A Particular Medullary-Spinal Inhibitory Pathway is Recruited for the Expression of Muscle Atonia During REM Sleep

Journal of Experimental Neuroscience
Volume 12: 1–3

DOI: 10.1177/1179069518808744

Sara Valencia Garcia, Pierre-Hervé Luppi and Patrice Fort

SLEEP Team, CNRS UMR 5292, INSERM U1028, Centre de Recherche en Neurosciences de Lyon (CRNL), Université Claude Bernard Lyon 1, Lyon, France.

ABSTRACT: Muscle atonia is a major pathognomonic sign of paradoxical sleep (PS; coined REM Sleep), during which dreams mainly occur. In the 1980s, an idiopathic syndrome called REM sleep behavior disorder (RBD) was described in patients endowed with loss of PS paralysis concomitant to abnormal movements, suggesting a dysfunction of PS networks. Another major clinical RBD feature is its prevalent phenoconversion into synucleinopathies as Parkinson's disease in a delay of 10-15 years after diagnosis. Thus, we undertook experiments in rats to disentangle brainstem networks involved in PS, including atonia. We first identified a contingent of pontine glutamate neurons recruited during PS with inputs to the ventromedial medulla (vmM) where they contact γ-aminobutyric acid (GABA)/glycine inhibitory neurons also activated during PS. Here, we further show that these vmM inhibitory neurons send efferents to somatic spinal motoneurons until lumbar levels. As reported for the pontine generator, the genetic inactivation of the vmM inhibitory neurons abolishes atonia during PS without effects on waking locomotion and is sufficient to recapitulate major RBD symptoms. These original data suggest that RBD may reflect a severe dysfunction and/or degeneration linked to a developing synucleinopathic attack targeting specifically neurons that generate PS-specific atonia.

KEYWORDS: Brainstem, glycine, GABA, REM sleep behavior disorder, rat, polysomnography

RECEIVED: September 26, 2018. **ACCEPTED:** October 2, 2018.

TYPE: Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by CNRS UMR5292, INSERM U1028, Université Claude Bernard Lyon I, and Agence Nationale de la Recherche (OPTOREM, ANR-13-BSV4-0003-01). SVG received PhD grants from Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation and Association France Parkinson. PF was supported by grants from Association France Parkinson and Société Francaise de Recherche et Médecine du Sommeil (SFRMS).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Patrice Fort, SLEEP Team, CNRS UMR 5292, INSERM U1028, Centre de Recherche en Neurosciences de Lyon (CRNL), Université Claude Bernard Lyon 1, Faculté de Médecine RTH Laennec, 7 rue Guillaume Paradin, 69008 Lyon, France. Email: patrice.fort@univ-lyon1.fr

COMMENT ON: Valencia Garcia S, Brischoux F, Clément O, et al. Ventromedial medulla inhibitory neuron inactivation induces REM sleep without atonia and REM sleep behavior disorder. *Nat Commun*. 2018;9:504. doi:10.1038/s41467-017-02761-0. PubMed PMID: 29402935. https://www.ncbi.nlm.nih.gov/pubmed/29402935

Commentary

In the 1950s, a particular brain state that was called paradoxical sleep (PS) or REM sleep was discovered in mammals. Distinctive from synchronized sleep (or slow-wave sleep [SWS]), PS is at electrophysiological level characterized by the time-locked occurrence of cortical mantle activation (low-amplitude, fast electroencephalography [EEG]), rapid eye movements (REM), and body paralysis with postural atonia (flat skeletal electromyography [EMG]) reflecting a sustained glycine-dependent hyperpolarization of brainstem/spinal somatic motoneurons. Among others, a crucial role of this motor inhibition is to protect the sleeper's body and PS itself, as suggested by neurological symptoms in patients suffering an idiopathic parasomnia called REM sleep behavior disorder (RBD). Indeed, RBD is endowed with loss of muscle atonia allowing patients to enact their dreams through unconscious, abnormal, violent movements with vocalizations, causing both injuries to themselves or bed partners and a chronic negative impact on PS amount and quality.[1] Of particular clinical interest, recent longitudinal studies evidenced a close etiological link between RBD and synucleinopathies since a vast majority of RBD patients develop such devastating neurodegenerative pathologies as Parkinson's Disease within a 10- to 15-year phenoconversion window.[2] In that context, we developed a wide range of innovative and complementary approaches in rats to decipher at anatomical, genetic, and functional levels the neuronal networks and neurobiological mechanisms underlying PS atonia, possibly incapacitated in RBD.[3,4]

In our first experimental work, we completed the characterization of the pontine sublaterodorsal nucleus (SLD), previously identified as the potential generator of PS atonia.[5] We demonstrated indeed that the genetic inactivation of glutamate neurotransmission issued from SLD neurons is sufficient for reducing PS amounts (by 30%) and mimicking RBD symptoms, ie generating a PS-like state (EEG activation, REMs) without atonia and depicting an abnormal amount of locomotor activity with fairly organized movements. We were able to show that SLD glutamate neurons send dense descending efferents to the ventromedial medulla (vmM) composed of the raphe magnus (RMg), gigantocellular alpha (GiA) and ventral reticular nuclei (GiV), whereas sparse pathways along the whole spinal cord were traced (Figure 1A). These original data identified clearly the vmM, rather than spinal cord, as the candidate to contain the glycine/gamma-aminobutyric acid (GABA) interneurons responsible for inhibiting somatic motoneurons during PS.[3] The next series of experiments were therefore designed to unravel the potential contribution of vmM in PS in rats.[4] Our first priority was to circumscribe the inhibitory

(glycine/GABA) premotoneurons that are specifically recruited during PS for atonia expression. Within brainstem and spinal cord, we thus mapped neurons co-expressing GlyT2 messenger RNA (mRNA; ie glycine in nature) and c-Fos protein (ie activated) during PS. In parallel, we plotted in brainstem neurons activated during PS anterogradely labeled after injection of fluorogold tracer into pools of lumbar motoneurons. As a result, we observed glycine premotor neurons in both vmM and spinal cord close to lumbar motoneurons. A vast majority of the vmM premotor neurons were activated during PS, whereas spinal ones were not but in response to forced locomotion during waking. Taken together, these compelling anatomical data underline vmM, not spinal cord,[6,7] as the best brainstem candidate for containing the contingent of neurons that mediate the PS-selective inhibition of somatic motoneurons. To assess this point at functional level, we infused *in situ* adeno-associated virus (AAV) carrying short-hairpin RNA (shRNA) against the vesicular transporter of GABA and glycine (vGAT) to knockout the local expression of vGAT and block permanently the fast inhibitory neurotransmission in vmM. Contrasting with data after the genetic inactivation of glutamate SLD neurons,[3] PS amounts were not significantly modified in shRNA-vGAT-treated rats (–15%) indicating that vmM is not crucial for generating the state *per se*. However, the feature of a PS bout according to polysomnographic/video recording appears strongly abnormal with loss of atonia (evidenced on nuchal electromyogram [EMG]) and the occurrence of movements of ears, nose or limbs, sometimes engaging several body's territories to mimic complex behaviors as pellet seeking in woodchips, crawling, or jumping. Most violent behaviors often induced a premature awakening, leading to shorter PS episodes. As suggested above by our anatomical data, this behavioral phenotype completes the demonstration that inhibitory vmM neurons play a decisive role in the physiological expression of PS-specific atonia. Moreover, their genetic inactivation is sufficient for reproducing a phenotype with oneiric behaviors resembling closely human RBD, as similarly observed after the genetic inactivation of glutamate SLD neurons.[3] It is therefore likely that human RBD may be linked to a pathological dysfunction targeting primarily all or part of the network formed by SLD and vmM to control brainstem and spinal somatic motoneurons during PS. In line with our basic research, recent clinical studies reported cases of acute RBD after lesions, inflammations, or ischemic stroke involving human brain structures equivalent to SLD and vmM in rats.[8,9]

Besides the contribution to atonia during PS, this study also unravels a surprising point regarding both neurochemical organization and functional implication of vmM. Indeed, different research groups have extensively studied over the last decade this large medullary region in the context of locomotion, control of speed and posture. These studies were focused on the contribution played by glutamate excitatory neurons encountered within the vmM, where they are intermingled with GABA/glycine neurons. These glutamate neurons are endowed with robust descending axonal pathways along the spinal cord to control networks recruited for locomotion and hindlimb/forelimb movements. These recent papers may lead to the misinterpretation that vmM exerts only an excitatory descending control over spinal networks.[10,11] In most of these studies, the potential contribution of inhibitory neurons was indeed consistently neglected, while these neurons also project in parallel not only to pools of somatic motoneurons but likely to most of spinal Rexed layers.[10,12] Of particular interest, none reported any modification in PS expression (ie oneiric behaviors) in response to manipulation of glutamate vmM neurons, indicating that they are not necessary for motor control during PS and that effects we report are indeed specific of GABA/glycine vmM neurons. Further supporting this conclusion, neurotoxic lesion of vmM with unspecific loss of reticular neurons reproduced similar effects during PS than after the selective inactivation of GABA/glycine neurons,[13] although we noticed that induced abnormal motor events were more frequent and violent (our unpublished data and Valencia Garcia et al[4]). The increased intensity in oneiric behaviors in lesions studies may support a minor contribution of glutamate vmM neurons in expression of PS atonia via spinal glycine premotoneurons, although they were disqualified for that role as they were never activated during natural PS.[4] A more likely explanation is that GABA/glycine vmM neurons may co-express neurotransmitters as neuropeptides (ie Enkephalins[14,15]) also contributing to and/or facilitating inhibitory processes leading to the PS-specific hyperpolarization of somatic motoneurons. In animals treated with shRNA-vGAT, only GABA/glycine neurotransmission is impaired, putative co-transmissions remain functionally intact. In lesioned rats, all neurotransmissions and connections emanating from vmM cell bodies are abolished that may thus explain more intense behavioral effects. An interesting direction would be to study the co-neurotransmitters expressed in inhibitory vmM neurons and their potential roles in PS.

In conclusion, we demonstrated that a specific medullary-spinal inhibitory pathway is recruited for the expression of muscle atonia during PS. The genetic inactivation of GABA/glycine vmM neurons at the origin of this pathway is sufficient to provoke the loss of paralysis during PS and the occurrence of abnormal motor behaviors. These symptoms mimic closely human RBD, allowing us to understand more precisely the etiology of this parasomnia, particularly regarding potential pathological mechanisms underlying the phenoconversion of RBD in synucleinopathies (Figure 1).

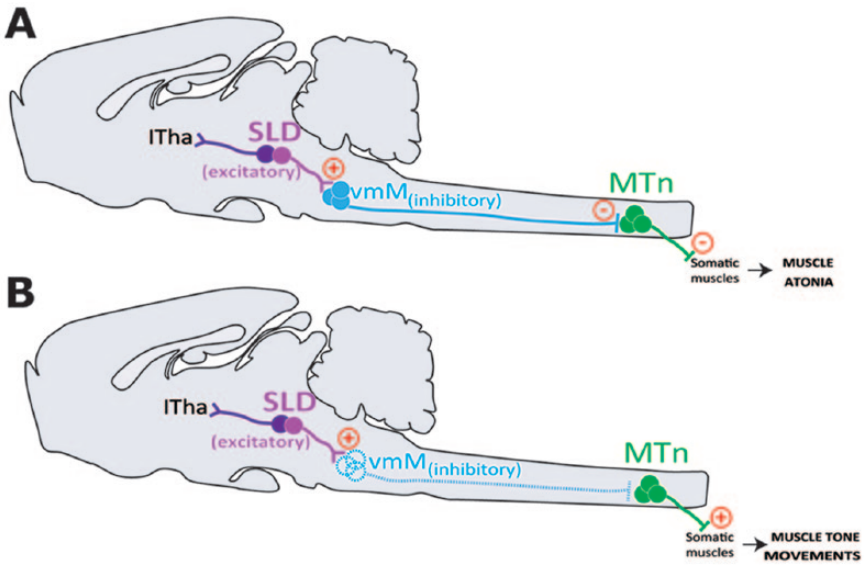

Figure 1. Schematic drawing of the neuronal networks controlling muscle atonia during PS (or REM sleep). (A) The sublaterodorsal nucleus (SLD) contains two neuronal populations, either ascending to the intralaminar thalamus (ITha) or descending to the ventromedial medullary reticular formation (vmM). The vmM contains GABA neurons (acid γ-aminobutyric acid)/glycine) activated during SP by the SLD, sending their axons to spinal somatic motoneurons (MTn). Atonia results from this cascade of synaptic events specific to PS. (B) Genetic dysfunction of excitatory SLD or inhibitory vmM neurons leads in both cases to the loss of muscle atonia during PS with the occurrence of abnormal locomotor activity.

Acknowledgements

The authors wish to thank Dr Frédéric Brischoux for his important contribution during vGAT-shRNA experiments.

Author Contributions

SVG and PF wrote the manuscrit; SVG made the Figure; SVG, PHL and PF designed the studies.

ORCID iD

Patrice Fort https://orcid.org/0000-0003-1211-8631

REFERENCES

1. Arnulf I. REM sleep behavior disorder: motor manifestations and pathophysiology. *Mov Disord*. 2012;27:677–689.
2. Howell MJ, Schenck CH. Rapid eye movement sleep behavior disorder and neurodegenerative disease. *JAMA Neurol*. 2015;72:707–712.
3. Valencia Garcia S, Libourel PA, Lazarus M, et al. Genetic inactivation of glutamate sublaterodorsal nucleus recapitulates REM sleep Behavior Disorder. *Brain*. 2017;140:414–428.
4. Valencia Garcia S, Brischoux F, Clement O, et al. Ventromedial medulla inhibitory neuron inactivation induces REM sleep without atonia and REM sleep behavior disorder. *Nat Commun*. 2018;9:504.
5. Clement O, Sapin E, Berod A, et al. Evidence that neurons of the sublaterodorsal tegmental nucleus triggering paradoxical (REM) sleep are glutamatergic. *Sleep*. 2011;34:419–423.
6. Krenzer M, Anaclet C, Vetrivelan R, et al. Brainstem and spinal cord circuitry regulating REM sleep and muscle atonia. *PLoS ONE*. 2011;6:e24998.
7. Vetrivelan R, Fuller PM, Tong Q , Lu J. Medullary circuitry regulating rapid eye movement sleep and motor atonia. *J Neurosci*. 2009;29:9361–9369.
8. Limousin N, Dehais C, Gout O, et al. A brainstem inflammatory lesion causing REM sleep behavior disorder and sleepwalking (parasomnia overlap disorder). *Sleep Med*. 2009;10:1059–1062.
9. Geddes MR, Tie Y, Gabrieli JD, et al. Altered functional connectivity in lesional peduncular hallucinosis with REM sleep behavior disorder. *Cortex*. 2016;74:96–106.
10. Brownstone RM, Chopek JW. Reticulospinal systems for tuning motor commands. *Front Neural Circ*. 2018;12:30.
11. Gatto G, Goulding M. Locomotion control: brainstem circuits satisfy the need for speed. *Curr Biol*. 2018;28:R256–R259.
12. Holstege JC. The ventro-medial medullary projections to spinal motoneurons: ultrastructure, transmitters and functional aspects. *Prog Brain Res*. 1996;107:159–181.
13. Holmes CJ, Jones BE. Importance of cholinergic, GABAergic, serotonergic and other neurons in the medial medullary reticular formation for sleep-wake states studied by cytotoxic lesions in the cat. *Neuroscience*. 1994;62:1179–1200.
14. Todd AJ, Spike RC, Russell G, Johnston HM. Immunohistochemical evidence that Met-enkephalin and GABA coexist in some neurones in rat dorsal horn. *Brain Res*. 1992;584:149–156.
15. Fort P, Rampon C, Gervasoni D, et al. Anatomical demonstration of a medullary enkephalinergic pathway potentially implicated in the oro-facial muscle atonia of paradoxical sleep in the cat. *Sleep Res Online*. 1998;1:102–108.