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Review

# $K^+$ Channel modulation causes genioglossus inhibition in REM sleep and is a strategy for reactivation☆,☆☆

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ABSTRACT

Rapid eye movement (REM) sleep is accompanied by periods of upper airway motor suppression that cause hypoventilation and obstructive apneas in susceptible individuals. A common idea has been that upper airway motor suppression in REM sleep is caused by the neurotransmitters glycine and γ-amino butyric acid (GABA) acting at pharyngeal motor pools to inhibit motoneuron activity. Data refute this as a workable explanation because blockade of this putative glycine/GABAergic mechanism releases pharyngeal motor activity in all states, and least of all in REM sleep. Here we summarize a novel motor-inhibitory mechanism that suppresses hypoglossal motor activity largely in REM sleep, this being a muscarinic receptor mechanism linked to G-protein-coupled inwardly rectifying potassium (GIRK) channels. We then outline how this discovery informs efforts to pursue therapeutic targets to reactivate hypoglossal motor activity throughout sleep via potassium channel modulation. One such target is the inwardly rectifying potassium channel Kir2.4 whose expression in the brain is almost exclusive to cranial motor nuclei.



## 1. Overview

Rapid eye movement (REM) sleep is associated with periods of respiratory variability and motor suppression that can significantly affect lung ventilation. In particular, suppression of pharyngeal muscle activity in REM sleep predisposes susceptible individuals to obstructive apneas and hypopneas (Eastwood et al., 2010). The influences of sleep state-dependent neuromodulators on hypoglossal motor activity, and the experimental model systems used to generate that information, have recently been summarized (Horner, 2008) and are not repeated here. Rather, this paper first emphasizes and distils two key points from that literature. We initially highlight that there is evidence for major suppression of pharyngeal motor excitability in REM sleep that occurs by a process that does not significantly involve the inhibitory amino acids glycine and γ-amino butyric acid (GABA); i.e., distinct from the processes inhibiting spinal motor pools. Importantly, we also emphasize that the responses to glycine and GABA receptor antagonism at the cranial motor pools indicate release of a tonic and state-independent inhibitory tone rather than a motor inhibitory mechanism of REM sleep. We also introduce that there is also a process of disfacilitation operating in REM sleep to suppress pharyngeal muscle activity, and identify the neuromodulators involved.

The main thrust of this review, however, is to summarize and identify a novel motor-inhibitory mechanism that suppresses hypoglossal motor activity in REM sleep. This is a muscarinic receptor mechanism linked to G-protein-coupled inwardly rectifying potassium (K) (GIRK) channels that is largely restricted to REM sleep. In the final section of this review we then outline how this discovery informs efforts to pursue therapeutic targets that can reactivate hypoglossal motor tone throughout sleep via potassium channel modulation. One such target is the inwardly rectifying potassium channel Kir2.4, expression of which in the brain is almost exclusive to the cranial motor nuclei.

## 2. Genioglossus motor suppression in REM sleep

### 2.1. Inhibition by glycine and/or GABA play a minimal role

Inhibitory glycine and GABA processes are importantly involved in the descending inhibition of spinal motor activity in REM sleep

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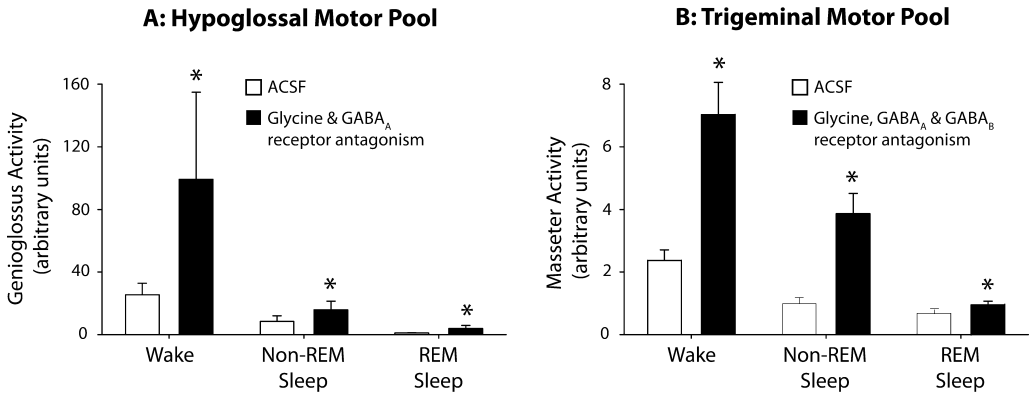


**Fig. 1.** Levels of genioglossus and masseter muscle activities before and after antagonism of glycine and GABA receptors at the hypoglossal (A) and trigeminal (B) motor pools. Data re-plotted from the original papers (Morrison et al., 2003b; Brooks and Peever, 2012) with permission of the authors. Note that blockade of glycine, $GABA_A$ and $GABA_B$ receptors, alone or in combination, activates hypoglossal and trigeminal motor activities across all sleep–wake states, but least of all in REM sleep. This pattern of response is evidence for the presence of a *tonic* and *state-independent* inhibitory tone that is released by glycine and GABA receptor antagonism. * Indicates significant effect of the intervention relative to artificial cerebrospinal fluid (ACSF) controls. All antagonists were delivered to the respective motor pools by reverse microdialysis at the following doses: strychnine (glycine receptor antagonist, 0.01–0.1 mM), bicuculline ($GABA_A$ receptor antagonist, 0.1 mM) and CGP52432 ($GABA_B$ receptor antagonist, 0.2 mM). The number of rats comprising each data set is 7 and 13 for the hypoglossal and trigeminal motor pools respectively. Values are plotted as mean + SEM. Levels of respiratory-related genioglossus activity and tonic masseter muscle activity are plotted in arbitrary units from the original papers; differences in relative values reflect different amplification and recording equipment between the independent laboratories.

(Chase et al., 1989; Soja et al., 1991; Siegel, 2000; Lu et al., 2006). Major suppression of cranial, including genioglossus, motor activity also occurs during periods of REM sleep. Accordingly, one idea has been that the pharyngeal motor inhibition of REM sleep is also caused by the actions of glycine and GABA released onto the cranial motoneurons that innervate the pharyngeal musculature, i.e., as occurs at spinal motor pools.

Whether inhibitory glycine and GABAergic receptor mechanisms contribute to the strong suppression of genioglossus activity in REM sleep has been debated. Studies have been performed using a pharmacologically-induced 'REM sleep-like state' produced by local application of the cholinergic agonist carbachol into the pontine reticular formation of decerebrated or anesthetized animals. Such studies first identified that there is minimal contribution of glycine and $GABA_A$ receptor mechanisms to depression of hypoglossal motor activity during the carbachol-induced REM sleep-like state (Kubin et al., 1993). Intracellular recordings, however, identified that a sample of hypoglossal motoneurons received post-synaptic glycinergic inhibitory potentials during the REM-like state (Yamuy et al., 1999; Fung et al., 2000). In studies performed at the hypoglossal motor pool during natural sleep, it was shown that although glycine and $GABA_A$ receptor mechanisms are functional at the hypoglossal motor nucleus *in-vivo* (Morrison et al., 2002; Liu et al., 2003) and across sleep–wake states (Morrison et al., 2003a,b), they contribute minimally to the major suppression of genioglossus activity in natural REM sleep. Of particular note, blockade of glycine and $GABA_A$ receptors, either alone or in combination, releases a tonic inhibition and activates hypoglossal motor activity across all sleep–wake states, but least obviously in REM sleep (Fig. 1A) (Morrison et al., 2003a,b). Thus, some other non-glycinergic and non-$GABA_A$ receptor-mediated mechanism appears to be more strongly involved in hypoglossal motor suppression in REM sleep.

At the trigeminal motor pool, blockade of glycine and $GABA_A$ receptors either alone or in combination also releases a tonic inhibition and activates motor tone across all sleep–wake states, but again least obviously in REM sleep (Brooks and Peever, 2008a). A similar result is likewise obtained with combined antagonism of glycine, $GABA_A$ and $GABA_B$ receptors at the trigeminal motor pool across sleep–wake states (Fig. 1B) (Brooks and Peever, 2012). The former paper (Brooks and Peever, 2008a), in particular, generated significant debate regarding the role of glycine and $GABA_A$ receptor-mediated inhibition of cranial motor activity (Berger, 2008; Chase, 2008; Funk, 2008; Kubin, 2008; Soja, 2008). The masseter muscle was the measured output variable in the studies by Brooks and Peever (2008a, 2012), with this muscle modulating jaw position and airway function (Hollowell et al., 1991; Hollowell and Suratt, 1991). Importantly, the trigeminal motor pool also innervates the palatal muscles that are relevant to the maintenance of upper airway patency and airway collapse during sleep (Tangel et al., 1991; Horner, 1996; Kuna and Remmers, 2000). Together, these observations both at the hypoglossal and trigeminal motor pools (Fig. 1) (Morrison et al., 2003a,b; Brooks and Peever, 2008a, 2012) argue against glycine and/or GABAergic mechanisms as a significant explanation for the inhibition of hypoglossal and trigeminal motor activity during REM sleep.

The data illustrated in Fig. 1 also support some of the original studies investigating mechanisms of suppression of trigeminal motor excitability in REM sleep, e.g., as indicated by modulation of the masseteric (jaw-closer) reflex (Soja et al., 1987). Those authors identified that neurotransmitters *other* than glycine and GABA significantly contributed to the REM sleep-related suppression in excitability of that cranial motor pool (Soja et al., 1987). Subsequent studies confirmed these findings because intracellular recordings from individual trigeminal motoneurons showed that the post-synaptic inhibitory potentials of REM sleep were abolished by local application of strychnine in only six of nineteen cells studied (Chase et al., 1989), i.e., some other inhibitory mechanism was operating in the majority of those cells sampled. The interested reader is directed to the following articles for further discussion of the pros and cons of reductionist and *in-vivo* experimental approaches to identify the mechanism of pharyngeal motor inhibition in REM sleep (Berger, 2008; Brooks and Peever, 2008b; Funk, 2008; Kubin, 2008).

### 2.2. Disfacilitation and REM sleep genioglossus motor suppression

A process of disfacilitation mediated by reduced excitatory inputs to cranial motor pools can contribute to decreased pharyngeal muscle activity in sleep, especially REM sleep (Kubin et al., 1998; Horner, 2009). Noradrenergic, serotonergic and glutamatergic state-dependent drives have been a particular focus of studies identifying mechanisms of suppression of hypoglossal motor activity in both natural sleep (Sood et al., 2005, 2006; Chan et al., 2006; Steenland et al., 2008), and experimental models using a