

Dispatches

Sleep: What Goes Up Must Come Down

The function of sleep is hotly contested. Two recent studies suggest that fly sleep may be required to rescale synapses in the brain.

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Sleep is required to maintain cognitive function but the reason is not understood. One model proposes that sleep provides the brain a time in which incoming sensory information is minimal, so memories acquired while awake can be stabilized or consolidated within the relevant neural circuits. How memories are consolidated from a labile short-term form to a more stable long-term representation is not well understood. However, in many species, even brief periods of sleep restriction impair cognitive function and the capacity to consolidate new memories [1]. Electrophysiological recordings in rats [2] have shown that activity patterns observed during wakeful experience are re-activated in the appropriate circuits during sleep, suggesting that sleep may promote off-line memory consolidation. Furthermore, reactivating a memory with a non-disruptive cue while asleep promotes memory consolidation in humans [3].

A second, more general model, that is not mutually exclusive to the memory consolidation hypothesis, proposes that sleep maintains cognitive function by promoting brain-wide 'synaptic homeostasis'. Cirelli and Tononi [4] proposed that many synapses in the brain are strengthened, or 'potentiated', by normal circuit use during wake. Slow-wave neural activity that is specific to sleep globally resets the synapses, returning the brain to a baseline state. Since memories are also believed to be formed by altering synaptic connections between neurons, the mechanism must be sophisticated enough to retain the memory-relevant synaptic changes while downscaling the others. In their most recent work, Gilestro, Tononi and Cirelli [5] extend

their synaptic homeostasis theory from humans [6] and rats [7] to the fruit fly, *Drosophila melanogaster*, which exhibits many of the behavioral hallmarks of mammalian sleep [8].

Gilestro *et al.* [5] examined the expression levels of synaptic marker proteins in the fly brain through the sleep/wake cycle and following sleep deprivation by quantifying immunoblots of brain extract and immunofluorescence signals in the intact fixed brain. They analyzed levels of the synaptic active zone protein bruchpilot (BRP), in addition to the synaptically localized discs-large (DLG), synapsin, syntaxin, and cysteine string proteins. In unperturbed flies, BRP levels were increased following six hours of spontaneous wake compared with six hours of spontaneous sleep.

To examine whether expression levels were further increased with forced waking, flies were sleep-deprived by mechanical stimulation, or by a novel method where a guest fly joined the host fly in the recording chamber. Both deprivation methods resulted in sleep loss and increased expression of each synaptic protein that was assayed. Longer periods of sleep deprivation by mechanical stimulation generally correlated with higher expression levels of each protein. Importantly, the authors verified that BRP accumulation was a result of sleep loss, and not mechanical stimulation. The flies that were most sleep-deprived following the same period of stimulation showed the highest BRP expression levels. The wake-induced increase of BRP is apparently reversed by sleep. BRP levels gradually decreased during the period the flies were asleep. The BRP decrease required sleep, and not simply passage of time, because it was prevented by keeping the flies awake.

These correlative data suggest that synaptic protein levels throughout the brain of the fly increase with wake and decrease with sleep. One might expect that specific areas of the brain with higher levels of activity during wake would be most likely to show sleep/wake-state-regulated changes in expression level. However, quantifying BRP intensity level by immunostaining and microscopy suggested a general increase throughout the brain. Gilestro *et al.* [5] quantified BRP labeling intensity in three regions of the central brain following sleep deprivation. BRP staining increased in the mushroom bodies (MB; Figure 1), an area necessary for olfactory memory [9] that also regulates sleep/wake amount [10,11]. Enigmatically, electrophysiological recordings of MB neurons in a restrained fly indicate that they have a very low spontaneous firing rate [12]. BRP expression level was also elevated by wake in the ellipsoid body, a region implicated in locomotor activity control [13]. This is not unexpected, because flies move frequently while awake. Lastly, BRP intensity increased in the antennal lobes, which contain the processes of olfactory sensory neurons, local neurons and projection neurons [9]. Presumably, the other primary sensory regions — the visual area of the optic lobes and the gustatory area of the subesophageal ganglion — also exhibit significant changes in BRP level. It will be important to determine whether increased BRP in these neurons alters the function of the relevant synapses, or whether the excess BRP is 'junk' that results from general circuit use that is cleared during sleep.

Nevertheless, the finding that synaptic proteins are elevated by wake and reduced by sleep in flies provides a plausible role for fly sleep in resetting synaptic weight. It will be interesting to test whether synaptic proteins are altered in mutant flies that sleep less, such as the

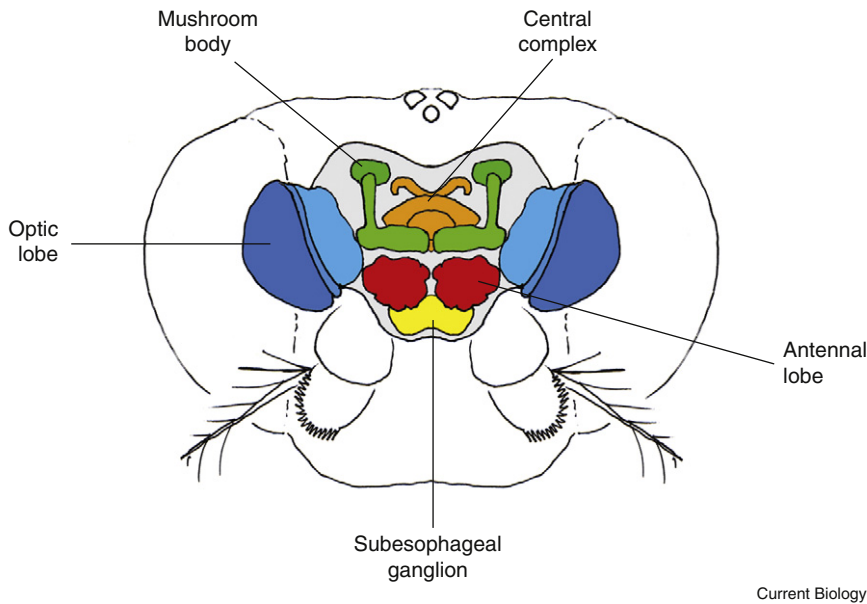


Figure 1. The major areas of organized neuropil in the fly brain.

The primary visual areas are the optic lobes (blue). Gustatory receptor neurons innervate the subesophageal ganglion (yellow). Olfactory sensory neurons project to the antennal lobes (red) and this information is relayed to the mushroom bodies (green). The central complex, including the ellipsoid and fan-shaped bodies (orange) controls locomotion. The LNV cell bodies (not shown) are located at the junction between the optic lobes and central brain. Donlea *et al.* [20] studied large LNV processes in the optic lobes. An outline of the head capsule is shown for orientation.

potassium channel mutant *shaker* [14], and in what direction. Additionally, it will be important to discern whether mutations in, or over-expression of, synaptic proteins alter sleep amount and brain function, such as memory formation and consolidation. Although the reported electrophysiological correlate for fly sleep [15] does not resemble mammalian slow-waves, it will also be interesting to determine whether such brain activity is required to reduce synaptic protein expression in flies.

Another question arising from the Gilestro study [5] is whether changes in synaptic protein levels are part of the homeostatic 'sleep-need' signal. The timing of sleep is thought to be regulated by the circadian clock, whereas the amount of sleep is controlled by an unknown homeostatic mechanism. The brain monitors 'sleep-need' similar to a thermostat that monitors temperature fluctuation and adjusts around a set-point. When deprived of sleep for one night, you sleep longer the following night to compensate. It is unclear how sleep-need is represented in the brain. If state-dependent changes in

synaptic proteins constitute part of the molecular sleep-homeostat, then the challenge will be to uncover how this information is monitored and transduced to brain regions that initiate sleep/wake. A subset of circadian pacemaker neurons known as the large ventral lateral neurons (LNV) promote wake in flies [16–19]. In a companion paper, Donlea *et al.* [20] overexpressed fluorescent GFP-DLG and GFP-synaptobrevin fusion proteins in the small and large LNV. They found a modest increase in the number of GFP-labeled synapses in the optic lobe (from large LNV projections) following 48 hours of sleep deprivation. They also found an increase in GFP labeling following 'social enrichment'. In this protocol, flies are either kept in isolation, or are enriched by housing in groups. Social enrichment increases sleep, and is thought to promote plasticity by providing a more stimulating environment for the fly. A hopeful interpretation of these data is that the LNV may monitor changes in synaptic protein expression and transduce the information to modulate sleep amount. It will be

important to test whether changes in synaptic proteins within the LNV are a critical part of the sleep homeostat.

If synaptic downscaling turns out to be a critical and conserved function of sleep that allows the brain to remain plastic and/or is a critical part of the sleep homeostat, the powerful genetics of the fly will surely aid discovery of the underlying mechanisms.

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Chromosome Segregation: Monopolin Goes Spindle

At anaphase onset the mitotic spindle undergoes dramatic changes in order to segregate sister chromatids. Surprisingly, the monopolin complex, best known for its role at kinetochores in meiosis, is now shown to localize to, and stabilize, the mitotic anaphase spindle.

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During meiosis, a single round of DNA replication is followed by two rounds of cell division (meiosis I and II), resulting in four daughter cells with half the chromosomes. Meiosis I is characterized by segregation of homologous chromosomes in a process known as reductional division and requires the attachment of sister kinetochores to microtubules organized by the same spindle pole. In the budding yeast *Saccharomyces cerevisiae*, a single microtubule contacts the pair of sister kinetochores in meiosis I [1], and the meiosis-specific monopolin-complex component Mam1 is essential for this mono-orientation of sister kinetochores [2] (Figure 1A). Mam1 forms a complex together with the casein-kinase-1 Hrr25 and the ubiquitous nucleolar proteins Csm1 and Lrs4 [3,4]. In the fission yeast *Schizosaccharomyces pombe*, kinetochores bind multiple microtubules, as in animal cells, and a monopolin complex comprising Pcs1 and Mde4 (orthologs of budding yeast Csm1 and Lrs4, respectively) prevents attachment of microtubules emanating from opposite poles to the same kinetochore (merotelic attachments) in mitosis and meiosis II [5] (Figure 1B). In an unexpected twist, Choi *et al.* [6] now report in this issue of *Current Biology* that the fission yeast monopolin complex is targeted by a Cdc14-like phosphatase [7,8] to the anaphase spindle where it contributes to spindle elongation and stabilization.

At the metaphase to anaphase transition of mitosis, remarkable changes in spindle morphology and dynamics take place. During metaphase, microtubules display high turnover, which favours kinetochore capture by kinetochore microtubules (kMTs). This increase in microtubule dynamics is promoted by the activity of the cyclin-dependent kinase Cdk1 [9,10]. Once all sister chromatids are attached to kMTs from opposite spindle poles (bioriented), the protease separase is activated. Separase dissolves sister chromatid cohesion, thus allowing shrinking kMTs to move the chromosomes towards the spindle poles, and cells advance into anaphase. Suddenly microtubules are stabilized, and the spindle midzone is formed by overlapping interpolar microtubules (iMTs) in the middle of the spindle where microtubule-bundling proteins, kinesin motor proteins and signalling molecules localize. Sliding forces produced by motor proteins of the midzone drive spindle elongation, separating the sister chromatids further apart [11,12].

In budding and fission yeast, anaphase spindle elongation is particularly drastic (as much as five fold), largely contributing to the physical separation of sister chromatids. Cdc14 is an important regulator of the anaphase spindle. Decreasing Cdk1 activity and increasing Cdc14 phosphatase activity trigger a program of dephosphorylation of Cdk1 targets that guides cells through anaphase and eventually to the G1 phase of the cell cycle (mitotic exit) [13,14]. In a Cdc14-dependent manner, several

microtubule-associated proteins are recruited to or regulated at the anaphase spindle to modulate microtubule behaviour. Assembly of the spindle midzone is directly triggered by the Cdc14-dependent dephosphorylation of the microtubule-bundler Ase1 [15]. Dephosphorylation of the DASH complex component Ask1 by Cdc14 is responsible for the reduced dynamics of iMTs in anaphase [10]. Regulation of the ortholog of the inner centromere protein (INCENP) Sli15 by Cdc14 targets the Aurora B chromosomal passenger complex to the anaphase spindle where it controls spindle stability [16].

The fission yeast Cdc14 homolog, Clp1 (also known as Flp1), also interacts with Aurora B kinase [17], suggesting that functions of Cdc14-like phosphatases are conserved between budding and fission yeast. Choi *et al.* [6] now identify the first Clp1 target involved in regulation of the anaphase spindle. They show that the fission yeast monopolin complex, which is entrapped in the nucleolus during interphase, becomes released as cells enter mitosis. Mde4 and Pcs1, as mentioned above, bind to kinetochores to ensure chromosome biorientation. Upon Clp1-dependent dephosphorylation of Cdk1 sites on Mde4, the monopolin complex localizes to the extending anaphase spindle. Surprisingly, Pcs1–Mde4 does not uniformly decorate the anaphase spindle as is the case, for example, for the DASH complex [18]. Instead, the monopolin complex seems to be excluded from the spindle midzone, a region of overlapping antiparallel iMTs. Whether the monopolin complex indeed binds preferentially to parallel microtubules, as suggested by Choi *et al.* [6], awaits experimental confirmation. Initial *in vitro* microtubule-binding experiments with recombinant Pcs1–Mde4 complex did not show microtubule-binding activity. Thus, it is presently unclear