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Current Opinion in Neurobiology

Fast and slow Ca^{2+}-dependent hyperpolarization mechanisms connect membrane potential and sleep homeostasis

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Several lines of evidence indicate that the sleep-wake state of cortical neurons is regulated not only through neuronal projections from the lower brain, but also through the cortical neurons' intrinsic ability to initiate a slow firing pattern related to the slow-wave oscillation observed in electroencephalography of the sleeping brain. Theoretical modeling and experiments with genetic and pharmacological perturbation suggest that ion channels and kinases acting downstream of calcium signaling regulate the cortical-membrane potential and sleep duration. In this review, we introduce possible Ca^{2+}-dependent hyperpolarization mechanisms in cortical neurons, in which Ca^{2+} signaling associated with neuronal excitation evokes kinase cascades, and the activated kinases modify ion channels or pumps to regulate the cortical sleep/wake firing mode.

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Current Opinion in Neurobiology 2017, **44**:212–221

This review comes from a themed issue on **Neurobiology of sleep**

Edited by **Yang Dan** and **Thomas Kilduff**

http://dx.doi.org/10.1016/j.conb.2017.05.007

Introduction

Sleep is a fundamental brain state that is observed across a wide range of animal species [1,2]. Despite differences in central nervous system architecture, essential properties of sleep appear to be broadly conserved across species. Sleep is a reversible and homeostatically regulated state of reduced responsivity to environmental stimuli. Although the physiological functions of sleep have not been fully explored, memory consolidation and other potential roles of sleep appear to be highly conserved from flies to humans [3]. This functional conservation suggests that at least some of the mechanisms that regulate sleep are closely related to the fundamental properties of neurons in addition to the neural-circuit architecture specific to each animal species.

Sleep-wake behavior is apparently controlled by systems operating in a variety of time scales [4,5]. In the behavioral response, the transition from wakefulness to sleep occurs within a minute. In this sense, the system that switches the entire brain (or at least most of the cortical regions) to a sleep state may operate in a time window of seconds. Once animals fall asleep, the sleep state continues for hours in great apes (*e.g.*, humans) or for a few minutes in most animals, including rodents (*e.g.*, mice). Thus, a system designed to measure the length of a sleep episode would operate on a time scale of minutes to hours. Furthermore, sleep is homeostatically regulated on a time scale of a few days: sleep deprivation on one day increases sleep pressure over the next few days.

What kind of mechanism can control processes with such a wide range of time scales to control sleep, and be evolutionarily conserved in so many species? In this review, we discuss the role of calcium-ion (Ca^{2+}) signaling in cortical neurons as a sleep regulator. Ca^{2+} is a crucial factor in regulating neuronal properties across different time scales, from milliseconds to hours, in different types of neurons [6]. On a millisecond time scale, an ion flux of Ca^{2+} directly depolarizes the membrane potential. An influx of Ca^{2+} also triggers neurotransmitter release at the synapse [7]. Intracellular Ca^{2+} further induces several molecular cascades that modulate neuronal properties over a longer time scale (nearly an hour), such as the formation of synapses [8]. Thus, the evolutionarily conserved and temporally diverse roles of Ca^{2+} signaling may have essential functions in sleep regulation. In addition, we introduce the concept of Ca^{2+}-dependent hyperpolarization of the neuronal membrane potential during sleep, which provides further mechanistic and quantitative insights connecting sleep and Ca^{2+} signaling.

Ca^{2+}-dependent hyperpolarization of cortical-membrane potential during non-rapid eye movement (NREM) sleep

Transitions between wake and sleep states are accompanied by qualitative alterations in global neuronal firing patterns in the cortex, which appear as changes in

electroencephalogram (EEG) patterns. In the sleep state, specifically, in non-rapid eye movement (NREM) sleep, the synchronous firing of cortical neurons produces an EEG pattern with a high amplitude and a low frequency, typically 0.5–4 Hz. The firing pattern becomes non-synchronous when animals go into rapid eye movement (REM) sleep or wake up; these states are characterized by an EEG pattern with a low amplitude and a high frequency, and the macroscopic firing pattern loses synchrony as individual cortical neurons process various environmental inputs. The synchronous firing of cortical neurons during NREM sleep has been observed across vertebrates from humans to lizards [9••].

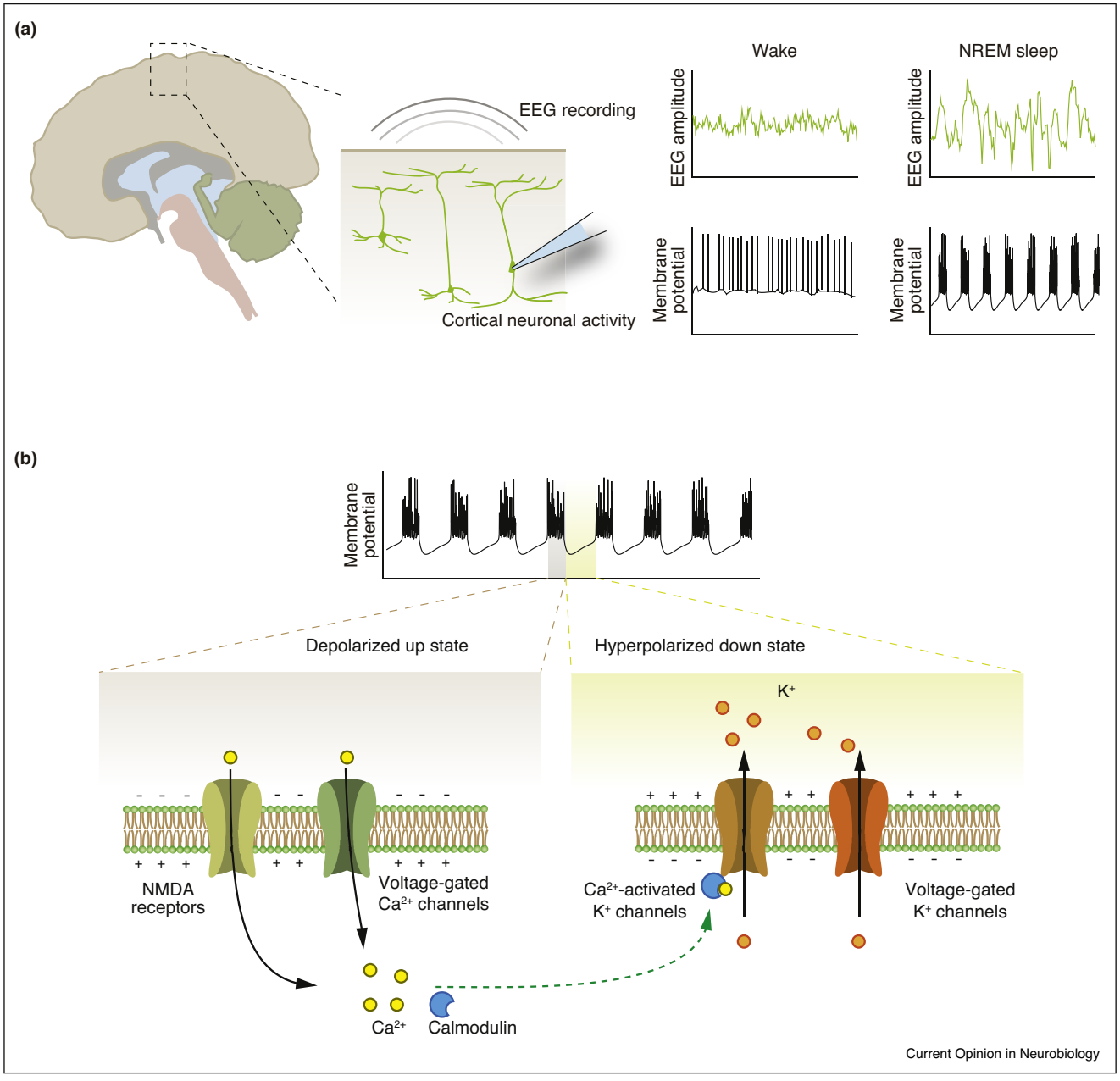
Intracellular and extracellular electrophysiological recordings have revealed that the EEG pattern during NREM sleep is composed of various types of cortical-neuron activities: thalamocortical neurons generate oscillations at 1–4 Hz, while cortical neurons generate slow (<1 Hz) oscillations [10]. Note that ascending arousal projections, rather than the thalamus, may be important for the 1–4 Hz EEG oscillations, given that lesions in the basal forebrain or parabrachial nucleus cause a loss of EEG power greater than 1 Hz, whereas a thalamic lesion only marginally affects the EEG pattern [11]. Slow-wave oscillations (less than 1 Hz) are likely to be generated by cortical-neuron activity. NREM sleep (also called slow-wave sleep) is associated with a characteristic firing pattern that appears as slow-wave oscillations. Slow-wave oscillations have two electrophysiological phases: the depolarized bursting phase, and the hyperpolarized silent phase [12,13] (Figure 1a). Importantly, the bursting phase is fragmented by insertions of the silent phase with a frequency that is well matched to the slow-wave oscillation EEG pattern (less than 1 Hz) during NREM sleep. This electrophysiological finding was recently confirmed by cell-type-specified *in vivo* membrane-potential imaging [14]. Neurons are hyperpolarized during the silent phase by hyperpolarization of the membrane potential, which is over 10 mV lower than a pyramidal neuron's typical resting potential. Several models have been proposed to explain this type of phasic firing, which has also been observed in non-cortical cells [15]. In sleep studies, researchers have used computational models of thousands of cortical neurons, called neuron-network models, to investigate the mechanism of bursting and silent phases. These models indicate that hyperpolarization occurs by cell-intrinsic regulation rather than by the intermittent input of synaptic repression [16–18]: bursting-phase firing triggers the silent phase via an activity-dependent potassium ion (K^+) current mediated by sodium ion (Na^+)-dependent K^+ channels or Ca^{2+}-dependent K^+ channels, depending on the assumption of the model. However, predictions provided by models with large numbers of neurons and parameters have limited generality: the huge parameter space prevents a comprehensive survey of parameter combinations, and hence of shared features that could give rise to slow-wave oscillation.

A recent study aiming to construct a generalized model of cortical firing, called an averaged-neuron model, supports the importance of cell-intrinsic Ca^{2+}-dependent K^+ currents [19••]. The averaged-neuron model applies a mean-field approximation to the neural-network model to generate a simple computational model. The averaged-neuron model assumes that all excitatory and inhibitory neurons are connected to each other, and the model is formed as though one neuron were connecting to itself with both excitatory and inhibitory input. Thus, this model has synaptic connections, but its output does not depend on the specific pattern of the neuron-network circuit. The averaged-neuron model produced a pattern of slow-wave oscillation with alternating bursting and silent phases in ~1000 sets of randomly generated parameters describing the conductance of ion channels/pumps. The hallmark of the slow-wave activity observed by EEG is not only the unique firing pattern, but also the synchrony of the firing pattern among cortical neurons. Because this model implicates the averaged-neuron behavior, slow-wave oscillations appearing in the model can be interpreted as synchronous bursting and resting repeats within a population of neurons connected to excitatory and inhibitory inputs. A systematic investigation of all of the obtained parameter sets for a slow-wave oscillation pattern indicated that Ca^{2+} influx upon excitation (through NMDA receptors and voltage-gated Ca^{2+} channels) and the activation of Ca^{2+}-dependent K^+ channels are the most critical parameters for inducing a hyperpolarized membrane potential during a silent phase (Figure 1b). Consistent with this view, the activation of Ca^{2+} pumps or exchangers might terminate the silent phase in this model. These theoretical models suggest that a primitive source of slow-wave oscillations in cortical neurons during NREM sleep may be the cell-intrinsic control of membrane potential, particularity though firing-induced K^+ outward currents such as the Ca^{2+}-dependent hyperpolarization mechanism.

Fast dynamics: the Ca^{2+}-dependent hyperpolarization mechanism acts through ion channels and pumps during NREM sleep

An important question is whether the mechanism of membrane-potential regulation, which is a fast, dynamic process characterized by neuron properties, plays an active role in the sleep phenotype, or whether the firing state of cortical neurons is subordinate to other regions of the brain in sleep control. There are several lines of evidence suggesting that the cortical-neuron assembly itself has an intrinsic ability to initiate the bursting and silent phases during NREM sleep (Figure 2). First, bursting and silent phases that alternate at 0.5–4 Hz can be reproduced in *ex vivo* cortical slices [18,20],

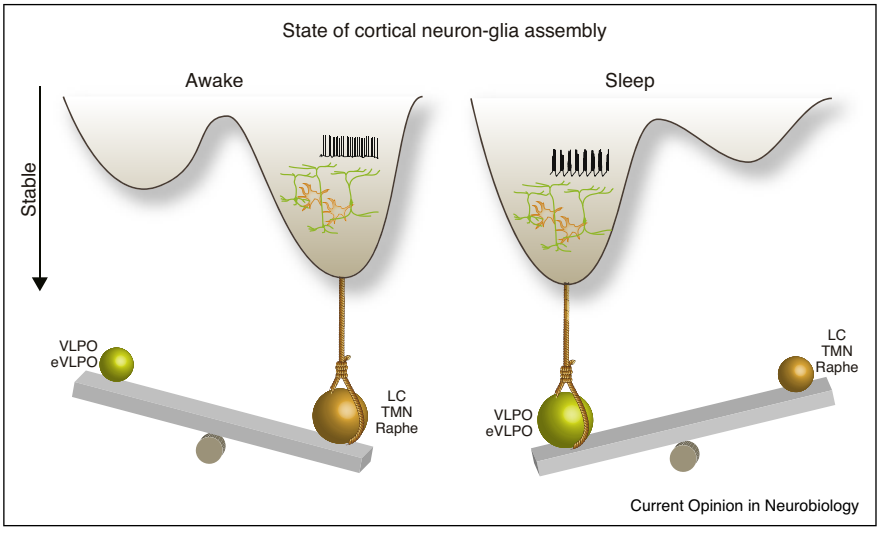
Figure 1



Regulation of cortical membrane potential during NREM sleep. **(a)** Sleep/wake states are characterized by different behaviors of EEG signals originating from the collective activity of the cortical neurons. Slow-wave EEG-signal oscillations during NREM sleep, particularly those <1 Hz, are thought to be produced by the intermittent firing of cortical neurons. **(b)** The intermittent-firing pattern is composed of alternating depolarized bursting and hyperpolarized silent phases. The silent-phase hyperpolarization may be maintained by Ca^{2+}-dependent K^+ channels activated by a Ca^{2+} influx accumulated during the preceding bursting phase.

indicating that inputs from other brain regions are not necessary for this slow-wave oscillation pattern. Second, the slow-wave oscillation pattern on EEG during NREM sleep is also observed in the local part of the cortex during REM sleep and in periods of wakefulness, and this is called local sleep [21,22]. Local sleep suggests that the state of the entire cortex during sleep is not fully defined downstream of other core sleep-regulating circuits, but that a part of the cortex can independently initiate synchronous slow-wave oscillations. The cortex-autonomous ability to alter sleep-related states may be involved in the ability of fur seals and birds to switch to

Figure 2



Intrinsic ability of cortical cells to transition between awake-firing and sleep-firing states. The cortical neuron–glia assembly is thought to have two stable states corresponding to an awake state and an NREM-sleep state with a characteristic slow-wave oscillation pattern. Wake-promoting and sleep-promoting centers of the brain may stabilize the state of cortical neurons, either in an awake or sleep state. VLPO: ventrolateral preoptic nucleus; eVLPO: extended ventrolateral preoptic nucleus; LC: locus coeruleus; TMN: tuberomammillary nucleus.

bilateral-hemispheric sleep on land and unihemispheric sleep when flying or swimming [23,24]. Based on this view of sleep, researchers have attempted to reconstruct sleep in cultured neurons and glial cells *in vitro* [25,26]. Notably, Jewett *et al.* recapitulated the homeostatic regulation of a sleep-like state *in vitro*: electrical stimulation to induce a wake-like firing state in cultured neurons was followed by an increase in the sleep-like firing state on the next day of stimulation, suggesting that the cultured neuron itself retains a homeostatic property that allows it to maintain constant excitability over a day. Taken together, the cortex-intrinsic change in state may provide a modified version of a flip-flop model: regions of the brain, including the ventrolateral preoptic nucleus (VLPO) and other sleep/wake centers, induce a bias in the firing state of the cortex, which itself can autonomically switch between states of sleep and wakefulness (Figure 2).

If slow-wave oscillations in the cortex are produced autonomously in the cortex, the next question is whether such an electrophysiological pattern itself actively regulates sleep duration in the entire organism. Massimini *et al.* showed that using 5-Hz transcranial magnetic stimulation of the local cortex to mimic the EEG pattern of NREM sleep deepened slow-wave EEG activity in a sleeping human subject [27]. Thus, the state of the local cortical neurons during sleep may actively control the animals' sleep. Several genetic studies support an active role for the cortical firing state; consistent with the computational prediction, researchers successfully produced mice with longer or shorter sleep durations by manipulating the Ca^{2+}-dependent hyperpolarization pathway, which is important for inducing a hyperpolarized silent phase. The pharmacological inactivation or CRISPR/Cas9-mediated knockout of ion channels to block Ca^{2+} entry (*i.e.*, NMDA receptors and voltage-gated Ca^{2+} channels), along with the downstream Ca^{2+}-dependent K^+ channels, shortens the daily sleep duration in mice [19••,28•]. Given the critical roles of Ca^{2+} in general neural function, impairing Ca^{2+}-dependent hyperpolarization pathways might produce a non-specific phenotype (*e.g.*, non-specific neural damage). However, knocking out plasma-membrane Ca^{2+} ATPase (PMCA) genes to inhibit Ca^{2+} efflux lengthens sleep duration. Therefore, the opposing sleep-duration phenotypes induced by inhibiting the Ca^{2+}-dependent hyperpolarization pathway or the Ca^{2+}-efflux pathway strongly suggest that these effects are not explained by a simple disruption of the physiological controls.

The importance of cortical ion flux was further highlighted by a study that focused on the extracellular ion environment. Ding *et al.* found that natural sleep is associated with a higher extracellular Ca^{2+} and magnesium ion (Mg^{2+}) concentration and a lower extracellular K^+ concentration [29••]. This concentration bias would support an influx of Ca^{2+} and efflux of K^+. Considering that astrocytes regulate the homeostasis of the extracellular ion environment, the importance of extracellular ion conditions implies the active involvement of not only neurons, but also glial cells for inducing the sleep state.

Indeed, other studies have investigated how adenosine signaling through gliotransmission affects cortical slow-wave oscillations [30] and promotes rebound sleep after sleep deprivation [31]. Moreover, optogenetically stimulating astrocytes induces synchronized slow-wave oscillation in cortical neurons *in vivo* [32•]. Thus, neuron–glia interactions would act cooperatively to modulate slow-wave oscillation patterns.

The cell-intrinsic properties that elicit slow-wave oscillation might be synchronized and organized through the neural-network architecture. Studies using optogenetics and designer receptors exclusively activated by designer-drug (DREADD) perturbation revealed a causal relationship between the sleep-wake transition and the activity of a subset of neurons. The core neuron subsets for sleep regulation are in the basal forebrain for NREM control [33] and in the pons/midbrain for REM control [34,35]. These dedicated neural circuits coordinate the state of the brain such that the state of the entire organism switches between sleep and wake states [36], and loss of this coordination at the whole-brain level may lead to the abnormal EEG patterns associated with psychiatric diseases [37,38].

Slow dynamics: the homeostatic regulation of sleep/wake cycles through Ca^{2+}-dependent phosphorylation cascades

The close relationship between membrane-potential control and sleep-wake dynamics presents a mystery as to the mechanism that coordinates the differences in time scales between ion-flux regulation, which occurs in the subsecond order, and the regulation of sleep homeostasis, which occurs over a period of hours. It is difficult to account for the slow sleep dynamics based only on ion-flux dynamics. A common biological architecture to provide homeostatic control over longer time scales is the use of signaling cascades. Interestingly, the Ca^{2+}-dependent hyperpolarization hypothesis of sleep control proposes that higher hyperpolarization activity (sleep state) can be triggered via Ca^{2+} influx by activating a Ca^{2+}-dependent pathway such as calcium/calmodulin-dependent kinase II (CaMKII) α/β [19••], which is important in synaptic learning processes [39]. Knocking out CaMKIIα/β shortens sleep duration, suggesting that CaMKIIα/β play a role in inducing sleep. The Ca^{2+}-dependent hyperpolarization hypothesis proposes that active neuron firing during an awake state causes an influx of Ca^{2+}, and a subsequent influx of CaMKIIα/β activates a Ca^{2+}-dependent hyperpolarization mechanism to induce NREM sleep [19••].

Compared with the duration of a single episode of wake or sleep, the dynamics of CaMKIIα/β activation itself upon neuronal excitation are relatively fast—within a minute, at least at synapses [40]. This time gap may be complemented by a kinase cascade downstream of CaMKIIα/β. Several studies on long-term potentiation (LTP) indicate that CaMKII further triggers the downstream mitogen-activated protein kinases (MAPKs) ERK1/2, the activity of which is sustained for over an hour [41,42]. Interestingly, a recent study demonstrated that knocking out ERK2 specifically in cortical neurons reduces the NREM-sleep duration, and that the level of phosphorylated (active) MAPK differs between NREM and wake states [43], although other studies indicate that MAPK activation is associated with REM rather than NREM-sleep [44,45]. Ca^{2+} also potentiates PKC activity, which may increase with sleep deprivation [46]. The activities of MAPK and PKC are altered by sleep-modulating substances (D_2 agonist, melatonin, and pentobarbital) [47]. These results suggest that the sleep-wake cycle affects the activity of kinases downstream of Ca^{2+} signaling.

Another candidate kinase cascade downstream of CaMKIIα/β was recently identified by a forward genetic screening of sleep-controlling genes in mice [48••]. NREM duration was significantly longer in mice with a *sleepy* mutation, which involves exon skipping across the SIK3 kinase gene, with the loss of several amino acids including a PKA-phosphorylation site. This phosphorylation inhibits SIK3 activity, suggesting that the *sleepy* SIK3 mutant is a gain-of-function mutant. Sleep deprivation did not appear to affect the level of PKA-responsible phosphorylation in SIK3. On the other hand, sleep deprivation increased the SIK3-activating phosphorylation level; thus, kinases other than PKA may be involved in the sleep deprivation-dependent phosphorylation of SIK3.

The role of PKA in regulating sleep/wake cycles appears to be the opposite of that of CaMKIIα/β, SIK3, and ERK1/2. For example, sleep deprivation reduces cAMP-PKA signaling in the hippocampus, thereby impairing memory consolidation [49–51]. Partially suppressing PKA activity increased fragmentation and EEG delta power during NREM sleep [52]. Since the downstream pathway of PKA in sleep regulation is still unknown, it will be interesting to investigate the molecular interactions between sleep-promoting kinases (*e.g.*, CaMKIIα/β, SIK3, and ERK1/2) and the awake-promoting kinase PKA in the context of sleep/wake cycles.

Connecting fast and slow dynamics: Ca^{2+}-dependent phosphorylation cascades homeostatically regulate the Ca^{2+}-dependent hyperpolarization mechanism

Genetic studies have identified genes that regulate sleep duration, but the molecular pathways that connect these genes are unknown. Sleep and sleep deprivation can affect numerous molecules such as ion channels, pumps, and kinases both directly and indirectly. It is not enough to measure protein activity upon sleep deprivation (or the natural sleep-wake cycle) or to quantify sleep phenotypes

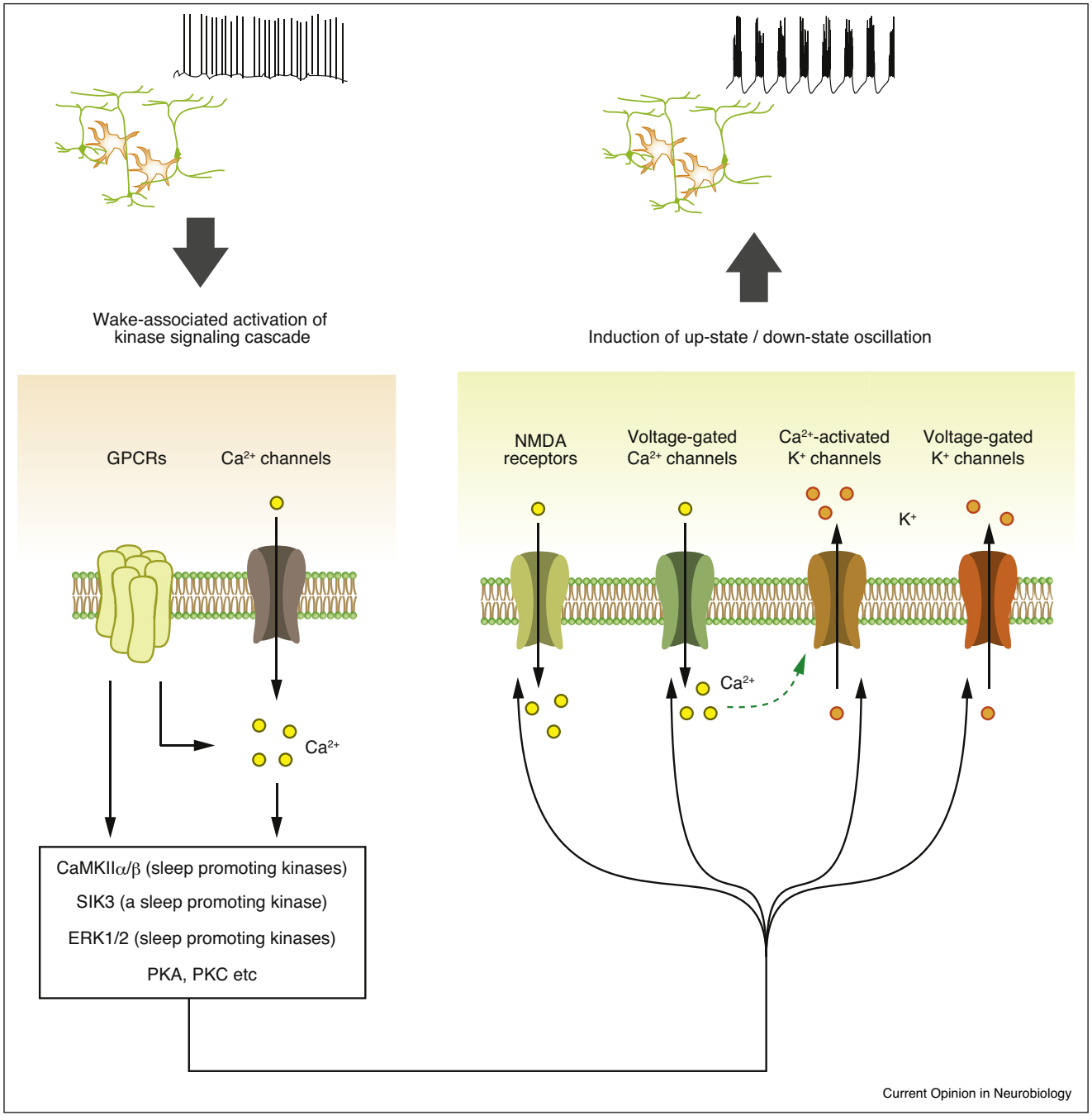
in animals with an impaired or enhanced activity of gene products: to understand the molecular network of sleep regulation, we need to illustrate a path from neuronal activity during the wake state to sleep-regulating factors. As a perspective for future studies, we here discuss a possible signaling cascade among sleep-regulating factors. Notably, the local power of the slow-wave oscillation in the cortex appears to respond to the intensity of synaptic transmissions processed during the wake state in the same local area [53–55], indicating that some part of the mechanism of sleep homeostasis is completed within the local cortical area or even within the individual neurons.

Slow-wave oscillation is typically observed in NREM sleep, which suggests that the ion channels involved in this firing pattern act differently during the NREM-sleep and wake states. Otherwise, an influx of Ca^{2+} upon neuronal firing during the awake state would suddenly induce Ca^{2+}-dependent hyperpolarization, and the neuron would not able to maintain an active awake state. Thus, it may be reasonable to assume that during NREM sleep, (1) Ca^{2+} channels are more permeable to Ca^{2+}, (2) Ca^{2+}-dependent K^+ channels become more sensitive and active in response to Ca^{2+} influx, and/or (3) Ca^{2+} pumps are less permeable to Ca^{2+}. If the state of ion channels and pumps is modified by highly excitable neuronal firing, then this activity-dependent modification would close the homeostatic loop: prolonged wakefulness promotes sleep. The question, then, is how the channels/pumps involved are regulated. The mechanism that switches cortical neurons between the awake and sleep states may involve the phosphorylation of ion channels or pumps. Although several studies have investigated phospho-proteomic changes in the sleep and awake states [56], a critical kinase substrate for homeostatic sleep regulation has yet to be identified. One simple hypothesis is that sleep-regulating kinases directly phosphorylate sleep-regulating ion channels or pumps. To homeostatically induce sleep, kinases that are activated downstream of increased excitability (*e.g.*, accumulated Ca^{2+} signals) may phosphorylate ion channels or pumps to accelerate Ca^{2+}-dependent hyperpolarization (Figure 3). Thus, the Ca^{2+}-dependent phosphorylation cascade may form a slow Ca^{2+}-dependent hyperpolarization mechanism. For example, the voltage-gated T-type Ca^{2+} channel CACNA1H ($Ca_v3.2$) is activated when phosphorylated by CaMKII [57]. Considering that both molecules promote sleep, based on the phenotypes of knockout mice, and that CaMKII is activated by synaptic input that is generally higher during the awake state, CaMKII might activate CACNA1H to promote wake-driven sleep. Identifying novel phosphorylation sites will provide potential links between sleep-regulating kinases and substrates. Advances in mass spectrometry allow us to identify phosphorylation and other modifications comprehensively even in complex membrane proteins. Blesneac *et al.* took this approach with CACNA1H [58] and identified tens of phosphorylation sites *in vivo*, some of which shifted the window current of this channel to make it more sensitive to membrane-potential depolarization. As the amount of molecular information increases, the next challenge is to evaluate which molecular pathway is responsible for regulating sleep duration *in vivo*.

The important feature of phosphorylation-dependent protein regulation is its reversibility. Thus, the importance of kinases in sleep regulation implies that their reverse enzymes, phosphatases, are also critical for regulation. Currently, no potent sleep-related phosphatases have been discovered in mammals; however, notably, phosphorylation can also be reversibly regulated by proteolysis-synthesis protein turnover. In other words, the degradation of old phosphorylated proteins and the synthesis of new proteins can supply unphosphorylated proteins with an effect similar to the dephosphorylation of phosphorylated proteins. Indeed, the transcription factor CREB, which is regulated by PKA, is involved in regulating sleep duration. Reducing CREB expression sharply increases the sleep time in mice [59], and the CREB, PKA, and MAPK activities change during the sleep-wake cycle [44]. In flies, PKA–CREB activity is important for sleep particularly in the mushroom body, a part of the brain that is critical for regulating sleep-wake behavior in the fly. As in mammals, blocking the CREB activity increases sleep in flies, [60] while PKA overexpression inhibits sleep [61]. These results suggest that elevated cAMP signaling modulates CREB activity to reduce the amount of sleep. Alternatively, the protein degradation pathway is also important for controlling sleep: a genetic study in flies identified the short-sleep *insomniac* gene, which encodes a protein regulating the Cullin-3 ubiquitin ligase complex [62]. We also note that ion-channel regulation at the transcription or translation level has been implicated as an underlying mechanism for periodic changes in membrane excitability during day/night cycles in mice and flies [63–65], and that some parts of the sleep/wake cycle may involve periodic regulation at the transcription or translation level.

Sleep duration and structure depends markedly on the developmental stage [66], and the Ca^{2+}-dependent hyperpolarization pathway may be involved in sleep regulation over the animal's life span. The NMDA receptor member *Nr3a* is known for its age-dependent expression in rodents, which peaks during a critical period of experience-dependent synaptic plasticity [67,68]. The *Nr3a* expression is low in adult rodents and humans. Notably, knocking out the *Nr3a* gene shortens sleep, but knocking out the other non-lethal NMDA receptor genes does not (*Nr2a*, *Nr2c*, *Nr2d*, and *Nr3a*) [28•]. *Nr3a*'s unique sleep-promoting role may be part of an underlying mechanism to ensure a higher amount of sleep during childhood and a lower amount after adolescence.

Figure 3



Possible relationship between sleep-regulating kinases, ion channels and pumps, and the neuronal firing-dependent control of membrane potential. Several kinases are activated through Ca^{2+} influx and other awake-associated signals. Kinases are *slow-regulation* factors in the sleep-wake cycle. The activated kinases modify ion channels involved in the slow-wave oscillation pattern, and the ion channels act as *fast-regulation* factors. This connection between slow and fast regulation factors provides a plausible mechanism for the homeostatic control of wake-induced sleepiness. GPCRs: G protein-coupled receptors.

Similarly, the expression of *Cacna1g* decreases in aged humans [69]. Knocking out *Cacna1g* shortens sleep duration; thus, decreased *Cacna1g* expression may decrease nighttime sleep duration in the elderly.

Evolutionary conservation of cell-intrinsic sleep regulation

Several studies in flies show that ion-channel activities are modulated during the sleep-wake cycle. One study

showed that a reduction of voltage-gated K^+ current (through *Shaker* and *Slab*) and induction of leak K^+ current (through *Sandman*) form the underlying mechanism for the dopamine-mediated hyperpolarization of the dFB, a sleep-promoting region of the brain in flies [70]. This study also connected the roles of the classical sleep-related mutants found in *Fmn* [71] and *Shaker* flies [72], and demonstrated that the Shaker molecule itself is dynamically regulated by the sleep-wake cycle. Another study identified a set of neurons responsible for sleep deprivation and subsequent rebound sleep in flies [73••]; interestingly, sleep deprivation results in an increase in Ca^{2+} levels and NMDA receptors in this neuron set. The study further demonstrated that Ca^{2+} is released from ER storage through the IP3 receptor in response to sleep deprivation. In a broad sense, mouse genetics focusing on cortical excitability overlap with fly genetics in identifying key players: intracellular Ca^{2+} signal, altered K^+ current, and kinases/phosphatases potentially regulate channel properties [60,61,74–76]. Although studies in flies suggest that these ion channels operate mostly in a defined set of sleep-regulating neurons, we would nonetheless point out that even in flies, the excitability of individual neurons in response to stimuli is reduced depending on the preceding awake period [77•]. This finding is consistent with local sleep in mammals; thus, neuron-intrinsic homeostasis may also be valid in flies. Another fly study showed that blocking the NMDA receptor decreases sleep, as was also observed in mammals, and that pan-neurons (rather than a specific set of neurons) appear to contribute to this effect [78••]. In addition, the Ca^{2+}-dependent phosphatase calcineurin promotes sleep in the fruit fly [75,76]. The NMDA receptor and Ca^{2+}-dependent phosphatase might act cooperatively rather than in opposition to induce sleep in fruit flies.

Conclusion

The genetic identification of sleep-regulating genes has provided a list of ion channels/pumps and kinases that may function pan-neuronally. Connecting the enzyme-substrate networks among these molecules may reveal a cellular cascade that is conserved across neuron subtypes and across phyla, in which case the molecular pathway should be understood in the context of neural networks. Revisiting the homeostatic action of mammalian sleep, sleep deprivation increases the power of slow-wave activity observed on EEG. This effect might be due to changes in the intraneuronal molecular properties of individual cortical neurons and to functional changes in the neural circuits that synchronize the cortical neuron state; it is highly possible that both mechanisms are important for brain function. To understand the mechanism of sleep control in a complex neural network, we believe that it is necessary to understand the properties of each neuron during the sleep-wake cycle, because the system behavior emerges from the properties of both the architecture and the components.

Conflict of interest statement

The authors have no conflict of interest regarding on this manuscript.

Acknowledgements

This work was supported by AMED-CREST, CREST, Brain/MINDS, the Basic Science and Platform Technology Program for Innovative Biological Medicine, JSPS (25221004, 23115006, 25830146, 16K14653), RIKEN, and the Takeda Science Foundation.

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. Siegel JM: **Sleep viewed as a state of adaptive inactivity**. *Nat. Rev. Neurosci.* 2009, **10**:747-753.

2. Siegel JM: **Do all animals sleep?** *Trends Neurosci.* 2008, **31**:208-213.

3. Krueger JM, Frank MG, Wisor JP, Roy S: **Sleep function: toward elucidating an enigma**. *Sleep Med. Rev.* 2016, **28**:46-54.

4. Olbrich E, Claussen JC, Achermann P: **The multiple time scales of sleep dynamics as a challenge for modelling the sleeping brain**. *Philos. Trans. A Math. Phys. Eng. Sci.* 2011, **369**:3884-3901.

5. Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J: **Sleep as a fundamental property of neuronal assemblies**. *Nat. Rev. Neurosci.* 2008, **9**:910-919.

6. Berridge MJ: **Neuronal calcium signaling**. *Neuron* 1998, **21**:13-26.

7. Greengard P: **The neurobiology of slow synaptic transmission**. *Science* 2001, **294**:1024-1030.

8. Hopf FW, Waters J, Mehta S, Smith SJ: **Stability and plasticity of developing synapses in hippocampal neuronal cultures**. *J. Neurosci.* 2002, **22**:775-781.

9. •• Shein-Idelson M, Ondracek JM, Liaw HP, Reiter S, Laurent G: **Slow waves, sharp waves, ripples, and REM in sleeping dragons**. *Science* 2016, **352**:590-595.
The authors recorded local field potential (LFP) in the dorsal forebrain area of reptiles, and monitored eye movement and other behavior. They found that reptiles showed two alternating types of sleep—with and without slow oscillation—in the LFP. Sleep without slow oscillation was accompanied by eye movement. Thus, the electrophysiological features of NREM and REM sleep are conserved across homeothermal and poikilothermal vertebrates.

10. Amzica F, Steriade M: **Electrophysiological correlates of sleep delta waves**. *Electroencephalogr. Clin. Neurophysiol.* 1998, **107**:69-83.

11. Fuller PM, Sherman D, Pedersen NP, Saper CB, Lu J: **Reassessment of the structural basis of the ascending arousal system**. *J. Comp. Neurol.* 2011, **519**:933-956.

12. Steriade M, McCormick DA, Sejnowski TJ: **Thalamocortical oscillations in the sleeping and aroused brain**. *Science* 1993, **262**:679-685.

13. Steriade M, Timofeev I, Grenier F: **Natural waking and sleep states: a view from inside neocortical neurons**. *J. Neurophysiol.* 2001, **85**:1969-1985.

14. Marshall JD, Li JZ, Zhang Y, Gong Y, St-Pierre F, Lin MZ, Schnitzer MJ: **Cell-type-specific optical recording of membrane voltage dynamics in freely moving mice**. *Cell* 2016, **167**:1650-1662 e1615.

15. Izhikevich EM: *Dynamical Systems in Neuroscience: The Geometory of Excitability and Bursting*. Cambridge, Massachusetts, USA: The MIT Press Location; 2007.

16. Compte A, Sanchez-Vives MV, McCormick DA, Wang XJ: **Cellular and network mechanisms of slow oscillatory activity (<1 Hz) and wave propagations in a cortical network model**. *J. Neurophysiol.* 2003, **89**:2707-2725.

17. Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ: **Model of thalamocortical slow-wave sleep oscillations and transitions to activated States**. *J. Neurosci.* 2002, **22**:8691-8704.

18. Sanchez-Vives MV, Mattia M, Compte A, Perez-Zabalza M, Winograd M, Descalzo VF, Reig R: **Inhibitory modulation of cortical up states**. *J. Neurophysiol.* 2010, **104**:1314-1324.

19. •• Tatsuki F, Sunagawa GA, Shi S, Susaki EA, Yukinaga H, Perrin D, Sumiyama K, Ukai-Tadenuma M, Fujishima H, Ohno R *et al.*: **Involvement of Ca^{2+}-dependent hyperpolarization in sleep duration in mammals**. *Neuron* 2016, **90**:70-85.

Based on a computational model of cortical-neuron spiking, a study by Tatsuki*et al.* proposed an NREM-sleep-promoting role for channels and pumps in the Ca^{2+}-dependent hyperpolarization pathway in cortical neurons. The authors generated 26 lines of knockout mice by an improved CRISPR method and characterized the sleep phenotypes of these lines by a respiration-based method. Their results confirmed the predictions of the Ca^{2+}-dependent hyperpolarization hypothesis, listed in this and another study [28], in which an NMDA receptor (*Nr3a*), Ca^{2+}-dependent K^+ channels (*Kcnn2* and *Kcnn3*), and voltage-gated Ca^{2+} channels (*Cacna1g* and *Cacna1h*) promote sleep, whereas Ca^{2+} ATPase (*Atp2b3*) suppresses sleep. Administering MK-801, an NMDA-receptor inhibitor (*Nr1*/*Nr2b*), also decreased sleep and increased cortical excitability. Tatsuki *et al.* also identified the first sleep-promoting kinases (CaMKIIα/β) in mammals and proposed that mammalian sleep/wake cycles are homeostatically regulated by a Ca^{2+}-dependent hyperpolarization pathway via Ca^{2+}-dependent phosphorylation cascades.

20. Sanchez-Vives MV, McCormick DA: **Cellular and network mechanisms of rhythmic recurrent activity in neocortex**. *Nat. Neurosci.* 2000, **3**:1027-1034.

21. Funk CM, Honjoh S, Rodriguez AV, Cirelli C, Tononi G: **Local slow waves in superficial layers of primary cortical areas during REM sleep**. *Curr. Biol.* 2016, **26**:396-403.

22. Vyazovskiy VV, Olcese U, Hanlon EC, Nir Y, Cirelli C, Tononi G: **Local sleep in awake rats**. *Nature* 2011, **472**:443-447.

23. Lyamin OI, Kosenko PO, Lapierre JL, Mukhametov LM, Siegel JM: **Fur seals display a strong drive for bilateral slow-wave sleep while on land**. *J. Neurosci.* 2008, **28**:12614-12621.

24. Rattenborg NC, Voirin B, Cruz SM, Tisdale R, Dell'Omo G, Lipp HP, Wikelski M, Vyssotski AL: **Evidence that birds sleep in mid-flight**. *Nat. Commun.* 2016, **7**:12468.

25. Jewett KA, Taishi P, Sengupta P, Roy S, Davis CJ, Krueger JM: **Tumor necrosis factor enhances the sleep-like state and electrical stimulation induces a wake-like state in co-cultures of neurons and glia**. *Eur. J. Neurosci.* 2015, **42**:2078-2090.

26. Hinard V, Mikhail C, Pradervand S, Curie T, Houtkooper RH, Auwerx J, Franken P, Tafti M: **Key electrophysiological, molecular, and metabolic signatures of sleep and wakefulness revealed in primary cortical cultures**. *J. Neurosci.* 2012, **32**:12506-12517.

27. Massimini M, Ferrarelli F, Esser SK, Riedner BA, Huber R, Murphy M, Peterson MJ, Tononi G: **Triggering sleep slow waves by transcranial magnetic stimulation**. *Proc. Natl. Acad. Sci. U. S. A.* 2007, **104**:8496-8501.

28. • Sunagawa GA, Sumiyama K, Ukai-Tadenuma M, Perrin D, Fujishima H, Ukai H, Nishimura O, Shi S, Ohno R, Narumi R *et al.*: **Mammalian reverse genetics without crossing reveals *Nr3a* as a short-sleeper gene**. *Cell Rep.* 2016, **14**:662-677.

See the annotation on Reference [19].

29. •• Ding F, O'Donnell J, Xu Q, Kang N, Goldman N, Nedergaard M: **Changes in the composition of brain interstitial ions control the sleep-wake cycle**. *Science* 2016, **352**:550-555.

This study revealed that the increase in extracellular K^+ during awake periods is independent of the AMPA signal. This K^+ increase was recapitulated in a brain-slice culture in which a cocktail of neurotransmitters increased extracellular K^+ even in the presence of tetrodotoxin. On the other hand, extracellular Ca^{2+} and Mg^{2+} levels decreased during periods of wakefulness. This causal relationship was further confirmed by the demonstration that an infusion of artificial cerebrospinal fluid (ACSF), which mimicks ionic sleep conditions promotes sleep, and wake-mimetic ACSF promotes wakefulness both for the local brain region and in the arousal state of the entire animal.

30. Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, Moss SJ, Haydon PG: **Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo**. *Proc. Natl. Acad. Sci. U. S. A.* 2009, **106**:15037-15042.

31. Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG: **Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss**. *Neuron* 2009, **61**:213-219.

32. • Poskanzer KE, Yuste R: **Astrocytes regulate cortical state switching in vivo**. *Proc. Natl. Acad. Sci. U. S. A.* 2016, **113**:E2675-E2684.

This study used in vivo Ca^{2+} imaging to show that the intracellular Ca^{2+} level of astrocytes increases prior to the start of the slow-oscillation firing of neuronal cells in the same region. This slow-oscillation neuronal state could be triggered by inducing astrocytic Ca^{2+} signaling via selective Arch expression in astrocytes. These results indicated that astrocyte activity shifts the neuron into a sleep-related firing state.

33. Xu M, Chung S, Zhang S, Zhong P, Ma C, Chang WC, Weissbourd B, Sakai N, Luo L, Nishino S *et al.*: **Basal forebrain circuit for sleep-wake control**. *Nat. Neurosci.* 2015, **18**:1641-1647.

34. Weber F, Chung S, Beier KT, Xu M, Luo L, Dan Y: **Control of REM sleep by ventral medulla GABAergic neurons**. *Nature* 2015, **526**:435-438.

35. Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S: **Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice**. *Science* 2015, **350**:957-961.

36. Weber F, Dan Y: **Circuit-based interrogation of sleep control**. *Nature* 2016, **538**:51-59.

37. Hughes JR, John ER: **Conventional and quantitative electroencephalography in psychiatry**. *J. Neuropsychiatry Clin. Neurosci.* 1999, **11**:190-208.

38. Huys QJ, Maia TV, Frank MJ: **Computational psychiatry as a bridge from neuroscience to clinical applications**. *Nat. Neurosci.* 2016, **19**:404-413.

39. Lisman J, Yasuda R, Raghavachari S: **Mechanisms of CaMKII action in long-term potentiation**. *Nat. Rev. Neurosci.* 2012, **13**:169-182.

40. Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R: **Activation of CaMKII in single dendritic spines during long-term potentiation**. *Nature* 2009, **458**:299-304.

41. Thomas GM, Huganir RL: **MAPK cascade signalling and synaptic plasticity**. *Nat. Rev. Neurosci.* 2004, **5**:173-183.

42. El Gaamouch F, Buisson A, Moustie O, Lemieux M, Labrecque S, Bontempi B, De Koninck P, Nicole O: **Interaction between alphaCaMKII and GluN2B controls ERK-dependent plasticity**. *J. Neurosci.* 2012, **32**:10767-10779.

43. Mikhail C, Vaucher A, Jimenez S, Tafti M: **ERK signaling pathway regulates sleep duration through activity-induced gene expression during wakefulness**. *Sci. Signal.* 2017, **10**.

44. Luo J, Phan TX, Yang Y, Garelick MG, Storm DR: **Increases in cAMP, MAPK activity, and CREB phosphorylation during REM sleep: implications for REM sleep and memory consolidation**. *J. Neurosci.* 2013, **33**:6460-6468.

45. Dumoulin Bridi MC, Aton SJ, Seibt J, Renouard L, Coleman T, Frank MG: **Rapid eye movement sleep promotes cortical plasticity in the developing brain**. *Sci. Adv.* 2015, **1**:e1500105.

46. Abrial E, Betourne A, Etievant A, Lucas G, Scarna H, Lambas-Senas L, Haddjeri N: **Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania**. *Int. J. Neuropsychopharmacol.* 2014, **18**.

47. Hong SI, Kwon SH, Hwang JY, Ma SX, Seo JY, Ko YH, Kim HC, Lee SY, Jang CG: **Quinpirole increases melatonin-augmented pentobarbital sleep via cortical ERK, p38 MAPK, and PKC in mice**. *Biomol. Ther. (Seoul)* 2016, **24**:115-122.

48. •• Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, Ma J, Nakane S, Tomita J, Ikkyu A *et al.*: **Forward-genetics analysis of sleep in randomly mutagenized mice**. *Nature* 2016, **539**:378-383.
Forward genetics screening in mice identified sleep-regulating genes. SIK3 kinase promotes NREM sleep, and the voltage-independent, non-selective cation channel NALCN inhibits REM sleep. The phosphorylation level at a specific site (T221) of SIK3 increased in response to sleep deprivation, although the upstream kinase is unknown.

49. Vecsey CG, Baillie GS, Jaganath D, Havekes R, Daniels A, Wimmer M, Huang T, Brown KM, Li XY, Descalzi G *et al.*: **Sleep deprivation impairs cAMP signalling in the hippocampus**. *Nature* 2009, **461**:1122-1125.

50. Havekes R, Bruinenberg VM, Tudor JC, Ferri SL, Baumann A, Meerlo P, Abel T: **Transiently increasing cAMP levels selectively in hippocampal excitatory neurons during sleep deprivation prevents memory deficits caused by sleep loss**. *J. Neurosci.* 2014, **34**:15715-15721.

51. Havekes R, Park AJ, Tudor JC, Luczak VG, Hansen RT, Ferri SL, Bruinenberg VM, Poplawski SG, Day JP, Aton SJ *et al.*: **Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1**. *eLife* 2016, **5**.

52. Hellman K, Hernandez P, Park A, Abel T: **Genetic evidence for a role for protein kinase A in the maintenance of sleep and thalamocortical oscillations**. *Sleep* 2010, **33**:19-28.

53. Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G: **Arm immobilization causes cortical plastic changes and locally decreases sleep slow-wave activity**. *Nat. Neurosci.* 2006, **9**:1169-1176.

54. Huber R, Esser SK, Ferrarelli F, Massimini M, Peterson MJ, Tononi G: **TMS-induced cortical potentiation during wakefulness locally increases slow-wave activity during sleep**. *PLoS One* 2007, **2**:e276.

55. Huber R, Ghilardi MF, Massimini M, Tononi G: **Local sleep and learning**. *Nature* 2004, **430**:78-81.

56. Suzuki A, Sinton CM, Greene RW, Yanagisawa M: **Behavioral and biochemical dissociation of arousal and homeostatic sleep need influenced by prior wakeful experience in mice**. *Proc. Natl. Acad. Sci. U. S. A.* 2013, **110**:10288-10293.

57. Iftinca MC, Zamponi GW: **Regulation of neuronal T-type calcium channels**. *Trends Pharmacol. Sci.* 2009, **30**:32-40.

58. Blesneac I, Chemin J, Bidaud I, Huc-Brandt S, Vandermoere F, Lory P: **Phosphorylation of the Cav3.2 T-type calcium channel directly regulates its gating properties**. *Proc. Natl. Acad. Sci. U. S. A.* 2015, **112**:13705-13710.

59. Graves LA, Hellman K, Veasey S, Blendy JA, Pack AI, Abel T: **Genetic evidence for a role of CREB in sustained cortical arousal**. *J. Neurophysiol.* 2003, **90**:1152-1159.

60. Hendricks JC, Williams JA, Panckeri K, Kirk D, Tello M, Yin JC, Sehgal A: **A non-circadian role for cAMP signaling and CREB activity in *Drosophila* rest homeostasis**. *Nat. Neurosci.* 2001, **4**:1108-1115.

61. Joiner WJ, Crocker A, White BH, Sehgal A: **Sleep in *Drosophila* is regulated by adult mushroom bodies**. *Nature* 2006, **441**:757-760.

62. Stavropoulos N, Young MW: **Insomniac and Cullin-3 regulate sleep and wakefulness in *Drosophila***. *Neuron* 2011, **72**:964-976.

63. Flourakis M, Kula-Eversole E, Hutchison AL, Han TH, Aranda K, Moose DL, White KP, Dinner AR, Lear BC, Ren D *et al.*: **A conserved bicycle model for circadian clock control of membrane excitability**. *Cell* 2015, **162**:836-848.

64. Belle MD, Diekman CO, Forger DB, Piggins HD: **Daily electrical silencing in the mammalian circadian clock**. *Science* 2009, **326**:281-284.

65. Whitt JP, Montgomery JR, Meredith AL: **BK channel inactivation gates daytime excitability in the circadian clock**. *Nat. Commun.* 2016, **7**:10837.

66. Roffwarg HP, Muzio JN, Dement WC: **Ontogenetic development of the human sleep-dream cycle**. *Science* 1966, **152**:604-619.

67. Perez-Otano I, Larsen RS, Wesseling JF: **Emerging roles of GluN3-containing NMDA receptors in the CNS**. *Nat. Rev. Neurosci.* 2016, **17**:623-635.

68. Wong HK, Liu XB, Matos MF, Chan SF, Perez-Otano I, Boysen M, Cui J, Nakanishi N, Trimmer JS, Jones EG *et al.*: **Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain**. *J. Comp. Neurol.* 2002, **450**:303-317.

69. Rice RA, Berchtold NC, Cotman CW, Green KN: **Age-related downregulation of the CaV3.1 T-type calcium channel as a mediator of amyloid beta production**. *Neurobiol. Aging* 2014, **35**:1002-1011.

70. Pimentel D, Donlea JM, Talbot CB, Song SM, Thurston AJ, Miesenbock G: **Operation of a homeostatic sleep switch**. *Nature* 2016, **536**:333-337.

71. Kume K, Kume S, Park SK, Hirsh J, Jackson FR: **Dopamine is a regulator of arousal in the fruit fly**. *J. Neurosci.* 2005, **25**:7377-7384.

72. Cirelli C, Bushey D, Hill S, Huber R, Kreber R, Ganetzky B, Tononi G: **Reduced sleep in *Drosophila* Shaker mutants**. *Nature* 2005, **434**:1087-1092.

73. •• 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.
Drosophila lines expressing dTrp1 under various GAL4 drivers were screened to identify a neuron subset (called R2 neurons). Inactivating R2 neurons caused the flies to become more resilient to sleep deprivation without changing the basal level of sleep duration. Sleep deprivation increased the excitability, intracellular Ca^{2+} level, and NR1-gene expression levels in R2 neurons. Conversely, knocking down the IP3 receptor in R2 neurons reduced the amount of recovery sleep needed, suggesting that the ER-mediated regulation of intracellular Ca^{2+} levels is involved in regulating sleep homeostasis.

74. Vanderheyden WM, Gerstner JR, Tanenhaus A, Yin JC, Shaw PJ: **ERK phosphorylation regulates sleep and plasticity in *Drosophila***. *PLoS One* 2013, **8**:e81554.

75. Tomita J, Mitsuyoshi M, Ueno T, Aso Y, Tanimoto H, Nakai Y, Aigaki T, Kume S, Kume K: **Pan-neuronal knockdown of calcineurin reduces sleep in the fruit fly, *Drosophila melanogaster***. *J. Neurosci.* 2011, **31**:13137-13146.

76. Nakai Y, Horiuchi J, Tsuda M, Takeo S, Akahori S, Matsuo T, Kume K, Aigaki T: **Calcineurin and its regulator sra/DSCR1 are essential for sleep in *Drosophila***. *J. Neurosci.* 2011, **31**:12759-12766.

77. • Bushey D, Tononi G, Cirelli C: **Sleep- and wake-dependent changes in neuronal activity and reactivity demonstrated in fly neurons using in vivo calcium imaging**. *Proc. Natl. Acad. Sci. U. S. A.* 2015, **112**:4785-4790.
Calcium imaging of Kenyon cells in the mushroom body with single-cell resolution during wake, sleep, and recovery sleep periods revealed that Kenyon cells in an individual fly respond heterogeneously to the same stimulus when the fly has been awake for a long period. This phenomenon may be related to local sleep in mammals.

78. •• Tomita J, Ueno T, Mitsuyoshi M, Kume S, Kume K: **The NMDA receptor promotes sleep in the fruit fly, *Drosophila melanogaster***. *PLoS One* 2015, **10**:e0128101.
Knocking down an NMDA receptor (*Nmdar1*) decreased the sleep amount. This result was further confirmed by the finding that administering MK-801 also decreased the sleep amount. This fruit-fly study was the first to demonstrate the effect of MK-801 on sleep duration.