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# Molecular mechanisms of (recovery) sleep: lessons from *Drosophila melanogaster*

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One of the key features of sleep is that if the duration of a waking period is prolonged, the following sleep period will be longer, including more slow-wave activity. This homeostasis is explained by production of sleep pressure that accumulates during the waking period. It is generally accepted that neuronal activity, in one way or other, is the driving force for accumulation of sleep pressure, both during spontaneous sleep-wake cycle and during prolonged wakefulness.

Prolonged wakefulness is associated with increased energy consumption, production of danger signals and modulations in neural plasticity. Data derived from experiments with *Drosophila melanogaster* introduces a fascinating window to the basic mechanisms of sleep and sleep homeostasis, and undoubtedly sheds light to the mechanisms of sleep regulation also in humans. However, the existence of substantial cortex, which is regarded as a key actor in mammalian NREM sleep regulation, will add to the complexity of the regulatory circuits.

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## What is sleep homeostasis?

The mathematical two-process model of sleep regulation is constructed of EEG recordings obtained from different species, and it describes the relationship between a period of wakefulness and the sleep following it. The model shows that the longer the previous waking period has been, the more sleep (in form of slow waves and duration of the sleep period) it will induce. It can accurately predict how much EEG delta power increases after a known period of (prolonged) wakefulness [1].

The concept of sleep pressure, or sleep need, describes the mechanism by which sleep is produced, either during spontaneous sleep-wake cycle (normal sleep) or after prolonged waking period (recovery sleep). The generally accepted idea is that neuronal activity, in one way or other, drives the accumulation of sleep pressure, but there is no consensus as to the details of this process, or rather, these processes.

## What in wakefulness produces recovery sleep?

### Energy consumption-related mechanism

*Reasoning for the concept*

Neuronal activity consumes energy — the more activity, the more energy is consumed. As neurons are more active during waking than sleep [2], the duration of waking will increase need for energy, and prolonged waking may expose brain for energy depletion. A molecule that would signal of energy shortage could also act as a sleep homeostat, or sleep-inducing factor. Indeed, such molecule exists: adenosine, a metabolite of ATP, increases in the basal forebrain in the course of prolonged wakefulness [3] and is able to increase sleep via adenosine A1 receptors [4,5]. In addition, many genes involved in energy metabolism are upregulated during waking [6••]. Increased oxidative phosphorylation in mitochondria increases reactive oxygen species (ROS), which can activate immune defense [7], bridging increased neuronal activity, energy production and immune defense.

### Neuronal plasticity-related mechanisms

*Reasoning for the concept*

Neuronal activity modifies the number and strength of synapses. Both forming of new synapses and keeping up neuronal activity consume energy. Moreover, continuous strengthening of synapses can develop into runaway potentiation [8••], which restricts neuronal plasticity and impairs memory and learning. For these reasons, synaptic strength needs to be re-scaled. The decrease in need to process (sensory) information during sleep appears to offer an optimal condition for the synaptic scaling that aims at restoring overall synaptic strength. Experimental evidence to support this view comprises of studies conducted on fruit flies and rodents as well as humans [8••].

Synaptic strength homeostasis, as defined by the synaptic homeostasis hypothesis (SHY), states that overall synaptic strength in the brain increases during waking and decreases during sleep [9,10••]. In effect, the scaling is

selective: electron microscope imaging of the synapses in mouse cortex and hippocampus demonstrated that axon-spine interface size decreased between wake and sleep in small and medium sized synapses (majority of the synapses), but not so much in large synapses [10••,11]. The overall weakening does not exclude the option that some synapses are fortified [8••].

One recent study showed that during sleep, AMPA receptors are removed from synapses and dephosphorylated, which weakens the synapses via mechanisms involving Homer1a, mGLUR1/5, noradrenaline and adenosine [12••]. In this scenario, the long form of Homer 1 that couples mGluR1/5 to IPR3 signaling pathway is replaced by the shorter form, Homer-1a, weakening the IPR3-mediated signaling during sleep. Homer-1a also activates the GluR1/5 signaling that is independent of agonist, which drives weakening of excitatory synapses during scaling down process. Noradrenaline prevents Homer-1a from binding to GluR1/5, while adenosine promotes it [12••,13].

Neuronal firing homeostasis expresses itself in observations that both after a period of excessive activity and quiescence, activity in neuronal circuits' returns to baseline levels [14]. Firing rates appear to be homogenized during sleep-wake cycle: neurons with high firing rates during waking decrease their firing in sleep, while those with low firing rates in waking, increase firing during sleep [15].

Understanding of some basic mechanisms of sleep homeostasis has derived from experiments conducted on *Drosophila melanogaster*. One sleep homeostasis-regulating area in the fly brain is localized in the central complex that is important for motor control and navigation [16••] (see Figure 1). The area also receives innervation from the monoaminergic systems, enabling state-dependent modulation of the activity of the circuitries [16••]. The core circuit consists of R2 cells of the ellipsoid body and dFSB neurons in the fan-shaped body. The R2 cells collect information from other brain areas, including Helicon cells that respond to visual input [17], and contribute to generating sleep need. The R2 cells increase their firing rate in the course of waking, inducing plasticity changes that potentially code the need for sleep. Manipulations of R2 cell activity affects sleep, suggesting that they convey this information to the dFSB cells, which then execute the sleep regulation.

The dFSB cells act as sleep switch, increasing their firing rate upon increasing sleep pressure. Two potassium channels, Shaker and Sandman, regulate the activity of the dFSB cells. Shaker is a voltage-gated potassium channels that regulates the repolarization efficacy of action potentials, promoting faster repolarization and faster spiking activity. Sandman is a leak-channel that leaks potassium out of the cell, hyperpolarizing it and decreasing its firing rate.

In waking, dFSB cells are in OFF-state, characterized by Shaker channel inactivity (promoted by dopamine) and Sandman channel integration to cell membrane, allowing potassium leak. In the course of waking, Shaker channel activates through its subunit, Hyperkinetic, allowing potassium flow through the channel and thus promoting firing of the dFSB cell. To the same effect plays internationalization of Sandman: potassium leak from the cells stops, and the dFSB neurons increase their firing rate, moving to ON state, which initiates sleep.

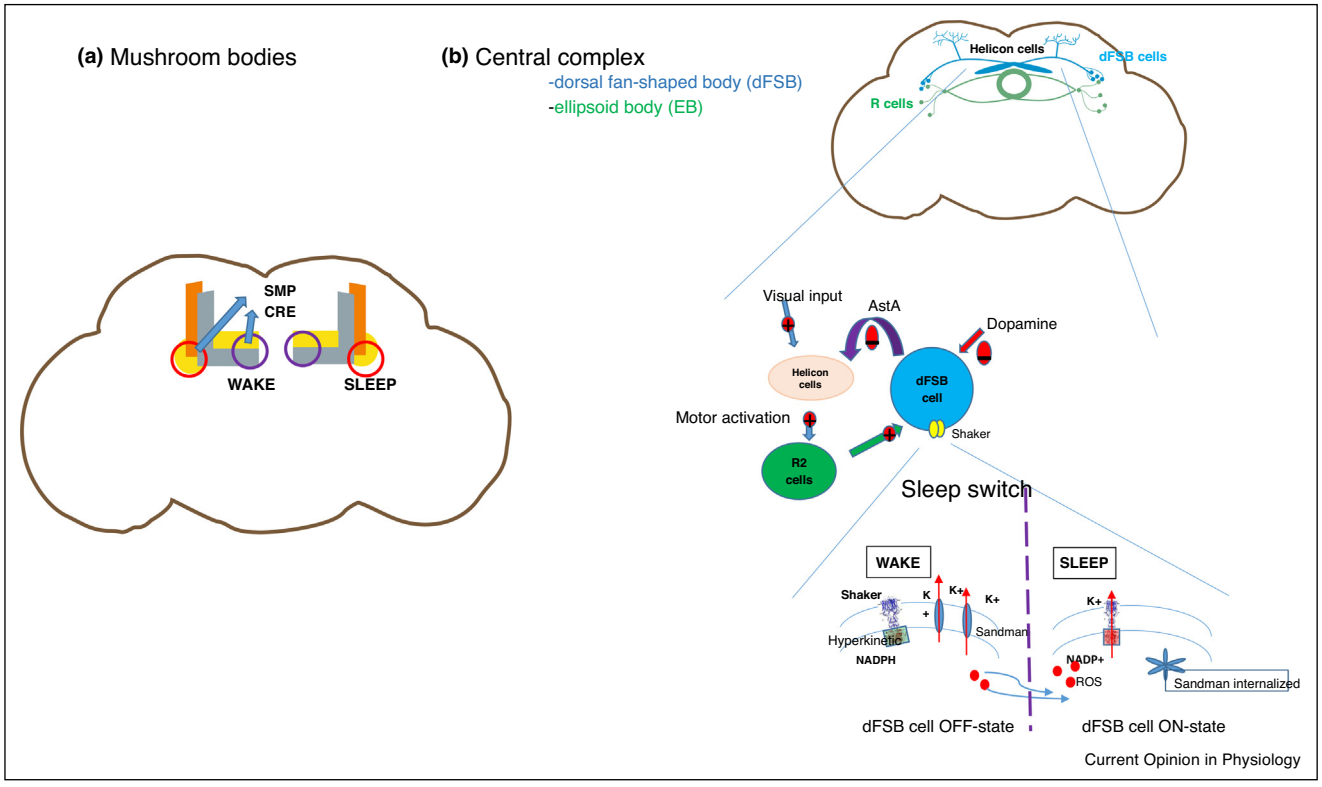
The role of hyperkinetic has been clarified only recently [19]: Hyperkinetic senses oxidation/reduction status of the cell through connection to NADPH/NADP + unit. The oxidation of NADPH to NADP + is promoted by reactive oxygen species (ROS), which are formed in mitochondria in oxidative phosphorylation. When NADPH is bound to Hyperkinetic, potassium current through Shaker is blocked.

In the course of waking and high energy demand, NADPH is oxidized to NADP + and Shaker current is activated, allowing activation of the dFSB cell. This mechanism offers a link between the activity of the dFB neurons and energy production, giving supports to the energy theory of sleep homeostasis.

The other fly brain area connected to homeostatic sleep regulation is the mushroom body, which is important in olfactory learning and memory [20], and has been suggested to correspond to hippocampus in mammals. They Keynon interneurons in the mushroom bodies receive sensory inputs and their axonal projections form two target areas of which one is involved in promotion of wakefulness and the other in promotion of sleep [21••]. Sleep-promoting and wake-promoting areas project to two brain areas, the crepine (CRE) and the superior medial protocerebrum (SMP), where also dFSB neurons have dendrites. It is thus possible that the Keynon-mediated signals are transmitted to the central complex via CRE and SMP and are integrated to sleep regulation by fFSB neurons [21••].

Interestingly, two separate areas, one involved in motor control and navigation, the other in olfactory learning and memory, form sleep homeostasis regulating circuits. It can be hypothesized that each activity unit, sensory, motor or integrative, forms local sleep regulation circuits for sleep homeostasis. These circuits may be integrated, although loosely, since profound sleep increases/decreases can be induced by manipulations of the circuits separately [18]. In mammals, sensory and motor information flows through thalamus, which also profoundly regulates vigilance states [22].

**Figure 1**




**(a) Mushroom body.** Of the three subpopulations of the Keynon cells axonal branches in the mushroom bodies, one promotes selectively sleep (yellow) and the other waking (grey). Of their postsynaptic output neurons, those that promote wakefulness are glutamatergic (purple rings) and those that promote sleep are cholinergic (red rings). Activity of the sleep promoting neurons is increased in sleep deprivation. Both project to the crepine (CRE) and the superior medial protocerebrum (SMP) areas, where they may interact with central complex neurons.
**(b) The central complex.** The increased firing of the R2 cells in the course of waking induces plastic changes (increase Ca2+ and dNR1) [18]. The information is conveyed to dFSB cells.
Two potassium channels, Shaker and Sandman, regulate the activity of the dFSB cells. Shaker is a voltage-gated potassium channels that regulates the repolarization efficacy of action potentials, promoting faster repolarization and faster spiking activity. Sandman is a leak-channel that leaks potassium out of the cell, hyperpolarizing it and decreasing its firing rate.
In the course of waking, Shaker channel activates through its subunit, Hyperkinetic that senses oxidation/reduction status of the cell through connection to NADPH/NADP+ unit.
The oxidation of NADPH to NADP+ is promoted by reactive oxygen species (ROS), which are formed in mitochondria in oxidative phosphorylation. Upon internationalization of Sandman, potassium leak from the cells stops, and the dFSB neurons increase their firing rate, moving to ON state, which initiates sleep.
The activity of R2 cells is promoted by Helicon cells that respond to visual input and play a permissive role in locomotion [17], adding a potential user-dependent aspect to sleep pressure accumulation. Further, the activation of dFSB cells during sleep inhibits Helicon cells, and thus indirectly also R2 cells, forming a negative feed-back circuit for the accumulation of sleep pressure.

## Defense-related mechanisms

### *Reasoning for the concept*

Paucity of sleep constitutes a threat for the organism, and as response, defense reactions are activated. Experimental evidence show that, indeed, prolonged wakefulness activates the elements of immune defense, first the innate immune defense including activation of cytokines [23], but later also the acquired immune system is affected [24]. Many of the immune response mediators are able to induce also sleep [23], in mammals as well as in fruit flies. A genome-wide screen in Drosophila identified a single gene, *nemuri*, that induced sleep. The expression of the gene was low under normal conditions but was induced by sleep deprivation and also by infection. The expression was localized to the dorsal fan-shaped body (dFSB), which is a key sleep regulatory are in the fly brain [25]. Nemuri acts also in connection with infection, evidently killing microbes and thus representing a molecule that bridges immune and sleep regulation, a role played by cytokines in mammals [25].

In mammalian brain, neuronal activity increases release of chemokines and cytokines, including TNFa and IL-1 — both known as sleep-inducing molecules. These immune

signaling molecules are also important modulators of synaptic functions and neural plasticity [26,27]. Thus, in addition to regulation of sleep, these molecules act as regulators of both neural plasticity and immune responses.

The so far identified sleep-inducing molecules appear to integrate functions of several categories: for example, cytokines in mammals, and the counterpart in Drosophila (nemuri), mediate immune responses, and simultaneously regulate sleep [25]. Further, adenosine plays a role not only in energy metabolism [3], but through ATP (particularly via P2X7 receptor), also in immune responses and neuronal events [28]. Particularly the gap between neural plasticity and defense mechanisms is narrowing: TNFa, one of the early responder of the innate immune defense, is also a key regulator of synaptic plasticity [29]. Thus it is questionable to what extent it is useful to view molecules participating in sleep homeostasis regulation as representatives of a specific, narrow functional category.

## Are the mechanisms of normal sleep induction and recovery sleep induction the same?

Several observations support the notion that the mechanisms to produce recovery sleep are at least partially different from those that regulate sleep homeostasis under natural sleep-wake cycle. Different brain sites [30], as well different brain cells [31] may be activated. In rats, depletion of basal forebrain cholinergic cells abolishes recovery sleep, while the natural sleep-wake cycle remains largely intact [32], and in Drosophila, only activation of the cholinergic cells, but not other wake-inducing cells, induced recovery sleep [33•]. In fruit flies, the TNFa homologue, Eiger, mediates sleep. Its knock-down in astrocytes, but not in neurons, reduces sleep duration. On the other hand, expression of the TNFa receptor family, Wengen, in neurons, but not in astrocytes, is needed for sleep-deprivation-induced increase in sleep [31].

It is probable that in the course of prolongation of the waking period, additional mechanism to induce sleep are recruited. Under natural sleep-wake cycle, sleep homeostasis could be mainly regulated by plasticity-related mechanisms, and modulations of mediator levels, such as TNFa, would be rather connected to plasticity mechanism than defense responses. To speculate further on this line, the mediators could be induced by neurons, but in the course of prolongation of the waking, glia cells would be recruited to produce these mediators in larger quantities in order to arouse immune responses.

## Is there wakefulness that does not induce recovery sleep?

Behavioral sleep studies have revealed that several species, when studied in their natural habitat, use survival strategies that include long periods of sustained wakefulness, in order to gain nutritional or reproductive advantage. In many cases, these prolonged periods of wakefulness do not induce recovery sleep [34], indicating that these species have strategies to bypass, at least temporarily, sleep homeostasis processes.

Experimental studies have confirmed that, indeed, such mechanisms exist, and also have started to identify mechanisms that induce recovery sleep and compare to those that do not.

One experiment, conducted on rats, showed that while pharmacological activation using different glutamate receptor agonists induced equal durations of prolonged wakefulness, only activation with NMDA, but not AMPA, induced NREM recovery sleep. Interestingly, NMDA increased EEG theta activity in waking, while AMPA did not, suggesting that the quality of waking was important for induction of recovery sleep [35]. Respective experiments in Drosophila showed that, although activation of three different neurotransmitter systems in fly promoted wakefulness, only cholinergic activation-induced wakefulness promoted recovery sleep, while activation of octopaminergic neurons suppressed homeostatic recovery sleep. Blocking the activity of the recovery sleep-inducing neurons did not modulate baseline sleep, indicating that recovery sleep regulation and baseline sleep regulation have at least partially separate mechanisms.

## Conflict of interest statement

Nothing declared.

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## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Borbely AA: **A two process model of sleep regulation**. *Hum Neurobiol* 1982, **1**:195-204.
2. Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G: **Cortical firing and sleep homeostasis**. *Neuron* 2009, **63**:865-878.
3. Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW: **Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness**. *Science* 1997, **276**:1265-1268.
4. Gass N, Porkka-Heiskanen T, Kalinchuk AV: **The role of the basal forebrain adenosine receptors in sleep homeostasis**. *Neuroreport* 2009, **20**:1013-1018.
5. Lazarus M, Oishi Y, Bjorness TE, Greene RW: **Gating and the need for sleep: dissociable effects of adenosine A1 and A2A receptors**. *Front Neurosci* 2019, **13**:740.

6. •• Cirelli C, Faraguna U, Tononi G: **Changes in brain gene expression after long-term sleep deprivation**. *J Neurochem* 2006, **98**:1632-1645
This is an important study to show the large extent of genes that are affected by sleep deprivation.

7. Kelley N, Jeltema D, Duan Y, He Y: **The NLRP3 inflammasome: an overview of mechanisms of activation and regulation**. *Int J Mol Sci* 2019, **20** http://dx.doi.org/10.3390/ijms20133328.

8. •• Tononi G, Cirelli C: **Sleep and synaptic down-selection**. *Eur J Neurosci* 2020, **51**:413-421 http://dx.doi.org/10.1111/ejn.14335 Epub 2019 Jan 23. Review
The authors give through and updated version of the SHY hypothesis.

9. Tononi G, Cirelli C: **Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration**. *Neuron* 2014, **81**:12-34.

10. •• de Vivo L, Bellesi M, Marshall W, Bushong EA, Ellisman MH, Tononi G, Cirelli C: **Ultrastructural evidence for synaptic scaling across the wake/sleep cycle**. *Science* 2017, **355**:507-510
This is the first direct evidence, obtained using electron microscopy, that synaptic weakening takes place in majority, but not all synapses during sleep.

11. Spano GM, Banningh SW, Marshall W, de Vivo L, Bellesi M, Loschky SS, Tononi G, Cirelli C: **Sleep deprivation by exposure to novel objects increases synapse density and axon-spine interface in the hippocampal CA1 region of adolescent mice**. *J Neurosci* 2019, **39**:6613-6625.

12. •• Diering GH, Nirujogi RS, Roth RH, Worley PF, Pandey A, Huganir RL: **Homer1a drives homeostatic scaling-down of excitatory synapses during sleep**. *Science* 2017, **355**:511-515
This is an interesting summary of the role of Homer1a in sleep regulation, as well as in downscaling of synapses in sleep.

13. Martin SC, Monroe SK, Diering GH: **Homer1a and mGluR1/5 signaling in homeostatic sleep drive and output**. *Yale J Biol Med* 2019, **92**:93-101.

14. Turrigiano G: **Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement**. *Annu Rev Neurosci* 2011, **34**:89-103.

15. Watson BO, Levenstein D, Greene JP, Gelinas JN, Buzsaki G: **Network homeostasis and state dynamics of neocortical sleep**. *Neuron* 2016, **90**:839-852.

16. •• Pimentel D, Donlea JM, Talbot CB, Song SM, Thurston AJF, Miesenbock G: **Operation of a homeostatic sleep switch**. *Nature* 2016, **536**:333-337
This is an important milestone in describing the role of potassium channels Shaker and Sandman and neurotransmitter dopamine in regulation of sleep homeostasis in Drosophila.

17. Donlea JM, Pimentel D, Talbot CB, Kempf A, Omoto JJ, Hartenstein V, Miesenbock G: **Recurrent circuitry for balancing sleep need and sleep**. *Neuron* 2018, **97**:378-389.e4.

18. Liu S, Liu Q, Tabuchi M, Wu MN: **Sleep drive is encoded by neural plastic changes in a dedicated circuit**. *Cell* 2016, **165**:1347-1360.

19. Xu P, Cox KH, Takahashi JS: **A hyperkinetic redox sensor drives flies to sleep**. *Trends Neurosci* 2019, **42**:514-517.

20. Cognigni P, Felsenberg J, Waddell S: **Do the right thing: neural network mechanisms of memory formation, expression and update in Drosophila**. *Curr Opin Neurobiol* 2018, **49**:51-58.

21. •• Sitaraman D, Aso Y, Jin X, Chen N, Felix M, Rubin GM, Nitabach MN: **Propagation of homeostatic sleep signals by segregated synaptic microcircuits of the drosophila mushroom body**. *Curr Biol* 2015, **25**:2915-2927
The authors describe the role of Mushroom body cells and circuits in Drosophila sleep regulation.

22. McCormick DA, Bal T: **Sleep and arousal: thalamocortical mechanisms**. *Annu Rev Neurosci* 1997, **20**:185-215.

23. Krueger JM, Majde JA, Rector DM: **Cytokines in immune function and sleep regulation**. *Handb Clin Neurol* 2011, **98**:229-240.

24. Aho V, Ollila HM, Rantanen V, Kronholm E, Surakka I, van Leeuwen WM, Lehto M, Matikainen S, Ripatti S, Harma M *et al.*: **Partial sleep restriction activates immune response-related gene expression pathways: experimental and epidemiological studies in humans**. *PLoS One* 2013, **8**:e77184.

25. Toda H, Williams JA, Gulledge M, Sehgal A: **A sleep-inducing gene, nemuri, links sleep and immune function in Drosophila**. *Science* 2019, **363**:509-515.

26. Donzis EJ, Tronson NC: **Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences**. *Neurobiol Learn Mem* 2014, **115**:68-77.

27. Santello M, Volterra A: **TNFalpha in synaptic function: switching gears**. *Trends Neurosci* 2012, **35**:638-647.

28. Miras-Portugal MT, Sebastian-Serrano A, de Diego Garcia L, Diaz-Hernandez M: **Neuronal P2X7 receptor: involvement in neuronal physiology and pathology**. *J Neurosci* 2017, **37**:7063-7072.

29. Steinmetz CC, Turrigiano GG: **Tumor necrosis factor-alpha signaling maintains the ability of cortical synapses to express synaptic scaling**. *J Neurosci* 2010, **30**:14685-14690.

30. Porkka-Heiskanen T, Strecker RE, McCarley RW: **Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study**. *Neuroscience* 2000, **99**:507-517.

31. Vanderheyden WM, Goodman AG, Taylor RH, Frank MG, Van Dongen HPA, Gerstner JR: **Astrocyte expression of the Drosophila TNF-alpha homologue, Eiger, regulates sleep in flies**. *PLoS Genet* 2018, **14**:e1007724.

32. Kalinchuk AV, McCarley RW, Stenberg D, Porkka-Heiskanen T, Basheer R: **The role of cholinergic basal forebrain neurons in adenosine-mediated homeostatic control of sleep: lessons from 192 IgG-saporin lesions**. *Neuroscience* 2008, **157**:238-253.

33. • Seidner G, Robinson JE, Wu M, Worden K, Masek P, Roberts SW, Keene AC, Joiner WJ: **Identification of neurons with a privileged role in sleep homeostasis in *Drosophila melanogaster***. *Curr Biol* 2015, **25**:2928-2938
This work suggests that the homeostatic response is site-specific and mediated by cholinergic cells, in *Drosophila melanogaster*.

34. Rattenborg NC, de la Iglesia HO, Kempenaers B, Lesku JA, Meerlo P, Scriba MF: **Sleep research goes wild: new methods and approaches to investigate the ecology, evolution and functions of sleep**. *Philos Trans R Soc Lond B Biol Sci* 2017, **372** http://dx.doi.org/10.1098/rstb.2016.0251.

35. Wigren HK, Schepens M, Matto V, Stenberg D, Porkka-Heiskanen T: **Glutamatergic stimulation of the basal forebrain elevates extracellular adenosine and increases the subsequent sleep**. *Neuroscience* 2007, **147**:811-823.