LETTER

Reply to Grace: Role of cholinergic neurons in rapid eye movement (REM) sleep control

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We thank Grace (1) for the opportunity to discuss the role of cholinergic neurons in rapid eye movement (REM) sleep further. Grace suggests that optogenetic activation of a population of neurons does not necessarily demonstrate their role in the endogenous system when interrogating complex neural circuitry. We agree that we do not prove necessity of cholinergic neurons in REM sleep generation, as we point out in our discussion, "Future studies that selectively inhibit cholinergic neurons in the PPT [pedunculopontine tegmentum] and LDT [laterodorsal tegmentum] of nonhypercholinergic mice are needed to determine if cholinergic neurons are necessary for REM sleep generation" (2). However, in our report we do demonstrate the sufficiency of PPT/LDT neurons to influence REM sleep initiation but not influence REM sleep duration, thus distinguishing the role of cholinergic neurons on these properties of REM sleep. In addition, activation of cholinergic PPT neurons during non-REM sleep induced REM sleep versus wakefulness. Our data are consistent with the role of cholinergic neurons in generating an activated brain state and many studies pointing to the role of cholinergic neurons in REM sleep regulation (reviewed in ref. 3).

Neurons change their signal strength by changing their firing rate. Optogenetics uniquely allows us to control the firing rate of a specific cell type, whereas pharmacological studies do not allow direct control of the firing rate of a subpopulation of neurons. We activated the PPT/LDT neurons using physiologically relevant firing rates, thus closely reproducing the normal properties of these neurons. Drugs can have noncell-type specific effects, diffuse to other brain regions, and do not have the temporal specificity of optogenetics to be able to activate or inhibit neurons during particular sleep states. Grace (1) suggests that cholinergic input to the subceruleus serves as positive feedback to reinforce REM sleep transitions but is not actively involved in the initial REM sleep induction (4). Single neuron recording studies from both PPT and the subceruleus simultaneously will help clarify the temporal sequence of neuronal activation necessary for REM sleep to occur. Further elucidation of the endogenous function of cholinergic PPT/LDT neurons in REM sleep regulation will come from the culmination of studies using multiple approaches.

1 Grace KP (2015) How useful is optogenetic activation in determining neuronal function within dynamic circuits? *Proc Natl Acad Sci USA* 112:E3755.
2 Van Dort CJ, et al. (2015) Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. *Proc Natl Acad Sci USA* 112(2):584–589.
3 McCarley RW (2007) Neurobiology of REM and NREM sleep. *Sleep Med* 8(4):302–330.
4 Grace KP, Vanstone LE, Horner RL (2014) Endogenous cholinergic input to the pontine REM sleep generator is not required for REM sleep to occur. *J Neurosci* 34(43):14198–14209.

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The authors declare no conflict of interest.

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