Archives Italiennes de Biologie, 149: 406-425, 2011.

The tongue and its control by sleep state-dependent modulators

R.L. HORNER

Department of Medicine and Department of Physiology, University of Toronto, Toronto, Ontario, Canada

ABSTRACT

The neural networks controlling vital functions such as breathing are embedded in the brain, the neural and chemical environment of which changes with state, i.e., wakefulness, non-rapid eye movement (non-REM) sleep and REM sleep, and with commonly administered drugs such as anaesthetics, sedatives and ethanol. One particular output from the state-dependent chemical brain is the focus of attention in this paper; the motor output to the muscles of the tongue, specifically the actions of state-dependent modulators acting at the hypoglossal motor pool. Determining the mechanisms underlying the modulation of the hypoglossal motor output during sleep is relevant to understanding the spectrum of increased upper airway resistance, airflow limitation, hypoventilation and airway obstructions that occur during natural and drug-influenced sleep in humans. Understanding the mechanisms underlying upper airway dysfunction in sleep-disordered breathing is also important given the large and growing prevalence of obstructive sleep apnea syndrome which constitutes a major public health problem with serious clinical, social and economic consequences.

Key words

Sleep • Hypoglossal Motor Nucleus • Tongue • Genioglossus Muscle • Medulla • Obstructive Sleep Apnea

Rationale for focus on the tongue musculature

The tongue is a moveable mass of muscular tissue that is attached to the floor of the mouth (Fig. 1). The tongue is the organ of taste, aids in the mastication and swallowing of food as well as contributing to effective ventilation by keeping the pharyngeal airspace open to allow for the effective passage of air into the lungs. The respiratory function of the tongue can be compromised during sleep as a consequence of the naturally occurring changes in brain state that lead to changes in the neural and chemical inputs to the hypoglossal motor pool, the source of motor output to the muscles of the tongue. Ingestion of commonly used drugs can also change the neural and chemical inputs to the hypoglossal motor pool, so also influencing the ability of the tongue to keep the upper airway open for effective breathing. Examples of such commonly used drugs that influence brain chemistry include anaesthetics, sedative hypnotics and ethanol, all of which can also significantly influence tongue muscle activity and can impair breathing in some individuals. Such agents can exert their influences on tongue muscle activity via direct actions on key components of the respiratory network, including the hypoglossal motor pool, as well as the state-dependent neural inputs that modulate hypoglossal motor activity as a function of changes in sleep-wake states. This paper reviews the control of the tongue during sleep by state-dependent neuromodulators.

Corresponding Author: Richard L. Horner, Ph.D., Room 3206 Medical Sciences Building, University of Toronto, 1 Kings College Circle, Toronto, Ontario, Canada M5S 1A8. - Tel.: +1 416 946 3781 - Fax: +1 416 971 2112 - Email: richard.horner@utoronto.ca

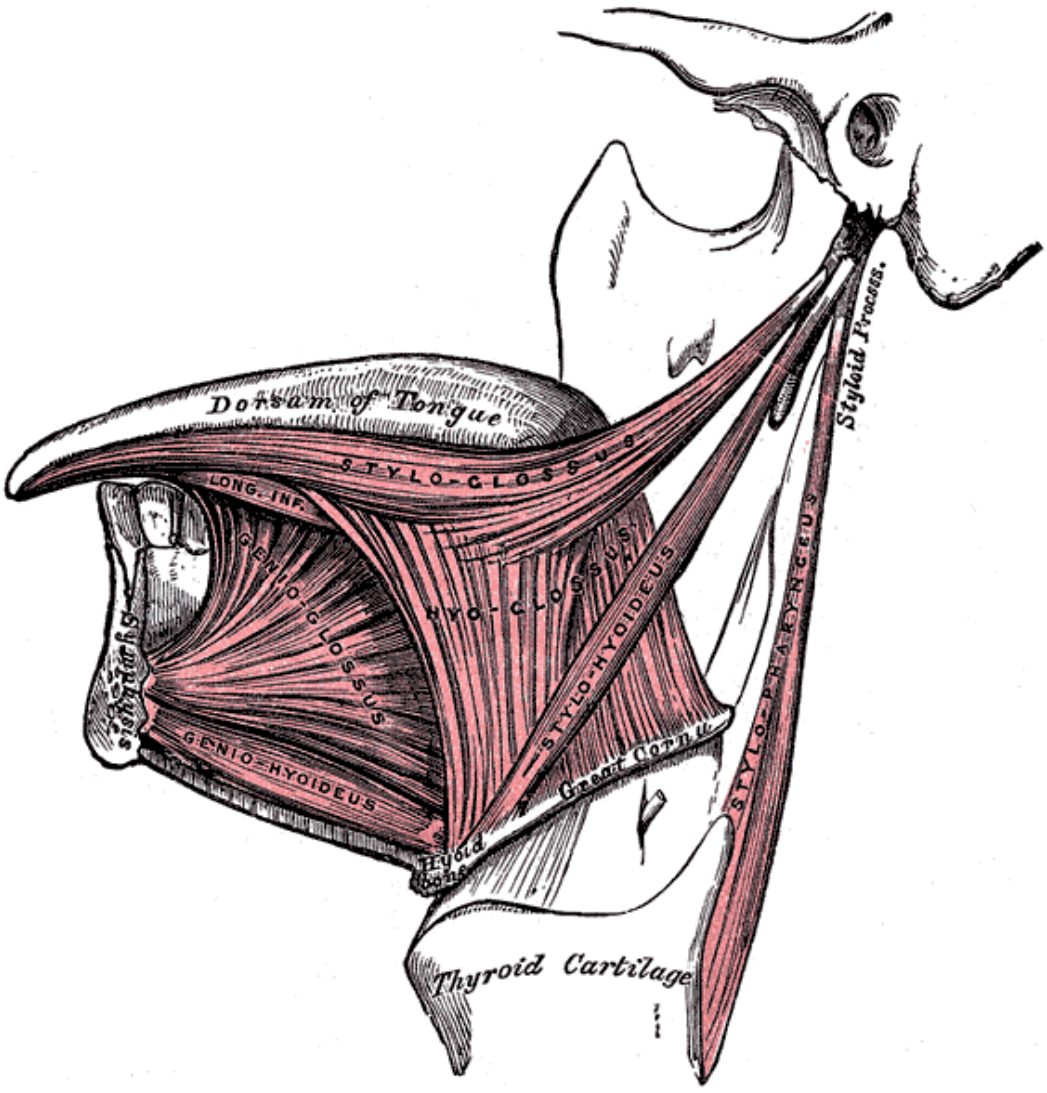

Fig. 1. - The major extrinsic muscles of the tongue and the regional anatomy of the upper airway showing that the hyoid bone effectively "floats" in a web of muscular attachments. This arrangement creates a compliant upper airspace that has major implications for its respiratory function in supine sleeping humans. *From* Gray H: Gray's Anatomy, image licensed under Creative Commons.

Structure of the tongue

The tongue is comprised of both intrinsic and extrinsic muscles, the former having their origin and insertion within the tongue, and the latter outside the tongue. The intrinsic muscles are comprised of the superior and inferior longitudinales, and the traverses and verticals linguae; the extrinsic muscles comprise the genioglossus, hyoglossus, styloglossus, and palatoglossus (Bailey and Fregosi, 2006). The hypoglossal motor pool gives rise to the hypoglossal nerve which innervates both the intrinsic and extrinsic tongue muscles (with the exception of the palatoglossus which is innervated by the pharyngeal plexus). Co-activation of these intrinsic and extrinsic tongue muscles during eupnea, hypercapnia, and hypoxia contributes to enlarging and/or stiffening the upper airspace with the mechanical consequence of an increased resistance to airway collapse (Horner, 1996; Fuller et al., 1999; Bailey et al., 2006). Decreased tongue muscle activity in sleep is critically involved in the pathogenesis of upper airway narrowing and closure during sleep in individuals with an airway that is anatomically vulnerable to collapse (Remmers et al., 1978). Nevertheless, decreased

activity of other pharyngeal muscles during sleep, such as those in the soft palate innervated by trigeminal motoneurons, also contributes to increased upper airway resistance and the predisposition to airway occlusion in sleep in such anatomically-vulnerable individuals (Tangel et al., 1991).

The particular vulnerability of the human upper airway to narrowing and obstructions during sleep has largely been attributed to differences in anatomy between humans and other land mammals. The two main reasons for the particular vulnerability of the *human* upper airway to narrowing and obstructions during sleep are briefly discussed below in order to emphasize the important role that the tongue has in keeping the upper airspace open, and to also place in context the influence of state-dependent modulators which act at the hypoglossal motor pool to modulate tongue muscle activity and therefore alter the ability of the upper airspace to act as an effective conduit for airflow. Ultimately, determining the mechanisms underlying the modulation of the hypoglossal motor pool during sleep is relevant to understanding the spectrum of increased upper airway resistance, airflow limitation, hypoventilation and airway obstructions that occur during natural and drug-influenced sleep in humans.

Consequences of the "floating" hyoid bone of humans

The hyoid bone has the unique distinction that it is the only bone in the human skeleton that is not articulated to any other bone by means of a joint. Several muscles and tendons are attached to the hyoid bone, so in effect this bone is tied to its various anchor points. Essentially, the hyoid bone "floats" in a web of muscular attachments stretching anterior, posterior, rostral and caudal (Fig. 1). Some of the muscles attached to the hyoid bone are those comprising the floor of the oral cavity and which attach to the mandible (geniohyoid, mylohyoid and the anterior belly of the digastric), while the hyoglossus muscle connects the hyoid bone to the body of the tongue proper. The stylohyoid muscle links the hyoid superiorly to the styloid process, that slender piece of bone that extends from the skull just below the ear and which also serves as an anchor point for several muscles associated with the tongue. The middle pharyngeal constrictor muscle arises from the whole length of the hyoid bone, from which the fibres wrap around to comprise the posterior wall of the pharyngeal airway where they insert into the midline fibrous raphé, so meeting the fibres originating from the muscle of the opposite side. Several muscles also link the hyoid bone inferiorly to the sternum (sternohyoid), the upper border of the scapula (omohyoid) and the thyroid cartilage which supports and protects the larynx and vocal cords (thyrohyoid muscle). By virtue of these connections and attachments to muscles linking the floor of the mouth, the tongue, the lateral and posterior pharyngeal walls, the larynx, sternum and scapula, the hyoid bone effectively braces the tongue, pharyngeal and laryngeal structures against each other so providing a broader range of movements and sound production capabilities that could not otherwise be achieved with a fixed structure (Fig. 1).

Although this anatomical arrangement of the hyoid bone in humans has the mechanical advantage of being permissive for the development of sophisticated motor behaviours such as speech, it also has the mechanical disadvantage of creating a more compliant architectural support for the airway walls. This compliant human upper airway is particularly vulnerable to collapse during sleep when muscle tone is reduced, especially in obese individuals in whom the weight of fat deposits in the neck can displace the hyoid bone in the posterior direction and further narrow the airspace behind the tongue (Koenig and Thach, 1988; Kuna and Remmers, 2000). The supine sleeping position that is peculiar to humans also renders the upper airspace vulnerable to collapse (Cartwright, 1984), a situation that likely involves tissue weight causing posterior movement of the hyoid.

Unlike humans, the hyoid bone in many other mammals is anchored to posterior structures by more rigid bony, rather than muscular, attachments. In the dog, for example, the hyoid is attached caudally to the thyroid cartilage via the thyrohyoid bone, and rostrally to the mastoid process of the skull via the stylohyoid bone and the short rod-like tympanohyoid cartilage (Evans and Christensen, 1979). This anchoring by fixed bony structures and cartilage (rather than via a web of soft tissue) provides for more structural stability, less mobility, and diminished capacity for vocalization, and these are likely some of the major reasons for the lack of airway obstructions in almost all animals other

than humans. Even obese mice (Brennick et al., 2009) and rats (Sood et al., 2007), and massively obese Vietnamese pot-bellied pigs have remarkably stable upper airspaces and do not have obstructive sleep apnea (Tuck et al., 1999; Tuck and Remmers, 2002a, b). The English bulldog, which has arisen as a product of selective breading rather than natural selection exhibits occasional obstructive apneas and hypopneas but these are largely confined to rapid eye movement (REM) sleep (Hendricks et al., 1987). In this case, the anatomical structures of this animal have been pushed to the limit by artificial selection, with the airway being severely crowded by the brachycephalic facial structure.

Consequences of the descent of the larynx in humans

The anatomical capacity for speech was actualized by descent of the larynx in homo sapiens which had the result of enlarging the separation of the tip of the soft palate from the epiglottis. These structures are much closer together in other species, as well as human babies, and can even overlap in some animals such as certain breeds of dogs and horses. The longer tract of tissue above the larynx of humans is well adapted to modify and expand the range of sounds produced by the vocal cords, by means of altering pharyngeal size and shape using the tongue and lips, and is responsible for producing the rich array of vocalizations that are uniquely human. The compromise, however, of this adaptation is that the large, bulky and muscular tongue now makes up the major anterior wall of the upper airspace, and this structure along with the compliant lateral pharyngeal walls can encroach on the available space, especially when supine and with the suction pressures generated during breathing.

Of additional relevance is that men, despite having overall larger airway volumes than women, on average have longer airways and thicker soft palates. Mathematical modeling in a reconstructed average male and female airway shows that these architectural features, particularly the longer airway, make the male airspace substantially more collapsible than its female counterpart (Malhotra et al., 2002); an essential difference being that one side of the airway wall is made up of a highly moveable structure, the tongue, with the male airway having a longer section of such a moveable wall. Such anatomical differences between men and women may contribute to the more collapsible airways of men, as assessed by the level off suction pressure required to cause airway collapse in the passive airway for any given degree of obesity (Jordan et al., 2005; Kirkness et al., 2008).

Importance of state-dependent inputs to the tongue

The upper airway is narrower and more collapsible in obstructive sleep apnea patients, and obesity is one of several factors that can cause this narrowing. Despite these factors, however, it is important to note that the airway remains open in wakefulness and closes *only* in sleep. Therefore, even in individuals with a narrow upper airway, airway closure is ultimately caused by the impact of state-dependent mechanisms on motor outflow to the pharyngeal muscles, whose tone is necessary and sufficient to keep the airway open in wakefulness. Accordingly, the next section reviews the neuronal groups involved in sleep-wake regulation and their potential for modulating tongue muscle activity. The final section details the state-dependent neural and chemical inputs to the hypoglossal motor pool and their effects on motor activity, as well as addressing the effects on hypoglossal motor activity of commonly used drugs.

Experimental models of tongue motor control by sleep state-dependent modulators

Many studies performed over the last 10 years have examined the control of hypoglossal motoneurons and hypoglossal motor output by sleep state-dependent modulators, both in *in vitro* and *in vivo* animals models, the latter including studies in natural sleep. Importantly, such studies in intact animals across natural sleep and awake states have provided the necessary extension of the prior fundamental studies of the cellular and network control of hypoglossal motoneurons performed *in vitro* (Bellingham and Funk, 2000; Berger, 2000; Rekling et al., 2000), and the *in vivo* studies performed in decerebrate or anesthetized animals in a pharmacologically-induced “REM sleep-like” state (Kubin et al., 1998). Because studies of the neurobiology of pharyngeal motor control across natural sleep-wake states have, so far, only been performed in rats which do not experience sleep-disordered breathing (the particular predisposition of humans to such problems was

outlined above), such studies need to be viewed in the context of identifying fundamental mechanisms of neural control that cannot otherwise be directly identified in humans.

Neurobiology of sleep and awake states

The rationale for why certain neuromodulators and neural systems acting at the hypoglossal motor pool are the focus of particular attention in this review is based on an understanding of the neural mechanisms underlying the control of sleep and awake states, and these are reviewed here.

Wakefulness-generating systems

Fig. 2A shows some of the major neuronal groups contributing to the ascending arousal system from the brainstem that activates the forebrain to produce electrocortical arousal in wakefulness. This ascending arousal system has two major pathways: The dorsal pathway from the cholinergic laterodorsal and pedunculopontine tegmental nuclei activates thalamic neurons and so influences electrocortical activity via glutamatergic thalamocortical projections (Jones, 2000; Saper et al., 2005). The ventral pathway traverses the hypothalamus and includes the aminergic arousal system, which originates from brainstem neuronal groups principally containing serotonin (dorsal raphé nuclei), noradrenaline (locus coeruleus), histamine (tuberomammillary nucleus) and dopamine (ventral periaqueductal grey, not shown in Fig. 2A). Orexin neurons from the perifornical region of the hypothalamus and cholinergic neurons from the basal forebrain also contribute to this ascending arousal system, and so contribute to cortical activation and wakefulness (Jones, 2000; Saper et al., 2005). Orexinergic innervation of brainstem arousal neurons also promotes wakefulness by stimulating and reenforcing the ascending wakefulness drive.

In addition to activating the thalamus and cortex, the ascending aminergic arousal system also sends inhibitory projections to non-REM sleep promoting neurons in the ventrolateral pre-optic area (VLPO) (Fig. 2A). This wiring circuit constitutes a "flip-flop" switch or bi-stable oscillator where reciprocal inhibition between arousal and sleep-active neurons prevents intermediate states, and serves to consolidate brain arousal in wakefulness by simultaneously inhibiting sleep-active neurons (McGinty and Szymusiak, 2000; Saper et al., 2001).

In summary, multiple arousal systems contribute to the maintenance of an alert, awake brain. Importantly, these neuronal systems are also positioned to influence the respiratory network and the hypoglossal motor pool by descending projections to the medulla (Fig. 2A), the functional and physiological implications of which are discussed below.

Non-REM sleep-generating systems

Non-REM sleep is actively generated by neurons in the VLPO, anterior hypothalamus and basal forebrain (only the VLPO is shown in Fig. 2B for clarity) (Jones, 2000; Saper et al., 2005). VLPO neurons become active in non-REM sleep, an effect strongly influenced by the thermal stimulus that accompanies the circadian-mediated decline in body temperature at normal bedtime (McGinty and Szymusiak, 2000). Importantly, activation of VLPO neurons leads to a direct suppression of cortical arousal via ascending inhibitory cortical projections, but VLPO neurons also promote sleep by descending inhibition of the aforementioned brainstem arousal neurons (Fig. 2B) (McGinty and Szymusiak, 2000; Saper et al., 2005). This neuronal inhibition of brainstem arousal is mediated by the inhibitory amino acid γ-amino butyric acid (GABA) (McGinty and Szymusiak, 2000; Saper et al., 2005), explaining the sedative-hypnotic effects of barbiturates, benzodiazepines, imidazopyridine compounds, ethanol and volatile anesthetics that enhance GABA-mediated neuronal inhibition via interactions with binding sites on $GABA_A$ receptors (Mihic et al., 1997; Mendelson, 2000; Orser, 2006; Franks, 2008; Franks and Zecharia, 2011). Imidazopyridine compounds such as zolpidem are benzodiazepine receptor ligands but differ structurally from benzodiazepines. Importantly, $GABA_A$ receptors are also strongly implicated in respiratory control and are present throughout the respiratory network (McCrimmon et al., 1995), excessive stimulation of which can promote respiratory depression (Robinson and Zwillich, 2000). The influence of GABA and commonly used drugs that exert a GABA-mimetic profile are reviewed in a later section of this paper.

Overall, this description of the change in neuronal activity patterns as a function of sleep-wake state

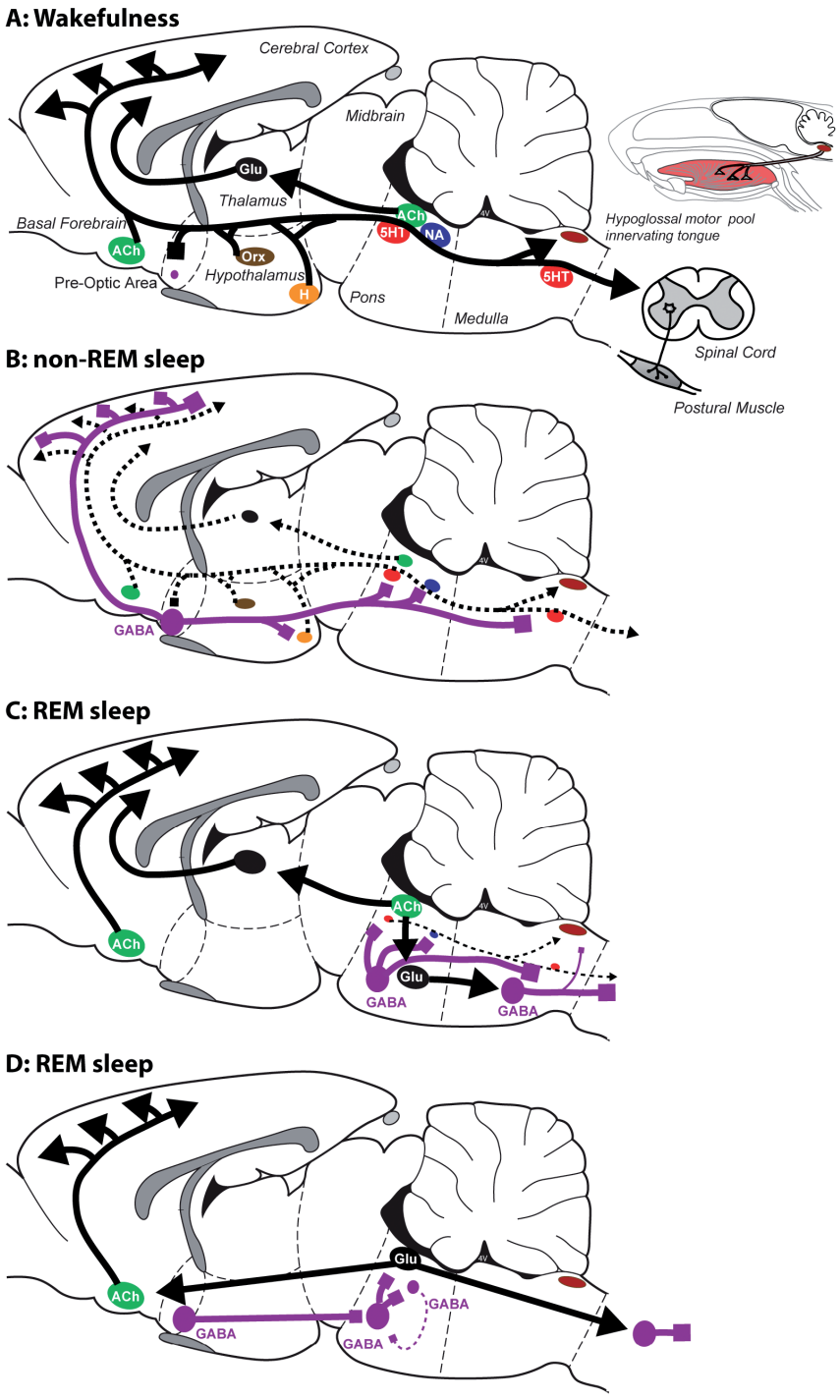

Fig. 2. - Wakefulness-generating (A), non-REM sleep-generating (B) and REM sleep-generating (C, D) neuronal systems in the brain. These neuronal systems are also positioned to influence respiratory network and hypoglossal motor activities via descending projections to the medulla. Solid lines indicate active neuronal groups and projections respectively, dashed lines and decreased symbol size indicates suppressed activity. → indicates excitatory projections, —■ indicates inhibitory projections. In this scheme the progressive suppression of hypoglossal motor output to the muscles of the tongue from wakefulness to non-REM sleep is mediated by disfacilitation, with further disfacilitation in REM sleep. REM sleep also recruits inhibitory pathways involving glycine and GABA, although the magnitude of their involvement in the suppression of tongue muscle activity in REM sleep (as opposed to spinal motor activity) is debated. See text for further details. *Abbreviations*: Ach, acetylcholine; 5HT, 5-Hydroxytryptamine (serotonin); GABA, γ-amino butyric acid; Glu, glutamate; H, Histamine; NA, noradrenaline; Orx, Orexin.

emphasizes that non-REM sleep is triggered by increased sleep-state dependent GABAergic neuronal activity, and is accompanied by a massed and coordinated withdrawal of activity of brainstem arousal neurons comprising serotonergic, noradrenergic, histaminergic and cholinergic neurons. GABAergic VLPO neurons therefore have a "non-REM-on" activity profile, whereas brainstem serotonergic, noradrenergic, histaminergic and cholinergic neurons have a "wake-on" activity profile and exhibit decreased activity from wakefulness to non-REM sleep. These changes in activity of brainstem arousal neurons are of particular relevance given the influence of these neuromodulators on hypoglossal motor activity (see below).

REM sleep-generating systems: aminergic-cholinergic interactions

In the aminergic-cholinergic explanation of REM sleep generation, decreased dorsal raphé and locus coeruleus activity, preceding and during REM sleep, progressively withdraws inhibition of pontine cholinergic neurons of the laterodorsal and pedunculopontine tegmental nuclei via reduced serotonin and noradrenaline acting on inhibitory serotonin 1A and α_2 receptors respectively (McCarley et al., 1995; Thakkar et al., 1998). Activation of these cholinergic neurons via this process of disinhibition then increases acetylcholine release into the pontine reticular formation (PRF) which is thought to then trigger REM sleep (Fig. 2C) (McCarley et al., 1995; Kubin, 2001; Lydic and Baghdoyan, 2005). Dorsal raphé and locus coeruleus neurons continue to remain silent in REM sleep via sustained GABAergic inhibition during the REM episode (Fig. 2C) (Nitz and Siegel, 1997a; Nitz and Siegel, 1997b). The central role of cholinergic neurons in generating REM sleep stems, primarily, from the evidence showing that pontine cholinergic mechanisms have the capacity to influence REM sleep expression. Such evidence includes enhancement of REM sleep following cholinergic stimulation of the pontine reticular formation (PRF), elevated acetylcholine concentrations at the PRF during REM sleep and following electrical stimulation of the pedunculopontine tegmental nucleus (PPTn), projections from the PPTn to the PRF, and the activity profile of REM sleep-active PPTn being consistent with their involvement in REM sleep (Morales et al., 1987b; Horner and Kubin, 1999; Hobson et al., 2000; Kubin, 2001; Hobson and Pace-Schott, 2002; Lydic and Baghdoyan, 2003, 2005; Steriade and McCarley, 2005).

REM sleep-generating systems: glutamatergic-GABAergic interactions

Despite the experimental support gathered over the last 25 years for the interaction of pontine monoaminergic (serotonergic and noradrenergic) and cholinergic neurons as being associated with REM sleep phenomena, new evidence implicates a core glutamatergic-GABAergic mechanism of REM sleep generation (Boissard et al., 2002; Lu et al., 2006), for reviews see (Luppi et al., 2006; Siegel, 2006; Fuller et al., 2007). In this explanation the brainstem aminergic and cholinergic groups are characterized as REM sleep modulators and not as REM sleep generators per se, a significant change in emphasis that has not yet been resolved (Siegel, 2006; McCarley, 2007). Fig. 2D illustrates the major features of this glutamatergic-GABAergic mechanism of REM sleep generation and the anatomical location of the pertinent neuronal groups involved. In this framework, activation of GABAergic REM sleep active neurons in the extended VLPO leads to inhibition of GABAergic inhibitory neurons in the pons (ventrolateral periaqueductal grey and lateral pontine tegmentum, vlPAG/LPT), which then leads to dis-inhibition of neurons in the sublaterodorsal tegmental nucleus (SLDn). The resulting activation of GABAergic SLDn neurons leads to feedback inhibition of vlPAG/LPT which acts to sustain the REM sleep episode. Importantly, the activation of glutamatergic SLDn neurons in REM sleep leads to cortical activation via ascending projections and activation of basal forebrain cholinergic neurons, and recruitment of descending inhibitory pathways that result in suppression of postural motor activity (see below). Accordingly, this glutamatergic-GABAergic mechanism of REM sleep generation is able to explain the manifestation of the cardinal signs of REM sleep independently of the aminergic-cholinergic interactions (Boissard et al., 2002; Lu et al., 2006; Luppi et al., 2006; Fuller et al., 2007).

Suppression of somatic spinal motor activity in REM sleep

Among other differences in the mechanisms of REM sleep generation between Figs. 1C and 1D, it is notable that the motor suppression of REM sleep is pro-

duced by a different pathway in each mechanism. In the aminergic-cholinergic mechanism of REM sleep generation, cholinergic stimulation of the PRF leads to motor suppression mediated by descending pathways involving glutamate-mediated activation of medullary reticular formation relay neurons (Siegel, 2000) that are inhibitory to spinal motoneurons via release of glycine (predominantly) and GABA (Fig. 2C) (Chase and Morales, 2000). A distinguishing feature of this scheme is the recruitment of inhibitory medullary reticular formation relay neurons, and it has been a long standing expectation that hypoglossal motoneurons must also be specifically inhibited in REM sleep by this same mechanism. Whether there is glycinergic or GABAergic inhibition of hypoglossal motoneurons in REM sleep is reviewed in the final section of this paper.

In contrast to the aminergic-cholinergic mechanism of REM sleep generation, the descending motor suppression pathways recruited in the glutamatergic-GABAergic mechanism does not require a relay in the medial medulla (Lu et al., 2006). Rather, the REM sleep-active pontine neurons are thought to produce atonia in spinal motoneurons via long glutamatergic projections to the ventral horn of the spinal cord where they activate local glycine and GABAergic interneurons that then inhibit motor activity (Lu et al., 2006). Currently it is not known if the pontine REM sleep-active glutamatergic neurons that send long descending axons to glycinergic inhibitory interneurons in the spinal cord (Fig. 2D) also send collaterals to cranial motoneurons such as the hypoglossal and trigeminal motor nuclei.

Summary

Following this review of the neural mechanisms underlying the control of sleep and arousal states it is clear that a number of neuromodulators and neural systems show changes in activity across sleep-wake states. Given the projections of such sleep-state dependent neural systems to respiratory neurons and respiratory motoneurons, including the hypoglossal motor pool which is the main focus of this review, there is a strong rationale to determine the functional implications of this changing neurochemical environment on the control of hypoglossal motor activity and this is the focus for the remainder of this paper.

Impact of sleep state-dependent neuromodulators on hypoglossal motoneurons and tongue muscle activity

Excitatory influences

There are excitatory serotonergic (Berger et al., 1992; Kubin et al., 1992; Al-Zubaidy et al., 1996; Woch et al., 1996) and noradrenergic influences on hypoglossal motoneurons (Aldes et al., 1992; Parkis et al., 1995; Al-Zubaidy et al., 1996; Rukhadze and Kubin, 2007), mediated principally by serotonin 2A receptors (Fenik and Veasey, 2003) and α_1 adrenergic receptors respectively (Funk et al., 1994; Parkis et al., 1995; Al-Zubaidy et al., 1996). Histaminergic neurons also project to medullary and pontine regions that contain respiratory neurons and motoneurons, including the hypoglossal motor pool (Watanabe et al., 1984; Iwase et al., 1993) where histamine also exerts excitatory effects via histamine type 1 receptors (Bastedo et al., 2009). The inputs from medullary raphé neurons also contain co-released thyrotropin releasing hormone and Substance P that are also excitatory to hypoglossal motoneurons (Johansson et al., 1981; Rekling, 1990; Kachidian et al., 1991; Bayliss et al., 1992). Glutamate is also co-released from pontine noradrenergic neurons (Liu et al., 1995). Interestingly for the trigeminal motor pool, noradrenaline increases motor activity indirectly by potentiating glutamate-mediated excitation while adrenergic stimulation by itself had no effect on motor output, as measured by masseter muscle activity (Schwarz et al., 2008).

Disfacilitation mediated by withdrawal of tonic excitatory influences in sleep

As Fig. 2 illustrates, the activity of brainstem serotonergic, noradrenergic and histaminergic neurons is highest in wakefulness, reduced in non-REM sleep and minimal in REM sleep. Brainstem reticular formation neurons are a likely source of glutamatergic inputs to the respiratory network (Orem and Kubin, 2000), and are positioned to also provide a source of tonic drive to respiratory neurons and motoneurons, with this influence altering from wakefulness to non-REM and REM sleep. Together, coordinated withdrawal of these excitatory inputs in sleep are therefore positioned to contribute to decreased genioglossus activity via a process of disfacilitation (Kubin

et al., 1998; Burgess et al., 2008; Horner, 2010). This section summarises the roles of serotonergic, noradrenergic, histaminergic and glutamatergic influences on hypoglossal motoneurons and tongue motor activity. For more detailed treatment the interested reader is referred to other reviews (Kubin and Davies, 2002; Horner, 2008, 2009).

Noradrenaline

Noradrenaline depolarizes and increases the excitability of hypoglossal motoneurons *in vitro* (Funk et al., 1994; Parkis et al., 1995; Al-Zubaidy et al., 1996). In intact animals, brainstem noradrenaline-containing neurons show declining discharge rates from wakefulness to non-REM and REM sleep (Fig. 2A-C). Fig. 3 shows the effects of α_1 receptor antagonism at the hypoglossal motor pool on tongue muscle activity across sleep-wake states. The decrease in respiratory-related and tonic tongue muscle activity following α_1 receptor antagonism at the hypoglossal motor pool in wakefulness indicates the presence of a functional endogenous noradrenergic drive to this motor pool when awake. The levels of respiratory-related tongue muscle activity in non-REM sleep were maintained chiefly by noradrenergic mechanisms because α_1 receptor antagonism decreased this activity to levels similar to those observed in REM sleep. That respiratory-related tongue muscle activity in sleep was minimal following α_1 receptor antagonism at the hypoglossal motor pool, and that there was then no subsequent significant change from non-REM to REM sleep, also implicates withdrawal of endogenous noradrenergic inputs to the hypoglossal motor pool as a major contributor to periods of tongue motor suppression in REM sleep (Chan et al., 2006; Horner, 2009). This latter result in natural REM sleep also fits with similar findings from the carbachol model of REM sleep in anesthetized rats, i.e., withdrawal of an endogenously active noradrenergic input contributes to suppression of hypoglossal motor activity (Fenik et al., 2005). Electrical or cholinergic stimulation of the pontine reticular formation also reduces both genioglossus activity and noradrenaline levels at the hypoglossal motor nucleus (Lai et al., 2001), consistent with the notion that suppression of motor activity following activation of the pontine REM sleep triggering region is associated with reduced excitatory noradrenergic inputs to the hypoglossal motor nucleus. The involvement of the locus coeruleus in facilitating somatic motor tone and the disfacilitation following reduction in noradrenaline release has recently been confirmed with optogenetic techniques (Carter et al., 2010; McGregor and Siegel, 2010).

Together, these observations do not support the old claim that active inhibition is the *only* explanation for motor suppression in REM sleep (Chase and Morales, 2000); disfacilitation also plays a significant and important role (Lai et al., 2001; Fenik et al., 2005; Chan et al., 2006; Carter et al., 2010; McGregor and Siegel, 2010).

Noradrenergic neurons of the locus coeruleus and sub-coeruleus show progressive decreases in activity from wakefulness to non-REM and REM sleep (Aston-Jones and Bloom, 1981). A significant noradrenergic innervation of the hypoglossal motor nucleus arises from sub-coeruleus (Aldes et al., 1992; Aston-Jones et al., 1995; Rukhadze and Kubin, 2007) with this region also providing an excitatory drive to spinal motoneurons (Guyenet, 1980; Liu et al., 1995). Although A1 and A7 neurons also have significant projections to the hypoglossal motor pool (Aldes et al., 1992; Rukhadze and Kubin, 2007), the sleep-state dependent activity of those neurons is not as well studied. When Fos protein expression is used as an indirect marker of the level of neuronal activity, the expression profiles suggest that A7 neurons have reduced or abolished activity in the carbachol model of REM sleep whereas A1 cells do not (Rukhadze et al., 2008). Fos protein expression in rostral A5 neurons is also reduced in the carbachol model of REM sleep (Rukhadze et al., 2008) in agreement with electrophysiological recordings although such recordings from a sample of A5 neurons also indicated that they did not project to hypoglossal motor pool (Fenik et al., 2002). This lack of electrophysiological evidence for projections to the hypoglossal motor pool, however, contrasts with projections from A5 to the hypoglossal motor nucleus identified by retrograde tracing (Rukhadze and Kubin, 2007). Overall, these data suggest that sub-coeruleus and perhaps A7 or A5 neurons are the most likely source of the endogenous excitatory noradrenergic drive modulating hypoglossal motor activity across sleep-wake states.

In summary, an endogenous excitatory noradrenergic drive contributes to the levels of tonic tongue motor activity and expression of respiratory-related activity in wakefulness, with this drive being

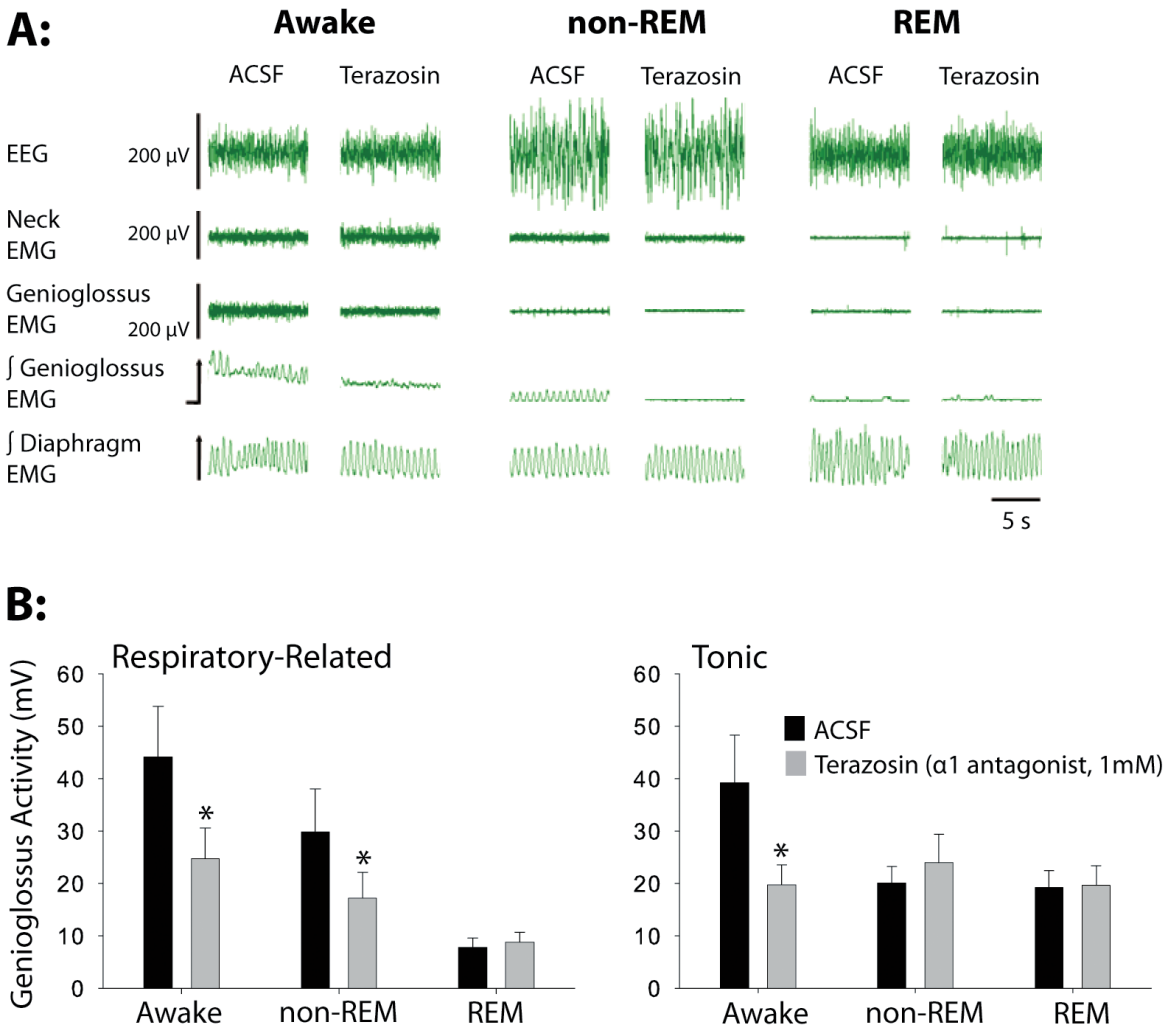

Fig. 3. - Example (A) and group data (B) showing the effects of α_1 receptor antagonism with terazosin delivered to the hypoglossal motor pool by reverse microdialysis in intact freely behaving rats in wakefulness and natural sleep. Compared to artificial cerebrospinal fluid (ACSF) controls, terazosin reduced respiratory-related and tonic genioglossus muscle activities in wakefulness, and also reduced respiratory-related genioglossus activity in non-REM sleep to levels indistinguishable from REM sleep; GG activity was lowest in REM sleep and there was no subsequent effect of terazosin. These data suggest a process of progressive disfacilitation of the hypoglossal motor pool from wakefulness to non-REM and REM sleep; see text for further details. Figure modified from Chan et al. (2006) and Horner (2009). *Abbreviations*: * indicates a significant difference from the respective ACSF controls.

withdrawn in non-REM sleep and REM sleep. Identification of this drive is significant because it is the first identification a neural drive contributing to the natural sleep state-dependent activity of a respiratory muscle.

Glutamate

Endogenous glutamatergic activity contributes a second identified tonic excitatory drive to the hypoglossal motor pool that increases tongue muscle activity in wakefulness, the withdrawal of which contributes to reduced activity in sleep (Steenland et al., 2008). This latter drive therefore constitutes the identification of a second neural substrate for the "*wakefulness stimulus*" to a respiratory muscle that modulates the level of tonic motor activity and the expression of respiratory-related activity. A similar tonic excitatory drive to the trigeminal motor

pool also contributes to masseter muscle activity in wakefulness, the withdrawal of which contributes to reduced activity in sleep (Burgess et al., 2008). The excitatory effects of glutamate at the hypoglossal motor pool are mediated by non-*N*-methyl-D-aspartate (non-NMDA) and NMDA receptor activation (Steenland et al., 2006; Steenland et al., 2008).

Serotonin

In the carbachol model of REM sleep in decerebrate cats, withdrawal of endogenous serotonin contributes significantly to decreased genioglossus muscle activity in sleep, especially REM sleep (Kubin et al., 1998). Despite robust genioglossus muscle activation with *delivery* of serotonin to the hypoglossal motor pool *in-vivo* in conscious rats (Jelev et al., 2001) and cats (Neuzeret et al., 2009), however, *endogenous* serotonin plays a much lesser role in the normal modulation of genioglossus muscle activity when tested in rats, at least as assessed by responses to application of serotonergic receptor antagonists (Sood et al., 2005), even in obese Zucker rats (Sood et al., 2007) that have a narrow pharyngeal airway that was postulated to require augmented motor tone to help maintain patency (Nakano et al., 2001; Brennick et al., 2006). Inhibition of serotonergic medullary raphé neurons, the source of serotonin and co-released Substance P and thyrotropin releasing hormone inputs to the hypoglossal motor pool (Manaker and Tischler, 1993), confirmed the lack of significant modulation of genioglossus activity across sleep-wake states by endogenous input from serotonergic neurons (Sood et al., 2006).

This lack of significant modulation of genioglossus activity by endogenous serotonin at the hypoglossal motor pool in intact, freely behaving rats across natural sleep-wake states (Sood et al., 2005; Sood et al., 2006; Sood et al., 2007) contrasts with several previous studies in anesthetized or decerebrate cats and rats in which an endogenous serotonergic drive to the hypoglossal motor nucleus was first demonstrated, by use of either serotonin receptor antagonism or inhibition/destruction of medullary raphé neurons (Kubin et al., 1992; Kubin et al., 1994; Woch et al., 1996; Dreshaj et al., 1998; Fenik and Veasey, 2003; Sood et al., 2003; Brandes et al., 2006; Sood et al., 2006; Zuperku et al., 2008). These latter studies, however, were all performed in the presence of vagotomy, and vagotomy augments the influence of serotonin at the hypoglossal motor nucleus (Sood et al., 2005; Sood et al., 2006).

If this minimal influence of endogenous serotonin on genioglossus muscle activity in intact awake and naturally sleeping animals also applies to humans, it may explain (at least in part) the lack of clinically significant effects on pharyngeal muscle activity and obstructive sleep apnea severity following manipulation of endogenous serotonin in humans (Hanzel et al., 1991; Berry et al., 1999; Kraiczi et al., 1999) and for reviews see (Hudgel and Thanakitcharu, 1998; Horner, 2001; Veasey, 2001, 2003; Smith and Quinnell, 2004; Veasey, 2005). There is one animal model, however, where serotonin appears to play an important role in the maintenance of pharyngeal muscle activity and an open upper airway. Pharyngeal dilator muscle activity and upper airway size are both diminished by systemic administration of the serotonin receptor antagonist ritanserin in awake bulldogs (Veasey et al., 1996). It remains to be determined whether this decreased pharyngeal dilator muscle tone after ritanserin is mediated by antagonism of an augmented serotonin drive to pharyngeal motoneurons that these bulldogs require to compensate for an already anatomically narrow airspace (Veasey et al., 1996), or whether the systemically administered ritanserin is acting elsewhere in the brain to modulate pre-motor regulatory inputs to the tongue.

The interested reader is referred to a recent review (Horner, 2008) for more detailed considerations of the evidence against serotonin having a significant role in maintaining upper airway patency in intact rodents *in vivo*, and an explanation for how serotonergic neurons can show sleep-state dependent activity, project to the respiratory network yet have minimal influence on resting respiratory motor activity.

Histamine

Histamine receptor types 1 and 2 are present in the medulla (Palacios et al., 1981; Traiffort et al., 1992; Iwase et al., 1993) and histaminergic neurons show sleep-state dependent activity (Figs. 1A-B). Like the serotonergic and noradrenergic neuronal components of the aminergic arousal system, therefore, sleep-state dependent changes in histamine are also positioned to modulate respiratory motor activity across sleep-wake states. Histamine at the hypoglossal motor pool increases tongue muscle activity, an

effect mediated by histamine type 1 (but not type 2) receptors (Bastedo et al., 2009). Despite robust genioglossus muscle activation with *delivery* of histamine receptor agonists to the hypoglossal motor pool *in-vivo* in conscious rats (Bastedo et al., 2009) and cats (Neuzeret et al., 2009), as with serotonin there was a lack of an endogenously active excitatory histaminergic drive when tested in rats (Bastedo et al., 2009).

Inhibitory influences

GABA and glycine are the main inhibitory neurotransmitters in the brain. Inhibitory GABA and glycine inputs to hypoglossal motoneurons have been characterized *in vitro* (Umemiya and Berger, 1995; Singer et al., 1998; O'Brien and Berger, 1999; Donato and Nistri, 2000), with co-release of both inhibitory neurotransmitters also occurring onto the same hypoglossal motoneuron (O'Brien and Berger, 1999). Glycine and $GABA_A$ receptor agonists at the hypoglossal motor pool produce robust suppression of tongue muscle activity during normal breathing and during reflex respiratory stimulation by hypercapnia, with antagonism of these receptors reversing the suppression (Morrison et al., 2002; Liu et al., 2003). The relevance of this inhibitory control of the hypoglossal motor pool by glycine and $GABA_A$ receptor mechanisms is reviewed below.

GABA and glycinergic influences in wakefulness and non-REM sleep

Glycine and $GABA_A$ receptors tonically modulate hypoglossal motor activity in freely behaving rats *in vivo*, as evidenced by robust increases in respiratory-related tongue muscle activity in wakefulness and non-REM sleep with glycine and $GABA_A$ receptor antagonism (Morrison et al., 2003b, a). This augmentation of respiratory-related activity at hypoglossal motoneurons may have been caused by blockade of end-inspiratory inhibition mediated by glycine and GABA (for more details see Horner, 2008). The augmentation of respiratory-related tongue muscle activity with $GABA_A$ receptor antagonism at hypoglossal motoneurons can be explained by a tonic GABA input that constrains the rhythmic activation of respiratory motoneurons via gain modulation (Zuperku and McCrimmon, 2002; Zuperku et al., 2008). Nevertheless, this increase in hypoglossal motor activity in wakefulness and non-REM sleep with $GABA_A$ receptor antagonism is not confined to cranial motoneuron pools expressing respiratory-related activity. Tonic increases in masseter muscle activity also occur with glycine and $GABA_A$ receptor antagonism at trigeminal motoneurons in wakefulness and non-REM sleep (Brooks and Peever, 2008). These data indicate that the hypoglossal and trigeminal motor pools are under a prevailing tonic $GABA_A$ and glycinergic inhibition that is readily observed in wakefulness and non-REM sleep.

REM sleep

REM sleep recruits powerful mechanisms that can abolish tongue muscle activity in between the large sporadic bursts that characterize the muscle twitches of REM sleep in this and other motor pools. The neuroanatomy of the REM sleep generating machinery (Figs. 1C-D), and studies in the carbachol model of REM sleep, provide evidence that spinal motoneurons are inhibited by glycine and $GABA_A$-receptor mechanisms in REM sleep (Morales et al., 1987a; Chase and Morales, 2000). However, whether glycine and GABAergic inhibition contributes significantly to the suppression of hypoglossal motor activity in REM sleep has been debated.

Iontophoresis of the glycine receptor antagonist strychnine onto hypoglossal motoneurons abolishes inhibitory post-synaptic potentials recorded during carbachol-induced "REM sleep" in decerebrate or anesthetized cats (Yamuy et al., 1999; Fung et al., 2000). Likewise, increased release of glycine and GABA occurs at both the hypoglossal motor nucleus and the lumbar ventral horn following cholinergic stimulation of the pontine reticular formation in decerebrate cats (Kodama et al., 2003). These latter measurements obtained by microdialysis over several minutes sampling duration, however, may have resulted from the sporadic release of glycine and GABA that could have been responsible for the transient inhibitory post-synaptic potentials recorded in the aforementioned studies with carbachol (Yamuy et al., 1999; Fung et al., 2000), i.e. not from tonic release throughout the REM episode.

In contrast to these *transient* glycine and $GABA_A$ inhibitory events at hypoglossal motoneurons in the carbachol model of REM sleep (Yamuy et al., 1999; Fung et al., 2000), microinjection of glycine or $GABA_A$ receptor antagonists into the hypoglossal motor nucleus failed to reduce the *tonic* suppres-

sion of hypoglossal nerve activity in the carbachol model (Kubin et al., 1993). Microdialysis perfusion of glycine or $GABA_A$ receptor antagonists (alone or in combination) into the hypoglossal (Morrison et al., 2003b, a) or trigeminal (Brooks and Peever, 2008) motor pools in naturally sleeping rats also failed to reverse the *tonic* suppression of tongue or masseter muscle activity in REM sleep. Nevertheless, in both cases glycine and $GABA_A$ receptor antagonism at the hypoglossal and trigeminal motor pools increased the amount and/or magnitude of the *transient* motor activations that also typify normal REM sleep (Morrison et al., 2003b, a; Brooks and Peever, 2008).

The above analysis highlights the differential effects of glycine and $GABA_A$ receptor antagonism on the *tonic* and *phasic* motor events of REM sleep; a minimal effect on the former and a measurable effect on the latter. This differential effect implies a functional role for glycine and $GABA_A$ receptor-mediated inhibition of hypoglossal and trigeminal motoneurons in natural REM sleep, albeit in a different form than previously proposed (Chase and Morales, 2000). An increase in transient motor activity in REM sleep after glycine and $GABA_A$ receptor antagonism may be explained by a suppression of the transient inhibitory potentials that normally serve to oppose the excitatory events that also accompany REM sleep. This "sculpting" of a background tonic excitation by time and amplitude-varying transient inhibitory potentials would have the net result of producing transient flurries of motor activity that are augmented by glycine and $GABA_A$ receptor antagonism (Horner, 2008). Nevertheless, these data refute the old claim that the hypoglossal and trigeminal motor pools receive tonic REM sleep-specific GABA and glycinergic inhibition throughout the REM episode that produces motor atonia.

Influence of drugs augmenting GABA inhibition

Direct application of the sedative hypnotics lorazepam and zolpidem to the hypoglossal motor pool leads to significant suppression of tongue muscle activity that is partially reversed by the $GABA_A$ receptor antagonist bicuculline (Park et al., 2008). This result is to be expected given that such agents enhance GABA-mediated neuronal inhibition via interactions with binding sites on $GABA_A$ receptors. Nevertheless, this suppression of tongue muscle activity indicates the direct effect of sedative-hypnotics on respiratory motoneuron activity. Interestingly, *systemic* administration of these agents, as well as pentobarbital which also augments the post-synaptic effects of GABA at the $GABA_A$ receptor, increases rather than suppresses tongue muscle activity (Younes et al., 2007; Park et al., 2008). In light of the opposite responses elicited by these agents when they are presented directly at the hypoglossal motor pool, this augmenting effect can only be attributed to mechanisms operating outside the hypoglossal motor pool, although the specific pathway has not yet been identified (for further discussion of likely candidate pathways see Park et al., 2008). Nevertheless, these results reveal the principle that the *net* effect of systemically applied $GABA_A$-receptor modulating sedatives on tongue muscle activity is a balance between an inhibitory effect acting locally at the hypoglossal motor pool and an augmenting effects acting via pre-motor inputs, with the balance in favor of the latter in rodents. These observations of dual opposing influences on hypoglossal motor output to tongue muscle are contrary to the prevailing notion of *only* inhibitory influences following sedative hypnotics such as lorazepam and zolpidem, and reveal novel mechanisms of integrative respiratory motor control operating *in-vivo*. Further consideration of these data and their implications are reviewed elsewhere (Horner, 2008).

Ethanol is another widely used drug in western society, with ethanol commonly worsening obstructive sleep apnea in humans. Ethanol exerts complex effects on the central nervous system, generally depressing neuronal function, at least in part via interactions with the $GABA_A$ receptor and exerting a GABA-mimetic profile. Since GABAergic neurons are importantly involved in the initiation and maintenance of non-REM sleep (see above), then a major component of the sedative effect of ethanol may be mediated via potentiating this sleep promoting (i.e., state-dependent) system. What is meant by the term "*state-dependent/arousal influences*" in this case is the excitability of those neuronal pools that comprise and contribute to the central nervous system states of brain arousal (encompassing wakefulness and sleep) that by their connectivity can bias the excitability of other neuronal systems (including the respiratory network), but with these state-

dependent systems not being classically involved in respiratory regulation *per se*. Recent evidence showed that administration of ethanol to rats, at a dose that produced physiologically-relevant blood alcohol concentrations for behavioral impairment in rats and humans, suppressed tongue muscle activity by primary influences on state-dependent aspects of central nervous system function (i.e., via sedation) independent of effects on the respiratory network *per se* (i.e., no effects at the hypoglossal motor pool) (Vecchio et al., 2010). Likely sources for the sedative effects of ethanol include inhibition of components of the reticular formation and ascending arousal systems (e.g., glutamatergic, serotonergic and/or noradrenergic inputs), or augmentation of the GABAergic sleep-promoting systems which provide major sources of drive to the hypoglossal motor pool modulating the expression of tonic and respiratory-related motor activities (see above).

Overall, these results identify the important principle that certain pharmacological agents can influence respiratory muscle activity by primary influences on state-dependent aspects of central nervous system function rather than motor control *per se*, and this separation had not been addressed in previous studies.

Summary

The hypoglossal and trigeminal motor responses to glycine and $GABA_A$ receptor antagonism at the respective motor pools indicates some functional role for these inhibitory amino acids in suppression of the transient motor events of REM sleep (Morrison et al., 2003a, b; Brooks and Peever, 2008). Glycine and $GABA_A$ receptor-mediated inhibition of these motor pools, however, cannot explain either the inability to reverse the atonia with glycine and $GABA_A$ receptor blockade, or the lack of respiratory-related tongue activity in REM sleep (Kubin et al., 1993; Morrison et al., 2003a, b; Brooks and Peever, 2008). Likewise the withdrawal of monoaminergic and glutamatergic inputs in sleep (see above) cannot fully explain the significant suppression of hypoglossal motor activity because re-application of an α_1 receptor agonist cannot mitigate the tongue muscle atonia (Chan et al., 2006), likewise for glutamate at the trigeminal motor pool (Burgess et al., 2008). The potential role of inhibitory metabotropic $GABA_B$ receptors at the hypoglossal motor nucleus (Okabe et al., 1994) has not yet been investigated in natural REM sleep to determine if this may contribute to motor suppression. $GABA_B$ receptors contribute to the motor suppression of REM sleep at the trigeminal motor pool, although there also it does not mitigate masseter muscle atonia (Brooks and Peever, 2010).

The potentially significant influence of cholinergic mechanisms at the hypoglossal motor pool also needs to be investigated in future studies. In anesthetized rats acetylcholine at the hypoglossal motor pool exerts a net inhibitory effect on tongue muscle due to a powerful muscarinic receptor-mediated inhibition that overwhelms a nicotinic receptor-mediated excitation (Liu et al., 2005), although whether such an inhibition contributes to the profound suppression of tongue muscle activity in REM sleep remains to be determined.

Overall, this review highlights that the hypoglossal motor pool is influenced by a variety of neuromodulators, some of which change dynamically across sleep and awake states to alter motoneuron excitability. Processes of *both* disfacilitation and inhibition operate to explain tongue motor suppression in REM sleep.

Acknowledgements

The author's work was supported by grants from the Canadian Institutes of Health Research and the Ontario Thoracic Society. The author also gratefully acknowledges development grants from the Canada Foundation for Innovation and the Ontario Research and Development Challenge Fund. RLH holds a Tier 1 Canada Research Chair in *Sleep and Respiratory Neurobiology*.

References

Al-Zubaidy Z.A., Erickson R.L., Greer J.J. Serotonergic and noradrenergic effects on respiratory neural discharge in the medullary slice preparation of neonatal rats. *Pflügers Arch.*, **431**: 942-949, 1996.

Aldes L.D., Chapman M.E., Chronister R.B., Haycock J.W. Sources of noradrenergic afferents to the hypoglossal nucleus in the rat. *Brain Res. Bull.*, **29**: 931-942, 1992.

Aston-Jones G. and Bloom F.E. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J. Neurosci.*, **1**: 876-886, 1981.

Aston-Jones G., Shipley M.T., Grzanna R. The locus coeruleus, A5 and A7 noradrenergic cell groups. In: Paxinos G. (Ed.), *The rat nervous system.* San Diego, Academic Press: 183-213, 1995.

Bailey E.F. and Fregosi R.F. Modulation of upper airway muscle activities by bronchopulmonary afferents. *J. Appl. Physiol.*, **101**: 609-617, 2006.

Bailey E.F., Huang Y.H., Fregosi R.F. Anatomic consequences of intrinsic tongue muscle activation. *J. Appl. Physiol.*, **101**: 1377-1385, 2006.

Bastedo T., Chan E., Park E., Liu H., Horner R.L. Modulation of genioglossus muscle activity across sleep-wake states by histamine at the hypoglossal motor pool. *Sleep*, **32**: 1313-1324, 2009.

Bayliss D.A., Viana F., Berger A.J. Mechanisms underlying excitatory effects of thyrotropin-releasing hormone on rat hypoglossal motoneurons in vitro. *J. Neurophysiol.*, **68**: 1733-1745, 1992.

Bellingham M.C. and Funk G.D. Cholinergic modulation of respiratory brain-stem neurons and its function in sleep-wake state determination. *Clin. Exp. Pharmacol. Physiol.*, **27**: 132-137, 2000.

Berger A.J., Bayliss D.A., Viana F. Modulation of neonatal rat hypoglossal motoneuron excitability by serotonin. *Neurosci. Lett.*, **143**: 164-168, 1992.

Berger A.J. Determinants of respiratory motoneuron output. *Respir. Physiol.*, **122**: 259-269, 2000.

Berry R.B., Yamaura E.M., Gill K., Reist C. Acute effects of paroxetine on genioglossus activity in obstructive sleep apnea. *Sleep*, **22**: 1087-1092, 1999.

Boissard R., Gervasoni D., Schmidt M.H., Barbagli B., Fort P., Luppi P.H. The rat ponto-medullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. *Eur. J. Neurosci.*, **16**: 1959-1973, 2002.

Brandes I.F., Zuperku E.J., Stucke A.G., Jakovcevic D., Hopp F.A., Stuth E.A. Serotonergic modulation of inspiratory hypoglossal motoneurons in decerebrate dogs. *J. Neurophysiol.*, **95**: 3449-3459, 2006.

Brennick M.J., Pickup S., Cater J.R., Kuna S.T. Phasic respiratory pharyngeal mechanics by magnetic resonance imaging in lean and obese Zucker rats. *Am. J. Respir. Crit. Care Med.*, **173**: 1031-1037, 2006.

Brennick M.J., Pack A.I., Ko K., Kim E., Pickup S., Maislin G., Schwab R.J. Altered upper airway and soft tissue structures in the New Zealand Obese mouse. *Am. J. Respir. Crit. Care Med.*, **179**: 158-169, 2009.

Brooks P.L. and Peever J.H. Glycinergic and $GABA_A$-mediated inhibition of somatic motoneurons does not mediate REM sleep motor atonia. *J. Neurosci.*, **28**: 3535-3545, 2008.

Brooks P.L. and Peever J.H. GABAergic and glycinergic control of upper airway motoneurons in rapid eye movement sleep. *Adv. Exp. Med. Biol.*, **669**: 259-262, 2010.

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

Carter M.E., Yizhar O., Chikahisa S., Nguyen H., Adamantidis A., Nishino S., Deisseroth K., de Lecea L. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.*, **13**: 1526-1533, 2010.

Cartwright R.D. Effect of sleep position on sleep apnea severity. *Sleep*, **7**: 110-114, 1984.

Chan E., Steenland H.W., Liu H., Horner R.L. Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. *Am. J. Respir. Crit. Care Med.*, **174**: 1264-1273, 2006.

Chase M.H. and Morales F.R. Control of motoneurons during sleep. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 155-168, 2000.

Donato R. and Nistri A. Relative contribution by GABA or glycine to Cl(-)-mediated synaptic transmission on rat hypoglossal motoneurons in vitro. *J. Neurophysiol.*, **84**: 2715-2724, 2000.

Dreshaj I.A., Haxhiu M.A., Martin R.J. Role of the medullary raphe nuclei in the respiratory response to CO_2. *Respir. Physiol.*, **111**: 15-23, 1998.

Evans H.E. and Christensen G.C. The skeleton. *Miller's Anatomy of the Dog*. Philadelphia, Saunders: 107-224, 1979.

Fenik V., Marchenko V., Janssen P., Davies R.O., Kubin L. A5 cells are silenced when REM sleep-like signs are elicited by pontine carbachol. *J. Appl. Physiol.*, **93**: 1448-1456, 2002.

Fenik P. and Veasey S.C. Pharmacological characterization of serotonergic receptor activity in the hypoglossal nucleus. *Am. J. Respir. Crit. Care Med.*, **167**: 563-569, 2003.

Fenik V.B., Davies R.O., Kubin L. REM sleep-like atonia of hypoglossal (XII) motoneurons is caused by loss of noradrenergic and serotonergic inputs. *Am. J. Respir. Crit. Care Med.*, **172**: 1322-1330, 2005.

Franks N.P. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.*, **9**: 370-386, 2008.

Franks N.P. and Zecharia A.Y. Sleep and general anesthesia. *Can. J. Anaesth.*, **58**: 139-148, 2011.

Fuller D.D., Williams J.S., Janssen P.L., Fregosi R.F. Effect of co-activation of tongue protrudor and retractor muscles on tongue movements and pharyngeal airflow mechanics in the rat. *J. Physiol.*, **519**: 601-613, 1999.

Fuller P.M., Saper C.B., Lu J. The pontine REM switch: past and present. *J. Physiol.*, **584**: 735-741, 2007.

Fung S.J., Yamuy J., Xi M.C., Engelhardt J.K., Morales F.R., Chase M.H. Changes in electrophysiological properties of cat hypoglossal motoneurons during carbachol-induced motor inhibition. *Brain Res.*, **885**: 262-272, 2000.

Funk G.D., Smith J.C., Feldman J.L. Development of thyrotropin-releasing hormone and norepinephrine potentiation of inspiratory-related hypoglossal motoneuron discharge in neonatal and juvenile mice in vitro. *J. Neurophysiol.*, **72**: 2538-2541, 1994.

Guyenet P.G. The coeruleospinal noradrenergic neurons: anatomical and electrophysiological studies in the rat. *Brain Res.*, **189**: 121-133, 1980.

Hanzel D.A., Proia N.G., Hudgel D.W. Response of obstructive sleep apnea to fluoxetine and protriptyline. *Chest*, **100**: 416-421, 1991.

Hendricks J.C., Kline L.R., Kovalski R.J., O'Brien J.A., Morrison A.R., Pack A.I. The English bulldog: a natural model of sleep-disordered breathing. *J. Appl. Physiol.*, **63**: 1344-1350, 1987.

Hobson J.A., Pace-Schott E.F., Stickgold R. Dreaming and the brain: toward a cognitive neuroscience of conscious states. *Behav. Brain Sci.*, **23**: 793-842; discussion 904-1121, 2000.

Hobson J.A. and Pace-Schott E.F. The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nat. Rev.Neurosci.*, **3**: 679-693, 2002.

Horner R.L. Motor control of the pharyngeal musculature and implications for the pathogenesis of obstructive sleep apnea. *Sleep*, **19**: 827-853, 1996.

Horner R.L. and Kubin L. Pontine carbachol elicits multiple rapid eye movement sleep-like neural events in urethane-anaesthetized rats. *Neuroscience*, **93**: 215-226, 1999.

Horner R.L. The neuropharmacology of upper airway motor control in the awake and asleep states: implications for obstructive sleep apnoea. *Resp. Res.*, **2**: 286-294, 2001.

Horner R.L. Neuromodulation of hypoglossal motoneurons during sleep. *Respir. Physiol. Neurobiol.*, **164**: 179-196, 2008.

Horner R.L. Emerging principles and neural substrates underlying tonic sleep-state-dependent influences on respiratory motor activity. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **364**: 2553-2564, 2009.

Horner R.L. Central neural control of respiratory neurons and motoneurons during sleep. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 237-249, 2010.

Hudgel D.W. and Thanakitcharu S. Pharmacologic treatment of sleep-disordered breathing. *Am. J. Respir. Crit. Care Med.*, **158**: 691-699, 1998.

Iwase M., Homma I., Shioda S., Nakai Y. Histamine immunoreactive neurons in the brain stem of the rabbit. *Brain Res. Bull.*, **32**: 267-272, 1993.

Jelev A., Sood S., Liu H., Nolan P., Horner R.L. Microdialysis perfusion of 5-HT into hypoglossal motor nucleus differentially modulates genioglossus activity across natural sleep-wake states in rats. *J. Physiol.*, **532**: 467-481, 2001.

Johansson O., Hokfelt T., Pernow B., Jeffcoate S.L., White N., Steinbusch H.W., Verhofstad A.A., Emson P.C., Spindel E. Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. *Neuroscience*, **6**: 1857-1881, 1981.

Jones B.E. Basic mechanisms of sleep-wake states. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 134-154, 2000.

Jordan A.S., Wellman A., Edwards J.K., Schory K., Dover L., MacDonald M., Patel S.R., Fogel R.B., Malhotra A., White D.P. Respiratory control stability and upper airway collapsibility in men and women with obstructive sleep apnea. *J. Appl. Physiol.*, **99**: 2020-2027, 2005.

Kachidian P., Poulat P., Marlier L., Privat A. Immunohistochemical evidence for the coexistence of substance P, thyrotropin-releasing hormone, GABA, methionine-enkephalin, and leucin-enkephalin in the serotonergic neurons of the caudal raphe nuclei: a dual labeling in the rat. *J. Neurosci. Res.*, **30**: 521-530, 1991.

Kirkness J.P., Schwartz A.R., Schneider H., Punjabi N.M., Maly J.J., Laffan A.M., McGinley B.M., Magnuson T., Schweitzer M., Smith P.L., Patil S.P. Contribution of male sex, age, and obesity to mechanical instability of the upper airway during sleep. *J. Appl. Physiol.*, **104**: 1618-1624, 2008.

Kodama T., Lai Y.Y., Siegel J.M. Changes in inhibitory amino acid release linked to pontine-induced atonia: an in vivo microdialysis study. *J. Neurosci.*, **23**: 1548-1554, 2003.

Koenig J.S. and Thach B.T. Effects of mass loading on the upper airway. *J. Appl. Physiol.*, **64**: 2294-2299, 1988.

Kraiczi H., Hedner J., Dahlof P., Ejnell H., Carlson J. Effect of serotonin uptake inhibition on breathing during sleep and daytime symptoms in obstructive sleep apnea. *Sleep*, **22**: 61-67, 1999.

Kubin L., Tojima H., Davies R.O., Pack A.I. Serotonergic excitatory drive to hypoglossal motoneurons in the decerebrate cat. *Neurosci. Lett.*, **139**: 243-248, 1992.

Kubin L., Kimura H., Tojima H., Davies R.O., Pack A.I. Suppression of hypoglossal motoneurons during the carbachol-induced atonia of REM sleep is not caused by fast synaptic inhibition. *Brain Res.*, **611**: 300-312, 1993.

Kubin L., Reignier C., Tojima H., Taguchi O., Pack A.I., Davies R.O. Changes in serotonin level in the hypoglossal nucleus region during carbachol-induced atonia. *Brain Res.*, **645**: 291-302, 1994.

Kubin L., Davies R.O., Pack L. Control of upper airway motoneurons during REM sleep. *News Physiol. Sci.*, **13**: 637-656, 1998.

Kubin L. Carbachol models of REM sleep: recent developments and new directions. *Arch. Ital. Biol.*, **139**: 147-168, 2001.

Kubin L. and Davies R.O. Mechanisms of airway hypotonia. In: Pack A.I. (Ed.), *Sleep Apnea: Pathogenesis, Diagnosis and Treatment*. New York, Dekker: 99-154, 2002.

Kuna S. and Remmers J.E. Anatomy and physiology of upper airway obstruction. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 840-858, 2000.

Lai Y.Y., Kodama T., Siegel J.M. Changes in monoamine release in the ventral horn and hypoglossal nucleus linked to pontine inhibition of muscle tone: an in vivo microdialysis study. *J. Neurosci.*, **21**: 7384-7391, 2001.

Liu R.H., Fung S.J., Reddy V.K., Barnes C.D. Localization of glutamatergic neurons in the dorsolateral pontine tegmentum projecting to the spinal cord of the cat with a proposed role of glutamate on lumbar motoneuron activity. *Neuroscience*, **64**: 193-208, 1995.

Liu X., Sood S., Liu H., Nolan P., Morrison J.L., Horner R.L. Suppression of genioglossus muscle tone and activity during reflex hypercapnic stimulation by $GABA_A$ mechanisms at the hypoglossal motor nucleus in-vivo. *Neuroscience*, **116**: 249-259, 2003.

Liu X., Sood S., Liu H., Horner R.L. Opposing muscarinic and nicotinic modulation of hypoglossal motor output to genioglossus muscle in rats in vivo. *J. Physiol.*, **565**: 965-980, 2005.

Lu J., Sherman D., Devor M., Saper C.B. A putative flip-flop switch for control of REM sleep. *Nature*, **441**: 589-594, 2006.

Luppi P.H., Gervasoni D., Verret L., Goutagny R., Peyron C., Salvert D., Leger L., Fort P. Paradoxical (REM) sleep genesis: the switch from an aminergic-cholinergic to a GABAergic-glutamatergic hypothesis. *J. Physiol. Paris*, **100**: 271-283, 2006.

Lydic R. and Baghdoyan H.A. Neurochemical evidence for the cholinergic modulation of sleep and breathing. In: Carley D.W., Radulovacki M. (Eds.), *Sleep-related breathing disorders: Experimental models and therapeutic potential*. New York, Dekker: 57-91, 2003.

Lydic R. and Baghdoyan H.A. Sleep, anesthesiology, and the neurobiology of arousal state control. *Anesthesiology*, **103**: 1268-1295, 2005.

Malhotra A., Huang Y., Fogel R.B., Pillar G., Edwards J.K., Kikinis R., Loring S.H., White D.P. The male predisposition to pharyngeal collapse: importance of airway length. *Am. J. Respir. Crit. Care Med.*, **166**: 1388-1395, 2002.

Manaker S. and Tischler L.J. Origin of serotoninergic afferents to the hypoglossal nucleus in the rat. *J. Comp. Neurol.*, **334**: 466-476, 1993.

McCarley R.W., Greene R.W., Rainnie D., Portas C.M. Brainstem neuromodulation and REM sleep. *Semin. Neurosci.*, **7**: 341-354, 1995.

McCarley R.W. Neurobiology of REM and NREM sleep. *Sleep Med.*, **8**: 302-330, 2007.

McCrimmon D.R., Mitchell G.S., Dekin M.S. Glutamate, GABA, and serotonin in ventilatory control. In: Dempsey J.A., Pack A.I. (Eds.), *Regulation of Breathing*. New York, Dekker: 151-218, 1995.

McGinty D. and Szymusiak R. The sleep-wake switch: A neuronal alarm clock. *Nat. Med.*, **6**: 510-511, 2000.

McGregor R. and Siegel J.M. Illuminating the locus coeruleus: control of posture and arousal. *Nat. Neurosci.*, **13**: 1448-1449, 2010.

Mendelson W.B. Hypnotics: Basic Mechanisms and Pharmacology. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 407-413, 2000.

Mihic S.J., Ye Q., Wick M.J., Koltchine V.V., Krasowski M.D., Finn S.E., Mascia M.P., Valenzuela C.F., Hanson K.K., Greenblatt E.P., Harris R.A., Harrison N.L. Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. *Nature*, **389**: 385-389, 1997.

Morales F.R., Boxer P., Chase M.H. Behavioral state-specific inhibitory postsynaptic potentials impinge on cat lumbar motoneurons during active sleep. *Exp. Neurol.*, **98**: 418-435, 1987a.

Morales F.R., Engelhardt J.K., Soja P.J., Pereda A.E., Chase M.H. Motoneuron properties during motor inhibition produced by microinjection of carbachol into the pontine reticular formation of the decerebrate cat. *J. Neurophysiol.*, **57**: 1118-1129, 1987b.

Morrison J.L., Sood S., Liu X., Liu H., Park E., Nolan P., Horner R.L. Glycine at the hypoglossal motor nucleus: genioglossus activity, CO_2 responses and the additive effects of GABA. *J. Appl. Physiol.*, **93**: 1786-1796, 2002.

Morrison J.L., Sood S., Liu H., Park E., Nolan P., Horner R.L. $GABA_A$ receptor antagonism at the hypoglossal motor nucleus increases genioglossus muscle activity in NREM but not REM sleep. *J. Physiol.*, **548**: 569-583, 2003a.

Morrison J.L., Sood S., Liu H., Park E., Nolan P., Horner R.L. Role of inhibitory amino acids in control of hypoglossal motor outflow to genioglossus muscle in naturally sleeping rats. *J. Physiol.*, **552**: 975-991, 2003b.

Nakano H., Magalang U.J., Lee S.D., Krasney J.A., Farkas G.A. Serotonergic modulation of ventilation and upper airway stability in obese Zucker rats. *Am. J. Respir. Crit. Care Med.*, **163**: 1191-1197, 2001.

Neuzeret P.C., Sakai K., Gormand F., Petitjean T., Buda C., Sastre J.P., Parrot S., Guidon G., Lin J.S. Application of histamine or serotonin to the hypoglossal nucleus increases genioglossus muscle activity across the wake-sleep cycle. *J. Sleep Res.*, **18**: 113-121, 2009.

Nitz D. and Siegel J. GABA release in the dorsal raphe nucleus: role in the control of REM sleep. *Am. J. Physiol.*, **273**: R451-455, 1997a.

Nitz D. and Siegel J.M. GABA release in the locus coeruleus as a function of sleep/wake state. *Neuroscience*, **78**: 795-801, 1997b.

O'Brien J.A. and Berger A.J. Cotransmission of GABA and glycine to brain stem motoneurons. *J. Neurophysiol.*, **82**: 1638-1641, 1999.

Okabe S., Woch G., Kubin L. Role of $GABA_B$ receptors in the control of hypoglossal motoneurons in vivo. *NeuroReport*, **5**: 2573-2576, 1994.

Orem J. and Kubin L. Respiratory physiology: Central neural control. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 205-220, 2000.

Orser B.A. Extrasynaptic $GABA_A$ receptors are critical targets for sedative-hypnotic drugs. *J. Clin. Sleep Med.*, **2**: S12-18, 2006.

Palacios J.M., Wamsley J.K., Kuhar M.J. The distribution of histamine H1-receptors in the rat brain: an autoradiographic study. *Neuroscience*, **6**: 15-37, 1981.

Park E., Younes M., Liu H., Liu X., Horner R.L. Systemic vs. central administration of common hypnotics reveals opposing effects on genioglossus muscle activity in rats. *Sleep*, **31**: 355-365, 2008.

Parkis M.A., Bayliss D.A., Berger A.J. Actions of norepinephrine on rat hypoglossal motoneurons. *J. Neurophysiol.*, **74**: 1911-1919, 1995.

Rekling J.C. Excitatory effects of thyrotropin-releasing hormone (TRH) in hypoglossal motoneurons. *Brain Res.*, **510**: 175-179, 1990.

Rekling J.C., Funk G.D., Bayliss D.A., Dong X.W., Feldman J.L. Synaptic control of motoneuronal excitability. *Physiol. Rev.*, **80**: 767-852, 2000.

Remmers J.E., deGroot W.J., Sauerland E.K., Anch A.M. Pathogenesis of upper airway occlusion during sleep. *J. Appl. Physiol.*, **44**: 931-938, 1978.

Robinson R.W. and Zwillich C.W. Medications, sleep and breathing. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 797-812, 2000.

Saper C.B., Chou T.C., Scammell T.E. The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosc.*, **24**: 726-731, 2001.

Rukhadze I. and Kubin L. Differential pontomedullary catecholaminergic projections to hypoglossal motor nucleus and viscerosensory nucleus of the solitary tract. *J. Chem. Neuroanat.*, **33**: 23-33, 2007.

Rukhadze I., Fenik V.B., Branconi J.L., Kubin L. Fos expression in pontomedullary catecholaminergic cells following rapid eye movement sleep-like episodes elicited by pontine carbachol in urethane-anesthetized rats. *Neuroscience*, **152**: 208-222, 2008.

Saper C.B., Scammell T.E., Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature*, **437**: 1257-1263, 2005.

Schwarz P.B., Yee N., Mir S., Peever J.H. Noradrenaline triggers muscle tone by amplifying glutamate-driven excitation of somatic motoneurones in anaesthetized rats. *J. Physiol.*, **586**: 5787-5802, 2008.

Siegel J.M. Brainstem mechanisms generating REM sleep. In: Kryger M.H., Roth T., Dement W.C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, Saunders: 112-133, 2000.

Siegel J.M. The stuff dreams are made of: anatomical substrates of REM sleep. *Nat. Neurosci.*, **9**: 721-722, 2006.

Singer J.H., Talley E.M., Bayliss D.A., Berger A.J. Development of glycinergic synaptic transmission to rat brain stem motoneurons. *J. Neurophysiol.*, **80**: 2608-2620, 1998.

Smith I.E. and Quinnell T.G. Pharmacotherapies for obstructive sleep apnoea: where are we now? *Drugs*, **64**: 1385-1399, 2004.

Sood S., Liu X., Liu H., Nolan P., Horner R.L. 5-HT at hypoglossal motor nucleus and respiratory control of genioglossus muscle in anesthetized rats. *Respir. Physiol. Neurobiol.*, **138**: 205-221, 2003.

Sood S., Morrison J.L., Liu H., Horner R.L. Role of endogenous serotonin in modulating genioglossus muscle activity in awake and sleeping rats. *Am. J. Respir. Crit. Care Med.*, **172**: 1338-1347, 2005.

Sood S., Raddatz E., Liu X., Liu H., Horner R.L. Inhibition of serotonergic medullary raphe obscurus neurons suppresses genioglossus and diaphragm activities in anesthetized but not conscious rats. *J. Appl. Physiol.*, **100**: 1807-1821, 2006.

Sood S., Liu X., Liu H., Horner R.L. Genioglossus muscle activity and serotonergic modulation of hypoglossal motor output in obese Zucker rats. *J. Appl. Physiol.*, **102**: 2240-2250, 2007.

Steenland H.W., Liu H., Sood S., Liu X., Horner R.L. Respiratory activation of the genioglossus muscle involves both non-NMDA and NMDA glutamate receptors at the hypoglossal motor nucleus in-vivo. *Neuroscience*, **138**: 1407-1424, 2006.

Steenland H.W., Liu H., Horner R.L. Endogenous glutamatergic control of rhythmically active mammalian respiratory motoneurons in vivo. *J. Neurosci.*, **28**: 6826-6835, 2008.

Steriade M. and McCarley R.W. Neuronal control of REM sleep. In: Steriade M. and McCarley R.W. *Brainstem control of wakefulness and sleep*. New York, Plenum Press: 461-511, 2005.

Tangel D.J., Mezzanotte W.S., White D.P. Influence of sleep on tensor palatini EMG and upper airway resistance in normal men. *J. Appl. Physiol.*, **70**: 2574-2581, 1991.

Thakkar M.M., Strecker R.E., McCarley R.W. Behavioral state control through differential serotonergic inhibition in the mesopontine cholinergic nuclei: a simultaneous unit recording and microdialysis study. *J. Neurosci.*, **18**: 5490-5497, 1998.

Traiffort E., Pollard H., Moreau J., Ruat M., Schwartz J.C., Martinez-Mir M.I., Palacios J.M. Pharmacological characterization and autoradiographic localization of histamine H2 receptors in human brain identified with [125I]iodoaminopotentidine. *J. Neurochem.*, **59**: 290-299, 1992.

Tuck S.A., Dort J.C., Olson M.E., Remmers J.E. Monitoring respiratory function and sleep in the obese Vietnamese pot-bellied pig. *J. Appl. Physiol.*, **87**: 444-451, 1999.

Tuck S.A. and Remmers J.E. Mechanical properties of the passive pharynx in Vietnamese pot-bellied pigs. I. Statics. *J. Appl. Physiol.*, **92**: 2229-2235, 2002a.

Tuck S.A. and Remmers J.E. Mechanical properties of the passive pharynx in Vietnamese pot-bellied pigs. II. Dynamics. *J. Appl. Physiol.*, **92**: 2236-2244, 2002b.

Umemiya M. and Berger A.J. Presynaptic inhibition by serotonin of glycinergic inhibitory synaptic currents in the rat brain stem. *J. Neurophysiol.*, **73**: 1192-1201, 1995.

Veasey S.C., Panckeri K.A., Hoffman E.A., Pack A.I., Hendricks J.C. The effects of serotonin antagonists in an animal model of sleep-disordered breathing. *Am. J. Respir. Crit. Care Med.*, **153**: 776-786, 1996.

Veasey S.C. Pharmacotherapies for obstructive sleep apnea: how close are we? *Curr. Op. Pulm. Med.*, **7**: 399-403, 2001.

Veasey S.C. Serotonin agonists and antagonists in obstructive sleep apnea: therapeutic potential. *Am. J. Respir. Crit. Care Med.*, **2**: 21-29, 2003.

Veasey S.C. Will we ever have an effective pharmacotherapy for obstructive sleep apnea? *Sleep*, **28**: 18-19, 2005.

Vecchio L.M., Grace K.P., Liu H., Harding S., Le A.D., Horner R.L. State-dependent versus central motor effects of ethanol on breathing. *J. Appl. Physiol.*, **108**: 387-400, 2010.

Watanabe T., Taguchi Y., Shiosaka S., Tanaka J., Kubota H., Terano Y., Tohyama M., Wada H. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res.*, **295**: 13-25, 1984.

Woch G., Davies R.O., Pack A.I., Kubin L. Behaviour of raphe cells projecting to the dorsomedial medulla during carbachol-induced atonia in the cat. *J. Physiol.*, **490**: 745-758, 1996.

Yamuy J., Fung S.J., Xi M., Morales F.R., Chase M.H. Hypoglossal motoneurons are postsynaptically inhibited during carbachol-induced rapid eye movement sleep. *Neuroscience*, **94**: 11-15, 1999.

Younes Y., Park E., Horner R.L. Pentobarbital sedation increases genioglossus respiratory activity in sleeping rats. *Sleep*, **30**: 478-488, 2007.

Zuperku E.J. and McCrimmon D.R. Gain modulation of respiratory neurons. *Respir. Physiol. Neurobiol.*, **131**: 121-133, 2002.

Zuperku E.J., Brandes I.F., Stucke A.G., Sanchez A., Hopp F.A., Stuth E.A. Major components of endogenous neurotransmission underlying the discharge activity of hypoglossal motoneurons in vivo. *Adv. Exp. Med Biol.*, **605**: 279-284, 2008.