Are All Motoneurons Created Equal in the Eyes of REM Sleep and the Mechanisms of Muscle Atonia?

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 RECENT STUDY BY BROOKS AND PEEVER[1] EXAMINES STATE-DEPENDENT CHANGES IN TRIGEMINAL MOTOR ACTIVITY IN FREELY BEHAVING RATS following microdialysis of glycine and $GABA_A$-receptor antagonists into the V nucleus. Authors challenge the concept established in the 60s[2] and entrenched in the 80s primarily with the intracellular recording studies of Morales, Chase and Soja[3-8] that the muscle atonia of REM sleep is produced by a common, brainstem-derived, REM or active-sleep specific, postsynaptic, glycine-mediated inhibition of somatic motoneurons.

The nervous system employs two main mechanisms to reduce activity. The first is through active GABA or glycinergic inhibition and the second is via disfacilitation, the withdrawal of excitatory input. As already mentioned, Brooks and Peever[1] have challenged the prevailing view that for most motoneurons active inhibition underlies REM atonia. This view is based primarily on the analysis of lumbar and V motoneurons, of which the latter were examined by Brooks and Peever, and is often extrapolated to all motoneurons that show REM sleep specific reductions in excitability.[9] Is there evidence of any exceptions to this standard? The best candidates are respiratory motoneurons. First, many muscles innervated by these motoneurons do not experience complete atonia; most of us continue to breathe, albeit somewhat erratically, during active sleep without becoming hypoxic. Their activity is, never-the-less, reduced in REM with different respiratory motoneurons showing a gradation in their sensitivity to REM sleep atonia. Phrenic motoneurons that drive the diaphragm (the main inspiratory pump muscle in mammals) are least sensitive, while expiratory pharyngeal (vagal), and XII motoneurons (controlling upper airway muscles) are the most suppressed.[9]

Significant effort has focused on understanding the mechanistic basis of the variable inhibition within respiratory motoneuron pools. Not only is this an important question of basic neurophysiology, it is of great interest from a clinical perspective due to the growing prevalence of sleep disordered breathing in human populations.[10] Sleep disordered breathing, which includes obstructive sleep apnea, manifests in part due to a differential suppression of airway compared to diaphragm muscle tone during sleep, especially REM sleep.[11] This gradation in sensitivity to REM sleep atonia could reflect variability in the magnitude of the REM-specific glycinergic inhibition. Indeed the proposal that all motoneurons, regardless of how they are categorized (respiratory and non-respiratory, spinal and cranial, antigravity) are controlled by a single common mechanism is appealing in its simplicity. Nevertheless, a substantial body of work by Leszek Kubin and colleagues[9] using the carbachol model of REM-sleep atonia, and more recently by Richard Horner[12,13] using a preparation developed to enable delivery of drugs via microdialysis in unrestrained rats (from which the one used by Brooks and Peever was derived) suggest that the depression of XII activity, and by extrapolation perhaps all respiratory motoneurons, is primarily due to disfacilitation rather than glycinergic inhibition.[14-20] The basic approach, like that used by Brooks and Peever, is to deliver an antagonist to the motoneuron pool and assess whether the magnitude of the REM-sleep induced atonia (measured at the relevant nerve for the carbachol model or muscle in freely moving rats) is influenced by the absence of that modulatory system. An altered atonia is taken as evidence that the modulator in question contributes. Debate about the relative roles of disfacilitation vs active inhibition in producing the REM-sleep atonia of respiratory muscles continues. In light of recent advances with these two models, however, the debate has expanded and in fact shifted to focus on which modulatory systems underlie the disfacilitation[13,15,17,18] (more on this to follow).

In contrast, and counter to the statement by Brooks and Peever, the fact that active sleep-specific glycinergic inhibition underlies the muscle atonia of nonrespiratory muscles, especially lumbar and V, has not been seriously debated for some time. In fact, until the study by Brooks and Peever in V motoneurons,[1] this view and the data supporting it have not been challenged. Thus, the provocative statement by Brooks and Peever, based on the analysis of V muscle output, that the role of synaptic inhibition in REM sleep atonia requires revisiting has attracted a lot of attention. Not only does it propose that glycinergic inhibition is not the sole modulator of the REM sleep atonia in V motoneurons, it suggests that inhibition is not involved and that an as yet unidentified mechanism, presumably involving disfacilitation, is solely responsible.

What is the basis for rejecting the long-standing focus on inhibitory processes? First, what are the weaknesses in the data

Submitted for publication July, 2008
Accepted for publication July, 2008
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supporting the longstanding view that synaptic inhibition mediates REM sleep atonia in V motoneurons? The hypothesis that inhibition is the sole contributor to REM sleep atonia[2] was consolidated based on heroic intracellular recording data that first demonstrated active sleep-specific IPSPs.[4-6] Intracellular recordings were then coupled with juxtacellular application of antagonists to provide compelling evidence that all changes in motoneuron properties measured during REM sleep could be reversed by the block of glycinergic inhibition with strychnine.[7,8,21] These data provided no evidence supporting a role for disfacilitation.

The only possible limitation of the data supporting active glycinergic inhibition is that observations are limited to the level of single motoneurons. Individual motoneurons were recorded intracellularly and the effect of juxtacellular strychnine (and other transmitter agonists/antagonists) on single cell properties were compared across states. These measurements have not been extended to the whole nerve or muscle level. Thus, a potential advantage of the method used by Brooks and Peever is the ability to assess motor output at the population level where perhaps subtle disfacilitation-mediated changes in motoneuron excitability that were difficult to detect at the single motoneuron level may emerge. While the rational for trying to assess the population level effects is reasonable, manipulation of neuromodulators that show state-dependent changes in activity such as norepinephrine (NE) or serotonin (5HT) NE would seem a more fruitful approach, as it is impossible to find significant fault with the intracellular data showing that glycinergic inhibitory mechanisms are activated in REM sleep. Regardless of drug selection, it is next necessary to ask whether manipulation of REM-specific glycinergic drives is achievable using microdialysis probes? The answer is no. As to why not, it is instructive to consider why juxtacellular application of strychnine was used in combination with intracellular recording in the first place, as this is technically more difficult than more general infusion of drugs via pressure injection or microdialysis. It reflects the organization of the V motor system in which the motor nuclei are in close spatial proximity to, and in fact partially overlap, local excitatory and inhibitory interneuronal networks and premotor systems. Juxtacellular iontophoresis of strychnine was used to ensure that drug action was limited to those synapses that participate in the control of REM sleep. Microdialysis of strychnine into (or nearby) the V nucleus as performed by Brooks and Peever will almost certainly modify activity of surrounding networks in multiple and unpredictable ways. Consider one example of many possible scenarios in which the dialyzed strychnine produced in waking and NREM an increased activation of V motoneurons through disinhibition of a premotor excitatory pool that is normally inhibited. In this case, the transition to REM sleep would still be associated with a drop in EMG. This drop, however, would reflect withdrawal of an excitation in NREM that is not normally present. It would not indicate that EMG activity can still be reduced in REM sleep in the absence of glycinergic inhibition. Nor would this provide evidence that an unknown REM-specific, but non-glycinergic inhibitory mechanism underlies REM sleep atonia. The possibility that something akin to this contributed to the responses of Brooks and Peever is suggested by the profound increase in EMG activity observed during NREM sleep in the strychnine treated animals. This increase is attributed by Brooks and Peever to the unmasking of a previously unidentified NREM-specific glycinergic inhibition. Intracellular recordings from V motoneurons, however, provide no evidence of such a barrage of excitatory inputs in NREM sleep,[5,22,23] an observation that was unfortunately missed by Brooks and Peever.

This is not the only case where Brooks and Peever misinterpret their data. For example it is difficult to understand the basis for concluding that the lack of EMG activity during nonphasic REM sleep is due to disfacilitation rather than active inhibition. The absence of any activity during this phase in the presence of strychnine could as easily reflect that there is insufficient excitation to overcome the degree of inhibition that they may have been unable to block. Brooks and Peever appear to argue that because the amplitude of the phasic REM bursts increases with strychnine infusion, which is the time when synaptic inhibition is strongest, the block of synaptic inhibition must have been sufficient to block the weaker inhibition during the nonphasic REM. This argument, however, does not make sense. It is irrelevant whether the strychnine could reduce the maximum inhibition. Synaptic inhibition is strongest during the phasic REM excitatory bursts,[24] but these excitatory inputs are also suprathreshold. Thus, *any* reduction in inhibition will appear as an increase in EMG burst amplitude. This is not equivalent to establishing that all inhibition during nonphasic REM has been blocked. Another point is that even if the block of inhibition during nonphasic REM sleep was complete, lack of EMG activity would not exclude glycinergic inhibition. EMG activity would only be detected if a suprathreshold excitatory input was also present. Finally, the conclusion that strychnine application revealed REM-specific phasic glycinergic inhibition requires revisiting. This is based on their earlier incorrect conclusion that there is no glycinergic inhibition in nonphasic REM sleep, which then leads to the conclusion that any strychnine-mediated increase in the amplitude of EMG bursts in phasic REM sleep could only reflect block of phasic inhibition that was synchronized with the phasic bursts of excitation.

Despite the limitations outlined above, a very intriguing and perhaps the most significant aspect of the study, as pointed out by Dr. Berger in his comments, was the observation that exogenous AMPA, even in the presence of strychnine and bicuculline, was unable to evoke EMG activity in REM sleep (Figure 9, Figures cited in this commentary refer to Figures in the original paper by Brooks and Peever). Compared to baseline EMG activity in NREM sleep, strychnine, bicuculline, and AMPA evoked a 980% increase in EMG output, but this disappeared with the transition to REM. Whether these data indicate that something other than glycinergic inhibition is involved in REM, however, is unclear. Brooks and Peever missed an important opportunity to enhance the significance of this surprising observation. First and foremost, a dose response for AMPA would have been beneficial. While 0.1 mM AMPA is a potent dose acutely, potency is likely to decrease with prolonged microdialysis due to AMPA receptor desensitization and internalization. Second, inclusion of raw data showing the true magnitude of the EMG activity in strychnine, bicuculline, and AMPA compared to baseline, as done for phasic REM bursts in Figure 9c (top left and right panels) would be extremely valuable. This is necessary because the level of EMG activity in NREM strychnine, bicuculline, and

AMPA (Figure 9B) does not appear to be enormously elevated over that observed in NREM when only strychnine and bicuculline are present (Figure 8D); i.e., how much of the 980% increase above baseline observed in NREM in strychnine, bicuculline, and AMPA is due to AMPA?

In light of all this, are the data provided by Brooks and Peever[1] compelling enough to suggest that the role of synaptic inhibition in the muscle atonia of REM sleep requires revisiting? Not yet. While the observation that exogenous AMPA can not evoke a motor response in REM after strychnine and bicuculline application is intriguing, underlying mechanisms must be resolved before these data are sufficient to question high resolution intracellular data. Also problematic is that limitations of the approach employed by Brooks and Peever were not fully acknowledged. Nor were their data interpreted in relation to the full scope and significance of the overwhelming database showing that active postsynaptic glycinergic inhibition accounts for all changes in motoneuron properties observed with the transition to REM.

Where does this leave us with respect to the relative roles of glycinergic inhibition vs disfacilitation in the REM sleep atonia of respiratory motoneurons? As mentioned above, recent debate has focused on XII motoneurons and which combination of modulators are most important in the disfacilitation, but the relative role of glycinergic inhibition is still under investigation[15] and ongoing consideration.[25] First it is worth pointing out that a detailed intracellular analysis of XII motoneuron properties across natural sleep-wake cycles combined with the local application of antagonists as performed for V motoneurons would likely resolve this debate very quickly. That these data are not available speaks to the difficulty of such experiments. Instead, the majority of relevant data have been generated using local injection of agents into the XII nucleus in the carbachol model of REM sleep atonia, or microdialysis of drugs into the XII nucleus of unrestrained rats, which, to be fair, are also extremely challenging paradigms. These preparations and approaches share some of the limitations outlined above in reference to that used by Brooks and Peever to explore mechanisms of atonia in V. Important differences, however, include that the organization of the XII nuclei is somewhat better suited for these approaches than V. XII nuclei are organized bilaterally around the midline enabling more discrete application. XII nuclei are also among the most homogeneous nuclei in the nervous system (<5% of neurons are interneurons[26]) limiting actions on local interneuronal networks. Its physiology is also better suited. Unlike V motoneurons which are completely silent in REM,[1] XII motoneurons and the genioglossus muscle typically retain at least minor activity in REM because output can be increased by elevating levels of inspired CO_2. Thus it is possible to assess the effects of most manipulations on XII output across sleep states. Also important is that antagonism in the region of the XII nucleus of noradrenergic and serotonergic modulatory inputs prior to REM (natural or in the carbachol model) onset dramatically reduces further reductions in muscle tone.[9,15-17] Positive evidence of disfacilitation was not provided by Brooks and Peever. Lastly, investigators are aware that the potential for antagonists to influence local premotor networks is a significant limitation. Kubin and others, for example, have recently gone to extensive lengths to address possible spillover of injected antagonists into nearby premotor fields by modeling the degree of diffusion and precisely measuring injected volumes in the carbachol model of REM sleep to avoid spread outside the motor nucleus. Under these conditions, NE, 5HT, and GABAergic mechanisms can account for all reductions in tone associated with REM.[15] They report no need to invoke glycinergic inhibition.

These data, however, are not sufficient to conclude that glycinergic inhibition is not involved in the atonia of REM sleep in XII motoneurons. Large scale infusion of antagonists still has the potential to influence activity of surrounding premotor networks that are not involved in the state-dependent control of motoneuron excitability. Thus, approaches like this are most problematic when examining the role of glycinergic and GABAergic systems as these will dramatically affect premotor network activity in diverse, unpredictable ways. In addition, while intracellular data from XII motoneurons are not available across natural state changes, data from the carbachol model of REM sleep clearly reveal REM-specific glycinergic IPSPs in XII motoneurons[27] similar to those recorded in nonrespiratory motoneurons in the same model.

Thus, the current picture is that data from local application of neuromodulatory antagonists into XII in the carbachol model[15] or actual REM[17] favor disfacilitation as the dominant mechanism underlying REM atonia in XII; intracellular recording data in the carbachol model support glycinergic inhibition.[27] Is there any way that these data may be reconciled? The impact of carbachol REM-specific glycinergic IPSPs on inspiratory activity in XII motoneurons has not been documented. Therefore one possibility is that respiratory inputs are unique and somewhat resistant to REM sleep-specific glycinergic inhibitory mechanisms, but more sensitive to disfacilitation. Several mechanisms could protect inspiratory inputs from REM-specific inhibition. Inspiratory EPSPs could be of large enough amplitude that, like the non-NMDA receptor mediated inputs that underlie muscle twitches of phasic REM,[23] they are not blocked by active sleep-specific inhibition. Second, inspiratory synaptic inputs could be spatially segregated to portions of the dendritic tree that do not receive active sleep-specific IPSPs.

Are either of these mechanisms likely? Large amplitude glutamatergic drive may help preserve inspiratory activity during REM sleep in phrenic motoneurons. However, it is unlikely a mechanism common to all respiratory motoneurons as many, including XII motoneurons, do not receive large amplitude inspiratory drive. The distribution of inspiratory inputs relative to active sleep-specific inputs is unknown. The rapid reversal of active sleep-specific IPSPs with injection of intracellular chloride suggests that they are located close to the cell soma,[6] where they would be most effective in inhibiting inputs. Thus, while spatial segregation can not be ruled out, it is unlikely. A definitive answer will await intracellular recordings from inspiratory XII motoneurons during actual wake-NREM-REM state transitions.

In summary, accumulating evidence suggests that disfacilitation contributes to the REM sleep atonia of some respiratory motoneurons. In contrast, despite the suggestion by Brooks and Peever to the contrary, their new data are not yet compelling enough to challenge data showing that active sleep-specific glycinergic inhibition is the mechanism controlling REM-sleep atonia in V and possibly all nonrespiratory motoneurons that are subject to state-dependent reductions in excitability.

DISCLOSURE STATEMENT

Dr. Funk has indicated no financial conflicts of interest.

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