This Week in The Journal

Unforeseen Side Effects of Optogenetic Halorhodopsin Activation

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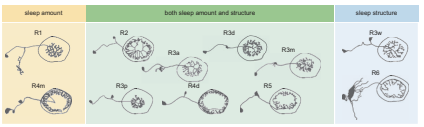
(see pages 685–692)

It is not hyperbole to say that optogenetics, the technology using light to control the excitability of genetically engineered neurons, has revolutionized neuroscience, by allowing the activation or silencing of specific neuronal populations in isolation, providing researchers the opportunity to dissect out the roles of specific cell classes in different brain functions. Halorhodopsin, the first inhibitory optogenetic tool to be introduced, works by activating an electrogenic light-sensitive chloride pump to hyperpolarize cells. This had previously been shown to load chloride ions into cells—a predictable outcome—but new work, using young adult mice broadly expressing halorhodopsin in pyramidal neurons of the neocortex driven by the Emx1 promotor, now has identified other rather surprising consequences of halorhodopsin activation.

Natural chloride loading, secondary to intense activation of GABA receptors, is followed by a surge in extracellular potassium (K^+), because chloride is removed by coupling it to potassium extrusion, using the potassium–chloride cotransporter KCC2. Similarly, Parrish et al. now show in acute brain slices a similar rise in extracellular K^+ that peaked at 8 mM a full minute after the end of illumination, which was also mediated by KCC2 activity. Unexpectedly, though, they also found a drop in extracellular K^+ during illumination due to redistribution of K^+ ions into neurons that were being hyperpolarized by halorhodopsin through their leak channels. Consequently, nearby nonpyramidal interneurons and glia displayed a slight hyperpolarization of about 2 mV due to the lower extracellular K^+ and its effect on their potassium equilibrium potential. This had a measurable reduction in the excitability of non-halorhodopsin-expressing fast-spiking interneurons during the period of illumination.

Next, the authors discovered a more surprising—and consequential—side effect of halorhodopsin activation: that prolonged periods of illumination can trigger cortical spreading depolarizations (CSD). CSDs, which have been associated with both migraine and seizure pathology, are commonly induced experimentally by increasing the extracellular K^+, leading to the suggestion that neuronal depolarization is a key prompt for their initiation. The current findings that CSD could be induced using halorhodopsin completely changes this view, because these events arise from a situation where the majority of neurons are hyperpolarized and the extracellular K^+ is actually lower than normal. The authors further showed that CSD also occurred *in vivo* in anesthetized halorhodopsin-expressing mice by illumination of the cortical surface. These CSDs appeared identical to CSDs elicited by the physical trauma of a pinprick to the cortical surface, an established technique to evoke the phenomenon. Together, the data confirm the theoretically predicted consequences of halorhodopsin activation on ionic concentrations and, importantly, reveal a new way to investigate the important yet poorly understood physiology underlying CSD.



Single subtype of ring neurons involved in the regulation of sleep amount (yellow), structure (blue), and both amount and structure (green) in *Drosophila* flies.

Thermogenetic Activation Reveals Fly Ring Neuron Roles in Sleep Patterns

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(see pages 764–786)

The beauty of studying the *Drosophila melanogaster* fruitfly lies in the elegance and simplicity of the strikingly small number of neurons that carry out the organism's functions. Combination *Drosophila* with powerful genetic technology allows researchers to make observations about functionally homologous neuronal populations in more complex organisms, including mammals. Now, a study by Yan et al. points the way toward the neural underpinnings of sleep-controlling neurons in flies with systemic effects. An important midline brain structure called the ellipsoid body (EB) plays roles in navigation, spatial orientation, arousal, and sleep, and the EB's high level of connectivity positions it as an integrative center for visual, mechanosensory, motor, and circadian information. Previous studies have helped identify functional roles for EB neurons, including recent investigations that have revealed 22 subpopulations of ring neurons connected to specific aspects of sleep. In the current study, the authors used 34 GAL4 drivers for subpopulations of ring neurons to drive thermogenetic activation of the warmth-sensitive ion channel dTrpA1. Flies were entrained on a 12 h light/dark cycle, and sleep was recorded for 3 d: at baseline, under warmth conditions (which led to labeled neuron firing), and recovery. The authors then examined the changes in total sleep as well as sleep structure including the number of sleep episodes, episode length, and behavioral transition probabilities P(doze), a measure of sleep drive, and P(wake), which correlates with arousal state or sleep depth. Activation of some drivers led to increased sleep and increased episode length but not episode number, as well as increased P(doze) and P(wake), suggesting that those labeled neurons played a role in sleep pressure and depth. Activation of other neuronal subpopulations reduced total sleep, decreased episode length, and increased P(doze) and P(wake), suggesting a contribution to increased sleep pressure and decreased daytime sleep depth. Analysis of nighttime neuronal activation was more complex, but revealed neurons that appeared to participate in sleep promotion by changing sleep depth. Other neurons seemed to contribute to promoting nighttime wakefulness and altered sleep structure. Still other neurons altered sleep structure without changing the total sleep time, indicating that these variables could be independently controlled. And some effects of thermogenetic activation were revealed only during the recovery day. In-depth analysis showed that some sleep effects required coactivation of multiple neuronal populations. Together, the data further extend the understanding of neuronal control of sleep in fruitflies—which may not be so simple after all.

This Week in The Journal was written by Stephani Sutherland

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