Adventures and Tribulations in the Search for the Mechanisms of the Atonia of REM Sleep

Commentary on Brooks PL and 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.

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The Background

Thirty years ago, the first report was published with intracellular recordings from motoneurons, trigeminal motoneurons, in chronically instrumented, behaving cats across the sleep-wake cycle.[1] A major observation was that synaptic activity, both excitatory and inhibitory, declined during rapid eye movement (REM) sleep in association with the characteristic motoneuronal hyperpolarization. However, subsequent intracellular recordings from motoneurons revealed that REM sleep was associated with the appearance of inhibitory postsynaptic potentials (IPSPs) of which some had uniquely large amplitudes.[2-7] The potentials were abolished by strychnine, an antagonist of chloride-dependent, fast inhibition mediated by glycine, when the drug was administered by iontophoresis into the vicinity of the recorded motoneuron.[8] These results have led to a widely accepted concept that postsynaptic, glycine-mediated inhibition is *the cause* of motoneuronal hyperpolarization.

In another study, using an indirect approach to assess the role of inhibitory amino acids, glycine, or GABA, in the REM sleep-related suppression of trigeminal motoneurons, evidence for a contribution of active inhibition was inconclusive. Concurrently, the study provided clear evidence that neurotransmitters other than glycine or GABA significantly contribute to the REM sleep-related depression of motoneuronal excitability.[9] Some of the potential mechanisms considered included a REM sleep-dependent loss of Ia afferent-mediated activation and REM sleep-related withdrawal of aminergic excitation of motoneurons.

Fifteen years ago, I coauthored a study titled "*Suppression of hypoglossal motoneurons during the carbachol-induced atonia of REM sleep is not caused by fast synaptic inhibition.*"[10] We used an unanesthetized, decerebrate cat carbachol model of the atonia of REM sleep in experiments designed to test whether antagonists of glycinergic or $GABA_A$-receptors microinjected into the hypoglossal motor nucleus can eliminate or diminish the depression of hypoglossal nerve activity elicited by microinjections of a cholinergic agonist, carbachol, in the dorsomedial pontine reticular formation. We found that each of the two antagonists used, strychnine and bicuculline, increased the baseline level of hypoglossal nerve activity and antagonized the reflexly elicited inhibition of hypoglossal motoneurons. We also found that neither antagonist diminished the magnitude of the depression of hypoglossal nerve activity during the atonia of REM sleep. While the properties of the carbachol models of REM sleep atonia are discussed elsewhere,[11,12] three aspects of the experimental design used in our 1993 study[10] are relevant for our comments on the article by Brooks and Peever.[13] First, by increasing the doses of the antagonists injected into the hypoglossal nucleus, we were able to ensure that we attained full occupancy of the relevant inhibitory receptors. Second, by using vagotomy and hypercapnic conditions, and taking advantage of the fact that hypoglossal motoneurons are rhythmically activated by central inspiratory drive, we established the conditions under which hypoglossal nerve activity was depressed during the carbachol-induced atonia but did not entirely disappear. This allowed us to continuously monitor motoneuronal activity during both the baseline conditions and the REM sleep-like atonia. Third, the experiments were conducted under neuromuscular paralysis and artificial ventilation, which eliminated any potential peripheral receptor contribution to the REM sleep-like depression of motoneuronal activity.

The Concepts Proposed by Brooks and Peever

Brooks and Peever[13] investigated the effect of strychnine or/and bicuculline on the suppression of activity in the masseter, a muscle innervated by trigeminal motoneurons, during REM sleep. The study was conducted in chronically instrumented, behaving rats, and the drugs were delivered into the trigeminal motor nucleus by reverse microdialysis. Some of the unique features of the study included experiments with combined delivery of both strychnine and bicuculline and an attempt to elevate the masseteric activity by co-perfusion with a glutamatergic receptor agonist throughout the sleep-wake cycle.

Brooks and Peever made the following statements about motoneuronal control during REM sleep:

1. Neither glycine nor GABA mediate the REM sleep atonia of trigeminal motoneurons.

2. Trigeminal motoneurons are tonically inhibited by glycine and GABA during wakefulness and NREM sleep, with the

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inhibition during NREM sleep being stronger than that during wakefulness.

3. Phasic glycinergic inhibition suppresses the expression of masseter twitches that characteristically occur with some delay after REM sleep onset.

The experiments are elegantly illustrated, and the results are consistent with the proposed concepts. Personally, I was pleased to see that our finding of a minimal contribution of amino acid-mediated inhibition to REM sleep atonia of hypoglossal motoneurons[10] may also apply to trigeminal motoneurons. The key question, however, is whether the evidence gathered by Brooks and Peever can sway the opinion of those convinced that active inhibition is the main cause of REM sleep atonia. Having examined the data, I concluded that the experiments did not provide conclusive evidence for any of the main statements.

Regarding the inability of the antagonists to eliminate the tonic depression of masseter activity during REM sleep, I note that the antagonist concentrations were relatively low (100 μM) and possibly not sufficient to fully antagonize all relevant receptors located on cell bodies and dendrites of trigeminal motoneurons. That this could be the case is suggested by at least three lines of evidence. First, in the key control experiment used to assess the effectiveness of the antagonists (Figure 10, Figures cited in this commentary refer to Figures in the original paper by Brooks and Peever), the concentrations of glycine and muscimol ($GABA_A$-receptor agonist) were such that they depressed masseter activity to a lesser degree than the magnitude of depression attained during REM sleep. Therefore, the subsequent demonstration that the two antagonists combined restored masseter activity only slightly above the pre-antagonist level does not represent a stringent enough test to conclude that the concentrations of the antagonists were sufficiently high, and their spread sufficiently wide, to fully antagonize the hypothetically strong endogenous inhibition of motoneurons during REM sleep. Second, the finding that neither one, nor the two antagonists combined, nor the two antagonists plus a glutamatergic agonist could bring masseter to tonic firing during REM sleep contrasts with the evidence from other studies showing that strychnine or bicuculline microinjected directly into the trigeminal or hypoglossal motor nucleus at concentrations 10-25 times higher than those used by Brooks and Peever can increase motoneuronal exctitability during the atonia of REM sleep.[9,10] It is technically difficult to measure the spread and tissue concentration of drugs focally applied into the brain *in vivo*. What is feasible is to determine whether a higher dose of antagonists would further increase masseter activity, indicating that more receptors could be blocked. In the absence of such experiments, the possibility remains that the antagonist concentrations were insufficient.

The concept that trigeminal motoneurons are more strongly inhibited by glycine and GABA during NREM sleep than in wakefulness is an interesting one, but the evidence for this is derived from relative measures of masseter activity. As a result of the greatly reduced activity during quiet wakefulness and NREM sleep compared to active wakefulness, the effect of the antagonist measured in relative units comes out as much stronger during non-REM sleep than during active wakefulness. However, the same data examined on the basis of the absolute activity increases caused by the antagonists (Figures 2B, 5B, 7B) would lead one to the opposite conclusion; namely, that the magnitude of endogenous GABAergic and glycinergic inhibition of trigeminal motoneurons is stronger during active wakefulness than during the other behavioral states.

Likely due to the use of too low concentrations of the antagonists, Brooks and Peever were unable to maintain tonic masseter activation during REM sleep and could only observe phasic muscle twitches. They found that perfusion with strychnine, but not bicuculline, increased twitch amplitude during REM sleep (Figures 2C and 5C). They interpreted this as evidence for a REM sleep-specific "phasic inhibitory drive" that is mediated by glycine and functions to suppress muscle twitches. This postulate was not supported by the results of the combined perfusion with strychnine and bicuculline, as there was no increase in twitch amplitude (Figure 7C). Considering that the masseter was inactive during REM sleep (with the exception of twitches), one must assume that the membrane potential of trigeminal motoneurons was below the firing threshold except when phasic synaptic volleys comprising both activation and inhibition reached motoneurons and were strong enough to transiently bring them to firing. Accordingly, one would expect that any manipulation that increases motoneuronal excitability (brings membrane potential closer to firing threshold) would increase the amplitude, frequency and/or duration of muscle twitches. This should also be the case for perfusion with a glutamatergic receptor agonist only. Such data were not presented in this study, but another related study shows that manipulations with glutamatergic receptors in the trigeminal motor nucleus can alter the amplitude and frequency of masseter twitches.[14] Thus, while the finding that strychnine and bicuculline increased different measures of masseter twitches during REM sleep provide reassurance that the antagonists had some effect, it does not constitute evidence for a REM sleep-specific, glycinergic "inhibitory drive" that is "phasic" and distinctly involved in the control of twitches during REM sleep.

The Wakefulness Stimulus for Motor Activation, and Where Do We Go From Here?

Having concluded that neither glycine nor GABA mediates the tonic inhibition of trigeminal motoneurons during REM sleep atonia, Brooks and Peever proposed that "a powerful, yet unidentified, inhibitory mechanism overrides motoneuron excitation during REM sleep." While this is an attractive mystery, the authors did not consider the alternative to an active, REM sleep-specific inhibition as a major cause of motoneuronal atonia that already has been proposed and tested.[15] Namely, the finding that a combined antagonism of endogenous noradrenergic and serotonergic activation of hypoglossal motoneurons (the condition mimicking the silencing of central noradrenergic and serotonergic neurons during REM sleep) occludes the effect of REM sleep-like state on this motoneuronal group[15] currently stands unchallenged as the evidence that REM sleep atonia can be fully explained as resulting from the withdrawal of excitation mediated by just two transmitters, norepinephrine and serotonin. This result, because it was obtained using an anesthetized rat model of REM sleep atonia, may be a simplification. It is likely that other neuronal groups that are silent under anesthesia but have wake-related activity in behaving mammals (e.g., orexin, histamine, dopamine, acetylcholine) also provide im-

portant components of what we would collectively describe as the wakefulness drive for motor activity. This combined excitation may be gradually withdrawn from motoneurons, to some degree during NREM sleep and then more so during REM sleep, with the distinct sources of wake-related activation probably making different partial contributions in different pools of orofacial and spinal motoneurons. This not withstanding, the role of the combined withdrawal of noradrenergic and serotonergic drive as a major cause of the atonia of REM sleep needs to be evaluated using various models because only for these two amines there is currently multifaceted experimental support for a major role of their withdrawal in the atonia of REM sleep.[15] Indeed, the decline of synaptic activity in trigeminal motoneurons during REM sleep [1] likely represents a decline of multiple wake-related, excitatory drives for motor activity.

Is there a Role for Amino Acid-Mediated, Tonic Inhibition of Motoneurons During REM Sleep?

The study of Brooks and Peever[13] importantly adds to the evidence that neither glycine nor GABA play a major role in the atonia of REM sleep in orofacial motoneurons.[9,10,15-17] Nevertheless, there is also compelling evidence that both hypoglossal and trigeminal motoneurons receive IPSPs during REM sleep. The evidence is based on intracellular recordings,[6,7] and indirectly supported by the finding that the antagonism of glycinergic and $GABA_A$-receptor-mediated inhibition superimposed on the antagonism of aminergic excitation unveils in hypoglossal motoneurons a small excitatory effect of the atonia of REM sleep (discussed in ref. 15). Thus, however small its role, there is a tonic inhibitory component in the effect of REM sleep on orofacial motoneurons.

In contrast to the results from orofacial motoneurons that consistently point to a small role of postsynaptic inhibition, the contribution of amino acid-mediated inhibition to hyperpolarization of spinal motoneurons and the REM sleep atonia of the trunk and limb muscles remains to be determined. Numerous studies described strychnine-sensitive IPSPs,[2-5] and in one study iontophoretic application of strychnine nearly abolished all changes indicative of reduced motoneuronal excitability during REM sleep.[18] However, the causality of the relationship between the occurrence of IPSPs and motoneuronal hyperpolarization has not been systematically tested, and the strong currents used in the iontophoresis experiments could have deleterious effects on motoneurons in addition to the intended antagonism of glycinergic receptors. Thus, based on the current evidence, one can neither refute nor accept the theory that glycinergic inhibition is the main cause of REM sleep atonia in spinal motoneurons. Therefore, the reference to "somatic motoneurons" in the title of Brooks and Peever article implying that their results apply to all motoneurons, both orofacial and spinal, is unwarranted. Indeed, efforts also need to continue to find the hypothetical pathways that mediate the active, REM sleep-specific inhibition of motoneurons and the glycinergic interneurons with REM sleep-specific activity required by the concept that glycinergic inhibition causes the atonia of REM sleep.

Given that serotonin and norepineprine levels decrease during REM sleep atonia in the region of the spinal ventral horn,[19] the withdrawal of noradrenergic and serotonergic excitation also may contribute to the REM sleep-related hyperpolarization of spinal motoneurons. How large this is remains to be determined. More studies of spinal motoneurons using a range of approaches like those that have been used to date in hypoglossal and trigeminal motoneurons are needed to resolve this question.

In summary, this field has generated abundance of evidence that is "consistent with" but only limited proof that is "beyond reasonable doubt." I am sure that the intent of Brooks and Peever was to obtain the latter and that their article will stimulate further pursuit of the mechanisms of REM sleep atonia.

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