Do Flies Count Sheep or NMDA Receptors to Go to Sleep?

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The drive to sleep increases the longer that we stay awake, but this process is poorly understood at the cellular level. Now, Liu et al. show that the plasticity of a small group of neurons in the *Drosophila* central brain is a key component of the sleep homeostat.

Thinking about another cup of coffee as you try to beat that grant deadline? One reason we resort to caffeine is because the urge to sleep becomes stronger the longer that we stay awake (Borbély, 1982). And to compensate for pulling an all-nighter, homeostatic mechanisms make us sleep deeper and longer than normal when we finally do sleep. So where is the sleep homeostat? And how does it count our waking hours? In this issue of *Cell*, Liu et al. (2016) identify a set of neurons in the *Drosophila* central brain which seem to lie at the core of the sleep homeostat: not only do these neurons regulate the drive to sleep, but they also increase their firing rate and change synaptic structure as sleep pressure rises (Figure 1).

One model for the sleep homeostat proposes that sleep-inducing substances called somnogens accumulate in specific regions of the awake brain and promote sleep once they reach high-enough levels (Brown et al., 2012). However, the half-lives of possible somnogens, such as adenosine (Porkka-Heiskanen et al., 1997), tend to be on the order of minutes while it takes hours to dissipate increased sleep pressure. This suggests that molecules like adenosine may be signals from the sleep homeostat rather than the homeostat itself.

With this in mind, Liu et al. (2016) set out to identify neurons—rather than molecules—that alter sleep homeostasis. They chose *Drosophila*, a widely used organism to investigate sleep (Hendricks et al., 2000; Shaw et al., 2000). One advantage of the fly is the relative simplicity of the brain, which makes it possible to ascribe important biological functions to small groups of neurons. There are also numerous tools to monitor and manipulate individual cells.

To identify potential homeostat neurons, the authors increased night-time neuronal activity in different sets of neurons and then measured the time flies spent asleep the following day. This powerful screen allowed the authors to compare how long an individual fly slept before, during, and after activating a discrete set of neurons. Liu et al. (2016) screened 500 different driver lines, most of which showed classical sleep homeostasis; thus, if neuronal activation kept flies awake during the night, those same flies showed a sleep rebound and slept longer the next day; conversely, if neuronal activation increased sleep during the night, most flies then slept less after activation ended. However, the authors found eight lines in which sleep increased both during and after neuronal activation, indicating that these neurons increase sleep drive. It is the relatively long-lasting effect on sleep after the end of neuronal activation that sets these neurons apart from previously described mammalian and fly neurons that are considered effectors of the homeostat rather than part of the central mechanism (Brown et al., 2012; Donlea et al., 2014).

Strikingly, all of the eight lines that increase sleep drive expressed in 16 R2 ring neurons, a group of cells in the ellipsoid body in the fly's central brain. But finding a group of neurons that affects a biological response by making them fire for 12 hr is very different from showing that they normally function in that process. Therefore, Liu et al. (2016) carefully tested how R2 neurons respond to sleep deprivation. Using electrophysiology, they show that R2 neurons increase their firing frequency between dawn and dusk as the pressure to sleep rises. Sleep deprivation increases their firing rate even further and also switches R2 neuron firing from single action potentials to bursting—as if the neurons are increasingly desperate to make flies fall asleep. Sleep deprivation also increases basal Ca^{2+} levels, Ca^{2+}-dependent transcription, and the intensity of a pre-synaptic marker, Bruchpilot, in R2 neurons (Figure 1). All of these returned to normal after flies were allowed to sleep, and similar changes were not seen in other brain regions, indicating that these are specific responses of R2 neurons to sleep deprivation. Ca^{2+}-dependent transcription in R2 neurons is also higher in 1-day-old flies than in older flies. This correlates with young flies sleeping for longer than adult flies (Shaw et al., 2000)—in an analogous manner to humans.

Liu et al. (2016) also show that the changes in the physiology of R2 neurons are not just impressive correlations of sleep deprivation but are intimately related to their ability to promote sleep. They reduced Ca^{2+} release from R2 neuron intracellular stores by knocking down expression of the IP3 receptor. This prevented sleep deprivation from increasing intracellular Ca^{2+} and altering pre-synaptic markers in R2 neurons and even reduced the amount of rebound sleep. Thus, changes in the physiology of R2 neurons in response to sleep deprivation seem to be required for sleep rebound. Finally, the

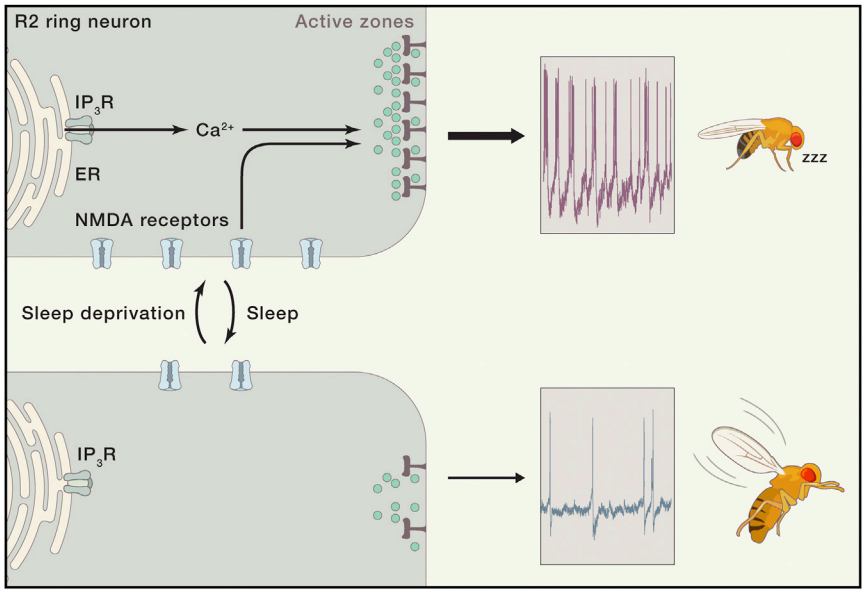

Figure 1. R2 Ring Neurons Function in Drosophila Sleep Homeostasis
Time spent awake and sleep deprivation cause intracellular Ca^{2+} levels and NMDA receptor abundance to rise in R2 ring neurons in the *Drosophila* central brain. This increases the number and size of R2 neuron pre-synaptic active zones and R2 firing rates, which promote sleep (top). These changes are plastic: sleep reverses these changes in R2 ring neurons to reduce sleep drive (bottom).

authors demonstrate that sleep deprivation also increases the number of actively translated NMDA receptor transcripts in R2 neurons. This increases NMDA receptor levels, although it is not yet clear if this is a transcriptional or post-transcriptional response. Importantly, they find that normal NMDA receptor levels are required for flies to sleep longer after sleep deprivation, but do not affect baseline sleep. Increased NMDA receptor levels in R2 neurons are therefore also a specific response to sleep deprivation and seem to be at least part of what these neurons count to determine sleep duration (Figure 1).

Sleep timing is not solely determined by the sleep homeostat. Circadian information is also crucial, as are factors such as anxiety and mental health (Brown et al., 2012). Even the amount of social interactions can have long-lasting effects on sleep duration in flies (Ganguly-Fitzgerald et al., 2006), although it is unknown if this affects the homeostat. Some of the most interesting questions opened up by the work of Liu et al. (2016) are what cells lie upstream of R2 neurons, what internal or environmental stimuli are relayed by these upstream neurons, and how these diverse stimuli are integrated. The conservation of neuronal mechanisms between flies and mammals suggests there should be an analogous neural circuit in mammals, which may even involve NMDA receptors, well-known regulators of plasticity in mammalian neurons (Malenka and Nicoll, 1993). Perhaps, one day, the insights of basic science into sleep will give us something more specific than a cup of coffee to help us beat that deadline. And maybe insomniacs won't need to count sheep either.

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