Unraveling the Mechanisms of REM Sleep Atonia

A Response to Kubin LK, Berger AJ, Funk GD, Soja P, and Chase MH. Critical Topics Forum. Sleep 2008;31:1473-91

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WE THANK THE EDITORS[1] FOR THIS FORUM AND THE COMMENTATORS[2-6] FOR THEIR DISCUSSION OF OUR WORK.[7] WE AGREE WITH THE COMMENTATORS that determining mechanisms of rapid eye movement (REM) sleep motor atonia is of major scientific importance and clinical relevance. The concept that REM atonia is under the control of one physiological mechanism and one neurotransmitter pathway has seduced many of us. The undoubted appeal of the current theory—that glycinergic inhibition of motoneurons is the only mechanism mediating REM atonia—is its inherent simplicity. However, the fact that several laboratories now provide evidence indicating that glycinergic inhibition plays only a minimal role in mediating REM atonia[7-9] and that other neurotransmitter pathways contribute to motor suppression in REM sleep[10-13] indicates that we need to reevaluate mechanisms of REM atonia. *If glycinergic inhibition is indeed the dominating force driving motor inhibition during REM sleep, then why does blockade of glycine receptors at the trigeminal motor pool not prevent REM atonia in masseter muscles?*[7] Our work demonstrates that antagonism of glycine and $GABA_A$ receptors at the trigeminal motor pool causes a profound increase in masseter muscle tone during both wakefulness and non-rapid eye movement (NREM) sleep; however, this same intervention does not prevent or even reverse REM atonia, despite the fact that it potently provokes muscle-twitch activity in REM sleep (Figure 1). In fact, REM atonia persists even when glycine and $GABA_A$ receptors are blocked and potent glutamatergic agonists are simultaneously applied to the trigeminal motor pool. We interpret these findings to indicate that (1) glycinergic- and $GABA_A$-mediated inhibition plays a minimal role in triggering REM atonia; (2) inhibition primarily functions to suppress muscle twitches during REM sleep; and, (3) a powerful, yet unidentified, inhibitory mechanism or mechanisms override excitation during REM sleep. Some commentators offer alternative possibilities for our experimental observations; we respond to these discussion points below.

MULTIPLE MECHANISMS MEDIATE REM ATONIA

The first issue we address is the notion that there is a consensus or lack of controversy concerning mechanisms generating REM atonia. Drs. Chase[3] and Soja[6] propose that hyperpolarization of motoneurons by glycine-mediated inhibitory postsynaptic potentials (IPSPs) is the exclusive factor producing REM sleep atonia. This assertion stems from their intracellular studies of motoneurons, which, although important for determining aspects of synaptic physiology, do not actually allow one to determine whether such mechanisms trigger physiological changes in muscle tone during REM sleep—this is one of the inherent limitations of this method. Nevertheless, if we assume that glycinergic inhibition is indeed the *only* mechanism responsible for producing REM atonia, then all lines of evidence should support this claim. However, multiple studies,[7-10,13] including the original intracellular studies themselves,[14,15] do not completely support this oversimplified perspective. Although intracellular recording experiments demonstrate that motoneurons are inhibited by glycine, there is evidence that non-glycinergic mechanisms are also involved. For example, in Chase et al's study,[14] strychnine blocked IPSPs in only 32% (6 of 19 cells) of recorded motoneurons during REM sleep; IPSPs remained, albeit with reduced amplitudes and frequencies, in 68% of recorded cells (see Figure 3 in Chase et al[14]). It is unlikely that residual IPSPs were the result of incomplete glycine-receptor antagonism because the dose of strychnine used (i.e., 15 mM) was orders of magnitude, i.e., 30,000 times, higher than required to block glycine receptors on motoneurons in vitro (i.e., 400 nM).[16,17] If glycinergic inhibition is the only mechanism hyperpolarizing motoneurons in REM sleep, then why do IPSPs remain? The most parsimonious explanation for these observations is that residual IPSPs are mediated by non-glycinergic inhibitory mechanisms. Although GABAergic neurotransmission contributes to motoneuron inhibition during REM sleep, this mechanism cannot explain residual IPSPs because antagonism of $GABA_A$ receptors only affects IPSP durations.[14] That both glycine and GABA influence motoneuron activity in REM sleep is not unexpected because glycine and GABA are co-released onto motoneurons,[16,17] which underscores the point that multiple transmitters affect motoneuron physiology during REM sleep. The mechanism or mechanisms responsible for residual IPSPs during REM sleep are unknown.

We also want to emphasize that there has never been a systematic study demonstrating that motoneuron hyperpolarization during REM sleep results from glycinergic IPSPs. In fact, the only reported study that addresses this issue demonstrates that strychnine reduces, but does not prevent, motoneuron hyperpolarization during REM sleep—a small but significant degree of hyperpolarization remains despite blockade of glycine receptors (see Figure 1 and accompanying statistics from Soja et al[15]). If glycinergic inhibition is the only mechanism respon-

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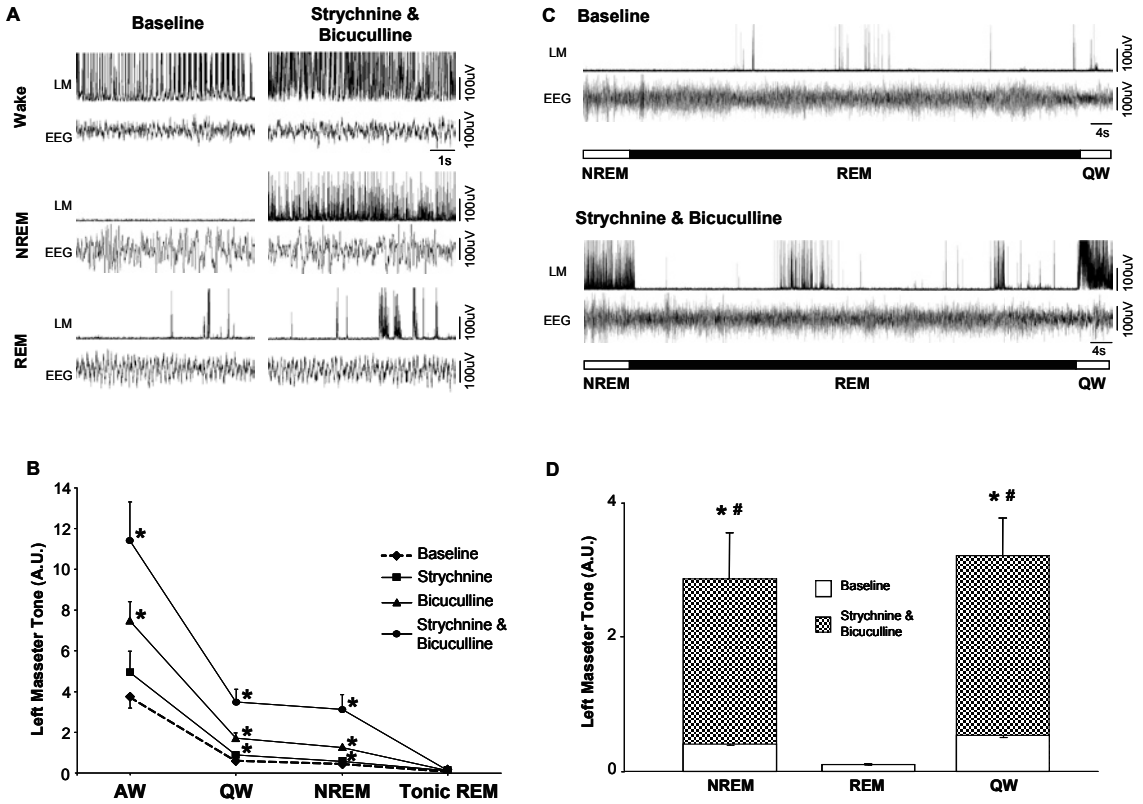

Figure 1—Blockade of glycine and $GABA_A$ receptors at the trigeminal motor pool does not prevent masseter muscle atonia during REM sleep. A. Typical EMG and EEG traces illustrating that strychnine/bicuculline perfusion into the left trigeminal motor pool increases left masseter muscle (LM) tone during waking and NREM sleep; this intervention only provokes muscle twitch activity during REM sleep, it does not reverse masseter REM atonia. B. Group data showing that 0.1mM strychnine/bicuculline perfusion increases LM tone in active (AW) and quiet waking (QW) and during NREM sleep; masseter activity in tonic REM sleep is unaffected. C. Example EEG/EMG traces from 1 rat showing how left masseter muscle activity changes during the transition into and out of REM sleep during baseline and strychnine/bicuculline perfusion. Traces illustrate that strychnine/bicuculline perfusion at the left trigeminal motor pool increases LM activity during the NREM and waking periods preceding and following REM onset, but that masseter atonia is unaffected by this intervention. D. Group data showing that strychnine/bicuculline perfusion increases basal LM tone in NREM and post-REM waking (QW), but not during REM sleep.
Note: * indicates $P < 0.05$ from baseline levels; # indicates $P < 0.05$ from tonic REM sleep; A.U., arbitrary units. All values are mean ± SEM. Figures are adapted from Brooks and Peever (2008).[7]

sible for hyperpolarizing motoneurons during REM sleep, then why does it remain after antagonism of glycine receptors? The authors of this paper themselves suggest that (1) "residual hyperpolarization may result from the presynaptic withdrawal of facilitatory influences (i.e., disfacilitation)" or that (2) "a non-glycinergic inhibitory neurotransmitter may be responsible for the residual tonic hyperpolarization."[15] The possible role of disfacilitation in motoneuron hyperpolarization during REM sleep has not been studied at the cellular level. Therefore, although intracellular studies illustrate that glycine inhibits motoneurons during REM sleep, this is *not the only factor* responsible for either generating IPSPs or hyperpolarizing motoneurons during REM sleep; other mechanisms play a role in generating inhibition/hyperpolarization—mechanisms that require identification.

It is important to realize that non-glycinergic mechanisms also regulate motor outflow to skeletal muscles during REM sleep. For example, Dr. Siegel's group has demonstrated that release profiles of several neurotransmitters change within hypoglossal and spinal motor pools during REM-like atonia. They report that glycine and GABA levels increase[12] and serotonin and noradrenaline levels decrease during REM atonia,[13] suggesting that both increased inhibition and decreased excitation contribute to reduced motoneuron excitability and loss of muscle tone during REM sleep. Furthermore, Drs. Horner and Kubin, as well as our laboratory, have demonstrated that reduced monoaminergic excitation at somatic motor pools contributes to REM sleep mechanisms of motor suppression.[10,11,18] In particular, withdrawal of an excitatory noradrenergic drive at trigeminal and hypoglossal motor pools underlies, at least in part, the reduction in masseter and genioglossus muscle tone in REM sleep.[11,18] However, reduced excitation is not the only mechanism responsible for muscle atonia during natural REM sleep because direct application of excitatory transmitter agonists (e.g., serotonin, noradrenaline, and glutamate) at trigeminal and hypoglossal motor pools cannot reverse REM atonia of masseter and genioglossus muscles, despite the fact that these same excitatory agents have potent stimulatory effects on muscle tone during both wakefulness and NREM sleep.[7,11,18-20] Because REM atonia persists even when glycine and $GABA_A$ receptors are blocked and glutamatergic agonists are simultaneously applied to the trigeminal motor pool, we conclude that a powerful,

yet unidentified, inhibitory mechanism or mechanisms override excitation during REM sleep.[7]

Together, the preceding lines of evidence demonstrate that *multiple mechanisms mediate motor outflow to skeletal muscles during REM sleep*. Such mechanisms include, but are not limited to, increased glycinergic and $GABA_A$-mediated inhibition,[7,8,14,15] as well as decreased noradrenergic and serotonergic excitation.[10,11,18] Although intracellular studies demonstrate that glycinergic mechanisms inhibit spinal and trigeminal motoneurons during REM sleep,[14,15,21] accumulating evidence[7-9] indicates that neither glycinergic nor $GABA_A$-mediated inhibition plays a critical role in triggering REM atonia. We echo the sentiments of Drs. Berger[2] and Kubin[5] who cautiously suggest that REM atonia is probably not mediated by a single pathway but rather by a multitude of neurotransmitter mechanisms.

TECHNICAL CONSIDERATIONS

Some commentators have suggested that certain technical limitations with our experimental model prevented us from making meaningful conclusions. Although we acknowledge that our experimental approach has some limitations, we disagree that they preclude us from making credible assertions; indeed, many laboratories use a similar approach to deduce mechanisms of brain function during sleep.[22-26] We justify the claim that our experimental paradigm can be used to generate interpretable and meaningful data by addressing commentators' concerns below.

Some commentators have suggested that we cannot interpret our data because the microdialysis technique affects all cell types within the trigeminal motor pool. We agree that microdialysis perfusion simultaneously affects thousands of cells, receptors, and synapses within the trigeminal nucleus. In fact, we consider this a distinct advantage of the technique because only studies that manipulate the activity of an entire motoneuron population (e.g., trigeminal motor pool) can ultimately determine mechanisms responsible for producing *physiological* changes in muscle tone during REM sleep. Intracellular studies that examine the activity of one motoneuron at a time can only *infer* that the pathway under study represents the actual mechanism operating within the entire motor pool. In our experiments, we acknowledge that our manipulations not only affect the 4,000 to 6,000 α-motoneurons,[27-29] which are the primary constituents of motor pools, but also the supporting neural elements such as interneurons and glia. However, multiple lines of evidence indicate that the *net effect* (i.e., masseter muscle tone) of neurochemical manipulation at the motor pool is mediated by trigeminal motoneurons. For example, we have previously shown that the microdialysis technique can be used to selectively block the AMPA–receptor-dependent excitation of motoneurons that causes the characteristic muscle twitches that punctuate REM atonia.[19] Specifically, we have shown that perfusion of a non-NMDA receptor antagonist at the trigeminal motor pool completely blocks masseter muscle twitches during REM sleep, whereas perfusion of an NMDA receptor antagonist does not. These findings fully agree with intracellular studies showing that REM sleep muscle twitches are mediated by AMPA–receptor-driven excitatory postsynaptic potentials onto identified motoneurons during REM sleep.[30] If our approach can successfully block the specific biochemical mechanisms that cause motoneurons to trigger REM muscle twitches, then why can this same approach not be used to determine whether glycinergic inhibition of motoneurons represents the mechanism underlying REM atonia?

Furthermore, if the microdialysis technique primarily affects motor outflow via interneuron-motoneuron interactions, then we would have expected changes in both left and right masseter muscle tone because trigeminal interneurons within the left motor pool also project to and densely innervate the right motor pool (and vice versa).[31,32] However, in two separate studies, we have shown that manipulation of neurotransmission in the left trigeminal motor pool never affects right masseter muscle tone (see Figure 1 from Brooks and Peever[7] and Figures 3 and 4 from Burgess et al[19]). Although we have little doubt that interneurons are affected by such interventions, we assert that changes in muscle tone following drug interventions are predominantly mediated by trigeminal motoneurons.

Importantly, Dr. Funk[4] noted that the hypoglossal motor pool is a homogenous nucleus that contains relatively fewer interneurons than the trigeminal motor pool. However, it is important to note that blockade of glycinergic and GABAergic transmission at the hypoglossal motor pool is also unable to reverse genioglossus atonia during natural or carbachol-induced REM sleep.[8,9] Therefore, two series of experiments, one in the homogenous hypoglossal nucleus and the other in the trigeminal motor pool, provide complementary evidence that blockade of glycinergic- and $GABA_A$-mediated inhibition does not prevent REM atonia.

Some commentators have suggested that indiscriminate diffusion of drugs into nuclei surrounding the trigeminal motor pool somehow affected the normal triggering of REM sleep mechanisms. Drug diffusion is a ubiquitous problem in most studies of this nature, including the iontophoresis method used in intracellular recording studies discussed above. The iontophoresis method affects not only the recorded motoneuron, but also local interneurons and glia; moreover, the strong currents used to eject drugs can also have deleterious side effects on synaptic physiology (see reviews of this method[33-35]). Therefore, similar criticisms apply to the intracellular recording method. In our experiments, we acknowledge that drugs may diffuse into tissue surrounding the target site; however, we present evidence illustrating that microdialysis perfusate primarily affects a small core of neurons in the trigeminal motor pool. First, we have shown[36] that drug perfusion in the rostral ventromedial division of the trigeminal nucleus only affects the activity of palatal muscles that are innervated by motoneurons in this region; it has no effect on masseter muscles, which are innervated by motoneurons in the adjacent dorsolateral division of the nucleus,[37-39] indicating that drug perfusion only affects a small core of tissue. Second, we showed that sleep-wake architecture, electroencephalographic spectral power, and right masseter muscle activity were unaffected by manipulation of neurotransmission at the left trigeminal motor pool,[7, 19] suggesting that drugs did not substantially diffuse and affect cells in the surrounding nuclei that mediate sleep and generalized muscle tone.[40] Lastly, if drug diffusion into surrounding nuclei somehow affected the normal triggering of REM sleep mechanisms, then why did blockade of glycine receptors in the homogenous

hypoglossal motor pool,[8] which is located in the caudal medulla and distant to sleep-regulating circuitry, not prevent the major suppression of genioglossus muscle tone in REM sleep?

Finally, some commentators have suggested that REM atonia was not reversed because glycine/$GABA_A$ receptors were insufficiently antagonized. We admit that we cannot unequivocally refute this possibility; however, our control experiments show that strychnine and bicuculline (1) completely reversed the major suppression of masseter muscle tone evoked by exogenous application of high doses of glycine and GABA receptor agonists (see Figure 10 from Brooks and Peever[7]), (2) increased (above baseline) masseter tone by 502% during waking and 643% during NREM sleep, and, (3) potently increased masseter muscle twitches during REM sleep without affecting REM atonia (Figure 1). Because IPSPs that hyperpolarize motoneurons are maximal when REM sleep muscle twitches occur[41] and because we show that muscle twitches increased after strychnine/bicuculline application, we contend that glycine/$GABA_A$ receptors were sufficiently antagonized. Furthermore, a landmark study from Dr. Kubin's laboratory shows that doses of strychnine and bicuculline significantly higher (2.5 mM)[9] than the concentrations we used (0.1 mM)[7] were also unable to prevent loss of genioglossus muscle tone in REM sleep when applied to the hypoglossal motor pool. Together, these findings indicate that glycinergic and $GABA_A$-mediated inhibition plays, at most, a minor role in producing REM atonia.

REEVALUATING MECHANISMS OF REM ATONIA

If glycinergic inhibition is indeed this pervasive force that drives REM atonia, then why does blockade of glycine receptors at the trigeminal motor pool not prevent it? Firstly, it is important to recognize that the intracellular method only samples a fraction of the motoneuron population and that this method has associated sampling biases, e.g., large cells are most easily recorded.[42,43] Therefore, one possibility is that glycinergic inhibition predominates in only a minority of motoneurons. This could explain why antagonism of glycine receptors abolished IPSPs in only 32% of recorded motoneurons (with IPSPs remaining in 68% of cells)[14] and why a significant degree of motoneuron hyperpolarization remained during REM sleep even though glycine receptors were antagonized by strychnine.[15] This assertion is also supported by the fact that not all motoneurons within a motor pool are controlled by the same neurochemical mechanisms. Even though the hypoglossal motor pool is considered a homogenous nucleus, there are in fact a variety of neurochemical inputs that differentially control motoneuron activity, e.g., some motoneurons receive inspiratory inputs, others expiratory inputs, and others tonic inputs.[42] It is therefore possible that glycinergic inhibition is responsible for suppressing motoneuron activity in only a restricted population of cells.

Dr. Funk[4] also raised the point that different motor pools may be controlled by different biochemical mechanisms during REM sleep, which could explain why postural muscle tone is lost while diaphragm muscle activity is spared during REM sleep; this concept may also explain why neck and lingual muscle activity are differentially affected by REM sleep.[44] However, we suggest that the most parsimonious explanation is that glycinergic inhibition primarily functions to suppress the sporadic muscle twitches that punctuate REM atonia. This assertion is consistent with both our work[7] and that from intracellular recording studies.[14,15] The data under discussion show that antagonism of glycine receptors at the trigeminal nucleus potently facilitates masseter muscle twitch activity during REM sleep, and intracellular studies show that the largest and most frequent glycine-sensitive IPSPs on motoneurons occur during periods of REM sleep with muscle twitches.[45,46] Therefore, we suggest that glycinergic inhibition ultimately functions to oppose the glutamatergic inputs[30] that trigger REM sleep muscle twitches.[7,19]

Although glycine inhibition is supposed to cause REM atonia, we found that blocking glycine receptors had no effect on masseter muscle tone. We suggest that the residual IPSPs and hyperpolarization remaining in motoneurons after glycine receptor antagonism[14,15] represent the inhibitory mechanism responsible for REM atonia. Accordingly, we conclude that glycinergic inhibition plays a negligible role in producing REM atonia—this phenomenon is primarily mediated by an unidentified inhibitory neurochemical substrate or substrates. We therefore suggest that the mechanisms mediating REM atonia require reevaluation.

UNRAVELING MECHANISMS OF REM ATONIA

We conclude by discussing some of our recent work that continues to identify mechanisms mediating REM atonia. As previously discussed, we recognize that our current experimental model[7] has limitations; therefore, we have developed two additional model systems that obviate such limitations and permit dissection of REM sleep mechanisms using unique approaches. Firstly, we have developed a transgenic mouse model for studying how loss of normal glycine- and $GABA_A$-receptor function affects REM sleep motor control in behaving mice[47, 48] This model was generated by inserting a mutant glycine receptor subunit into the mouse genome, which results in a 70% and 90% reduction in normal glycinergic- and $GABA_A$-mediated inhibition onto presumptive spinal motoneurons.[49] Even though somatic motoneurons are virtually unable to respond to glycinergic/GABAergic inhibition, our electrophysiological and behavioral data show that REM atonia is perfectly preserved—the only disruption in muscle tone in REM sleep was a marked exaggeration of REM sleep muscle twitches.[47,48] Such observations are consistent with the paper under forum discussion.[7]

We have also established an experimental model that uses protein-silencing methods to engineer trigeminal motoneurons so that they no longer express the critical chloride transporter KCC2 (i.e., potassium chloride cotransporter 2) that mediates the inhibitory effects of GABA and glycine.[50,51] We used antisense oligonucleotide technology to delete KCC2 from trigeminal motoneurons and found significant increases in masseter muscle tone during both waking and NREM sleep; however, this same intervention did not prevent REM atonia. Blockade of glycinergic and GABAergic inhibition only caused potent increases in muscle twitch activity in REM sleep. These findings are entirely consistent with the paper under forum discussion.[7]

In summary, we have three separate but complementary experimental paradigms for manipulating glycinergic- and $GABA_A$-mediated inhibition at the trigeminal motor pool;

whether we disrupt inhibition via pharmacological, genetic, or protein-silencing methods, the results remain the same—depriving motoneurons of glycine- and $GABA_A$-mediated inhibition does not prevent REM sleep atonia. Lastly, we do not doubt or even dispute the results from Drs. Chase and Soja's intracellular studies showing that motoneurons are influenced by glycinergic inhibition during REM sleep[14,15]; however, we question the degree to which glycinergic inhibition contributes to motor atonia in REM sleep. Accordingly, we suggest that glycine is but one of many biochemical pathways responsible for controlling muscle tone in REM sleep. We therefore conclude that there is indeed considerable uncertainty, and therefore no consensus, concerning the mechanisms that underlie REM atonia.

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DISCLOSURE STATEMENT

The authors have indicated no financial conflicts of interest.

REFERENCES

1. Lydic R. The motor atonia of REM sleep: a critical topics forum. Sleep 2008;31:1471-2.
2. Berger AJ. What causes muscle atonia in REM? Sleep 2008;31:1477-8.
3. Chase M. Confirmation of the consensus that glycinergic postsynaptic inhibition is responsible for the atonia of REM sleep. Sleep 2008;31:1487-91.
4. Funk GD. Are all motoneurons created equal in the eyes of REM sleep and the mechanisms of muscle atonia? Sleep 2008;31:1479-82.
5. Kubin L. Adventures and tribulations in the search for the mechanisms of the atonia of REM sleep. Sleep 2008;31:1473-6.
6. Soja PJ. Glycine-mediated postsynaptic inhibition is responsible for REM sleep atonia. Sleep 2008;31:1483-6.
7. Brooks PL, Peever JH. Glycinergic and GABA(A)-mediated inhibition of somatic motoneurons does not mediate rapid eye movement sleep motor atonia. J Neurosci 2008;28:3535-45.
8. Morrison JL, Sood S, Liu H, et al. Role of inhibitory amino acids in control of hypoglossal motor outflow to genioglossus muscle in naturally sleeping rats. J Physiol 2003;552:975-91.
9. Kubin L, Kimura H, Tojima H, Davies RO, Pack AI. Suppression of hypoglossal motoneurons during the carbachol-induced atonia of REM sleep is not caused by fast synaptic inhibition. Brain Res 1993;611:300-12.
10. Fenik VB, Davies RO, 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 2005;172:1322-30.
11. Chan E, Steenland HW, Liu H, Horner RL. Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. Am J Respir Crit Care Med 2006;174:1264-73.
12. Kodama T, Lai YY, Siegel JM. Changes in inhibitory amino acid release linked to pontine-induced atonia: an in vivo microdialysis study. J Neurosci 2003;23:1548-54.
13. Lai YY, Kodama T, Siegel JM. 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 2001;21:7384-91.
14. Chase MH, Soja PJ, Morales FR. Evidence that glycine mediates the postsynaptic potentials that inhibit lumbar motoneurons during the atonia of active sleep. J Neurosci 1989;9:743-51.
15. Soja PJ, Lopez-Rodriguez F, Morales FR, Chase MH. The postsynaptic inhibitory control of lumbar motoneurons during the atonia of active sleep: effect of strychnine on motoneuron properties. J Neurosci 1991;11:2804-11.
16. Jonas P, Bischofberger J, Sandkuhler J. Corelease of two fast neurotransmitters at a central synapse. Science 1998;281:419-24.
17. O'Brien JA, Berger AJ. Cotransmission of GABA and glycine to brain stem motoneurons. J Neurophysiol 1999;82:1638-41.
18. Mir S, Yee N, Brooks PL, Peever JH. Noradrenergic control of motoneurons during natural sleep in rats. Sleep 2006;29:A27.
19. 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 2008;28:4649-60.
20. Jelev A, Sood S, Liu H, Nolan P, Horner RL. Microdialysis perfusion of 5-HT into hypoglossal motor nucleus differentially modulates genioglossus activity across natural sleep-wake states in rats. J Physiol 2001;532:467-81.
21. Soja PJ, Morales FR, Baranyi A, Chase MH. Effect of inhibitory amino acid antagonists on IPSPs induced in lumbar motoneurons upon stimulation of the nucleus reticularis gigantocellularis during active sleep. Brain Res 1987;423:353-8.
22. Coleman CG, Baghdoyan HA, Lydic R. Dialysis delivery of an adenosine A2A agonist into the pontine reticular formation of C57BL/6J mouse increases pontine acetylcholine release and sleep. J Neurochem 2006;96:1750-9.
23. Sood S, Morrison JL, Liu H, Horner RL. Role of endogenous serotonin in modulating genioglossus muscle activity in awake and sleeping rats. Am J Respir Crit Care Med 2005;172:1338-47.
24. Reid MS, Nishino S, Tafti M, Siegel JM, Dement WC, Mignot E. Neuropharmacological characterization of basal forebrain cholinergic stimulated cataplexy in narcoleptic canines. Exp Neurol 1998;151:89-104.
25. Kumar S, Szymusiak R, Bashir T, Rai S, McGinty D, Alam MN. Effects of serotonin on perifornical-lateral hypothalamic area neurons in rat. Eur J Neurosci 2007;25:201-12.
26. Tao R, Ma Z, McKenna JT, et al. Differential effect of orexins (hypocretins) on serotonin release in the dorsal and median raphe nuclei of freely behaving rats. Neuroscience 2006;141:1101-5.
27. Martin MR, Caddy KW, Biscoe TJ. Numbers and diameters of motoneurons and myelinated axons in the facial nucleus and nerve of the albino rat. J Anat 1977;123:579-87.
28. Watson CR, Sakai S, Armstrong W. Organization of the facial nucleus in the rat. Brain Behav Evol 1982;20:19-28.
29. Chen KN, Wen CY, Shieh JY, Tseng TM. The somatotopy of the masticatory neurons in the rat trigeminal motor nucleus as revealed by HRP study. Proc Natl Sci Counc Repub China B 1988;12:146-55.
30. Soja PJ, Lopez-Rodriguez F, Morales FR, Chase MH. Effects of excitatory amino acid antagonists on the phasic depolarizing events that occur in lumbar motoneurons during REM periods of active sleep. J Neurosci 1995;15:4068-76.
31. McDavid S, Lund JP, Auclair F, Kolta A. Morphological and immunohistochemical characterization of interneurons within the rat trigeminal motor nucleus. Neuroscience 2006;139:1049-59.

32. Ter Horst GJ, Copray JC, Van Willigen JD, Liem RS. Contralateral projections of cells in the motor trigeminal nucleus of the rat. Neurosci Lett 1990;113:260-6.
33. Bloom FE. To spritz or not to spritz: the doubtful value of aimless iontophoresis. Life Sci 1974;14:1819-34.
34. Hicks TP. The history and development of microiontophoresis in experimental neurobiology. Prog Neurobiol 1984;22:185-240.
35. Robinson DL, Hermans A, Seipel AT, Wightman RM. Monitoring rapid chemical communication in the brain. Chem Rev 2008;108:2554-84.
36. Schwarz P, Peever JH. Dopaminergic modulation of upper airway motoneurons controlling tensor palatini muscle tone in rats. Sleep 2008;31:A11.
37. Sasamoto K. Motor nuclear representation of masticatory muscles in the rat. Jpn J Physiol 1979;29:739-47.
38. Keller JT, Saunders MC, Ongkiko CM, et al. Identification of motoneurons innervating the tensor tympani and tensor veli palatini muscles in the cat. Brain Res 1983;270:209-15.
39. Mizuno N, Konishi A, Sato M. Localization of masticatory motoneurons in the cat and rat by means of retrograde axonal transport of horseradish peroxidase. J Comp Neurol 1975;164:105-15.
40. Lu J, Sherman D, Devor M, Saper CB. A putative flip-flop switch for control of REM sleep. Nature 2006;441:589-94.
41. Pedroarena C, Castillo P, Chase MH, Morales FR. The control of jaw-opener motoneurons during active sleep. Brain Res 1994;653:31-8.
42. Peever JH, Shen L, Duffin J. Respiratory pre-motor control of hypoglossal motoneurons in the rat. Neuroscience 2002;110:711-22.
43. Ritter AM, Mendell LM. Somal membrane properties of physiologically identified sensory neurons in the rat: effects of nerve growth factor. J Neurophysiol 1992;68:2033-41.
44. Lu JW, Mann GL, Ross RJ, Morrison AR, Kubin L. Differential effect of sleep-wake states on lingual and dorsal neck muscle activity in rats. Respir Physiol Neurobiol 2005;147:191-203.
45. Lopez-Rodriguez F, Chase MH, Morales FR. PGO-related potentials in lumbar motoneurons during active sleep. J Neurophysiol 1992;68:109-16.
46. Lopez-Rodriguez F, Morales FR, Soja PJ, Chase MH. Suppression of the PGO-related lumbar motoneuron IPSP by strychnine. Brain Res 1990;535:331-4.
47. Brooks PL, Peever JH. Clonazepam reduces myoclonic activity during REM sleep in a mouse model of REM sleep behavior disorder. Sleep 2008;31:A4.
48. Brooks PL, Tse G, Peever JH. Impaired GABAergic and glycineric neurotransmission induces REM-sleep behaviour disorder (RBD) in transgenic mice. Sleep 2007;30:A11.
49. Becker L, von Wegerer J, Schenkel J, Zeilhofer HU, Swandulla D, Weiher H. Disease-specific human glycine receptor alpha1 subunit causes hyperekplexia phenotype and impaired glycine- and GABA(A)-receptor transmission in transgenic mice. J Neurosci 2002;22:2505-12.
50. Rivera C, Voipio J, Payne JA, et al. The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 1999;397:251-5.
51. Ueno T, Okabe A, Akaike N, Fukuda A, Nabekura J. Diversity of neuron-specific K+-Cl- cotransporter expression and inhibitory postsynaptic potential depression in rat motoneurons. J Biol Chem 2002;277:4945-50.