Mechanisms of upper airway hypotonia

The onset of sleep is associated with a reduction in upper airway patency and an increase in resistance, an effect observed in normal humans and animals, and typically present in snorers and patients with obstructive sleep apnea (OSA)/hypopnea syndrome (1–5). Patients with OSA commonly have structural abnormalities that result in a narrowed upper airway and collapsible pharyngeal walls. During sleep, the airway cross-sectional area decreases considerably and the patients experience repeated, clinically significant, obstructive apneas or hypopneas. However, when OSA patients are awake, their airway remains patent (except during swallowing, speech, etc.). This sleep–wake–state dependence of the disorder points to the involvement of neural mechanisms. The marked increase in resistance is attributed to a sleep-related alteration in the neural control of upper airway striated muscles. In particular, decrements in the activity of upper airway dilator muscles, that is, those that counteract the collapsing force of the negative pressure generated in the airway during inspiration, play a permissive role in sleep-related airway obstructions.

Keywords

acetylcholine, acute effects, adherence, adipokines, American Academy of Sleep Medicine, apnea-hypopnea index, auto-CPAP testing, awakening, bariatric
INTRODUCTION
The onset of sleep is associated with a reduction in upper airway patency and an increase in resistance, an effect observed in normal humans and animals, and typically present in snorers and patients with obstructive sleep apnea (OSA)/hypopnea syndrome (1–5). Patients with OSA commonly have structural abnormalities that result in a narrowed upper airway and collapsible pharyngeal walls. During sleep, the airway cross-sectional area decreases considerably and the patients experience repeated, clinically significant, obstructive apneas or hypopneas. However, when OSA patients are awake, their airway remains patent (except during swallowing, speech, etc.). This sleep-wake–state dependence of the disorder points to the involvement of neural mechanisms. The marked increase in resistance is attributed to a sleep-related alteration in the neural control of upper airway striated muscles. In particular, decrements in the activity of upper airway dilator muscles, that is, those that counteract the collapsing force of the negative pressure generated in the airway during inspiration, play a permissive role in sleep-related airway obstructions.

The cross-sectional area and resistance of the upper airway are dynamic variables, being determined at any point in time by (i) the mechanical properties of the airway walls (chap. 1); (ii) mechanical events (pressure gradients and flows; see chap. 2); (iii) adhesive forces generated between the airway walls whenever they come in contact (6–9); and (iv) the pattern and magnitude of activity in upper airway muscles. The stiffness and size of the upper airway depend on both the level of upper airway muscle tone and airway position.

The neural and mechanical factors that determine upper airway patency are strongly interrelated (10,11). Consequently, the occurrence and time course of obstructive episodes cannot be uniquely predicted from the behavior of individual upper airway muscles during sleep. Electromyographic studies are informative, however, in that they help to link the mechanical and neural events, provide an indirect insight into the central mechanisms that control upper airway muscles, and allow one to compare the results from humans to those from experimental animals in which one can often test fundamental hypotheses in a manner not possible in human subjects. We start this chapter with the description of sleep-wake–cycle related alterations in the activity of upper airway muscles in normal humans, OSA patients, and experimental animals.

In addition to their respiratory function, upper airway muscles are involved in a number of other behaviors that are predominantly automatic, such as swallowing or coughing (12) or entirely voluntary (e.g., phonation). This multifunctionality is achieved through appropriate central neural connections that produce distinct patterns of upper airway muscle activity needed for different behaviors. At least four functionally distinct central neuronal systems control upper airway motoneurons: (i) those controlling centrally generated automatic behaviors (pattern generators) including breathing; (ii) those transmitting reflexes from peripheral receptors; (iii) those related to the level of sleep and arousal (state dependent); and (iv) those for volitional control. Distinct alterations occur in each of these systems in relation to sleep, with additional differences observed between slow-wave sleep (SWS) and rapid eye movement (REM) sleep. In the sections “State-Dependent Central Control of Upper Airway Motoneurons” and “State Dependence of Upper Airway Reflexes”, we discuss the effects of sleep on these distinct systems. Finally, in the section “Effects of Recurrent Disruptions of Sleep and Breathing on Upper Airway Motoneurons”, we consider selected mechanisms by which sleep-disordered breathing may cause long-term changes in the central neural control of upper airway muscles.

The effects of sleep on the upper airway have been extensively reviewed; here, we emphasize only the selected aspects of this broad subject. For further reading, we recommend...
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the earlier version of this chapter (13) and the following reviews that cover related and complementary topics (14–22).

EFFECTS OF SLEEP ON UPPER AIRWAY MUSCLES
Mechanistic Background
Upper airway obstructions in OSA patients occur most commonly in the pharynx (1,23–25) (reviewed in Ref. 26). This is due to the lack of rigid structures supporting this segment of the airway. In the pharynx, three distinct soft tissues, the posterior pharyngeal wall, the soft palate, and the base of the tongue, are pulled inward and toward each other by the centripetal force generated by the negative pressure during inspiration (Fig. 1). As the base of the tongue and the posterior pharyngeal wall are drawn towards the airway lumen, the soft palate, like a wedge, moves to fill the remaining space. This reduces the airway cross-sectional area and facilitates airway obstruction. Opposing this effect are the muscles that can stiffen the pharyngeal airway and cause a centrifugal movement of the pharyngeal walls (dilate the airway). Some of the most important upper airway muscles exhibiting this function are shown in Figure 1, together with the prevailing directions of their actions. In addition, the muscles that pull the airway downward (e.g., sternohyoid and sternothyroid) protect against obstructions by increasing the distance between the soft palate and the base of the tongue (27–29).

The nose and larynx, although not typical sites of airway obstruction, are important determinants of the magnitude of the negative pressure generated in the pharynx because they

Figure 1
Sagittal cross-section of the upper airway rendered from a scan of the upper airway of an obstructive sleep apnea patient (courtesy of Dr. Richard J. Schwab). The scheme emphasizes that three soft tissue elements, the tongue, the posterior pharyngeal walls, and the soft palate are pulled toward each other by intraluminal negative pressure. Dashed arrows show approximate directions and sites of attachment of the forces exerted by major muscles that counteract this negative pressure and, therefore, act as pharyngeal dilators. Note that the pharyngeal constrictors stiffen the posterior pharyngeal wall and their dilative or constrictive action is position dependent.
act as pressure dividers between the nasal, pharyngeal, and laryngeal compartments. Nasal resistance is about half the total airway resistance (30). Since the walls of the nasal passages are relatively noncompliant, the neurally generated changes in nasal resistance are small (31). Instead, various nonneural factors (e.g., mucosal congestion) may have a large impact on this component of airway resistance (32). An increase in nasal resistance increases the magnitude of the negative inspiratory pressure in the pharynx. This favors a collapse of the pharyngeal walls and may lead to airway obstruction. While opening the mouth may terminate an obstruction by relieving the negative pressure in the pharynx (33), it also allows the base of the tongue to come closer to posterior pharyngeal walls, thereby increasing airway resistance (34).

In contrast to the nasal airway, dynamic changes in neuromuscular activity in the larynx may have large effects on upper airway resistance (35), and can increase or decrease the magnitude of the negative pressure in the pharyngeal portion of the airway. However, active laryngoconstriction is not a part of the clinical picture in OSA. Rather, the larynx and the nose are important sources of afferent information for the reflex control of upper airway muscles. This is because the afferent information from receptors located in the nose and larynx has more prominent reflex effects on upper airway muscle activity than that from pharyngeal receptors (section “State Dependence of Upper Airway Reflexes”). Thus, the airway compartment where obstruction is most likely to occur is flanked by less compliant compartments that provide sensory information important for the reflex control of upper airway muscle tone.

Sleep-Related Changes in the Activity of Individual Upper Airway Muscles in Healthy Subjects

Table 1 lists 24 upper airway muscles, from the nares through the pharynx and larynx, which contribute to the control of airway patency. For each muscle, the table provides the origin of its motor innervation, the predominant action on airway patency, and the relative levels and patterns of activity during quiet wakefulness, SWS, and REM sleep. Most commonly, the sleep-related changes in the tone of individual upper airway muscles are described relative to the average tone observed during a period of quiet wakefulness preceding sleep. In normal subjects, this approach is limited by the often highly variable activity of some muscles during wakefulness and/or a very low level of baseline activity during quiet waking (33,38,42,72). In humans, to quantify upper airway electromyographic (EMG) signals, the level of activity during different states of sleep and wakefulness is also often expressed relative to the amount of activity generated during a maximal voluntary activation (39,43). Similarly, in experimental animals, sleep–wake changes of upper airway muscle tone are often quantified relative to the level of activity during quiet wakefulness prior to sleep onset (56), or relatively to the average level of activity recorded over long periods of undisturbed wakefulness (57). Theoretically, all these approaches are suitable for quantitative comparisons of the levels of muscle activity across behavioral states and among subjects. However, replication and comparison of data from different laboratories are hampered by the lack of well-established approaches to the selection of the data segments used for analysis, widely varying methods of EMG signal acquisition and processing, and limited recognition of the need to properly subtract electrical noise from genuine EMG. Particularly, the latter may cause large errors and variability when upper airway muscle activity is very low (e.g., in normal subjects during SWS). In addition, when high amplifier gains are used to compensate for low level of baseline activity, saturation of the signal will often occur during large phasic burst of activity. Saturated records are then excluded from analysis, which, in turn, biases EMG quantification and precludes quantitative comparisons among conditions, subjects, and laboratory settings. Thus, there is a need for standardization of the methods of EMG quantification.

A study with recording from lingual muscles in rats in which these confounding technical issues were minimized revealed that lingual EMG was minimal or absent during SWS and highly phasic during both active wakefulness and REM sleep. The phasic bursts of activity in REM sleep steadily increased with the duration of the state, with their amplitudes becoming as
Table 1  Sleep-Related Changes in the Average Level of Activity in Upper Airway Muscles in Normal Subjects

<table>
<thead>
<tr>
<th>Muscle Name</th>
<th>Presumed Action on Upper Airway; (bold = dilating action)</th>
<th>Nucleus Providing Motor Innervation</th>
<th>Pattern of Waking Activity</th>
<th>Change During SWS</th>
<th>Change During REM sleep</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alae nasi</td>
<td>Widens nares</td>
<td>VII</td>
<td>I + T</td>
<td>↓↓ or ↓↓</td>
<td>↑↑ or ↓↓</td>
<td>h: (31,36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E + T</td>
<td>↓↓ or ↓↓</td>
<td>irregular</td>
<td>r: (37)</td>
</tr>
<tr>
<td>Tensor veli palatini</td>
<td>Moves soft palate up and posteriorly</td>
<td>V</td>
<td>T + I</td>
<td>↓↓ or ↓↓</td>
<td>↓↓</td>
<td>h: (5,38,42)</td>
</tr>
<tr>
<td>Levator veli palatini</td>
<td>Moves soft palate up</td>
<td>V</td>
<td>I + T or silent</td>
<td>↓</td>
<td>h: (40,41)</td>
<td></td>
</tr>
<tr>
<td>Palatopharyngeus</td>
<td>Moves soft palate down; pharynx up</td>
<td>XI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palatoglossus</td>
<td>Moves tongue up and posteriorly</td>
<td>XII</td>
<td>I + T</td>
<td>↓</td>
<td>h: (41)</td>
<td></td>
</tr>
<tr>
<td>Stylloglossus</td>
<td>Moves tongue up and posteriorly</td>
<td>XII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genioglossus</td>
<td>Moves tongue down and anteriorly</td>
<td>XII</td>
<td>I + T or variable</td>
<td>↓ or = or ↑</td>
<td>↓ or = or ↓↓ or ↑↓</td>
<td>h: (5,33,38,42-50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>↓↓ or ↑</td>
<td></td>
<td>c: (51,75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>↓</td>
<td>↓↓</td>
<td>d: (52,53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>↓↓ or =</td>
<td>↓↓</td>
<td>g: (54,55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I or I + T or variable</td>
<td>↓ or ↓↓</td>
<td>↓↓ or = or ↑↑</td>
<td>r: (56-61)</td>
</tr>
<tr>
<td>Hyoglossus</td>
<td>Moves tongue down and posteriorly</td>
<td>XII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stylopharyngeus</td>
<td>Moves up and widens pharynx</td>
<td>IX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngeal constrictors (eight muscles)</td>
<td>Stiffen posterior pharyngeal wall; reduce pharyngeal circumference; move hyoid bone posteriorly</td>
<td>N. ambiguus (vagal)</td>
<td>E or silent</td>
<td>↓ or ↓↓</td>
<td>silent or irregular</td>
<td>h: (39,40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>↓ or ↓↓</td>
<td>=</td>
<td>r: (62)</td>
</tr>
<tr>
<td>Digastric anterior</td>
<td>Moves hyoid bone anteriorly</td>
<td>VII</td>
<td>T</td>
<td>↓↓</td>
<td>c: (63)</td>
<td></td>
</tr>
<tr>
<td>Digastric posterior</td>
<td>Moves hyoid bone posteriorly and laterally</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Muscle Name</th>
<th>Presumed Action on Upper Airway; (bold = dilating action)</th>
<th>Nucleus Providing Motor Innervation</th>
<th>Pattern of Waking Activity</th>
<th>Change During SWS</th>
<th>Change During REM Sleep</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geniohyoid</td>
<td>Moves <strong>tongue down</strong>; hyoid bone up</td>
<td>XII</td>
<td>I + T</td>
<td>↓↓</td>
<td>↑ or =</td>
<td>h: (64,65)</td>
</tr>
<tr>
<td>Stylohyoid</td>
<td>Moves hyoid bone up and posteriorly</td>
<td>VII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mylohyoid</td>
<td>Stiffens floor of the mouth</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omohyoid superior (and Thyrohyoid)</td>
<td>Moves <strong>hyoid bone down</strong></td>
<td>C₁₋₃ via XII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sternohyoid</td>
<td>Moves <strong>hyoid bone down</strong></td>
<td>C₁₋₃ via XII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sternothyroid</td>
<td>Moves <strong>thyroid cartilage down</strong></td>
<td>C₁₋₃ via XII</td>
<td></td>
<td></td>
<td></td>
<td>r: (60)</td>
</tr>
<tr>
<td>Posterior cricoarytenoid</td>
<td><strong>Abducts vocal folds</strong></td>
<td>N. ambiguus (vagal)</td>
<td>I + post-I + T</td>
<td>↓</td>
<td></td>
<td>h: (67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I + T</td>
<td>↑</td>
<td></td>
<td>c: (51,68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I + T</td>
<td>=</td>
<td></td>
<td>l: (69)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I + T</td>
<td>=</td>
<td></td>
<td>r: (70,71)</td>
</tr>
<tr>
<td>Lateral cricoarytenoid</td>
<td><strong>Adducts vocal folds</strong></td>
<td>N. ambiguus (vagal)</td>
<td>E</td>
<td>↓↓</td>
<td></td>
<td>r: (70,71)</td>
</tr>
<tr>
<td>Arytenoid</td>
<td><strong>Adducts vocal folds</strong></td>
<td>N. ambiguus (vagal)</td>
<td>E or I/E + T</td>
<td>↓↓</td>
<td>silent or irregular</td>
<td>h: (72)</td>
</tr>
<tr>
<td>Thyroarytenoid</td>
<td><strong>Relaxes vocal folds</strong></td>
<td>N. ambiguus (vagal)</td>
<td>E + T</td>
<td>↓↓</td>
<td>silent or irregular</td>
<td>h: (73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I or silent</td>
<td>=</td>
<td></td>
<td>l: (69)</td>
</tr>
<tr>
<td>Cricothyroid</td>
<td><strong>Tenses vocal folds</strong></td>
<td>N. ambiguus (vagal)</td>
<td>I + T</td>
<td>↑</td>
<td></td>
<td>h: (74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I + T</td>
<td>=</td>
<td></td>
<td>l: (69)</td>
</tr>
</tbody>
</table>

For each muscle, if available, data from sleep studies in humans are listed first, followed by chronically instrumented animals (h, human; c, cat; d, dog; g, goat; l, lamb; r, rat). Changes are described qualitatively relative to the activity during quiet wakefulness. Symbols: = - no change; ↑ or ↓ - small increase or decrease; ↑↑ or ↓↓ - large increase or decrease; ¶ - phasic REM sleep with intense eye movements. Abbreviations: E, expiratory; I, inspiratory; I/E, phase-spanning; T, tonic.
high as during wakefulness by the end of some REM sleep episodes (57). Figure 2 shows the
average levels of lingual EMG 2 minutes before and after state transitions. Due to averaging
across multiple segments of records aligned by the time of state transition, the characteristic
phasic nature of activity during REM sleep is smoothed out, revealing a gradual increase in the
mean level of activity with the duration of the state. Quantitatively similar sleep–wake changes
occurred at different recording sites within the tongue regardless of whether the sites were
located near the base or near the tip of the organ, and inspiratory modulation of lingual EMG
was extremely rare at all recording sites (58). Although not previously quantified, similar non-
respiratory bursts were reported as characteristic of lingual EMG during REM sleep in healthy
humans (44), cats (75), and rats (56,59), and were observed in the arytenoideus muscle of
humans (72). Recordings from the diaphragm of cats hyperventilated to apnea during SWS also
show that phasic excitatory inputs of nonrespiratory origin impinge on phrenic motoneurons
during REM sleep, and that their intensity gradually increases with the duration of the state (76).

Some tonic activity and a degree of inspiratory modulation are frequently present in
EMG records from the genioglossus and other pharyngeal muscles during both quiet wakeful-
ness and SWS in humans (38,45,77). However, relative to maximal voluntary activation or non-
respiratory phasic bursts of activity, the baseline level of activity during quiet wakefulness in
healthy humans is very low (39,42,72). In rats and probably all other mammals, large phasic
bursts of lingual muscle activity occur mainly in association with ingestive behaviors, rather
than with respiratory rhythm (78). Measurable levels of inspiratory modulation of that activity
were reported at normocapnic levels in some studies with normal rats (e.g., Refs. 56,60,79), but
in other studies inspiratory modulation of lingual EMG was extremely rare and present only
intermittently (57,58). Similarly, phasic respiratory modulation of genioglossal EMG was absent
in wakefulness in all six normal children studied as a control group in one study (49). Sauer-
land and Harper (45) suggested that the magnitude of respiratory modulation of the tongue
EMG varies with the lingual recording site, but this was not the case when systematically
studied in normal rats (58).

Table 1 shows that the muscles that have airway dilatory function have been studied
more extensively than constrictors. Although many studies report a decrement in upper airway
muscle activity during SWS and a further decrement during at least some periods of REM
sleep, this has not been consistently observed in those muscles that have been evaluated in
many laboratories and experimental settings; for example, the genioglossus. No relationship
between the sleep–wake patterns of activity can be identified among different muscles when
they are grouped according to either the source of their motor innervations or the prevailing
pattern of respiratory modulation (inspiratory or expiratory). Inconsistencies may be related to
differences in the baseline levels of tonic and respiratory-modulated activities in different
studies. These levels depend, in part, on the route of breathing and subject position, and may
be affected by other technical aspects of the experimental protocol (e.g., the level of chemical
respiratory drive and mechanical loading).

Upper Airway Muscle Activity in OSA Subjects

OSA subjects have a higher level of activity in their upper airway dilator muscles during wake-
fulness than normal individuals (whose upper airway is fully patent in the absence of upper
airway muscle tone) (42,43,48,50,66,80–82). This compensatory increase is not fully maintained
during sleep in either humans or the English bulldog, a natural animal model of OSA (83).
Consequently, the absolute magnitude of the sleep-related decrease of upper airway muscle
activity at sleep onset and increase in airway resistance is higher in sleep apneics than in normal
subjects (42,43,49). Simultaneous recordings of upper airway muscle activity and airflow con-
sistently show that flow limitations and occlusions occur at the time when upper airway muscle
activity reaches its nadir (e.g., Refs. 1,84,85). Conversely, increasing the upper airway motor
tone by electrical stimulation improves airway patency and can prevent obstructions (86–91).
There also appear to be fewer discrepancies concerning the levels of upper airway motor tone
Figure 2  The average time course of lingual muscle activity during state transitions in the rat. The top panels show average levels of lingual EMG determined during successive 10-second intervals over 2 minutes before and 2 minutes after different state transitions, as indicated above the panels. The activity was normalized within each animal and recording session by its average level during wakefulness. The bottom panels show the corresponding average time course of the cortical delta power (increases during SWS) normalized by its average level during SWS. Lingual activity is nearly abolished following entry into SWS precluding observation of any significant depression at the beginning of rapid eye movement REM sleep (A1 and B1). Following the onset of REM sleep, lingual EMG gradually increases and within 2 minutes reaches a level close to that during wakefulness. As a result of this increase, awakening from REM sleep is associated with only a small change in lingual EMG (C1). Abbreviations: EMG, electromyogram; W, wakefulness; SWS, slow-wave sleep; REMS, rapid eye movement sleep. Source: Modified from Ref. 57.
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during SWS and REM sleep among the studies with OSA patients than among the studies with normal subjects. The most plausible explanation is that the elevated level of baseline activity in OSA patients during wakefulness enables observation of consistent decrements of activity during both SWS and REM sleep. Similarly, in experimental animals with noncollapsible airways, upper airway muscle tone consistently decreases at SWS onset and then further declines at REM sleep onset when the baseline tone is experimentally increased by various means, including increased chemical drive for breathing (54,55–79), direct infusions of excitatory drugs into the vicinity of upper airway motoneurons (61,92), or vagotomy (92,93). In contrast, with no tone-enhancing interventions, upper airway muscle tone is often entirely abolished in normal subjects during SWS and no further decrements (48,49,56), or even increases (57,58,66), are observed during subsequent REM sleep. However, contrary to this scheme, a recent study reported significant decrements of genioglossal EMG at REM sleep onset that were of similar magnitude in OSA patients and control subjects, both studied while continuous positive airway pressure (CPAP) was applied to minimize the difference in passive upper airway resistance between the groups (50). The magnitude of these decrements was rather small, less than 1% of the maximal activity recorded during voluntary tongue protrusion or swallow, suggesting that highly standardized experimental conditions need to be used to detect changes in upper airway muscle tone at REM sleep onset in subjects with a fully patent upper airway.

Thus, the levels of upper airway muscle activity in wakefulness and the pattern of changes in upper airway motor tone across the sleep–wake cycle differ between healthy subjects and those with a compromised upper airway. While in the former, activity is often abolished during SWS, in the latter the increased level of activity in wakefulness is at least partially carried over into SWS and a nadir occurs during REM sleep. These differences are schematically depicted in Figure 3A, and exemplified by data from control dogs and English bulldogs.

![Figure 3](image)

**Figure 3** The pattern of pharyngeal dilator muscle activity across the sleep–wake cycle is different in healthy subjects and obstructive sleep apnea (OSA) patients with anatomically narrow upper airways that are predisposed to collapse. (A) The bars schematically represent the average levels of total activity present in a pharyngeal dilator (such as the genioglossus) during quiet wakefulness, slow-wave sleep (SWS), and rapid eye movement (REM) sleep in OSA patients (dark bars) and normal subjects (light bars). The scale adopted in this scheme is based on reports that the level of upper airway dilator tone during wakefulness is 3–4 times higher in OSA subjects than in normals (43,66), and that OSA patients have the lowest upper airway dilating tone during REM sleep, whereas healthy subjects often exhibit a nadir during SWS (57,66,48). The pattern also assumes that OSA is associated with a suppression of the large phasic bursts of activity that occur during REM sleep in normal subjects and experimental animals (44,57,58). (B) Data supportive of the pattern presented in A derived from recordings of the sternohyoid muscle activity in English bulldogs with a compromised upper airway and normal dogs. Source: Plotted based on numeric data from Ref. 66.
with anatomically compromised upper airway (66) (Fig. 3B). Interestingly, the nonrespiratory bursts of activity reported in some studies of REM sleep in normal human subjects (44,72) are either absent or excluded from analysis in studies of OSA subjects. Absence of such bursts would suggest that, in OSA, the need to maintain a steady airway-dilating tone leads to a suppression of the central sources responsible for the phasic bursts of activity during REM sleep. This possibility is incorporated in the scheme presented in Figure 3A, but the presence of this effect remains hypothetical.

The neural basis of the increased upper airway muscle tone in wakefulness in OSA subjects is unknown. According to one hypothesis, it is caused by a reflex mechanism whereby airway mechanoreceptors sensitive to negative pressure are more strongly stimulated in an anatomically narrow airway and provide a stronger reflex excitation to airway dilator motoneurons (42,43) (upper airway reflexes are discussed in the section “State Dependence of Upper Airway Reflexes”). The observation that positive pressure applied to the upper airway results in a larger drop in upper airway muscle activity in OSA patients than in normal subjects has been interpreted as evidence that the former have either a higher level of activity in upper airway receptors sensitive to negative pressure or a potentiated transmission along the reflex pathway from these receptors to airway dilator motoneurons (42,43). However, direct comparisons of the gain of upper airway reflex responses to negative airway pressure revealed no significant differences between OSA patients and control subjects (93,94). Hyperventilation also causes a larger increase in upper airway resistance in OSA patients than in normal subjects (32,95). This may be taken to suggest that OSA patients have enhanced transmission of chemical respiratory drive to upper airway motoneurons. One alternative, or complementary, explanation of the increased level of activity in upper airway muscles in wakefulness in OSA patients would be that the apparently stronger reflex effects are a result of central changes that increase the excitability of the motoneurons that innervate upper airway dilators. In support of this, rats exposed to chronic intermittent hypoxia, a model of recurrent nocturnal hypoxic episodes experienced by OSA patients, have increased density of noradrenergic terminals and increased expression of the excitatory α1-adrenergic receptors in the XII motor nucleus (96). As discussed in the section “Norepinephrine”, norepinephrine is a major source of a wake-related drive to upper airway motoneurons. Another explanation of the increased upper airway muscle activity during wakefulness in OSA patients would be that the perception of upper airway resistance triggers the increase. These latter mechanisms would, by definition, not function during sleep, thus contributing to the larger sleep-related decreases in upper airway motor tone in OSA patients than in normal subjects.

Sleep-Like Effects on Upper Airway Motor Tone in Reduced Animal Models
Interpretation of the mechanisms underlying EMG changes with the natural sleep–wake cycle in instrumented humans and animals is complicated by a host of feedback loops provided by mechano- and chemoreceptor reflexes that act to maintain ventilation at a level that meets metabolic demands, and the neuromechanical interactions between the respiratory pump and upper airway muscles mediated by mechanical events in the airway. Dissociation between reflexes and central effects of behavioral states on upper airway activity can be achieved in animals with neuromuscular blockade and artificial ventilation at constant parameters. Such an approach has been used to study the central neural effects of REM sleep on upper airway motoneurons because REM sleep-like neural phenomena can be triggered in intact, decerebrate, or anesthetized experimental animals by microinjections of a cholinergic agonist, carbachol, into a discrete region of the dorsomedial pontine reticular formation (92,93,97–101). In particular, the REM sleep-like effects that can be repeatedly elicited by carbachol in anesthetized rats exhibit a remarkable similarity to episodes of natural REM sleep, as evidenced by simultaneous recordings from central respiratory neurons, respiratory motoneurons, cortex, and hippocampus (100,101). Studies with carbachol models have led to fundamental new findings about the neurochemical basis of REM sleep influences on upper airway motoneurons (section “Tonic, State-Dependent Inputs”).
Since the baseline activity of most upper airway muscles is low and erratic at normocapnic levels in normal humans and intact animals, an elevated end-expiratory CO$_2$ level and/or vagotomy (which releases upper airway motoneurons from tonic vagal inhibition) are often used in acute animal experiments. This, in combination with neuromuscular paralysis and artificial ventilation, creates conditions in which reflex influences on respiratory output are minimized and kept constant, while the baseline level of activity in nerves innervating upper airway muscles is elevated. The latter is similar to the conditions in subjects with a compromised upper airway and facilitates observation of the suppressant effects of REM sleep on motoneuronal activity.

Studies with the carbachol model in the decerebrate cat demonstrate that the magnitude of the suppressant effects activated by cholinergic stimulation in the pons varies greatly among different pools of upper airway motoneurons (93,102). As shown in Figure 4, the activity of XII inspiratory-modulated motoneurons that innervate the genioglossus and expiratory-modulated vagal motoneurons that innervate the pharyngeal constrictors is almost abolished (depressed

![Figure 4](https://example.com/figure4.png)

**Figure 4** Stereotyped pattern of suppression of activity in respiratory motoneurons during the rapid eye movement (REM) sleep-like atonia produced by a pontine injection of carbachol. (A) Continuous recording of moving averages of phrenic (PHR), recurrent laryngeal (RL), hypoglossal (XII), vagal pharyngeal (Phar) nerves, and blood pressure (BP) during pontine microinjection of carbachol and subsequent reversal of the effect by pontine injection of atropine (injections at arrows). Note the small change in the magnitude of PHR and RL nerve activities and the strong decrements of the activities in the nerves innervating pharyngeal muscles (XII and Phar). (B) Mean data from 10 to 18 experiments, with the levels of activity in individual nerves expressed as a percentage of those prior to carbachol injections (dashed line). The inspiratory and expiratory components of RL nerve activity (RL$_i$ and RL$_e$) are only moderately depressed in contrast to the large depression of the inspiratory component of XII and the expiratory component of Phar activity (Phar$_e$). The effects are purely central because the experiments were conducted on paralyzed, vagotomized, and artificially ventilated, decerebrate cats. In such experiments, one can observe a depression of phrenic nerve activity that, due to chemical feedbacks, does not occur in spontaneously breathing animals. Source: Modified from Ref. 102.
to 10–15% of the control level), whereas that of inspiratory and postinspiratory laryngeal motoneurons is suppressed much less (to 75–80% of the control level). For laryngeal motoneurons, this result is compatible with that of the EMG studies of the cricothyroid muscle in chronically instrumented intact cats (68). The strong suppression of XII motoneurons is similar to the changes with sleep in electromyographic activity in naturally sleeping rats and goats in which the baseline genioglossal activity was increased by hypercapnia or hypoxia (54,55,79). The suppression of vagal pharyngeal motoneurons is similar to that observed in pharyngeal constrictors in OSA patients (82). An important conclusion from carbachol studies in decerebrate cats, in which compensatory reflexes are eliminated, is that there is a centrally determined differential pattern of the suppressant effects of REM sleep on the activity of different pools of upper airway motoneurons.

STATE-DEPENDENT CENTRAL CONTROL OF UPPER AIRWAY MOTONEURONS

The activity of upper airway motoneurons is the net result of excitatory and inhibitory inputs (drives) that are synaptically transmitted to motoneurons from various premotor sources. Two components are often distinguished in the baseline activity of individual upper airway muscles: (i) a tonic one that is present continuously throughout the respiratory cycle and (ii) a phasic one that is bound to a particular portion of the respiratory cycle, such as inspiration, the postinspiratory period, or the late expiratory period (Fig. 5A). In different muscles, the tonic and phasic components are expressed to various degrees, and with a pattern characteristic of that muscle. For example, the tensor veli palatini and digastricus muscles show only tonic activity, if any, under normocapnic conditions in wakefulness, but respiratory modulation appears when the respiratory drive is increased by hypercapnia or airway occlusion (38,42). In the genioglossus of healthy humans, the phasic inspiratory and tonic components together represent less than 2% of a maximal spontaneous activity (49), or 1–11% of the peak activity generated during a maximal voluntary tongue protrusion effort (43,103). Nevertheless, the presence of tonic and phasic components demonstrates that XII motoneurons of healthy humans receive inputs from both respiratory-modulated and tonic central neurons under unstimulated baseline conditions.

The mechanisms responsible for the inspiratory modulation of activity are different in motoneurons that innervate upper airway and respiratory pump muscles. In phrenic and intercostal motoneurons (as well as most central respiratory neurons, and expiratory-modulated orofacial motoneurons), respiratory modulation results from a combination of excitatory inputs arriving to motoneurons during their active phase of the respiratory cycle and inhibitory inputs during their inactive phase (104,105). In contrast, in inspiratory-modulated laryngeal (106), facial (107), and XII (108) motoneurons, there is little or no phasic inhibition during expiration. This lack of expiratory inhibition in inspiratory-modulated upper airway motoneurons allows upper airway motoneurons to respond to a variety of nonrespiratory inputs and is probably related to their primary involvement in various nonrespiratory orofacial behaviors and their only accessory function in ventilatory control.

In addition to the central respiratory and tonic drives, upper airway motoneurons are under reflex control, with reflexes from central and peripheral chemoreceptors and mechanoreceptors of the respiratory tract being particularly important (21,22,109). These reflex drives are principally tonic, but the phasic nature of the mechanical events in the respiratory system and the transmission of these afferent inputs through central respiratory neurons result in respiratory modulation of reflexes. Thus, central phasic respiratory, central tonic and reflex drives represent three functionally distinct inputs to upper airway motoneurons (Fig. 5A); these inputs may be affected differently by changes in sleep–wake states. The relative contribution of each of these three drives to the membrane potential (subthreshold excitability changes) and activity (once the firing threshold is reached) probably varies among motoneurons innervating different upper airway muscles. Panels B–D of Figure 5 show, schematically, three cases in which, due to different basal levels of the distinct drives impinging on a motoneuron and
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differential effects of sleep on these drives, transitions to sleep lead to different net changes in motoneuronal activity. Thus, the interpretation of changes in firing rate of individual cells, including motoneurons and different pools of respiratory muscles, requires additional knowledge of the relative changes in functionally distinct drives that collectively determine changes in activity across sleep–wake states.

Effects of Sleep on Central Respiratory Neurons
The respiratory drive to the respiratory motoneurons of the spinal cord (diaphragm, intercostals, and abdominals) originates in two groups of medullary respiratory cells called the dorsal
and ventral respiratory groups, with a smaller contribution from respiratory neurons of the parabrachial region of the pons (see Refs. 19,105,110,111 for reviews of the location of, and connections among, brainstem respiratory neurons). The excitatory drive from central respiratory neurons to respiratory motoneurons is mediated by glutamate through non-NMDA (N-methyl-D-aspartic acid) and, to a lesser extent NMDA, receptors (112–114).

In contrast to extensive studies of bulbospinal respiratory neurons that mediate respiratory drive to spinal motoneurons, the location and characteristics of those respiratory-modulated neurons that provide the respiratory drive to upper airway motoneurons are less well established. For XII motoneurons, inspiratory neurons with axonal projections to the XII motor nucleus are mainly located outside the regions containing bulbospinal respiratory neurons. Many are scattered in the reticular formation ventrolateral to the XII nucleus (115–117), which is the same intermediate medullary reticular region in which cells projecting to orofacial motor nuclei were found in retrograde tracing studies (118–120). Consistent with identification of glutamate as the main transmitter that mediates inspiratory drive to respiratory motoneurons, most anatomically identified XII premotor cells located in the reticular formation ventrolateral to the XII motor nucleus are glutamatergic, but many are also cholinergic (121,122).

To date, no respiratory neurons positively identified as having connections with upper airway motoneurons have been studied across the natural sleep–wake cycle. Consequently, our predictions regarding the behavior of such neurons can be only extrapolated from studies of respiratory premotor neurons for respiratory pump muscles and studies of respiratory neurons with identified axonal projections conducted in reduced carbachol models of REM sleep.

**Central Respiratory Neuronal Activity During SWS**

Studies in chronically instrumented cats by Orem and collaborators (123–125) and other groups (126–128) demonstrated that SWS is associated with a small or moderate reduction in the peak firing rate of inspiratory and expiratory neurons located in the region of the ventral respiratory group. In most cells having a strong and stable respiratory activity, the average peak firing rate was reduced by 10–20%, but in about 5–15% there was an increase. The average reduction corresponded to the reduction in the tidal volume and could be related to the slowing of the respiratory rhythm, a characteristic of SWS, with the same number of action potentials generated over a longer period. In contrast to the strongly respiratory-modulated neurons, cells with a weak and variable respiratory modulation during quiet wakefulness had a relatively large sleep-related reduction in peak firing rate (124). The efferent connections of the respiratory neurons recorded in chronically instrumented, behaving animals were not identified, but there can be little doubt that among those with strong respiratory modulation many had descending projections to spinal respiratory motoneurons. The weakly respiratory-modulated cells, whose activity was strongly depressed during SWS, could be premotor to upper airway motoneurons, respiratory pump motoneurons, or both (116). In addition, some could be vagal motoneurons located adjacent to the ventral respiratory group, or central neurons that were not functionally associated with any respiratory motor output, but rather involved in mediating the respiratory modulation of cardiovascular or other functions. Thus, there are relatively consistent decreases in the activity of medullary respiratory cells during SWS that are large only in neurons having a weak respiratory modulation. A subset of those cells may contribute to the decrements in respiratory modulation of the activity of upper airway motoneurons and muscles described in the sections “Sleep-Related Changes in the Activity of Individual Upper Airway Muscles in Healthy Subjects” and “Upper Airway Muscle Activity in OSA Subjects”.

Many respiratory-modulated neurons located in the pontine parabrachial region changed their firing rate when the animal entered SWS; increases or decreases in activity were observed with similar prevalence, with the average being a small decrease (129,130). The parabrachial region gives origin to projections to the orofacial motor nuclei containing upper airway motoneurons (118–120,131–133), but the interpretation of the sleep–wakefulness data is difficult without the knowledge of the efferent connections of the recorded neurons.
Thus, the small or moderate decrease in the activity of central respiratory neurons during SWS supports the conclusion that at least a part of the decrement in the activity of upper airway motoneurons during this state is caused by the withdrawal of their respiratory drive. The magnitude of this effect in different motoneuronal groups will depend on the relative contributions of the central respiratory and other drives to the excitability of a given motoneuronal group.

Central Respiratory Neuronal Activity During REM Sleep

In contrast to SWS, during REM sleep, the average level of activity of medullary respiratory neurons of the cat increases (134–137). This occurs on the background of a highly variable respiratory rhythm and tidal volume characteristic of this state. In individual respiratory cycles, respiratory cell activity may be higher or lower than the average, but the net change is toward a significant increase (Fig. 6A). The variability at the respiratory neuronal level grossly correlates with the breath-to-breath changes in the magnitude of diaphragmatic activity and changes in tracheal pressure (135), which is expected because many of the medullary respiratory cells studied were probably the main source of respiratory drive to respiratory pump muscles.

Correlation of the cell firing rate with the intensity of ponto-geniculo-occipital (PGO) waves suggests that one excitatory component of the effects of REM sleep on medullary respiratory cell activity is related to the pontine networks generating those waves (134). An extrapolation based on this analysis demonstrated that, during a hypothetical period of REM sleep with no PGO waves (tonic REM sleep), respiratory neurons of the medulla were still under both excitatory and suppressant influences. Respiratory cycles with excited and suppressed central respiratory neuronal activity tend to be clustered together, resulting in a quasi-rhythmic alteration between periods of enhanced and suppressed activity (135). Thus, during REM sleep, medullary respiratory neurons are subjected to both excitatory and inhibitory influences whose magnitudes show rapid fluctuations. A cluster of several breaths with a suppressed central respiratory activity may represent a period of high vulnerability to an obstructive or central hypopnea.

Figure 6  Rapid eye movement (REM) sleep exerts a powerful excitatory effect on the activity of most brainstem respiratory neurons. (A) Changes in the peak firing rate of medullary respiratory neurons recorded from chronically instrumented, behaving cats normalized to the average activity during slow-wave sleep (SWS). Source: From Ref. 137; courtesy of the American Academy of Sleep Medicine. (B) Recording from a medullary inspiratory nerve that had identified axonal projections to the XII motor nucleus obtained from a urethane-anesthetized rat during the pontine injection of carbachol that produced a characteristic, REM sleep-like depression of XII nerve activity. Note that the cell shows no decrease in activity even at the time of maximal suppression of XII nerve activity. This indicates that the REM sleep-like depression of XII nerve activity is caused by mechanisms other than a reduced inspiratory drive from medullary respiratory neurons. Source: Modified from Ref. 117.
apneic event. In addition, in subjects with an anatomically compromised upper airway, a mismatch in either the timing or strength of the central respiratory inputs to respiratory pump motoneurons relative to activation of upper airway motoneurons may facilitate upper airway closure due to a transiently inadequate muscular compensation for the airway-collapsing effect of negative inspiratory pressure (138).

The data from intact, chronically instrumented cats are supported by recordings obtained from reduced carbachol models of the REM sleep-like state (section “Sleep-Like Effects on Upper Airway Motor Tone in Reduced Animal Models”). In decerebrate cats, the peak firing rate of bulbospinal inspiratory and expiratory neurons of the ventral respiratory group shows, on an average, a small decrease following pontine injections of carbachol that produce REM sleep-like postural atonia. The magnitude of this decrease is much less than the simultaneously recorded decrements of phrenic, intercostal, or XII nerve activities (139). Moreover, about 20% of medullary inspiratory cells increase their activity following pontine carbachol injections, even though the respiratory motor outputs are consistently depressed (93,102). Similarly, disproportionately small decrements occur during pontine carbachol-induced decrements in XII nerve activity in a population of inspiratory neurons located in the region of the ventral respiratory group that send axons to the XII motor nucleus (117). In that study on urethane-anesthetized rats, XII nerve activity was reduced by 50% following pontine carbachol injections, whereas the peak activity of some XII premotor inspiratory cells increased and, on an average, it was reduced by only 5% (Fig. 6B). These results from cells that are likely a major source of inspiratory drive to XII motoneurons suggest that, similar to spinal respiratory motoneurons, the inspiratory drive to upper airway motoneurons is not appreciably reduced during REM sleep.

Overall, studies in naturally sleeping cats and in carbachol models show that there are both excitatory and suppressant effects on central respiratory neurons during REM sleep. The relative magnitudes of these two effects may vary in individual neurons, and probably also with experimental conditions. The studies in the reduced models in which reflex compensations were eliminated unequivocally demonstrate that both the excitatory and suppressant inputs impinging on central respiratory neurons during REM sleep are centrally generated. The mismatch between the small suppression of central respiratory activity and a much larger decrement in the activity of both respiratory pump and upper airway motoneurons indicates that nonrespiratory pathways contribute significantly to the suppression of activity at the motoneuronal level.

Similar to medullary respiratory neurons, many pontine parabrachial respiratory and nonrespiratory neurons increase their activity during REM sleep (129,140). Cells showing either increases or decreases during natural REM sleep behaved accordingly during the REM sleep-like state produced by pontine injections of carbachol in chronically instrumented, intact cats (140).

In summary, the central respiratory drive only intermittently decreases during natural REM sleep. Thus, while we can explain, at least in part, the increments in upper airway motor tone during REM sleep on the basis of increases in the activity of central respiratory neurons, the decrements seen in upper airway muscle activity during this stage of sleep are unlikely to be caused by changes in the central respiratory activity.

**Tonic, State-Dependent Inputs**

Several neurochemically distinct central neuronal groups have predictable and well-characterized changes in activity during the sleep–wake cycle and have direct connections with upper airway motoneurons (Fig. 7). The level of activity in norepinephrine (NE) and serotonin (5-HT)-containing neurons of the brainstem decreases during SWS and even more so during REM sleep (141–144). Similarly, orexin-containing cells located in the perifornical region of the posterior hypothalamus have maximal activity during active wakefulness, moderate level of activity during quiet wakefulness, minimal or no activity during SWS, and remain silent during REM sleep with the exception of occasional bursts of activity (145–147). Histaminergic cells...
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located in the hypothalamic tuberomammillary region also have maximal activity during wakefulness, reduced activity during SWS, and become silent during REM sleep (148). In contrast, the activity of a subpopulation of pontine acetylcholine (ACh)-containing neurons selectively increases during REM sleep, whereas other pontine cholinergic neurons increase their activity during both REM sleep and wakefulness. In all pontine ACh cells, the lowest activity occurs during SWS (101–149,153). If neurons have sleep-related decreases in activity and make excitatory connections with upper airway motoneurons, such excitation would be withdrawn; this is called disfacilitation. On the other hand, if neurons have increased activity during sleep and make inhibitory connections with motoneurons, they may contribute to decrements in upper airway motor tone through the mechanism of state-specific inhibition.

Sleep-Related Withdrawal of Excitatory Drives (Disfacilitation)

Both NE and 5-HT-containing afferent fibers are present in all orofacial motor nuclei (154–159). The net effect of applying 5-HT or NE onto orofacial, including upper airway, motoneurons is excitation (160–168), even though both amines may act through numerous receptor subtypes, of which only some produce excitatory effects (see Refs. 169–172 for reviews of NE and 5-HT receptors).

Norepinephrine

Brainstem NE-containing cells have gradually decreasing firing rates with the progression from active wakefulness to REM sleep (142,143), and project to orofacial motoneurons (132,133,157,173–175). Noradrenergic projections to orofacial motor nuclei originate in NE cells of the subcoeruleus region and the A5 and A7 groups, rather than the locus coeruleus (120,133,173,174). The activity of both subcoeruleus and A5 neurons ceases during REM sleep (143,175) and indirect evidence based on the expression of the immediate early gene, c-Fos, shows that the activity of pontine A7 and dorsal medullary A2 neurons also is suppressed during REM sleep, whereas that of ventrolateral medullary A1 neurons is not (176). In decerebrate

Figure 7 Activity patterns in neurochemically distinct populations of central neurons that have predictable changes in activity in relation to sleep–wake states and both directly and indirectly influence the activity of upper airway motoneurons.
cats, the levels of NE are reduced in the XII nucleus region when motoneuronal activity is depressed by electrical stimulation within the dorsomedial pontine REM sleep-triggering region (177).

The effects of NE on motoneurons may be exerted through multiple receptors located either postsynaptically on motoneurones or presynaptically on interneurons or afferent pathways to the motor nuclei. In XII motoneurones, postsynaptic excitatory effects are mainly mediated by $\alpha_1$-adrenoceptors (178). mRNA for all $\alpha_1$-adrenoceptor subtypes ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$) and binding sites for $\beta$-adrenoceptors are present in the orofacial motor nuclei (179–182), but, when studied at the single cell level, most XII motoneurones expressed mRNA for essentially one adrenergic receptor, the $\alpha_{1B}$ subtype (183). Thus, it appears that other subtypes are mainly located on astrocytes or cells other than motoneurones. Presynaptic effects may be mediated by $\alpha_2$-adrenoceptor subtypes, which suppress the release of various endogenous transmitters (184,185); therefore, the direction of their effect will depend on the nature of the affected afferent pathway. In situ hybridization and immunohistochemical studies show that, of the three $\alpha_2$-adrenoceptor subtypes ($\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$), only the $\alpha_{2C}$ subtype is present in upper airway motor nuclei in sizable amounts (186–189). In immature XII motoneurones, $\alpha_2$-adrenoceptors may be located postsynaptically on motoneurones and cause inhibition of their activity (178,190), but this effect sharply decreases with development (185,191). Thus, in the mature brain, the excitatory effects of NE on upper airway motoneurones are predominantly mediated by $\alpha_{1B}$-adrenoceptors, whereas $\alpha_2$-adrenoceptors may exert a more prominent role at various premotor sites, including those relevant for the control of respiratory rate (192–195).

In urethane-anesthetized rats, microinjections into the XII nucleus of prazosin, a selective antagonist of $\alpha_1$-adrenoceptors, causes a profound (about 80%) decrease of respiratory-modulated XII nerve activity, indicating that, under these experimental conditions, XII motoneurones receive a strong endogenous NE excitatory drive (196). When REM sleep-like atonia is elicited by pontine carbachol after injections of prazosin into the XII nucleus, XII nerve activity still exhibits a small decrement, showing that the REM sleep-like depression of XII motoneuronal activity cannot be fully accounted for by the withdrawal of NE-mediated excitation. However, following combined microinjections into the XII nucleus of prazosin and methysergide, an antagonist of serotonergic excitation, pontine carbachol elicits no further suppression of XII nerve activity (196) (Fig. 8). Considering that both NE and 5-HT cells have reduced activity in SWS and then become silent during REM sleep, these findings from anesthetized rats suggest that aminergic disfacilitation can importantly contribute to decrements of upper airway motor tone in both states of sleep. Furthermore, this result demonstrates that, at least in the anesthetized rat model, the REM sleep-related depression of XII motoneuronal activity can be fully explained as caused by a combined withdrawal of only two excitatory inputs: noradrenergic and serotonergic. In unanesthetized, naturally sleeping rats, local antagonism of noradrenergic excitation in the XII nucleus region also causes a profound suppression of lingual EMG, especially during wakefulness (198). Thus, at least in rats, noradrenergic activation is a powerful endogenous source of wake-related drive in XII motoneurones. Notably, in cats, serotonergic and histaminergic excitation is relatively much stronger than that mediated by NE (199). In addition to NE and 5-HT, transmitters such as histamine and orexins are likely to mediate the state-dependent, wake-related excitatory drive to upper airway motoneurones in humans and experimental animals, with relative contributions from those different sources being different in different species (200).

**Serotonin**

The serotonergic input to orofacial motoneurones originates in the pallidus, obscurus, and parapyramidal nuclei of the medullary raphe (132,133,201–203). These neurons, distributed along the midline of the medulla and physiologically identified as 5-HT containing, are most active during wakefulness, moderately active during SWS, and have dramatically decreased activity during REM sleep (204). Indeed, the REM sleep-related decreases in the activity of caudal medullary raphe neurons appear to be more profound than those in more rostral medullary

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raphe cells (see Figure 16 in Ref. 144). Thus, a significant portion of the excitatory input that they provide to upper airway motoneurons must be withdrawn during the transition from SWS to REM sleep. The excitatory effects of 5-HT onto upper airway motoneurons are mediated by multiple 5-HT receptors.

Both 5-HT_{2A} and 5-HT_{2C} receptor subtypes, both excitatory, are present in all orofacial motor nuclei, as demonstrated by receptor autoradiography (205), receptor immunohistochemistry (206,207), and the presence of the corresponding receptor mRNA (208–212). These receptors are coupled to phosphoinositol second messenger systems and increase motoneuronal activity by directly (postsynaptically) depolarizing the membrane through both closure of K⁺ channels and opening other cation channels. Pharmacological studies also show that the excitation of orofacial motoneurons is mediated by more than one 5-HT_{2} receptor subtype (164,165,213).

As expected from the recordings of 5-HT neurons during natural REM sleep, the medullary 5-HT neurons that have axonal projections to the XII nucleus are also silenced during decerebrate cats during the REM sleep-like atonia elicited by pontine carbachol (214). To mimic the withdrawal of the serotonergic input and assess its impact on the activity of XII motoneurons, 5-HT receptor antagonists were delivered directly into the XII nucleus or administered systemically. Studies of this type yielded varying estimates of the magnitude of the endogenous

Figure 8 The rapid eye movement (REM) sleep-like depression of XII nerve activity elicited by pontine carbachol does not occur following microinjections into the XII nucleus of a combination of antagonists of \( \alpha_{1} \)-adrenergic and serotonergic receptors (prazosin and methysergide). The antagonists eliminate the endogenous excitatory drive that normally maintains activity in XII motoneurons, as indicated by the greatly reduced level of XII nerve activity at the beginning of the record in B compared to that in A. The absence of any additional depression in XII nerve activity after the antagonists when an REM sleep-like episode is elicited by pontine carbachol (in B) provides evidence that REM sleep-like depression of XII nerve activity is caused by a combined withdrawal of noradrenergic and serotonergic excitation. Note that, while carbachol does not depress XII nerve activity in B, the characteristic increase in hippocampal theta activity and slowing of the respiratory rate in response to carbachol are intact. The absence of any additional depression is not simply secondary to the low level of XII nerve activity following the antagonist injections into the XII nucleus because the same result is obtained when XII nerve activity is increased by concomitant microinjection of bicuculline into the XII nucleus (197). Source: Modified from Ref. 196.
serotonergic excitatory drive to upper airway motoneurons. In decerebrate cats, in which XII nerve activity was initially enhanced by vagotomy and hypercapnia (165), and in English bulldogs (215), in which sternohyoid EMG was elevated compared to normal dogs (66) (Fig. 3B), pharmacological antagonism of 5-HT₂ receptors caused significant reductions of motoneuronal activity of the order of 50%. In two studies in anesthetized and vagotomized rats, a single microinjection of various antagonists of 5-HT₂ receptors reduced XII nerve activity by about 60% (216, 217). In a third study, also in anesthetized and vagotomized rats, multiple microinjections of a broad-spectrum 5-HT receptor antagonist, methysergide, placed along the entire rostrocaudal extent of the XII nucleus caused a lesser reduction, 15–30% (196). Then, in a study in chronically instrumented, unanesthetized rats with vagi intact and microperfusion of a portion of the XII nucleus with a 5-HT₂ receptor antagonist, mianserin, the genioglossal EMG was not significantly altered (218). To explain the latter negative result, the authors proposed that vagotomy enhances the endogenous serotonergic excitatory drive in XII motoneurons. However, the alternative explanation is that focal microperfusion within the XII nucleus with an antagonist is unable to demonstrate the presence of endogenous serotonergic excitation when the antagonist is delivered to only a fraction of the nucleus and acts on only a small subset of XII motoneurons that are spontaneously active. Thus, while other data indeed point to relatively weaker exogenous and endogenous excitatory effects of 5-HT on XII motoneurons in rats than in other species (196, 199), the negative finding in behaving rats can be explained without invoking the possibility that vagotomy enhances the XII motoneuronal activity by increasing the endogenous 5-HT₂ drive to XII motoneurons.

In unanesthetized, decerebrate and vagotomized cats, the neural nature of the endogenous 5-HT drive to XII motoneurons was demonstrated by microdialysis studies in which the extracellular level of 5-HT in the XII nucleus region decreased during both the REM sleep-like atonia (177, 219) and pharmacological inhibition of 5-HT-containing cells (219). In the same model, microinjections of 5-HT into the XII nucleus attenuated the REM sleep-like depression of XII nerve activity elicited by pontine carbachol (220). Similar findings were then reported in chronically instrumented and naturally sleeping rats in which administration of 5-HT into the XII nucleus region by reverse microdialysis attenuated the REM sleep-related depression of genioglossal muscle activity (61). Notably, in both studies, activation of XII motoneurons with exogenous serotonergic agonists was not sufficient to fully eliminate the depressant effect of REM sleep. Similarly, in urethane-anesthetized rats, multiple microinjections of methysergide placed along the entire rostrocaudal extent of the XII nucleus only partially attenuated the depression of XII nerve activity during the REM sleep-like atonia elicited subsequently (196). Thus, the withdrawal of serotonergic excitatory effects mediated by 5-HT₂ receptors makes a measurable, but only partial, contribution to the REM sleep-related decrements of XII motoneuronal activity.

In humans, attempts to treat sleep-disordered breathing by enhancing the central levels of 5-HT with reuptake blockers or L-tryptophan have yielded mixed results (chap. 29) (221). If in humans, as in rats (196), the endogenous noradrenergic drive to upper airway motoneurons is stronger than serotonergic drive, then pharmacological treatments that enhance noradrenergic transmission could prove more effective than treatments that enhance serotonergic transmission. However, any treatment that increases aminergic transmission is likely to have serious side effects, including profound suppression of sleep; this is a major obstacle for practical applications of a pharmacotherapy for OSA based on the fundamental findings showing that a combined withdrawal of noradrenergic and serotonergic excitation is the main cause of sleep-related upper airway hypotonia.

In addition to 5-HT₂ receptors, ligand binding and/or mRNA studies demonstrate that other 5-HT receptors, such as 5-HT₁A, 5-HT₁B, 5-HT₁D, and 5-HT₃, are present in the XII (210–212, 222–224) and other orofacial motor nuclei (225). However, in adult animals, neither agonists nor antagonists of 5-HT₁A receptors have any functional effects on the activity of orofacial motoneurons when the drugs are applied directly onto motoneurons (164, 165, 213, 226–227), and there is no evidence for the presence of 5-HT₁A receptor protein (228) or mRNA (210, 212) in upper airway
motor nuclei of adult rats. (5-HT$_{1A}$) receptors and their mRNA are present in XII motoneurons of neonatal rats, but the expression of these receptors decreases considerably by postnatal day 28, and 5-HT$_{1A}$ receptor agonists do not affect XII motoneuronal activity in mature rats (212,226,229)).

In contrast to 5-HT$_{1A}$ receptors that disappear from the XII nucleus in mature animals, 5-HT$_{1B}$ receptors are present and active in the XII nucleus in both neonatal and adult rats. Since these receptors are often localized presynaptically and modulate the release of various transmitters from axon terminals, the direction and magnitude of their effect depend on the set of afferent pathways to motoneurons that are active under the given experimental conditions. Accordingly, stimulation of 5-HT$_{1B}$ receptors caused an enhancement (230) or reduction (226,231,232) of the excitability of XII motoneurons. The receptors may be also located postsynaptically on XII motoneurons, as suggested by the presence of their mRNA in a majority of XII motoneurons (212), and 5-HT$_{1B}$ receptor-like immunoreactivity in most large cells located in the XII motor nucleus (233). In anesthetized rats and decerebrate cats, microinjection of a 5-HT$_{1B}$ agonist into the XII nucleus results in a moderate suppression (about 20%) of XII nerve activity (226), and in vitro studies reveal only a small change in XII motoneuron membrane resistance (232). During sleep, when 5-HT-containing neurons have reduced activity or become silent, reduced stimulation of 5-HT$_{1B}$ receptors located within the XII nucleus may cause some disinhibition of XII motoneuronal activity.

Although some studies reported expression of 5-HT$_{3}$ receptor mRNA in XII motoneurons (224), other studies concluded that it occurred at a very low level or not at all (210), and microinjections of 5-HT$_{3}$ receptor agonists and antagonists into the XII nucleus had no effect on XII nerve activity (234). In contrast, systemic administrations of 5-HT$_{3}$ receptor agonists enhanced XII motoneuronal activity and reduced the incidence of central sleep apneas (234,235). These effects could be mediated by stimulation of cell bodies of vagal afferents located peripherally in the nodose ganglion. Microinjection and mRNA studies suggest that 5-HT$_{4}$ and 5-HT$_{5}$ receptors also are present in XII motoneurons (211), but their functions remain to be determined.

5-HT neurons have extensive axonal projections within the brainstem (236). Thus, the sleep–wake cycle variations in the release of 5-HT may affect upper airway motor tone by acting not only on motoneurons but also at numerous premotor sites. In particular, 5-HT terminals make synaptic contacts with central respiratory neurons (237). In contrast to orofacial motoneurons, the net effect of 5-HT on medullary respiratory neurons is inhibitory (192,238); the inhibition is due, at least in part, to a direct action mediated by 5-HT$_{1A}$ receptors on inspiratory (239) and expiratory (240) neurons. Some expiratory, post-inspiratory, and inspiratory neurons located in the pre-Bötzing complex region may be also excited by 5-HT, presumably through 5-HT$_{1A}$ receptors (192,241–243). If inhibitory effects of 5-HT dominate at the level of premotor inspiratory neurons, whereas excitatory 5-HT effects dominate at the level of motoneurons, the sleep-related decrement in the activity of brainstem 5-HT neurons may exert simultaneously two opposite effects on the respiratory motor output. The central respiratory drive to motoneurons may be enhanced as a result of sleep-related withdrawal of inhibitory effects of 5-HT exerted at premotor levels. At the same time, withdrawal of the direct serotonergic excitation of motoneurons will lead to reduced motoneuronal excitability. Thus, an increase in the central respiratory drive may compensate, to some extent, for the sleep-related withdrawal of the tonic excitatory drive occurring at the motoneuronal level. The two opposing effects may have different magnitudes in different pools of respiratory motoneurons; this may determine the net effect of sleep on the activity of different motoneuronal pools.

Both neuroanatomical and neurophysiological data show that substantial differences exist in the density of the innervation and magnitude of the serotonergic effects on different motoneuronal pools. These differences are of interest because they may be related to the differences in the effect of sleep on different upper airway muscles (section “Effects of Sleep on Upper Airway Muscles”). In the XII nucleus, serotonergic terminals are more frequently closely apposed to the distal dendrites than to the proximal dendrites or cell bodies of XII motoneurons (155,244), and the same is observed for thyrotropin-releasing hormone.
(TRH)-containing terminals (TRH is extensively co-localized with 5-HT in cell bodies and terminals of serotonergic neurons (245)). In addition, the density of 5-HT and TRH terminals is higher in the vicinity of laryngeal, than XII or facial, motoneurons (159,245), suggesting that 5-HT may exert stronger effects on laryngeal than other orofacial motoneurons. In support of this, the duration of the excitatory postsynaptic potentials produced in trigeminal (masseter and mylohyoid) motoneurons by electrical stimulation within the medullary raphe was positively correlated with the density of serotonergic innervation (246), and the magnitude of the effect of iontophoretically applied 5-HT on masseter and digastric motoneurons was proportional to the density of serotonergic terminals in the corresponding portions of the trigeminal motor nucleus (162). However, the sensitivity of XII motoneurons to iontophoretically applied 5-HT was higher than that of laryngeal motoneurons. Relative to their control firing rates, XII motoneurons were excited more strongly, and the excitatory effect of 5-HT developed faster in XII than laryngeal motoneurons (247). One reason for the discrepancy between the density of synaptic contacts and the magnitude of the pharmacological effect of 5-HT may be the presence of 5-HT terminals that do not make identifiable synaptic contacts and, therefore, participate in what is called “volume transmission” (248). In this type of transmission, neurotransmitters are released into the extracellular space and must diffuse much larger distances to reach their receptors than in the case of classical synapses. Such “free” 5-HT terminals likely account for about 75% of 5-HT terminals in the XII nucleus (155), compared with less than 25% of such terminals in the trigeminal nucleus (249). Therefore, volume transmission appears to be the prevalent form of serotonergic transmission in the XII motor nucleus. This would be consistent with 5-HT (and NE) playing the role of a global, state-dependent modulator of the excitability of XII motoneurons (19,165,196). Indeed, the evidence to date shows that the aminergic excitatory drive may represent a major component of what has been long referred to as the “wakefulness stimulus for breathing” (15,200,250,251).

Neuromodulatory Peptides: Thyrotropin-Releasing Hormone, Substance P, and Orexins

Medullary 5-HT neurons contain, and may co-release from their axon terminals, various combinations of peptide transmitters, including TRH and substance P (SP) (252–255). Both SP- and TRH-containing terminals (245,256–260) and neurokinin (for SP) and TRH receptors (223, 261,262) are present within orofacial motor nuclei, and both peptides are excitatory to upper airway motoneurons (166,263–265). The effects of SP on XII motoneurons are similar to those of NE, with both acting through the same intracellular pathway (266).

It has been proposed that the release of peptides from the axon terminals of raphe neurons requires higher levels of activity than does the release of 5-HT (267), and that complex pre- and postsynaptic interactions may take place among the co-released transmitters and peptides at the site of their release (268,269). It is not known under what conditions TRH and SP can have endogenous excitatory effects on upper airway motoneurons. The studies with antagonists required to address this question have not been performed due to the lack of suitable compounds. In association with strong motor and respiratory efforts, the firing rate of caudal raphe cells increases by 50–100% above the 1–4 Hz typically seen during quiet wakefulness; in some cells, the firing behavior becomes less regular and occurs in bursts temporally related to rhythmic movements (270). This raises the possibility that TRH and/or SP are released at the time of enhanced respiratory effort, such as during exercise or arousals caused by airway occlusion. The powerful effects of these peptides when applied onto upper airway motoneurons warrant more research as they may have therapeutic potential in OSA.

Upper airway motoneurons also receive wake-related excitation from the neurons located in the perifornical region of the posterior hypothalamus that synthesize the excitatory peptides, orexins. These neurons project to orofacial motor nuclei (271,272); all motoneurons express orexin receptors (273,274) and these receptors mediate excitatory effects (275). Since orexin cells have elevated activity mainly during active wakefulness (Fig. 7), the loss of orexinergic excitation may be primarily responsible for the reduced upper airway motor tone during quiet
wakefulness when compared with active wakefulness. Multiple antagonists of the two known orexin receptors have been developed because of their potential usefulness in the treatment of insomnia. Although anatomical data show that NE and 5-HT neurons are the prime targets of orexinergic excitatory projections, pharmacological activation of orexin and other cells located in the perifornical hypothalamus strongly activates XII motoneurons even when 5-HT, and α-adrenergic receptors are blocked at the level of the XII nucleus (276). This suggests that the direct activation of XII motoneurons by orexin can occur independently of aminergic activation. As such, orexin neurons will represent another endogenous source of the “wakefulness stimulus” for upper airway motoneurons. In addition, considering that orexin cells generate bursts of activity during REM sleep (145,147), they may be responsible for the phasic activation of XII motoneurons during this state (57,58). Data also implicate orexin neurons in mediation of respiratory CO₂ sensitivity (277).

**Acetylcholine**

Brainstem ACh-containing neurons, especially those in the pons, have state-dependent activity patterns (101). Some are maximally active during both wakefulness and REM sleep, some have relatively selective activity increases during REM sleep (Fig. 7), and some exhibit distinct phasic bursts in association with the phasic events of REM sleep. There are both pre- and postsynaptic cholinergic effects on orofacial motoneurons (278–280); this raise the possibility that ACh may play an important role in regulating the excitability of upper airway motoneurons in a state-dependent manner. Postsynaptic excitatory effects are mediated in VII and XII motoneurons by nicotinic cholinergic receptors (279,280). mRNAs for ACh receptors, both nicotinic and muscarinic, are also expressed in XII premotor neurons (122) and in other neurons of the ventral respiratory group (101,281). The presence of muscarinic M₃ receptor mRNA in both glutamatergic and cholinergic XII premotor neurons of the intermediate region of the medullary reticular formation suggests that these receptors mediate presynaptic inhibition of inspiratory drive to XII motoneurons (113,121,122). This has been demonstrated in in vitro recordings from XII motoneurons in medullary slices from juvenile rats; the addition of M₃ muscarinic cholinergic receptor agonists to the bath suppressed spontaneous and evoked excitatory postsynaptic potentials mediated by glutamate (Fig. 9A) (278). Since the effect occurred without changes in the postsynaptic properties of the motoneurons and was associated with a reduction in the frequency, but not amplitude, of spontaneous postsynaptic currents, it was probably due to decreased glutamate release from presynaptic terminals. Unlike the permissive (disfacilitation) mechanisms discussed in the preceding sections, the presynaptic ACh effect would be actively inhibitory and could cause suppression of motoneuronal activity during REM sleep, or both wakefulness and REM sleep. Both nicotinic excitation and muscarinic suppression of genioglossal EMG were also observed in in vivo studies in urethane-anesthetized rats in which various cholinergic agonists and antagonists were microperfused into the XII nucleus and the adjacent dorsomedial medullary regions (282). The suppressant effects mediated by muscarinic receptors were more powerful than nicotinic excitation (Fig. 9B).

Cholinergic projections to the XII nucleus are bilateral and originate from the dorsal pons and intermediate medullary reticular region (121,122,283). The projections from the pons appear to be relatively scanty and primarily come from two distinct subregions of the pontine cholinergic cell fields, the pars-compacta of the pedunculopontine tegmental nucleus and α part of the laterodorsal tegmental nucleus (283). Considerably more cholinergic cells with axonal projections to the XII nucleus are found in the medulla (121,122). Based on recordings from cholinergic pontine cells across the sleep–wake cycle (150,151), one may assume that those that send axons to the XII nucleus have reduced activity during SWS, or both SWS and REM sleep. The sleep–wake behaviors of the many cholinergic XII premotor neurons located in the medullary reticular formation are unknown and need to be determined in order to gain further insight into their role in motoneuronal control within the framework of sleep-related upper airway hypotonia.
Sleep-Related Active Inhibition of Motoneurons

The active, postsynaptic inhibition of motoneurons mediated by amino acids such as glycine or GABA is widely seen as a major mechanism responsible for the REM sleep-related atonia of postural muscles. Glycine has been identified as the main mediator of the REM sleep-specific, inhibitory postsynaptic potentials present in lumbar postural motoneurons throughout (284–286), or at least at the transition into (287,288), the atonia of REM sleep. Consistent with the concept of active inhibition that causes the opening of Cl⁻ channels, the membrane resistance...
of lumbar motoneurons decreases during natural REM sleep (287, 289), and the postural atonia produced by pontine carbachol (290). Experiments using various experimental models and methodologies have led to the widely held view that REM sleep activates a descending inhibitory system that originates in the dorsomedial pontine reticular formation (corresponding to the pontine sites where carbachol is effective) and uses two successive synaptic relays, one in the ventromedial medullary reticular formation and another at the spinal segmental level. In support of this, neurons with REM sleep–specific increases in activity are found in the ventromedial medullary reticular formation (291–295), and stimulation of cells in this region with glutamate evokes a generalized postural atonia (296).

In support of a key role of glycine in mediating REM sleep-specific inhibitory potentials in motoneurons, iontophoretic applications of strychnine, a glycine receptor antagonist, nearly abolished such potentials together with the concomitant decreases of motoneuronal membrane resistance and membrane hyperpolarization in lumbar motoneurons of chronically instrumented, intact cats (286). The near abolition of membrane potential changes remains to be reconciled with the evidence that multiple non-glycinergic pathways that have state-dependent activity impinge on motoneurons and also must be assumed to contribute to REM sleep-related depression of motoneuronal activity. For example, similar to orofacial motoneurons, spinal motoneurons are excited by the transmitters and peptides released from the wake-active and REM sleep-silent brainstem NE and 5-HT neurons (263, 297–300). Accordingly, one would expect that, when aminergic neuronal firing ceases during REM sleep, this should cause a disinhibition and hyperpolarization of spinal motoneurons in addition to the hyperpolarization mediated by glycine.

As in spinal motoneurons, glycine-mediated inhibitory postsynaptic potentials also occur in XII and trigeminal motoneurons during the carbachol-induced atonia of REM sleep (63, 302, 303), and increased levels of both glycine and gamma-aminobutyric acid (GABA) were detected in the XII nucleus region in association with the motor atonia produced by electrical stimulation within the pontine REM sleep-triggering region (304). These findings have been interpreted as evidence that glycine, with or without GABA, is the cause of suppression of orofacial motoneuronal activity during REM sleep. However, in anesthetized rats, withdrawal of aminergic excitation was found to be a sufficient explanation of the mechanism underlying the REM sleep-related suppression of XII motoneuronal activity (196), whereas the contribution of glycine-mediated inhibition was found to be minimal (197). Indeed, not a single study of those that directly addressed the question of causality between the presence of glycine-mediated inhibition and suppression of motoneuronal activity during REM sleep supported the glycine hypothesis. In the first study of this kind, large injections of bicuculline or picrotoxin (to antagonize GABA_1 receptors) or strychnine (a blocker of glycine receptors) into the trigeminal motor nucleus of cats reduced the suppressant effect of REM sleep on reflexly evoked motoneuronal responses by less than 20% (305). Despite this result, the authors proposed that glycine was a major, albeit not a sole, contributor to the depression of trigeminal motoneuronal activity during REM sleep. In another study, in unanesthetized, decerebrate cats, the REM sleep-like depression of spontaneous activity of XII motoneurons elicited by pontine carbachol was not reduced by microinjections into the XII nucleus of either strychnine (Fig. 10) or bicuculline, whereas a reflexly evoked inhibition elicited by electrical stimulation of the lingual nerve was abolished (301). This study led to the conclusion that active inhibition plays a minimal role in the suppression of XII motoneuronal activity during the REM sleep-like atonia. Evidence for the lack of a major role of active inhibition in suppressing the XII motoneuronal activity during REM sleep was subsequently also obtained in chronically instrumented and naturally sleeping rats, in which microperfusion of either strychnine or bicuculline into the XII nucleus region did not prevent the REM sleep-related depression of genioglossal muscle activity (306). Similarly, the REM sleep atonia of jaw muscles (innervated by trigeminal motoneurons) also could not be reduced by strychnine or bicuculline in naturally sleeping rats (307). Together, these data demonstrate that, while glycine-mediated inhibitory postsynaptic potentials occur during REM sleep in XII and other motoneurons, their contribution to motoneuronal hyperpolarization and depression of activity in XII and trigeminal motoneurons is
negligible. Therefore, although all upper airway motor nuclei receive dense glycinergic innervation (308,309), a major role of this neurotransmitter is probably to mediate reflex inhibitory influences and coordinate the complex activity patterns that upper airway motoneurons generate in association with ingestive behaviors and phonation (310–312), rather than to cause the REM sleep-related hypotonia. In view of these findings, a combined withdrawal of noradrenergic and serotonergic excitation currently stands as both a necessary and sufficient mechanism by which to explain the REM sleep-like depression of XII and trigeminal motoneuronal activity (196,200). Any additional effects mediated by withdrawal of transmitters and peptides such as histamine and orexins remain to be investigated (98,199,272,276,313), whereas the effects mediated by inhibitory amino acids are minimal and dispensable (196,197,301).

STATE DEPENDENCE OF UPPER AIRWAY REFLEXES

Important excitatory effects on upper airway dilator muscles originate from central and peripheral arterial chemoreceptors sensitive to changes in pH, P_{CO_2}, and P_{O_2} from pulmonary receptors and from receptors located in the upper airway that respond to changes in airway pressure, muscle tension, temperature, and various chemical stimuli. The reflex effects of chemoreceptors and pulmonary receptors occur concurrently in upper airway and respiratory pump muscles, whereas at least certain aspects of the afferent inputs that originate in the upper airway seem to be specifically concerned with the control of upper airway muscles. Numerous studies of upper airway mechanoreceptor reflexes in humans have considered their importance in the maintenance of upper airway motor tone in OSA (16,43,50). These results need to be related to the extensive information about the physiology of airway receptor reflexes derived from studies in experimental animals (22,109,314). Here, we will focus primarily on reflexes from upper airway mechanoreceptors, rather than chemoreflexes.
Upper Airway Mechanoreceptors and Their Reflex Effects

Of the diverse types of receptors in the upper airway, only few show a slow adaptation to changes in intraluminal pressure suitable for mediating the tonic excitation that negative airway pressure imparts on airway motoneurons. These slowly adapting receptors are stimulated by both negative pressure and low-amplitude, high-frequency (±2.5 cm H₂O, 10–30 Hz) pressure vibrations like those generated in the airway during snoring (315). Interestingly, a subset of laryngeal and nasal (trigeminal) pressure receptors excited by negative pressure in the airway is also excited when upper airway muscles contract (316,317). Such receptors are called “drive” receptors because they are stimulated by a change in the configuration of the soft tissue in the airway wall that results from muscle contraction. The actual location of pressure and “drive” receptors within the airway tissue must be superficial, because both their activity and reflex effects on upper airway motoneurons are abolished by anesthetics applied to the airway mucosa (318,319). The relative proportions of receptors sensitive to pressure, muscle contraction, or both probably depend on the level of upper airway muscle tone; enhanced muscle tone, by increasing the tissue tension, would reduce the threshold for stimulation of the “drive” receptors by changes in both the intraluminal pressure and laryngeal muscle contraction (315).

The activity from upper airway mechanoreceptors is transmitted to the brainstem through the vagus (superior laryngeal branch carrying laryngeal primary sensory afferents), glossopharyngeal (pharynx), and trigeminal (nose) nerves. Although it is necessary to sever all three afferent pathways to abolish the excitatory effects of negative airway pressure on upper airway motoneurons (318,320,321), the most powerful effects originate from the laryngeal region (322). The principal site of central termination of upper airway afferents is the nucleus of the solitary tract [nucleus tractus solitarii (NTS)], with additional projections to the trigeminal sensory nucleus (see Ref. 21 for a review). In addition to slowly adapting airway mechanoreceptors, the superior laryngeal nerve carries afferents from receptors of many other modalities, which mediate reflexes that protect the airway from irritants and invasion by foreign particles. Stimulation of these afferents inhibits the central respiratory rhythm and activates coughing and/or swallowing (35,314,323).

Increased osmolarity of the fluid in the larynx increases upper airway mechanoreceptor sensitivity, resulting in an increased magnitude of the reflex excitatory effects of negative pressure on upper airway motoneurons (324,325). This effect may complement the increases of upper airway muscle activity reflexly evoked by stimulation of laryngeal chemoreceptors (326). Although the latter receptors are primarily responsible for airway protective reflexes (314), their moderate stimulation may facilitate upper airway motor tone.

Application to the airway lumen of steady negative pressure or pressure oscillations similar to those seen during snoring produces a reflex enhancement of upper airway motoneuronal activity (2,45,322,327–330). Conversely, receptor unloading by the application of positive pressure to the airway, neuromuscular paralysis, and upper airway anesthesia reduce genioglossal EMG and XII nerve activity and increases upper airway resistance (328,331–333). Such studies demonstrate the presence of an endogenous reflex excitatory drive that presumably originates in the slowly adapting airway pressure receptors.

Upper airway mechanoreceptor reflexes comparable to those in experimental animals operate also in humans. Application of negative pressure to the airway (334–341), high-frequency pressure oscillations (342) or airway occlusions (33,338,343) produce reflex increases in the activity of upper airway muscles. These responses are reduced following anesthesia of the superficial receptors of the glottic, pharyngeal, and nasal airway (340,344). In OSA patients, upper airway anesthesia also attenuates the increase in genioglossal muscle activity that occurs during obstructive events in SWS (345). The magnitude of the reflex excitatory effects of negative pressure in individual airway muscles depends on the route of respiration (321,338,343) and the phase of the respiratory cycle (346).

In humans, when short pulses of negative pressure are applied to the upper airway in wakefulness, the shortest latency of the excitatory response evoked in the genioglossus is compatible with a reflex pathway contained within the lower brainstem and involving few central...
relay neurons (336,346,347). Thus, reflex pathways similar to those described in anesthetized animals are likely to be responsible for the reflex effects observed in humans. One caveat with studies in awake subjects, however, is that the central pathways mediating the effects of airway receptors may be more complex than those activated in anesthetized animals, and may include components activated by the conscious sensation of the stimulus and/or may be transmitted centrally by neurons in the reticular formation that are active during wakefulness but silent and unresponsive to sensory stimuli during sleep and anesthesia. The application of negative pressure stimuli to the mouth in awake humans produces a complex cortical evoked potential whose earliest component has latency similar to that of the excitation evoked in the genioglossus (348). Thus, the initial portion of the genioglossus response produced during wakefulness is unlikely to be behaviorally controlled, but its later components may have a behavioral component, or be modified by the conscious sensation of the stimulus. Presumably, such behavioral contributions would be markedly attenuated during sleep.

In addition to classic input–output relationships between receptors and effectors, laryngeal afferents can produce an increased activity of upper airway dilator motoneurons that substantially outlasts the duration of the stimulus. Application of negative pressure pulses to the airway enhances genioglossal EMG or XII nerve activity for prolonged periods (318,328,333). Henke and Sullivan (342) provide a record from an OSA patient in whom a brief period of oscillating pressure applied to the airway during REM sleep produced a prolonged increase of genioglossal EMG and resolution of the apnea with no evidence of arousal. A prolonged activation can be elicited in experimental animals after a brief electrical stimulation of the superior laryngeal nerve (244,349). In the cat, such a post-stimulatory enhancement of XII nerve activity lasts several minutes (Fig. 11A) and occurs without changes in phrenic nerve activity. The latter feature distinguishes this effect from other forms of respiratory potentiation (350). The effect is

![Figure 11](https://example.com/figure11.png)

**Figure 11**  Stimulation of laryngeal afferents has excitatory effects on hypoglossal (XII) motoneurons that outlast the period of stimulation. (A) Compressed record of integrated activity of the phrenic and hypoglossal (XII) nerves before, during, and after an electrical stimulation of the superior laryngeal nerve (SLN) with a ~15-second train of pulses. The tonic level and the magnitude of inspiratory modulation of XII nerve activity remain elevated beyond the period of stimulation and exponentially return toward the control over a period of several minutes while the magnitude of phrenic nerve activity remains almost unchanged (except during the period of stimulation when it is abolished). Such a long-term potentiation of upper airway motor tone initiated by laryngeal afferents may have an important stabilizing effect on airway patency. (B) Another test with SLN stimulation in the same experiment (the time scale is expanded compared with A). A brief train of stimuli was applied to the lingual nerve 11 respiratory cycles after the termination of SLN stimulation. The enhancement of XII nerve activity produced by SLN stimulation is "reset" by lingual nerve stimulation. Thus, the level of activity in XII motoneurons can change rapidly due to opposing influences exerted by SLN and lingual nerve afferents. **Source:** Unpublished data from a decerebrate, vagotomized, paralyzed, and artificially ventilated cat from Ref. 355.
also different from the long-term potentiation of both diaphragmatic and upper airway respiratory motoneuronal activity by repeated hypoxic episodes or repeated applications of 5-HT or NE (351–353; see Refs. 19,20 for reviews) because it is not abolished by 5-HT antagonists (244).

One function of such a long-lasting enhancement following laryngeal stimulation may be to stabilize upper airway tone and airway patency. Interestingly, however, the increase of XII nerve activity initiated by a brief stimulation of laryngeal afferents can be instantly terminated (reset) by stimulation of lingual nerve afferents (Fig. 11B). This confirms the central nature of the enhancement phenomenon and reveals a potentially important “negative” contribution of trigeminal afferents to the control of upper airway motor tone. Thus, stimulation of various upper airway receptors may exert both immediate and prolonged effects on upper airway motor tone. Application of oscillatory pressure stimuli to the airway during sleep is a potentially attractive strategy that will take advantage of upper airway reflexes to prevent airway obstructions in OSA patients (342,354).

**Sleep-Dependent Modulation of Upper Airway Reflexes**

There is evidence that reflexes from upper airway mechanoreceptors can prevent upper airway hypotonia during sleep (356,357). At the same time, some attenuation of reflex transmission may occur during sleep as a result of sleep-related decrements in the excitability of motoneurons (Fig. 4D). Reflex transmission may also be altered by other central mechanisms related to changes in behavioral states.

In cats, during REM sleep but not SWS, reflex transmission from trigeminal tooth pulp afferents to second-order trigeminal sensory neurons was suppressed by pre- and/or postsynaptic mechanisms (358,359); however, another study in cats reported an increase in reflex transmission during SWS and REM sleep (360). In rabbits, reflex activation of the genioglossus is reduced during both SWS and REM sleep to a larger degree than that expected from the concomitant suppression of the spontaneous activity of the muscle (361). The transmission in spinothalamic and spinocerebellar pathways is either unchanged or facilitated during REM sleep (362,363), and the tactile receptive fields and response magnitudes of a majority of spinal dorsal horn neurons are increased (364). Similarly, the monosynaptic excitatory postsynaptic potentials recorded from lumbar motoneurons are larger during REM sleep than during SWS or wakefulness (365). These results do not uniformly support the common notion that sleep causes suppression of central transmission in reflex pathways, a phenomenon that needs to be distinguished from sleep-related suppression of motor responses at times when motoneuronal activity is reduced (e.g., during sleep, or attentive immobility during wakefulness).

In the respiratory system, some sleep-related attenuation of various respiratory reflexes often has been reported (366,367), but a distinction between a genuine state-dependent suppression of reflex transmission occurring centrally and an attenuation secondary to reduced excitability at the motoneuronal level has rarely been made (ref. 361 describes an attempt to make such a distinction). In the case of those cardiorespiratory output measures that are consistently maintained throughout wake and sleep (e.g., respiratory rhythm, diaphragmatic activity, heart rate, or arterial blood pressure), the effects of sleep on reflex transmission are small. For example, negative pressure pulses applied to the airway during expiration produce a similar reflex prolongation of expiration in wakefulness and SWS, and there is only a small attenuation of this response during REM sleep (323). The CO₂ threshold for the resumption of rhythmic respiratory activity does not change across the states of sleep and wakefulness (368,369), and the ventilatory sensitivity to CO₂ is the same during SWS and wakefulness in goats (55,370). In one study, apneas and bradycardias in response to instillation of water or inflation of a balloon in the larynx were more pronounced during REM sleep than in SWS when arousal did not occur, whereas arousals themselves were more likely to occur in SWS than in REM sleep (371). The gain of the arterial baroreceptor reflex controlling the heart rate does not differ between wakefulness and SWS in normal mice (372). The average diaphragmatic response to airway occlusion is reduced during REM sleep when compared with SWS, but a further analysis of the
breath-to-breath variability of the magnitude of this effect has revealed that it is an intermittent, and not a tonic, phenomenon (373). Such a reflex suppression may be related to certain phasic aspects of REM sleep, rather than to the neural mechanisms maintaining this state.

With regard to reflex responses of upper airway muscles to airway negative pressure, the data are conflicting. Most studies show that these reflexes were present, albeit attenuated, in the genioglossal muscle activity during SWS, or both SWS and REM sleep, in chronically instrumented animals (52,53,374), normal humans (342,366,375), and OSA patients (342,376). In some studies, the genioglossal muscle response to negative pressure was nearly abolished during SWS (334,337,347), but in another study in normal humans, the genioglossus excitatory response to an inspiratory resistive load was absent during wakefulness, but present during SWS (38). Some studies suggest that the reflexes are blunted in awake OSA patients (339), whereas others identify these reflexes as a major mechanism that maintains airway patency (377). In normal subjects, the dependence of upper airway reflexes on body position is stronger than the effects of sleep. In the supine position, genioglossal activation by upper airway negative pressure is stronger during SWS than in wakefulness, but the difference disappears in the lateral position (378).

Upper airway receptors also importantly contribute to arousal from sleep (379–384), thus showing that sensory modalities from the upper airway are being effectively transmitted to the brain systems that elicit arousal and maintain wakefulness. One group concluded, however, that the sensory input from the upper airway makes a negligible contribution to arousal in severe snorers and OSA patients (385,386). This may be related to the damaged sensory endings and other morphological abnormalities in upper airway mucosa and muscles observed in healthy snorers and OSA patients (81,387–392).

Neurons with state-dependent changes in activity and widespread projections within the brainstem (discussed in the section “Sleep-Related Changes in the Activity of Individual Upper Airway Muscles in Healthy Subjects”) may control the central transmission of reflexes in a state-dependent manner. Electrophysiological and neuropharmacological studies indicate that afferent pathways to XII motoneurons are modulated presynaptically by 5-HT and other “state-dependent” transmitters (230–232,277), but the evidence comes from in vitro studies in which the functional identity of the pathways studied was unknown. In an in vivo study, microinjections of 5-HT into the XII nucleus did not change the response of XII motoneurons to negative pressure pulses applied to the upper airway (393), suggesting that the transmission of this particular reflex is not modulated by 5-HT in the XII nucleus.

Reflex transmission may be modulated in a state-dependent manner at the site where peripheral receptor afferents make the first synaptic contact in the CNS. There are 5-HT and NE terminals and receptors in the NTS, the main projection site of viscerosensory afferents (173,179,186–189,214,222,223,394–396). Stimulation of cells in the medullary raphe, as well as microinjections of serotonergic agonists into the NTS, suppresses transmission in pathways mediating cardiovascular reflexes (397,398). There is also evidence that substance P, a peptide often co-localized with 5-HT, modulates transmission of certain vagal reflexes (399,400). Since inhibitory 5-HT and NE receptors predominate in the NTS, visceral reflex transmission at the level of the first central synapse may be relatively more suppressed during wakefulness than in sleep. In rats subjected to chronic intermittent hypoxia, there is a documented sprouting of both noradrenergic and serotonergic terminals in the XII nucleus (96), but the effect is probably not limited to this motor nucleus and may involve increased aminergic innervation of those brainstem regions that transmit reflex effects from the upper airway.

In summary, reflexes from airway mechanoreceptors probably play an important role in the control of upper airway motoneurons during both wakefulness and sleep. Their magnitude measured at the motoneuronal or motor output levels will vary with the sleep–wake cycle as a result of both state-dependent changes in upper airway motoneuronal excitability and sleep-specific central processes that may modulate transmission in selected reflex pathways. Furthermore, OSA-related changes in the motor and sensory innervation of the upper airway and in central reflex-transmitting pathways may alter the magnitude of upper airway reflexes in a disorder-specific manner.
EFFECTS OF RECURRENT DISRUPTIONS OF SLEEP AND BREATHING ON UPPER AIRWAY MOTONEURONS

Severe sleep deprivation may have a distinct effect on the control of orofacial motoneurons, as evidenced by the observation that it impairs motor aspects of speech (401). Sleep-related decrements in the activity of upper airway motoneurons and the frequency and severity of obstructive apneic episodes are exacerbated by sleep deprivation (402–405). Although the neurochemical basis of this is unknown, the effect may be mediated by hypothalamic neurons whose excitability varies with the accumulation of sleep debt and circadian time (406–409). A determination of the interactions between the mechanisms of sleepiness and those controlling upper airway motoneurons is critical for our understanding of the pathophysiology of OSA.

A chronic need to cope with recurring nocturnal hypoxemia may also cause changes in upper airway motor control, the central control of sleep, and metabolic regulation. Severe chronic intermittent hypoxia reduces the responsiveness of XII motoneurons to stimulants (410), increases the drive for sleep (411), and alters contractile properties of upper airway muscles (412). In rats, exposure to moderate chronic intermittent hypoxia similar to that experienced by OSA patients causes changes in hypothalamic gene expression important for both metabolism and the regulation of sleep (413), and, in obese mice, chronic intermittent hypoxia leads to hyperlipidemia (414). The mechanisms underlying these changes remain to be elucidated, but they probably include alterations in central aminergic transmission that, in turn, may cause altered functioning of many brain systems involved in cognitive, affective, and autonomic regulation.

SUMMARY AND CONCLUSIONS

In this chapter, we have discussed selected physiological mechanisms that, under normal conditions, are important for the maintenance of upper airway motor tone during wakefulness, and contribute to the loss of this tone during sleep. Such mechanisms have been presented as distinct “drives” that undergo state-specific changes upon transitions from wakefulness to SWS and REM sleep, as determined primarily in animals and humans with normal upper airways. It is likely that a reduced airway patency in OSA patients leads to a host of reflex and/or behavioral changes in the control of upper airway motor tone, including exaggerated decreases of this tone during sleep that appear in association with an increased baseline level of activity in wakefulness. Unfortunately, attempts to distinguish between the physiological mechanisms that are normally present and the pathological mechanisms specific to OSA are hampered by the limited availability of adequate animal models (415). With the limited availability of English bulldogs (83) and other large mammalian species (416–418), the focus has been on rodents in which one can mimic certain aspects of OSA through genetic manipulations (419–421) or interventions such as cyclical intermittent hypoxia (410–414,422–423; reviewed in Refs. 18,20). Even though rodents do not have nocturnal airway occlusions, only in rodents will one be able to conduct comprehensive studies of the neuroanatomical, neurochemical, and molecular alterations in the neural control of the upper airway that may occur in OSA. It would be desirable to develop rodent models with chronic upper airway obstructions, possibly using a combination of genetic manipulations and surgical interventions.

Current treatments for OSA aim to enlarge the airway. The most common treatment, CPAP, does just this, thereby eliminating, rather than taking advantage of, physiological reflexes that naturally enhance upper airway dilator muscle activity in response to negative pressure in the airway. Despite several attempts, no satisfactory treatment for OSA has yet been developed on the basis of extensive information about the physiology and pharmacology of the central control of upper airway muscles. The data reviewed in this chapter suggest that increasing the noradrenergic, serotonergic, histaminergic, and/or orexinergic activation of upper airway motoneurons during sleep and enhancing the reflex excitatory drives to upper airway motoneurons represent potential strategies for the development of such new treatments.
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