This Week in The Journal

Cholinergic Neurons' Local Connections Promote Wakefulness

Janneke C. Zant, Tae Kim, Laszlo Prokai, Szabolcs Szarka, James McNally, et al.

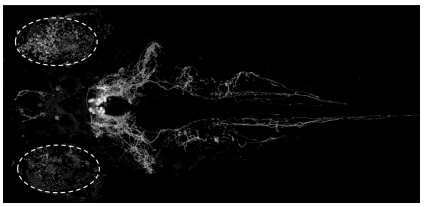
(see pages 2057–2067)

Wakefulness is maintained by the ascending reticular activating system in the brainstem and its targets in the basal forebrain (BF). BF neurons project throughout the neocortex and promote the low-voltage, high-frequency oscillations that characterize the awake state. A long-standing hypothesis supported by much evidence proposes that cholinergic neurons in the BF are the primary drivers of cortical activation. Indeed, a recent study demonstrated that optogenetic activation of BF cholinergic neurons triggers and prolongs wakefulness. But the same study found that optogenetic activation of BF glutamatergic or parvalbumin-expressing GABAergic neurons also promoted wakefulness. Moreover, cholinergic BF neurons excited local parvalbumin-expressing neurons (via nicotinic receptors) and inhibited local glutamatergic neurons [via muscarinic receptors (Xu et al., 2015, Nat Neurosci 18: 1641)]. Hence, the roles of different classes of BF neurons in driving wakefulness remain unclear.

To further elucidate the roles of BF neurons in wakefulness, Zant et al. developed an opto-dialysis probe that allowed them to measure local changes in acetylcholine levels and apply acetylcholine receptor antagonists while optically stimulating mouse BF cholinergic neurons. Besides promoting transitions from slow-wave sleep to wakefulness and increasing the total amount of time awake, stimulation of cholinergic neurons increased local acetylcholine levels. More remarkably, reverse dialysis of nicotinic and muscarinic receptor antagonists into the BF blocked the effects of optical stimulation.

These results are surprising, because they suggest that BF cholinergic neurons promote wakefulness not by activating neocortical neurons but by altering the activity of local GABAergic and glutamatergic neurons. Future work should therefore investigate whether cholinergic projections to the cortex also contribute to wakefulness, or whether they subserve other functions. Cholinergic projections might, for example, make cortical neurons more sensitive to non-cholinergic inputs, or they may primarily promote attention or alertness when an animal is already awake.

More broadly, this work demonstrates that optical stimulation alone will be insufficient to work out the complex wiring of neural circuits controlling sleep or other behaviors. The newly developed opto-dialysis probe should prove useful in future efforts to elucidate the effects of optical activation on local circuits.



qrfp-expressing neurons project in the hypothalamus, hindbrain, forebrain, and down the spinal cord in a zebrafish larva. Autofluorescence occurs in the eyes (surrounded by dashed ovals). See Chen, Chiu et al. for details.

QRFP Increases Sleep in Zebrafish

Audrey Chen, Cindy N. Chiu, Eric A. Mosser, Sohini Kahn, Rory Spence, et al.

(see pages 1823–1840)

Transitions from wakefulness to sleep are driven largely by the accumulation of adenosine in the basal forebrain and cortex; but other factors, such as light and hunger, also influence sleep and wakefulness. Many of these factors exert their influence via hypothalamic nuclei. For example, orexin-producing hypothalamic neurons are regulated by metabolic indicators such as glucose and leptin, and they excite wake-active histaminergic, cholinergic, and noradrenergic neurons. Therefore, orexinergic neurons have been proposed to promote wakefulness when an animal is hungry. Another hypothalamic neuropeptide, QRFP, also promotes arousal and feeding in mice (Takayasu et al., 2006, Proc Natl Acad Sci U S A 103: 7438) and may therefore share functions with orexin. But studies of QRFP in rodents have produced some conflicting results, and effects on sleep were not reported.

To further investigate the role of QRFP, Chen, Chiu et al. asked what this neuropeptide does in the simpler nervous system of zebrafish. Like in mammals, *qrfp* expression in zebrafish brains was restricted to the hypothalamus, where it colocalized with markers of glutamate transmission in neurons that projected within the hypothalamus and to the forebrain and hindbrain. Reducing *qrfp* expression increased locomotor activity both during the day (the normal active period in fish) and at night, and it reduced the number of sleep bouts (defined in zebrafish as being inactive for $\geq$1 min) during the day. Likewise, mutating QRFP receptors increased locomotor activity and reduced sleep during the day. In contrast, universal overexpression of *qrfp* decreased daytime locomotor activity without affecting sleep.

These results indicate that QRFP influences sleep and arousal, but has opposite effects on locomotor activity in zebrafish (where it reduces activity) and mice (where it increases activity). This difference may in part stem from the fact that mice are nocturnal while zebrafish are diurnal. Interestingly, in both species, the effects of QRFP appear to be stronger during the light period. Future work should investigate whether interactions between QRFP and other hypothalamic peptides such as melatonin, which is high at night whether or not a species is nocturnal, may explain some of these results. It will also be important to determine QRFP's role in feeding in zebrafish, its role in sleep in rodents, and the effects of light on these roles in both species. In addition, identifying inputs to *qrfp*-expressing neurons may provide additional insight about how external factors affect sleep/wake transitions.

This Week in The Journal is written by Teresa Esch, Ph.D.