring NREM and REM sleep.

The cessation of spontaneous discharge during the transition from NREM to REM sleep was anticipated because hyperpolarization shifted the membrane potential further from its "threshold" voltage and, in addition, there was a potent current shunt generated by IPSPs that were present during this state.[12,27,34] Our determination of state-related changes in hypoglossal motoneuron discharge mirrors the pattern of activity that has been reported for EMG activity of the genioglossus muscle in humans,[5] except for the fact that there was no evidence of respiratory-related activity of hypoglossal motoneurons in this and another study.[35] Hypoglossal motoneurons are the final common pathway for execution of the volitional and reflexive motor function that are task dependent according to specific conditions of behavioral states.[36] In addition, factors such as posture,[37] vestibular stimuli,[38] vagotomy,[8] and metabolic stress (hypoxia/hypercapnia)[21] also influence the degree to which individual motoneurons participate in respiratory and/or nonrespiratory functions.[36] The spontaneous discharge recorded in seven of 12 hypoglossal motoneurons (58%) during NREM sleep is consistent with another intracellular study that reported that 53% of the hypoglossal motoneurons that were recorded were nonrespiratory modulated.[25] Our data are also in agreement with human findings that a majority (38 of 51, or 75%) of motor units of the genioglossus muscle exhibit tonic discharges that are nonrespiratory related during NREM

A. NREM Sleep   **B. REM Sleep**

20 mV

10 ms

0.3 nA   2 nA

Figure 5—Examples of the increase in rheobase for motoneurons recorded during nonrapid eye movement (NREM) sleep (rheobase = 0.3 nA) **(A)** compared to that during rapid eye movement (REM) sleep (rheobase = 2 nA) **(B)**. Top trace, intracellular potentials; bottom trace, current monitor.

sleep.[39] Furthermore, spontaneous discharge, as illustrated in Figure 3, was different from the patterns of respiratory-modulated discharge described in animals[28] and humans.[39]

In conjunction with membrane hyperpolarization, a significant increase in the rheobase of hypoglossal motoneurons during REM sleep was observed in the current report as well as in studies of spinal motoneurons.[32,33] It is evident that both hyperpolarization and the current shunt produced by the REM

sleep-specific IPSPs contributed to the suppression in excitability and discharge of hypoglossal motoneurons during REM sleep, as demonstrated by our determination of the cells' rheobase. Because rheobase is a direct measure of membrane excitability,[16] the cessation of tonic motoneuron discharge during REM sleep establishes the mechanistic basis for atonia of the lingual musculature and hence collapsibility of the upper airway that occurs during REM sleep, which is a critical factor in the production of airway obstruction in humans that results in OSA.[4]

The presence of IPSPs that we observed during REM sleep is additional direct evidence that an active inhibitory process is responsible for the suppression of motoneuron discharge during this state.[18] The chloride channel-dependent nature of the REM sleep-specific IPSPs in hypoglossal motoneurons corresponds with findings that similar REM sleep-specific IPSPs bombard trigeminal, digastric, and spinal motoneurons during REM sleep.[19,40,41] These chloride-dependent IPSPs have been demonstrated to be mediated by strychnine-sensitive glycine receptors.[20,27,40] In humans with OSA, strychnine treatment is relatively efficacious in eliminating apnea, which supports the critical role of glycine receptor-mediated active inhibition during REM sleep.[42] Functional anatomical (c-fos) studies have also demonstrated that glycinergic premotor interneurons that innervate hypoglossal motoneurons are activated during REM sleep, which provides an anatomical basis (i.e., source) for the inhibitory glycinergic inputs (IPSPs) to hypoglossal motorneurons.[43] Note that REM sleep-specific IPSPs were not present during NREM sleep or wakefulness. If they had been present during these states, they would have been readily detected because the membrane potential during wakefulness and NREM sleep was more depolarized (-52 to -64 mV) than during REM sleep and the equilibrium potential for chloride is approximately -70 mV in motoneurons as calculated by the Nernst equation.[44]

Because chloride-dependent IPSPs could be activated by both glycine and gamma-aminobutyric acid A ($GABA_A$) receptors,[45] it is possible both these receptors are involved in mediating the REM sleep-specific IPSPs. *In vitro* voltage clamp studies have shown that both glycine- and $GABA_A$-receptor mediated miniature inhibitory postsynaptic currents (mIPSCs) are present in hypoglossal motoneurons,[30] although the likelihood of their being coreleased is challenged by another study that demonstrates differences between these two mIPSPs on the basis of their rise time, decay time, and their sensitivity to specific blockers.[29] This study demonstrated that the chloride-mediated mIPSCs, as recorded in hypoglossal motoneurons *in vitro*, are mainly dependent on the activation of glycine rather than $GABA_A$ receptors. In addition, REM sleep-specific IPSPs (that appear to be identical to those we observed in this study) that are recorded from spinal motoneurons have been shown to be completely blocked by the glycine antagonist, strychnine, not by $GABA_A$ antagonists (picrotoxin or bicuculline).[20] A similar complete blockade of strychnine-sensitive REM sleep-specific IPSPs that produce the carbachol-induced REM sleep atonia has also been documented in hypoglossal and masseter motoneurons.[27,40]

The findings that hypoglossal motoneuron hyperpolarization, an increase in rheobase, cessation of discharge, and the presence of IPSPs are phase-locked to REM sleep, and are not present during NREM sleep or wakefulness, confirm that postsynaptic inhibitory mechanisms are responsible for the suppression of genioglossal muscle activity during REM sleep. Therefore, postsynaptic (active) inhibition is the essential mechanism that underlies genioglossal muscle atonia during REM sleep. The current findings are in accordance with recent reports of a REM sleep related decrease in the antidromic field potential of hypoglossal nucleus in intact, unanesthetized animals.[9,35] Furthermore, previous findings that glycinergic postsynaptic processes and a reduction in the excitability of hypoglossal motoneurons occur during pharmacologically (carbachol) induced REM sleep-like state in anesthetized cats.[12,27]

With regard to the circuitry of motor inhibition during REM sleep, it has been shown that there are REM-generator neurons in the pons that are responsible for producing REM sleep. These neurons, which selectively discharge during REM sleep, have also been shown to activate glycinergic premotor cells in the nucleus reticularis gigantocellularis and nucleus magnocellularis of the ventromedial reticular formation.[1] These glycinergic premotor neurons project monosynaptically to motoneurons in the brainstem and spinal cord,[1] wherein they produce potent postsynaptic inhibitory processes, as observed in this and other studies.[10,19,31–34,41] Functionally, the inhibition of motoneurons at all levels of the neuraxis during REM sleep has been suggested to be a beneficial survival adaption that prevents animals from performing motor acts when they are essentially blind and are unaware of potentially harmful stimuli or environmental situations.[1]

It has been proposed that a multitude of synaptic mechanisms may contribute to REM sleep atonia of the genioglossus and other motoneurons.[2,46–49] These include (1) postsynaptic inhibition as observed in this and previous studies,[1,10,12,20,27,49] (2) disfacilitation caused by the withdrawal of premotor, aminergic inputs,[7,50] and (3) presynaptic and postsynaptic inhibition that is mediated by muscarinic receptors.[6,51] Microinjections of antagonists of norepinephrine (prazosin) and serotonin (methysergide) alone or in combination into the hypoglossal nucleus in anesthetized rats have shown that the withdrawal of both aminergic excitatory drives participate in the carbachol-induced depression of extracellularly recorded hypoglossal nerve activity.[50] It is of note that the animals used in the latter studies were bilaterally vagotomized and anesthetized, and REM sleep was induced by carbachol. These procedures increase aminergic excitatory drives, which resulted in an increase in hypoglossal motoneuron activity.[8] We believe that aminergic disfacilitation is important when there is an elevated level of excitability of hypoglossal motoneurons, which occurs in response to metabolic stress stimuli such as hypoxia.[21] Therefore, aminergic disfacilitation is likely to be involved in mediating REM atonia, especially under pathophysiological conditions such as OSA.[1] However, there is electrophysiological evidence that is incompatible with a disfacilitation hypothesis of aminergic inputs during naturally occurring REM sleep. For instance, both norepinephrine and serotonin are known to reduce the amplitude of post-spike AHP of brainstem and spinal motoneurons[52–54] via a closure of potassium channels.[55] However, the expected effect of a withdrawal of serotonin and/or norepinephrine on the AHP of action potentials is opposite to the observed decrease in AHP of motoneurons during this state.[12,20,27] In addition, there is no

effect of serotonin blockade on the genioglossal EMG activity across the sleep-waking states under normoxic conditions; serotonin's excitatory action on genioglossal EMG becomes apparent only under hypercapnic condition.[8] Finally, clinical studies have demonstrated that serotonergic enhancement strategy is ineffective in reducing the apnea-hypopnea index of patients with OSA during REM sleep.[56]

Limitations

Generally, good-quality intracellular records can be obtained from a limited number of positively identified motoneurons per animal. However, we have shown that a sample size similar to that used in the current study is sufficient to produce meaningful data: we have also demonstrated, statistically, the presence of significant differences between study parameters. In addition, the number of neurons recorded was comparable to the number studied in similar intracellular experiments dealing with other brainstem and spinal cord motoneurons.[20,31,32] We also believe that from a functional perspective there is no sampling problem. For example, despite the fact that the hypoglossal nucleus is composed of motoneurons that innervate the intrinsic and extrinsic lingual muscles,[57] bilateral stimulation of the hypoglossal nerve produces protrusion of the tongue and hence increases upper airway patency.[58] Similarly, coactivation of tongue protrudor and retractor muscles or independent activation of protrudor muscles improves upper airway flow mechanics.[59] In humans with OSA, hypoglossal nerve stimulation has been shown to increase the inspiratory airflow during sleep.[60]

CONCLUSION

Based on electrophysiological and functional neuroanatomical studies from our laboratory and other reports, active postsynaptic inhibitory glycinergic drives are confirmed to play a direct role in producing the suppression of hypoglossal motoneuron discharge and atonia of genioglossal muscle that occurs during REM sleep. Disfacilitatory drives may arise under pathological conditions such as OSA.[1]

ABBREVIATIONS

AHP, after-hyperpolarizing potential
c-fos, a proto-oncogene
EEG, electroencephalogram
EMG, electromyogram
EOG, electro-oculogram
IPSP, inhibitory postsynaptic potential
K-citrate, potassium citrate
KCl, potassium chloride
LGN, lateral geniculate nucleus
NREM, non-rapid eye movement
OSA, obstructive sleep apnea
PGO, ponto-geniculo-occipital
REM, rapid eye movement

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DISCLOSURE STATEMENT

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