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Preview

Life Without Dreams: Muscarinic Receptors Are Required to Regulate REM Sleep in Mice

Mitsuaki Kashiwagi[1] and Yu Hayashi[1,*]
[1]International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai Tsukuba, Ibaraki 305-8575, Japan
*Correspondence: hayashi.yu.fp@u.tsukuba.ac.jp
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Mammalian sleep comprises REM and NREM stages, but the regulation mechanisms are unclear. In this issue of *Cell Reports*, Niwa et al. (2018) comprehensively knocked out cholinergic receptors in mice and found that muscarinic signaling is crucial for REM sleep and possibly important for NREM sleep.

REM sleep is characterized by rapid eye movement, loss of muscle tone, and cortical activation, and is frequently accompanied by vivid dreams. Despite 65 years of intensive studies to identify the neural substrates and functions of REM sleep, both have remained largely unknown.

Jouvet and colleagues conducted transection studies using cats and identified brainstem areas crucial for generating REM sleep (Jouvet, 1962). The same group and others found that infusing the cholinergic receptor agonist carbachol into an area in the brainstem pons strongly induces REM sleep, an effect largely dependent on muscarinic receptors (Sakai and Onoe, 1997). These findings led researchers to hypothesize that acetylcholine has critical roles in generating REM sleep. The monoaminergic system has also attracted attention because it almost completely ceases firing during REM sleep, and monoamine oxidase inhibitors, which increase extracellular levels of monoamines, strongly reduce REM sleep. These observations led to the "reciprocal-interaction model"; i.e., reciprocal interactions between the cholinergic and monoaminergic systems determine the onset and termination of REM sleep (Hobson and Pace-Schott, 2002). Lesions of the major source nuclei of acetylcholine and monoamines in the pons, however, have little effect on REM sleep, suggesting that neither system plays a major role in REM sleep regulation (Lu et al., 2006). Therefore, the contribution of cholinergic systems to REM sleep regulation remains controversial.

In this issue of *Cell Reports*, Niwa et al. (2018) assessed the roles of cholinergic systems for sleep/wake regulation using a novel approach. Niwa and colleagues utilized a CRISPR/Cas9-system-based highly efficient genome editing method that they previously established to comprehensively knock out cholinergic receptors and examined the effect on sleep. They found that the metabotropic cholinergic receptors, i.e., muscarinic receptors, have a crucial and redundant role: *Chrm1* knockout (KO) reduced both non-REM (NREM) and REM sleep, whereas *Chrm3* KO reduced only NREM sleep. Furthermore, REM sleep was almost completely abolished when both *Chrm1* and *Chrm3* were knocked out. These findings provide evidence that cholinergic signaling is indeed essential for generating REM sleep. As the mice used in this study lack *Chrm1* and *Chrm3* globally, however, it is unclear whether muscarinic receptors in the pons or a different area are crucial for REM sleep.

REM sleep is homeostatically controlled, and selective REM sleep deprivation in rats is lethal, leading to the hypothesis that REM sleep plays an essential role for survival (Kushida et al., 1989). Thus, it is surprising that REM sleep can be chronically diminished to this level in the *Chrm1*/*Chrm3* double KO (dKO) mice.

Is REM sleep essential? Conventional REM sleep deprivation methods, such as forced awakening, are stressful and reduce NREM sleep as well. Thus, they cannot completely distinguish the effect of REM sleep loss from NREM sleep loss, or even elevated stress. The large reduction in REM sleep in this dKO mouse implies that REM sleep is not essential, at least for living in a domesticated environment. Another possibility is that the "need" for REM sleep is decreased or diminished in the dKO mice. That is, these mice might be able to circumvent the need for REM sleep. Classical studies implicated a role for REM sleep to support the subsequent expression of NREM sleep (Benington and Heller, 1994). This was further supported by findings in mice with genetic manipulation of REM sleep that REM sleep inhibition or induction leads to reduced or increased, respectively, slow wave activity (thought to reflect sleep depth and quality) in the subsequent NREM sleep (Hayashi et al., 2015). This might predict that the dKO mice may not require REM sleep since they can express sufficient NREM sleep without any help of REM sleep. If the need for REM sleep is truly decreased in dKO mice, these mice would be a good model for evaluating the function of REM sleep.

The sleep phenotype of the dKO mice must be carefully interpreted. In this study, the vigilance state was defined by electroencephalography (EEG) and electromyography, which are gold standards in this field. Clearly, REM sleep is severely impaired in dKO mice as delta oscillations

(0.5–4.0 Hz), a feature of NREM sleep, are always high during sleep. Further investigation is required, however, to conclude that dKO mice truly have a single sleep stage. For example, if the states are defined by the standard cortical EEG-based classification, then the monotreme echidna (*Tachyglossus aculeatus*) only has an NREM sleep-like state. Importantly, however, when the field potential signals are obtained from the brainstem, the echidna's sleep can be classified into two distinct stages corresponding to apparent NREM and REM sleep (Siegel et al., 1996). Similarly, the dKO mice in this study might have a sleep stage that compensates for the loss of EEG-defined REM sleep and can only be detected by measurements from subcortical areas, brainstem, or the autonomic nervous system.

Another important finding of this paper is that, in contrast to the conventional view that cholinergic signaling promotes either wake or REM sleep, cholinergic signaling might also be involved in promoting NREM sleep, as both *Chrm1* and *Chrm3* single-KO mice exhibited reduced NREM sleep. This mechanism should be further investigated at the circuit level. Interestingly, cholinergic signaling is also required for maintaining sleep in fruit flies (Shi et al., 2014). Thus, this study might support the evolutionarily conserved sleep-promoting mechanism between mouse and fruit fly.

Finally, as a technical consideration, the authors developed a tetracycline-responsive genetic tool termed tTR that works as a tTA repressor. By combining tTA and tTR, they genetically labeled a subpopulation of neurons whose feature is $A \cap \overline{B}$ by preparing A-tTA;B-tTR double transgenic mice. The tTR technology also works well *in vitro*. The tTR system provides a powerful tool that can be used to investigate various phenomena at systemic to single cell levels.

In summary, Niwa et al. (2018) used various state-of-the-art genetic approaches to provide insight into the roles of cholinergic neurons in regulating REM and NREM sleep. The finding that REM sleep was nearly abolished in mice with double knockout of the muscarinic receptors Chrm1 and Chrm3 raises exciting questions to be addressed in future studies of the neural circuitry regulating REM sleep and the function of REM sleep.

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