Glycine-Mediated Postsynaptic Inhibition is Responsible for REM Sleep 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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OVER FORTY YEARS AGO INVESTIGATORS HYPOTHESIZED THAT THE REDUCTION IN THE CONTRACTION OF HINDLIMB AND FORELIMB MUSCLES AND THE suppression of spinal reflexes during REM sleep was due to the postsynaptic inhibition of motoneurons.[1] Although it was recognized that the responsible processes could only be determined by recording intracellularly during naturally occurring states of sleep and wakefulness, it was considered to be technically impossible due to difficulties inherent in maintaining viable intracellular recordings of motoneurons in a behaving animal. These technical issues have been expounded on elsewhere.[2]

Nevertheless, beginning in 1978, Chase and colleagues[3,4] developed a chronic preparation whereby intracellular recordings could be obtained from identified motoneurons during naturally occurring states of sleep and wakefulness. They determined that postsynaptic inhibition was responsible for the suppression of motoneuron discharge during REM sleep.[5-7] Chase et al.[8] and Soja et al.[9] then developed a five-barreled multipipette that was combined with an intracellular recording electrode in order to eject neurotransmitters and their antagonists juxtacellularly onto the surface of intracellularly recorded motoneurons during sleep and waking states. Soja et al. discovered that glycine was the inhibitory neurotransmitter mediating the postsynaptic inhibition that was responsible for atonia during REM sleep.[8-10]

Concurrently, studies from multiple laboratories demonstrated that neurons in or in the vicinity of the nucleus pontis oralis initiate the processes that are responsible for the postsynaptic inhibition of motoneurons during REM sleep.[11,12] These cells activate premotor inhibitory interneurons in the region of the nucleus gigantocellularis which, in turn, discharge selectively during REM sleep.[13-18] They innervate motoneurons and promote their postsynaptic inhibition during REM sleep.[9,17-22]

Nevertheless, in 2008, Brooks and Peever[23] claimed that a "considerable controversy" exists regarding the neural mechanisms underlying REM sleep atonia (although they did not provide any references or data to support this claim). Brooks and Peever also did not question either the techniques or the consensus results of intracellular studies performed by various investigators.[5-7,24,25]

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In order to resolve this so-called controversy, Brooks and Peever[23] employed electromyogram (EMG) recordings to document atonia (loss of muscle tone) as the experimental endpoint to assess whether antagonists of the inhibitory neurotransmitters glycine and/or GABA were responsible for REM-related atonia. Experimentally, they used reverse- microdialysis procedures to infuse neurotransmitters and antagonists into the trigeminal motor nucleus and adjacent areas, in rats, for periods of two to four hours, during sleep and wakefulness.

Numerous factors were not considered by Brooks and Peever regarding their methods that include, but are not limited to, the rate and range of diffusion of dialyzed substances, the lack of dose-response studies, and actions of substances dialyzed over protracted time periods that affect neural networks other than motoneurons within and outside the trigeminal motor nucleus. Thus, there appears to be a significant misunderstanding of the limitations of the techniques that were employed and important misinterpretation of the results that were obtained in the attempt of Brooks and Peever to develop a cellular mechanistic explanation regarding REM atonia. In the studies by Brooks and Peever,[23] their misperceptions were further confounded by *mis*representations of *fact* from other cited work. A few of these issues will be pointed out here.

Specifically, Brooks and Peever found that masseter muscle atonia during tonic periods of REM sleep was not altered by the administration of strychnine, a glycine receptor antagonist, or the $GABA_A$-receptor antagonist bicuculline. They concluded that neither glycine nor GABA were responsible for atonia during the tonic periods of REM sleep (although they found that glycine-mediated postsynaptic inhibition was the key process that resulted in atonia during phasic REM periods). Why might this be and why are their data directly contradicted by many studies conducted over decades[1,8,10,26-28] that have demonstrated convincingly that glycine-mediated postsynaptic inhibitory processes are responsible for atonia during the tonic as well as phasic periods of REM sleep?

Perhaps the single most important issue here that should be understood by readers interested in REM atonia are the insurmountable problems associated with the experimental methods used by Brooks and Peever, 2008, that do not allow them to detect and differentiate between state-dependent drives and waking receptor activation in identified motoneurons, that previous *in vivo* intracellular recording experiments amply provide.[3-10,27-32] The work by Chase and colleagues clearly demonstrates increases in membrane conductance that are reflected by observed decreases in motoneuron input resistance and membrane time constant, which in turn, are due to increased strychnine-sensitive, large-

amplitude, spontaneous, inhibitory potentials that bombard motoneurons *de novo* during REM sleep. One should bear in mind that these spontaneous glycine-mediated IPSPs are strategically directed to the soma and proximal dendrites of motoneurons to obliterate spike genesis, which in turn, is reflected peripherally as REM atonia.[6-8,10,28] This direct inhibitory mechanism is a most effective means of suppressing motor outflow during tonic and phasic portions of REM sleep as opposed to any other less effective mechanisms such as presynaptic inhibition,[33] or monoaminergic disfacilitation of reticulospinal origin occurring remotely on the dendritic tree.[23]

Compounding the insurmountable problem of distinguishing between postsynaptic inhibition and disfacilitation in Brooks and Peever's study is their apparent naive portrayal of the trigeminal motor nucleus as consisting only of motoneurons without any other neural elements (e.g., afferent input, excitatory interneurons, inhibitory interneurons, or gamma-motoneurons) or input from other areas of the brain that their dialyzed drugs would have certainly exerted profound indirect effects upon and which are independent of pathways for REM atonia, e.g., those that mediate the complex patterns of trigeminal motor activities that are responsible for chewing, biting, swallowing, vocalization.[34] Their methods do not address these points and therefore their study (and others using this approach[35,36]) have inherent design shortcomings that ultimately confound their results and prohibited them from relating their data to state-dependent synaptic control mechanisms.

The points above cannot be overemphasized, as Brooks and Peever dismiss glycine mediated postsynaptic inhibition as a causal, root mechanism for REM atonia and suggest that another transmitter (acetylcholine) or mechanism(s), e.g., disfacilitation via the withdrawal of monoaminergic influences due to REM-related cessation of reticulospinal neurons are needed to explain REM atonia.[23] These are not tenable alternative hypotheses because when intracellularly recorded REM-sleep specific glycine-mediated IPSPS are blocked completely by juxtacellularly applied strychnine, all of the indicators of classical postsynaptic inhibition observed during REM sleep (*vs.* wakefulness or NREM sleep) before strychnine is ejected juxtacellularly, i.e., decreased input resistance, decreased membrane time constant, increased rheobase, increased membrane potential-hyperpolarization, and decreased AHP amplitude, are also blocked. Indeed, during REM sleep, in the presence of the juxtacellular release of strychnine, intracellularly monitored motoneuron activity resembles that observed during preceding episodes of wakefulness or quiet wakefulness.[10,28] There is no evidence of disfacilitation and, most importantly, no noticeable difference in the quantity or magnitude of spontaneous EPSPs that influence motoneurons following blockade of glycine receptors with strychnine.[10,28] Clearly, these data refute the notion that REM atonia is due to some unknown "biochemical substrate" as promoted by Brooks and Peever.[23]

In several key instances, Brooks and Peever[23] misquote the literature when they seek to support the following statement regarding their principal finding: "*Our most fundamental observation is that REM sleep atonia could not be reversed by either glycine or $GABA_A$ antagonists.*" A critical point that substantiates their claim, they state, is the fact that *"…trigeminal motoneurons are hyperpolarized by large amplitude IPSPs that are reduced (but not eliminated) by antagonism of glycine receptors (Soja et al., 1987; Chase et al., 1989)."* However, the record should be corrected here, as their inaccurate statement leads the reader to a false conclusion by claiming that REM-sleep-specific IPSPs *"are reduced (but not eliminated) by antagonism of glycine receptors,"* implying that because REM-specific glycine-mediated IPSPs are not eliminated, other processes must be involved. To support this critical point, they improperly cite the work of Soja et al. 1987,[9] which was an extracellular study dealing with changes in reflex amplitude during REM sleep: no spontaneous REM-specific IPSPs were recorded in this reflex study.[9]

The other paper referenced by Brooks and Peever was the combined intracellular/microiontophoretic study of Chase et al.,[8] in which the data contained therein directly contradicts the statements and the conclusions of Brooks and Peever. Specifically, Chase et al.[8] did not find, as claimed by Brooks and Peever, that the REM specific IPSPs were "reduced (but not eliminated) by antagonism of glycine receptors." In fact, and it could not be clearer in the Chase et al. article[8] referenced by Brooks and Peever, when describing the actions of strychnine on REM-specific IPSPs, that: "strychnine was effective in blocking, *completely*, all IPSPs." The word "completely" was included, in italics, in the original publication.[8] Additionally, in their study, Chase et al[8] found that neither of the GABA antagonists, picrotoxin nor bicuculline methiodide, *blocked* REM-specific IPSPs, only strychnine was able to block them, *completely*, throughout both the tonic and phasic periods of REM sleep.[8] Although the GABA antagonists bicuculline and picrotoxin did not block the REM-specific IPSPs, these agents were found to shorten the decay phase of all IPSPs bombarding motoneurons,[10] a finding also observed for picrotoxin on IPSPs evoked in motoneurons by single inhibitory interneurons studied in the acute anesthetized cat preparation[37] and in hypoglossal motoneurons in vitro.[38]

Consequently, Brooks and Peever's "most fundamental observation" is not supported by intracellular data as they claim, and their "most fundamental observation" is actually invalidated by the results of these[8,10] and other studies.[28] Unfortunately, they do not acknowledge these data, but state that cholinergic neurons in the pedunculopontine tegmental nucleus may be the "source of motoneuron inhibition during REM sleep because "Neurons in the region are maximally active during REM sleep[39,40] and they project to and inhibit motoneurons (Bellingham and Berger, 1996; Liu et al. 2005)."

Regarding the speculative role of pedunculopontine neurons in promoting REM atonia as proposed by Brooks and Peever, they cite the work of el Mansori et al.[39] who actually described cells that exhibited high rates of discharge during NREM as well as REM sleep; they were not "maximally active during REM sleep." Even more importantly, these cells projected to and promoted the excitation of *thalamic* neurons; they did not project to and/or inhibit motoneurons.[39] In the cited Steriade et al. article,[40] cells were studied in the pedunculopontine nucleus that increased their firing rates during wakefulness as well as during REM sleep, not selectively during REM sleep, as stated by Brooks and Peever. These cells also projected to the thalamus, not to motoneurons, as claimed by Brooks and Peever, and they promoted the excitation, not the inhibition of their target

cells. Hence, these studies clearly do *not* support Brooks and Peever's alternative source of motoneuron inhibition during REM sleep.[23]

The other two referenced papers were cited presumably to build on the alternative idea that the aforementioned cells "project to and inhibit motoneurons." However, Bellingham and Berger[41] never stated, discussed, or even inferred that the cholinergic neurons that they investigated provided postsynaptic inhibitory projections to motoneurons. Actually, their paper investigated muscarinic presynaptic depression of excitatory synaptic inputs, not postsynaptic inhibitory mechanisms.[41] The other referenced study by Liu et al.[42] was carried out in urethane-anesthetized, tracheotomized and vagotomized rats and dealt with muscarinic-mediated GG (genioglossus) suppression masking nicotinic excitation," which has little to do with pedunculopontine neurons "that project to and/or inhibit motoneurons." In addition to misquoting the literature as highlighted above, Brooks and Peever do not mention the electrophysiological, morphological and lesion data regarding the descending neural pathways that underlie motoneuron inhibition, as reviewed elsewhere.[26,27]

Brooks and Peever[23] continue by stating that, "*The most concrete demonstration that glycine and GABA$_A$-receptors were antagonized stems from the fact that strychnine and bicuculline provoke muscle twitch activity in REM sleep.*" It is not clear how strychnine and bicuculline could possibly "provoke" muscle activity during REM sleep.

Brooks and Peever[23] then suggest that activation of GABA$_B$ receptors may be an alternative basis for the presence of tonic inhibition during REM sleep, although they do acknowledge that "it is unknown whether GABA$_B$-receptors are activated on motoneurons during natural sleep, wake, or motor behaviors." Indeed, few, if any, data actually exist to suggest that GABA$_B$-receptors could account for the motor atonia of REM sleep.

Despite the impeccable experimental quality and difficulty of the Brooks and Peever study,[23] a number of alternate possibilities can explain their findings. Their protracted reverse-microdialysis of drugs inextricably results in complex drug actions on alpha-motoneurons, gamma-motoneurons, excitatory as well as inhibitory interneurons, excitatory and inhibitory projections from adjacent sites, as well as cells in the spinal cord, brainstem and/or forebrain projections to the motor nucleus, etc. Arguably, "forward", as opposed to "reverse" microdialysis techniques would have provided Brooks and Peever a more useful approach toward investigating the neurotransmitter basis for masseter muscle atonia that occurs during REM sleep.[23] Indeed, recent studies using conventional ("forward") microdialysis techniques have demonstrated *increased* levels of glycine in the hypoglossal motor pools and lumbar spinal cord ventral horn during naturally occurring or carbachol-induced REM sleep,[43,44] findings that further substantiate the intracellular studies discussed here.[8,10,28]

On the other hand, intracellular recording techniques afford one with incredible power of resolution to directly investigate the mechanism of motor atonia occurring in identified motoneurons in the trigeminal and other motor nuclei. Applying drugs directly on the somatic and proximal dendrites of these recorded cells using microiontophoretic procedures as documented for hypoglossal and lumbar motoneurons, albeit time-consuming and labor-intensive, circumvents the problems of interpretation presented above that undermine the work of Brooks and Peever.[23] With microiontophoresis, drug action is limited to REM sleep-specific synapses impinging on the neuron under study and hence, the action of specific antagonists, in this case, strychnine and bicuculline, can be used effectively to determine the role of glycine and GABA in mediating postsynaptic inhibition of motoneurons during REM sleep atonia.

CONCLUSIONS

In conclusion, the literature reviewed here and the points identified for re-clarification in the report by Brooks and Peever[23] confirm that REM atonia can be accounted for completely by a process of postsynaptic inhibition that is mediated by the neurotransmitter glycine. The experimental design and methods of procedure used are critical to obtain data that are meaningful and devoid of critical problems of interpretation.

DISCLOSURE STATEMENT

Dr. Soja has indicated no financial conflicts of interest.

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