Title
Soporific signaling: how flies sleep through the night

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Soporific Signaling: how flies sleep through the night.

A good night's sleep is hard to come by. All too often, my nights are spent looking up at the ceiling thinking about questions that I'm not sure that I want answered: Will my grant application finally be funded? Will my graduate student ever finish her thesis? Will that last experiment finally satisfy the reviewer? One thing that I do know is that I am not alone. Increasingly large numbers of people do not get enough sleep at night and pay the consequences. We try to make up for this lack of sleep by drinking our coffee during the day and taking sleeping pills the following night. Even if we do manage to fall asleep, staying asleep for the duration of the night becomes a challenge. As many will attest, this problem only gets worse as we age. While I suspect that the main solution for our sleepless society will involve lifestyle changes, there is a large and growing market for new classes of sleep aids. The development of new therapeutic targets for sleep disorders will require a better understanding of both the circuitry and signaling molecules that are involved in the control of sleep. Given the magnitude of our modern society's problem staying asleep, new research into these topics has to be particularly welcome. The current study by Foltenyi and colleagues[1] demonstrate a novel role for EGFR and ERK signaling in sleep regulation and consolidation in *Drosophila*.

In recent years, there has been much progress toward developing a mechanistic understanding of sleep. For example, our understanding of the underlying circuitry in mammals has steadily improved[2]. Discovering a role for the hypocretin/orexin in the regulation of sleep and Narcolepsy has been a major step forward[3,4]. That being said, we are still a long way from a molecular/genetic understanding of sleep. It is not clear that many sleep researchers would even accept the premise that sleep is a process that can be understood at this level. The use of *Drosophila* as a model for understanding the molecular basis of behavior has proven fruitful, with the success of this organism in circadian rhythms research providing a particularly compelling example. In the present study[1], the authors employ this model organism to explore the role of the transforming growth factor-α (TGF-α) signaling cascade in the control of sleep. Of course, for flies to become an effective model system in this research area, we have to be convinced that flies sleep, or at least undergo a process that is biologically similar to sleep in humans.

For more clinically-oriented researchers, the sleep state is defined by EEG recordings of cortical activity coupled with measurements of muscle tone. These electrophysiological correlates of sleep provide more quantitative measures than behavioral markers. They allow the discrimination of quiet inactivity from sleep as well as measurement of the different stages of sleep (REM/NREM). These objective criteria have proven extremely useful for the study of sleep in humans and other mammals. However, even among vertebrates, EEG correlates of sleep and wakefulness has to be considered in the context of the ecological niche of a particular species[5]. From a biological point of view, it seems unnecessarily narrow to define sleep based solely on patterns of cortical activity.

If behavioral criteria are used, a number of studies have found evidence that fruit flies exhibit canonical features of sleep. *Drosophila* exhibit a consolidated state of

behavioral inactivity, an increased arousal threshold during these inactive periods, and the ability to rapidly reverse the inactivity, i.e. to wake up[6,7]. Importantly, if deprived of sleep, the flies respond by sleeping more, experiencing a "sleep rebound." This homeostatic drive to recover sleep after deprivation is one of the hallmark features of sleep. Fruit flies even respond to the same pharmacological agents known to modulate arousal in humans including caffeine[6,7], and methamphetamine[8]. A recent study reports that older flies show fragmentation in sleep episodes similar to many aging humans[9]. Accepting the premise that shared features exist between the sleep states in insects and mammals enables us to apply the power of *Drosophila* genetics to the problem of uncovering the basic biological mechanisms controlling sleep.

Taking advantage of this strategy, the authors of the present study[1] examined the TGF-α signaling cascade. Previous work has demonstrated that TGF-α is rhythmically transcribed and secreted by cells within the main mammalian circadian oscillator, the suprachiasmatic nucleus[10]. In the fruit fly, members of the TGF-α family (e.g. Spitz) bind the epidermal growth factor receptor (EGFR). Activation of EGFR requires the processing protein Star and Rhomboid family (Rho), which are integral membrane proteases that cleave membrane bound TGF- α ligands to produce a soluble form of the signaling molecule. Triggering the EGFR pathway, in turn, activates extracellular signal-regulated kinase (ERK). ERK is familiar to neuroscientists as a kinase implicated in the regulation of plasticity in the adult nervous system including the photic regulation of circadian timing[11].

In the fruit fly, EGFR is widely expressed in the nervous system. The study by Foltenyi and colleagues[1] demonstrate that overexpression of EGFR signaling components, Rho and Star, cause an acute, reversible and dose-dependent increase in sleep that tightly parallels an increase in phosphorylated ERK (pERK). Unfortunately, they were unable to measure endogenous levels of pERK (due to a lack of sensitivity in the assay) to confirm that sleeping flies exhibit more pERK than awake flies. The ability of a dominant negative EGFR to block activation of ERK argues that the manipulation is specific to the EGFR pathway. In contrast to the increase in sleep observed after Rho overexpression, inhibiting its expression leads to a significant decrease in sleep. Importantly, this decrease in sleep was due to a dramatic shortening of the duration of sleep episodes accompanied by an elevation of sleep bout number. This observation suggests that the mutant flies have an increased need for sleep, but are unable to stay asleep. This sleep pattern that is similar to insomnia in humans (Fig. 1).

Part of the significance of this work[1] is that the authors were able to demonstrate anatomical specificity in their manipulations of this signaling pathway. The brain regions involved in the influence of this signaling cascade on sleep are the pars intercerebralis (PI) and tritocerebrum (TriC). The cells of the PI contain Rho and generate the EGFR ligands that activate ERK in the receiving cells within the TriC. The authors identified the PI as the region responsible for EGFR ligand secretion by demonstrating that the cells in that region express endogenous Rho, and that inhibiting Rho in this region resulted in decreased sleep. In insects, the PI contains neurosecretory cells that have been compared to the vertebrate hypothalamus. The TriC region was identified by the robust pERK

expression that was stimulated in response to the overexpression of the EGFR processing components Rho and Star. The authors argue that Rho and Star overexpression only enhances EGFR signaling in cells that endogenously express the ligand. Future studies will need to determine the effects of electrically silencing neurons in the PI or TriC regions on sleep.

Surprisingly, given the role of TGF-α in the SCN[10], EGFR/ERK signaling did not appear to alter the circadian timing of sleep. Circadian rhythms in *Drosophila* are driven in large part by a population of ventral lateral neurons (LNv) that express the neuropeptide pigment dispersing factor (PDF). Basic circadian properties were not altered in flies with Rho knocked down or in flies over-expressing Rho or Star. Inhibiting Rho expression in the LNv cells with Pdf-Gal4 driver did not change sleep patterns. Therefore, the effects observed on sleep regulation by EGFR/ERK signaling in the PI are most likely driven by a signal coming from a region of the brain that lies downstream of circadian control.

What are the identities of the downstream targets for EGFR/ERG signaling in the TriC region of the fly brain? A recent study showed that ERK directly phosphorylates the potassium channel Kv4.2[12]. Phosphorylated ERK appears to be expressed in the processes but not in the soma of the TriC neurons. Thus, the final target of this signaling pathway may well be changes in electrical activity/synaptic transmission in these neurons. This suggestion fits nicely with recent work in *Drosophila* indicating that a mutation in the potassium channel Kv1.4 also produces abnormalities in sleep maintenance[13].

Researchers using *Drosophila* to study the regulation of sleep have progressed to the point where they have begun to identify the regions and signaling pathways involved in the control of sleep and daily rhythms. However, much work remains to develop a circuit level understanding for the control of sleep. Questions will need to be addressed about the relationship between the neurons in the mushroom bodies[14,15], the PI, and the LNv, which are all implicated in the control of daily rhythms in sleep. With the improvements in the ability of this field to manipulate specific cell populations, we are likely to see continued progress in this area. This is good news for the prospect of developing future therapeutic targets for sleep disorders and, in time, this research may help us get a good night's sleep.

Fig. 1: Schematic illustrating the proposed role of extracellular signal regulated kinase (ERK) in the regulation of sleep in *Drosophila*. a) Rho-mediated activation of ERK signaling increases sleep duration. During the night, Rho activation in the pars intercerebralis (PI) leads to the production and secretion of an EGFR ligand. The resulting phosphorylation of EGFR activates ERK in the tritocerebrum (TriC). While the final targets of this signaling pathway are not known, the phosphorylated ERK appears to stay in the processes of the TriC neurons and may well regulate electrical activity and synaptic transmission in these neurons. b) During wakefulness, Rho signaling in the PI is proposed to be downregulated resulting in basal levels of ERK signaling. Inhibition of Rho expression in PI neurons results in decreased sleep levels with short, fragmented sleep bouts. This observation suggests that these mutant flies have an increased need for sleep, but are unable to do so (i.e. an insomniac fly).

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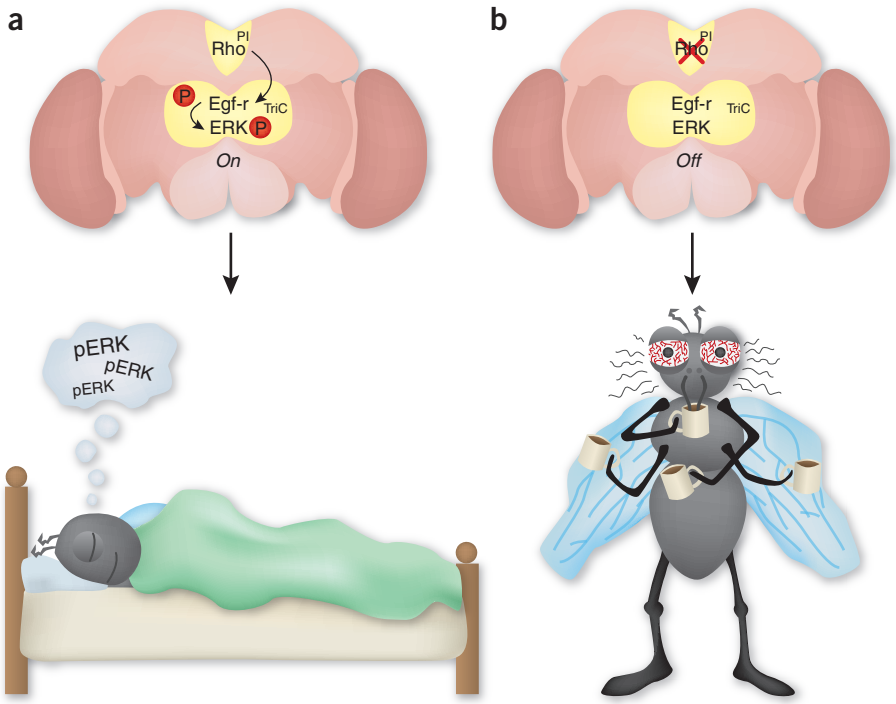
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