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Review

The systems biology of sleep: toward integrative understanding of molecular and circuit-based mechanisms of sleep

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Sleep, a universal biological phenomenon, is regulated by multiscale processes, from molecular mechanisms to cellular networks. While the underlying mechanisms, particularly those governing sleep homeostasis, were poorly understood, recent technological breakthroughs have facilitated the identification of molecular and circuit-based mechanisms. Advances in mouse genetics, including next-generation genetics that bypass the need for crossing and postnatal gene knockout methods, enable the comprehensive identification of molecular components for sleep regulation by combining them with noninvasive, large-scale sleep measurements. Elucidated mechanisms include Ca^{2+}-related and protein kinase/phosphatase-mediated signaling, supporting the phosphorylation hypothesis of sleep. The molecular signaling forms 'cellular sleepiness' in sleep regulatory neurons to modulate neuronal activity. These integrated understandings of multiscale mechanisms will lead to a system-level understanding of sleep regulation.

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Introduction

A system-level understanding of biological phenomena, the approach proposed in systems biology [1], is often summarized as comprising the following four steps: 1) identification of system structure, 2) analysis of system dynamics, 3) control of the system by perturbing system components, and 4) design and reconstruction of the system based on the elucidated principles [2]. In essence, systems biology is an attempt to understand complex biological phenomena by investigating the components of a system and their interactions.

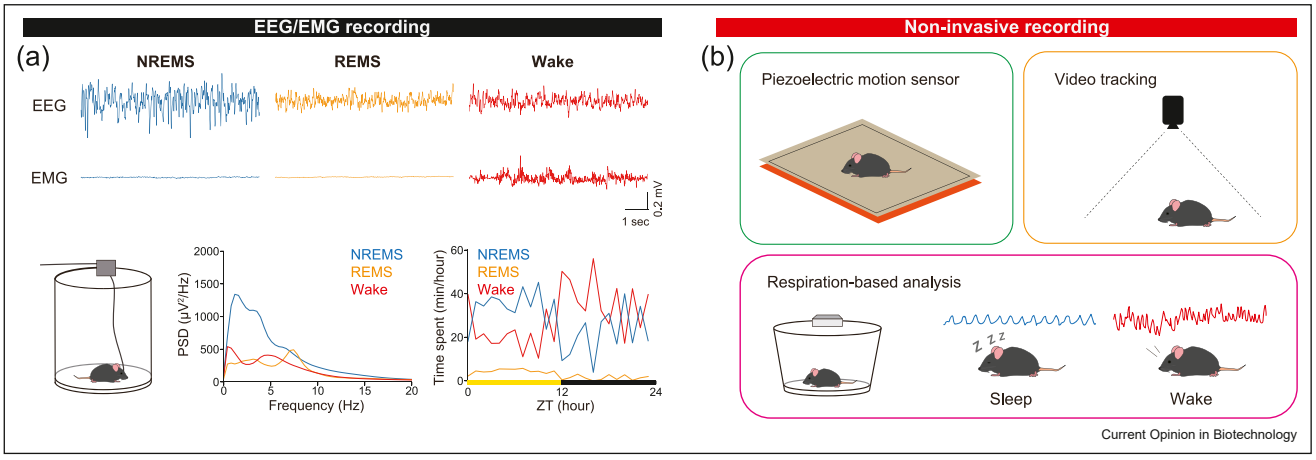
Sleep is an attractive field for systems biology: it is a complex biological function involving multiscale interactions — from intracellular molecular networks to multicellular neuronal communications — and is modulated by both animals' internal states and external environmental inputs. This multiscale regulatory network, spanning from individual neurons to brain circuits and even the influence of external signals, is well suited for analysis using a systems biology approach.

Behaviorally, sleep is observed not only in mammals but also in invertebrates like flies [3,4] and nematodes [5], and even cnidarians [6,7]. Electrophysiologically, two distinct stages of sleep, nonrapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS), are observable in mammals, birds, and reptiles [8,9]. While NREMS is characterized by increased slow-wave activity (SWA) in the delta frequency range (0.4–4 Hz) on the electroencephalogram (EEG), REMS is characterized by theta activity (5–10 Hz) on the EEG and skeletal muscle atonia (Figure 1a).

Chronic sleep deprivation (SD) leads to life-threatening consequences [10,11]. To prevent such outcomes, animals possess a robust homeostatic sleep control system, whereby heightened sleep needs accumulated during wakefulness subsequently induce sleep. For example, following acute SD (e.g. for six hours), mice show increased SWA, an indicator of heightened sleep need during the subsequent deeper and longer sleep called recovery sleep. Sleep homeostasis is a key characteristic of sleep and modulates sleep together with the circadian rhythm [12]. Despite the well-characterized phenomenology of sleep homeostasis, its molecular and cellular underpinnings remain poorly understood.

In recent years, high-throughput sleep quantification methods, combined with cutting-edge genetic perturbations — particularly in mice — have enabled the systematic and comprehensive identification of molecular

Figure 1



Phenomenology of sleep and noninvasive sleep recordings. **(a)** Representative EEG/EMG signatures, the spectral power distribution of the EEG, and hourly sleep/wake amounts in a C57BL/6N mouse under a 12-hour light/12-hour dark cycle. **(b)** Three typical methods for noninvasive sleep recording. The mouse illustration was modified from Openclipart (https://openclipart.org/).

and circuit-level components of sleep regulation. Building on the identification of sleep regulatory components, the dynamics of sleep regulation (i.e. the homeostatic control of sleep) are now being elucidated. Mechanistically, the homeostatic mechanism is multiscale, where individual neurons may sense the sleep need depending on wake-related activity through changes in intracellular signaling. Protein kinases and phosphatases in neurons play a key role in the intracellular molecular signaling of sleep, and the phosphorylation signaling subsequently alters neuronal activity through downstream effectors in sleep regulatory neurons, ultimately leading to the induction of sleep. In this review, we outline recent advances in the identification of sleep regulatory components and their dynamics through the integration of molecular and circuit-level understandings. Furthermore, we discuss future perspectives for controlling and engineering the sleep phenomenon based on the elucidated principles.

Noninvasive sleep recording with high scalability

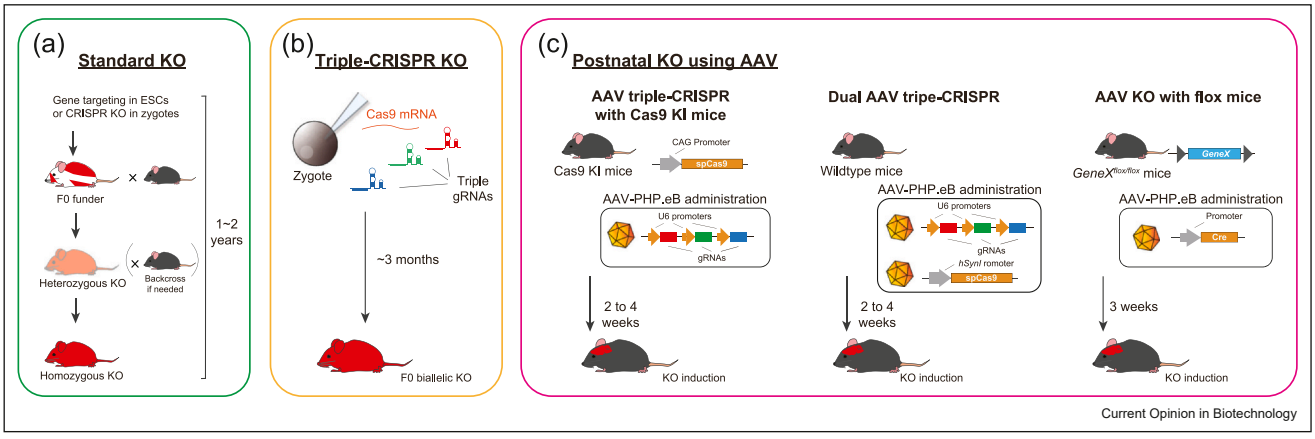
To systematically identify molecular and circuit-based regulators of sleep, it is important to evaluate sleep in a high-throughput and quantitative manner. EEG combined with electromyography (EMG) has been the gold-standard method for sleep stage classification since the finding that human EEG changes depending on sleep/wake states [13,14]. Rodent EEG recordings necessitate the surgical implantation of electrodes into the skull, which demands technical expertise and limits parallel recordings on a large scale. Additionally, EEG/EMG data sometimes need to be manually annotated to classify sleep/wake states by visual inspection, although automated and semiautomated annotation tools have also been developed.

To overcome this bottleneck, noninvasive sleep recordings capable of high-throughput measurement have been developed (Figure 1b). Because behavioral quiescence is a key characteristic of sleep, rodent sleep can also be evaluated by continuous immobility. One approach is to employ piezoelectric sensors to capture changes in mechanical pressure from body movements, including thoracic movements, and annotate sleep accordingly [15,16]. This noninvasive method is used for large-scale screening of sleep [17,18]. Video tracking also offers a way to quantify mouse behavior. Immobility-defined sleep (periods of continuous immobility lasting 40 seconds or more) can be evaluated by video tracking and serves as a surrogate measure for sleep duration in mice [19–22]. In addition to these motion detection approaches, we developed a respiratory-based sleep phenotyping system for noninvasive, fully automated sleep/wake staging [23]. In this system, mice are housed in semiclosed chambers, and the respiratory signals are detected through air pressure changes. Based on the respiratory pattern, sleep/wake states can be automatically staged with 95.3% accuracy. Because EEG signals contain neurophysiological information reflecting neuronal activity during sleep, noninvasive methods do not fully replace EEG/EMG for sleep assessment. Nonetheless, their high scalability, low technical requirements, and fully automated phenotyping contribute to large-scale systematic analysis.

Identification of molecular components of sleep regulation

Sleep is genetically controlled, providing a rationale for systematic genetic screening to uncover its molecular mechanisms. The screening of sleep-relevant genes has employed various strategies, including the identification

Figure 2



KO strategies to search for sleep regulatory genes. **(a)** Conventional method for generating KO mice. Multiple crossings are required to analyze the adult sleep phenotype of homozygous KO mice. **(b)** One-step biallelic KO by the triple-CRISPR method. To achieve biallelic KO in the F0 generation, triple gRNAs targeting single genes were injected into zygotes with Cas9 mRNA. The established KO mice can be rapidly analyzed for the adult sleep phenotype without further breeding (within approximately 3 months). **(c)** Approaches for AAV-mediated postnatal KO using Cas9 KI mice (left), dual AAVs (center), and flox mice (right). KO can be achieved through postnatal AAV administration, and the sleep phenotype can be tested several weeks thereafter. ESC, embryonic stem cells.
The mouse illustration was modified from Openclipart (https://openclipart.org/).

of naturally occurring mutations in humans and genome-wide association studies [24]. For the mouse model, forward genetic screens were conducted using random mutagenesis [21,25].

A straightforward way to demonstrate a specific gene's function is to analyze the phenotypic outcomes when the gene is downregulated. The emergence of genome editing technologies, particularly the CRISPR/Cas9 system [26], has made such reverse genetic screening approaches practically feasible. This strategy can be further accelerated by bypassing the time- and space-consuming crossing steps typically required to establish gene knockout (KO) mouse lines (Figure 2a). Specifically, by achieving high-confidence phenotypic analysis directly in F0 animals generated via CRISPR/Cas9-mediated gene disruption, large-scale genetic screening becomes more scalable and efficient. In principle, if CRISPR/Cas9-mediated genome editing is highly efficient and precise, it is conceivable that almost all somatic cells in F0 mice derived from CRISPR/Cas9-treated zygotes are in a biallelic KO state. Multiplex strategies using multiple guide RNAs (gRNAs) targeting the same gene improve the efficiency of gene editing [27,28]. The triple-CRISPR method was introduced for the nearly perfect (96%–100%) production of biallelic KO mice in the first F0 generation, in which three different gRNAs targeting different regions of a single gene are injected into zygotes along with mRNA encoding the Cas9 protein (Figure 2b). Notably, performing this on C57BL/6 zygotes allowed for the direct use of the first generation of KO mice in phenotypic analysis, eliminating the need for any crossing. Off-target effects are a potential limitation of the CRISPR/Cas9 system, and this is also a concern for multiplexing strategies. Practically, potential off-target effects can be excluded by performing independent experiments using different gRNA sets [23,29].

This 'next-generation mammalian genetics' (highly efficient production technologies for obtaining whole-body genetically modified mice without crossing) [23,30] has enabled the large-scale search for sleep regulatory genes. While it remains technically challenging to target the entire genome, this method makes it relatively feasible to perform systematic KOs across all members of specific gene families. Leveraging this strategy, we systematically investigated the roles of Ca^{2+}-related ion channels, pumps, and kinases in sleep regulation. Based on a computational model recapitulating the electrophysiological characteristics of sleep, multiple Ca^{2+} channels, Ca^{2+}-activated channels, and Ca^{2+}-pumps are predicted to be involved in sleep regulation. Accordingly, 26 genes encoding Ca^{2+}-related proteins were knocked out by the triple-CRISPR method, and the sleep phenotype of resultant KO mice was evaluated [23,29], demonstrating that the Ca^{2+} pathway including Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) α/β is involved in sleep regulation [29]. Using the same approach, we further knocked out 14 genes encoding acetylcholine receptors. Among these, double KO mice for muscarinic acetylcholine receptors 1 and 3 (CHRM1/3) exhibited a nearly complete loss of REMS [31]. Additionally, a recent study with a large set of KO mice (38

genes) demonstrated that protein kinase A (PKA) and protein phosphatase PP1 have wake-promoting and sleep-promoting roles, respectively [32].

Whole-body KO potentially has developmental effects and is sometimes not feasible due to lethality. Conditional KOs (tissue/cell-type specific KOs or inducible KOs) allow for precise analysis of gene functions. These are conventionally achieved by crossing LoxP-flanked mice of the targeted genes (flox mice) with mouse lines that express Cre recombinase in a tissue/cell-type-specific manner (Cre mice) [33], or by the use of inducible Cre recombinase (e.g. CreERT2) [34]. Recently, however, adeno-associated virus (AAV) greatly facilitated the systematic induction of postnatal gene silencing. For example, an AAV-mediated RNAi screen for SWA-regulatory K^+ channels was conducted by injecting AAV2 expressing shRNA into the parietal cortex [35]. Particularly, AAV-PHP.eB is notable for its ability to cross the blood–brain barrier and efficiently deliver genes to the central nervous system (CNS) in C57BL/6 mice [36]. This capability for systemic AAV delivery (e.g. via retro-orbital injection) offers a non-invasive, stable way to deliver genes to the CNS. We established AAV vectors that can express triple gRNAs under the control of U6 promoters (Figure 2c). Administering the AAV via retro-orbital injection to Cas9 knock-in (KI) mice can induce postnatal KO. This 'AAV triple-CRISPR' approach is also extended to the dual AAV approach, in which Cas9 is expressed via another AAV with a neuron-specific promoter [32]. Alternatively, the administration of AAV expressing Cre recombinase to flox mice can induce postnatal KO without the need for crossing [22]. These AAV-based postnatal KOs have recently revealed the roles of neuronal protein phosphatases, calcineurin and PP2A, in sleep regulation. KOs of PPP3CA/PPP3CB (catalytic subunits of calcineurin) [32,37,38], PPP3R1 (regulatory subunit of calcineurin) [32,37,38], and PP2ACA/PP2ACB (catalytic subunits of PP2A) [39] have been reported. Generally, KOs of these phosphatases result in reduced basal sleep with blunted homeostatic response to SD.

Phosphorylation hypothesis of sleep

Key insights from genetic studies for sleep in mice are that not only ion channels, ion pumps, and neurotransmitter receptors, but also protein kinases and phosphatases mediating intracellular signaling are involved in sleep regulation (Figure 3a). As mentioned above, the systematic production of KO mice and the analysis of sleep phenotype revealed that multiple kinases and phosphatases (CaMKII, PP1, and calcineurin) have sleep-promoting roles, while PKA has a wake-promoting function. In addition, forward genetics of randomly mutagenized mice identified *Sleepy* mutant, which exhibits increased sleep duration due to a splicing mutation (gain-of-function mutation) in the salt-inducible kinase 3 (SIK3) [25]. Subsequent studies also revealed the kinase-mediated signaling, including liver kinase B1 (LKB1) and histone deacetylases HDAC4/5, to form transcriptional regulation of sleep [40,41]. Additionally, whole-body and cortical neuron–specific KO mice of extracellular signal–regulated kinase 1 and 2 (ERK1/2) exhibited reduced sleep duration [42]. The roles of protein phosphorylation/dephosphorylation in sleep regulation are evident, as upregulating or downregulating core sleep regulatory phosphatases (PP1 and calcineurin) can dynamically alter total sleep duration, causing changes from approximately 4–17 hours (normal sleep duration is typically 11–12 hours) [32,37,38]. Notably, the downregulation of calcineurin reduces SWA, a hallmark of NREMS-relevant EEG with a blunted response to SD, suggesting the active role in sleep homeostasis.

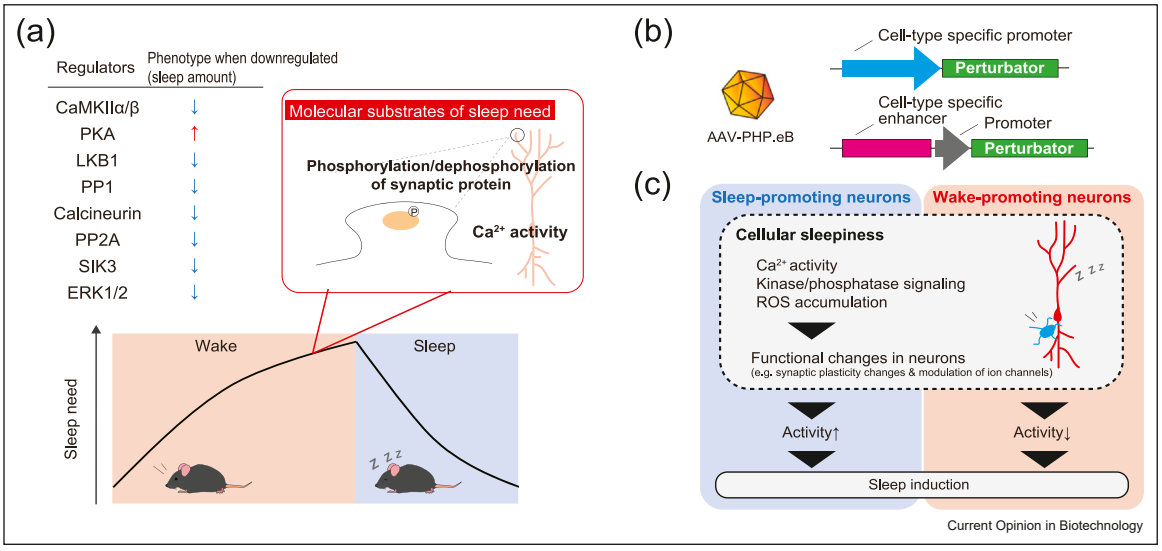
Recent phosphoproteomics analyses also revealed the involvement of brain phosphorylation/dephosphorylation signaling in sleep regulation. Phosphorylation states of synaptic proteins change depending on the sleep–wake cycle and sleep need accumulation in sleep-deprived animals [43–45]. Based on these findings, the 'phosphorylation hypothesis of sleep' has been proposed as a scenario of sleep regulation, in which protein phosphorylation imbalance caused by wakefulness serves as one of the integrators of sleep need, thereby promoting sleep [46] (Figure 3a). The activity of sleep regulatory kinases/phosphatases presumably changes depending on sleep need, thereby modifying the phosphorylation states of various effector proteins to affect transcription [40,41], synaptic plasticity, or the function of ion channels. Although the full picture of the sleep/wake-dependent kinase/phosphatase activity is still unknown, the sleep-promoting kinase CaMKII is known to be activated by increased neuronal spikes, which could be related to wakefulness [46].

Integration of molecular and circuit-based mechanisms of sleep

The phosphorylation hypothesis suggests that protein kinase/phosphatase activity mediates the sleep need in individual neurons. On the other hand, there are multiple sleep regulatory neuronal circuits in the brain. How is the molecular signaling implemented across these sleep regulatory neurons? Here, we focus on the cellular mechanisms of NREMS.

Many subcortical neurons have been shown to promote NREMS when stimulated [47–49]. These sleep-promoting neurons are considered to form sleep-promoting circuits that inhibit wake-promoting neurons or activate other sleep-promoting neurons. Recent studies also shed light on the roles of the cerebral cortex, the epicenter of

Figure 3



Molecular and circuit-based mechanisms of sleep regulation. **(a)** Typical sleep-regulatory protein kinases/phosphatases and proposed molecular mechanisms of sleep need accumulation in the brain. **(b)** Targeted gene delivery to neurons with AAV. Perturbators can be neurogenetic tools (e.g. optogenetic and chemogenetic tools) or proteins that alter the molecular signaling of sleep. **(c)** Possible mechanisms for sensing sleep need in sleep-promoting and wake-promoting neurons. Changes in intracellular signaling depending on sleep need alter the cellular state ('cellular sleepiness') through downstream effectors. CaMKIIα/β, Ca^{2+}/Calmodulin-dependent protein kinase II α and β; PKA, protein kinase A; LKB1, liver kinase B1; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; SIK3, salt-inducible kinase 3; ERK1/2, extracellular signal–regulated kinase 1 and 2; ROS, reactive oxygen species.
The mouse illustration was modified from Openclipart (https://openclipart.org/).

EEG, in sleep regulation. It was recently reported that silencing a subset of layer 5 cortical neurons by ablating their presynaptic machinery reduces both the NREMS amount and SWA, as well as the homeostatic response to SD [50]. In addition to early reports on nitric oxide synthase (nNOS)-expressing GABAergic interneurons [51,52] and neocortical somatostatin (SST)-expressing interneurons [53], a recent study employed c-Fos-based activity tagging to identify GABAergic cells that respond to sleep loss in the prefrontal cortex [54]. This revealed that SST-expressing interneurons, which project to the lateral preoptic hypothalamus and the lateral hypothalamus, are reactive to SD and regulate both sleep-preparatory behavior and recovery sleep. Furthermore, a whole-brain analysis at single-cell resolution using CUBIC (*c*lear, *u*nobstructed *b*rain *i*maging *c*ocktails and *c*omputational analysis), a tissue-clearing method [55,56], revealed that cortical parvalbumin (PV)-expressing GABAergic neurons respond to both SD and the administration of MK801 (a pharmacological agent that induces wakefulness) [57]. The inhibition and activation of the PV-expressing neurons result in the suppression and promotion of recovery sleep, respectively.

Because cell-type specificity in the brain is defined by molecular markers, transgenic mouse lines (e.g. conditional mouse lines such as Cre mice) have been necessary for precise cell targeting. However, the use of AAV-based gene delivery has expanded the flexibility of cell targeting. Remarkably, by changing the promoter and incorporating the cell-type-specific enhancer into the AAV vector, even the systemic administration of AAV-PHP.eB enables the targeted gene delivery to specific cells in the brain (Figure 3b). Recently, a large set of AAV-compatible enhancers that can target various cells in the neocortex has been reported [58]. In principle, the AAV vector incorporates enhancers defining specific neocortical cells with a promoter to enable targeted expression. These AAV-based tools would be able to target specific cells without the need for Cre mice. This strategy was also extended to other brain regions, including the striatum, and to the spinal cord [59,60]. While there are some limitations, including the coverage of labeling, unexpected off-target expression, and lot-to-lot variation, AAV-based approaches without the need for transgenic animals could greatly accelerate the search for the cellular basis of sleep regulation.

A key question is how sleep-regulatory neurons sense changes in sleep need to alter their cellular activity. Given that intracellular signaling mediates sleep regulation, individual neurons, particularly sleep regulatory neurons, code sleepiness through changes in the molecular signaling. The sleep-need-dependent changes in cellular state, defined as 'cellular sleepiness', are measurable through intracellular molecular states

(e.g. protein phosphorylation state). These molecular changes could be converted to alter neuronal activity to induce sleep in animals through downstream effectors (Figure 3c).

Ca^{2+} signal and related protein phosphorylation signaling could be considered sources of 'cellular sleepiness'. The intracellular Ca^{2+} level in a fly's brain neuron responsible for sleep homeostasis rises during prolonged wakefulness, leading to changes in neuronal plasticity depending on sleep need [61]. For the sleep-promoting kinase CaMKIIβ that is activatable by Ca^{2+} signal, we screened a sleep-promoting phosphomimic mutation at its autophosphorylation site [62]. This sleep-promoting effect is observable when the mutant is expressed in a subset of excitatory neurons. In the study reporting the roles of sleep-regulatory PV-expressing neurons [57], CaMKIIα is suggested to be the intracellular machinery that senses an increase in sleep need through its autophosphorylation and induces sleep by modulating neuronal activity. Furthermore, a recent study identified the role of thalamic nucleus reuniens (RE) neurons projecting to the zona incerta in sleep promotion with homeostatic regulation [63]. Interestingly, the inhibition of CaMKII in the RE neurons mitigates the homeostatic response to SD, through its effects on synaptic plasticity. SIK3 and its related signaling pathway were shown to regulate sleep amount in the hypothalamus, while the cerebral cortex-specific activation of this pathway increased SWA rather than sleep amount, suggesting differential regulatory roles among the brain regions [40,41]. The changes in intracellular molecular signaling including protein phosphorylation could alter synaptic plasticity by affecting scaffolding proteins or modulate the function of ion channels, although the precise mechanism remains a subject for future research.

Recent studies also revealed that cellular stress, including mitochondrial or cytosolic reactive oxygen species (ROS) in sleep-promoting neurons, induces sleep in flies and mice [64,65]. In the midbrain sleep-promoting neuron in mice, wake-dependent accumulation of H_2O_2 potentiates neuronal activity, which is partly mediated by the transient receptor potential melastatin 2 (TRPM2) channel [65]. 'Cellular sleepiness' may also be caused by the fatigue of the arousal system. The arousal-promoting locus coeruleus (LC), which releases noradrenaline/norepinephrine (NE), regulates NREMS–REMS cycles [66] and even mediates homeostatic sleep drive [67]. Prolonged stimulation of the LC diminishes neuronal activity and reduces NE release, which can increase the propensity for sleep [67]. This functional change is mediated by an autoinhibitory NE receptor (α2A adrenergic receptor) expressed in the LC. In summary, wakefulness-related changes in molecular signaling serve as a driving force of sleep homeostasis to alter neuronal activity in various neuronal subsets.

Currently, the molecular and circuit-level mechanisms of sleep regulation are not fully integrated, except for a few pioneering studies. Specifically, mechanistic insight into how molecular changes contribute to sleep-need-dependent changes in neuronal activity is still limited. To address these challenges, future studies are warranted to integrate highly scalable region/cell-targeting methods (e.g. AAV-based tools) with the silencing or restored expression of molecular regulators of sleep. Furthermore, integration with single-cell/spatial transcriptome analysis will provide new insights in these areas. Transcript profiling can reveal the molecular markers of identified sleep regulatory neurons [68], as well as information regarding the sleep regulatory signaling and the effectors that are dominant within specific neurons.

Future perspectives

In the system-level understanding proposed in systems biology, the aspects of control and design of biological phenomena are emphasized. Understanding the basic principles of sleep regulation at the molecular and cellular levels and the integration may provide hints for these aspects. As evidenced by the cortical regulation of sleep homeostasis, sleep may be regulated through cell-autonomous mechanisms within the cerebral cortex. In awake and behaving animals, SWA locally occurs in the neocortex in a use-dependent manner [69], while the intracellular mechanism that drives this cellular homeostasis is still unclear. Interestingly, even two-dimensional primary cultures of cortical neurons exhibit neuronal firing patterns resembling those observed during NREMS [70,71]. Although such models do not fully reproduce autonomous sleep-wake cycles, they may offer a minimal element of cell-autonomous sleep homeostasis and allow for the synthetic approach of molecular pathways and neuronal circuits of sleep regulation.

Anesthesia also provides an intriguing way to control sleep. General anesthesia is widely employed to induce surgical unconsciousness. Although general anesthesia is distinct from natural sleep, phenomenological links to NREMS exist. For instance, SWA emerges during the induction of general anesthesia. Additionally, general anesthesia induced by sevoflurane, an inhalational anesthetic, is sufficient to mitigate the NREMS increase following SD, suggesting that this agent modulates sleep homeostasis [72]. Interestingly, urethane, a general anesthetic, can induce cyclic alternations of NREMS-like and REMS-like states [73], although there are some differences in local field potential activity [74]. This suggests that urethane can manipulate or mimic the sleep regulatory mechanisms. Indeed, recent evidence suggests that neuronal circuits underlying general anesthesia and pharmacological sedation are shared with

those of natural sleep [75–77]. Additionally, a recent study demonstrated that the type 1 ryanodine receptor (RyR1), a Ca^{2+} release channel expressed on the endoplasmic reticulum membrane, is a direct molecular target of isoflurane, an inhalational anesthetic [78]. The activation of RyR1 by isoflurane is pertinent to its anesthetic/sedative effects, suggesting a potential link with the Ca^{2+}-related pathway of sleep regulation. Thus, anesthetics are already established agents that possibly control sleep homeostasis through the manipulation of both molecular and circuit-based mechanisms.

Conclusion

Compared to several decades ago, when sleep remained a 'black box', recently developed technologies, including the generation of KO without the need for crossing and AAV-mediated postnatal KO, have facilitated the identification of molecular principles of sleep regulation. These molecular insights are now being integrated with circuit-based mechanisms, particularly how 'cellular sleepiness' scales up to modulate animal sleep/wake states. These understandings of system dynamics bring us closer to a system-level understanding of sleep, including its control and design. The challenge in realizing the systems biology of sleep will also provide principles to understand other organism-level phenomena.

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CRediT authorship contribution statement

Hiroyuki J Kanaya: Conceptualization, Investigation, Visualization, Validation, Writing – original draft, Writing – review & editing, Funding acquisition. **Koji L Ode**: Conceptualization, Investigation, Validation, Writing – original draft, Writing – review & editing, Funding acquisition. **Hiroki R Ueda**: Conceptualization, Investigation, Validation, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Supervision.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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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. Kitano H: **Systems biology: a brief overview**. *Science* 2002, **295**:1662-1664.
2. Ukai H, Ueda HR: **Systems biology of mammalian circadian clocks**. *Annu Rev Physiol* 2010, **72**:579-603.
3. Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A, *et al.*: **Rest in Drosophila is a sleep-like state**. *Neuron* 2000, **25**:129-138.
4. Shaw PJ, Cirelli C, Greenspan RJ, Tononi G: **Correlates of sleep and waking in *Drosophila melanogaster***. *Science* 2000, **287**:1834-1837.
5. Raizen DM, Zimmerman JE, Maycock MH, Ta UD, You YJ, Sundaram MV, *et al.*: **Lethargus is a *Caenorhabditis elegans* sleep-like state**. *Nature* 2008, **451**:569-572.
6. Nath RD, Bedbrook CN, Abrams MJ, Basinger T, Bois JS, Prober DA, *et al.*: **The jellyfish Cassiopea exhibits a sleep-like state**. *Curr Biol* 2017, **27**:2984-2990 e3.
7. Kanaya HJ, Park S, Kim JH, Kusumi J, Krenenou S, Sawatari E, *et al.*: **A sleep-like state in Hydra unravels conserved sleep mechanisms during the evolutionary development of the central nervous system**. *Sci Adv* 2020, **6**:eabb9415.
8. Low PS, Shank SS, Sejnowski TJ, Margoliash D: **Mammalian-like features of sleep structure in zebra finches**. *Proc Natl Acad Sci USA* 2008, **105**:9081-9086.
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.
10. Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB: **Physiological correlates of prolonged sleep deprivation in rats**. *Science* 1983, **221**:182-184.
11. Sang D, Lin K, Yang Y, Ran G, Li B, Chen C, *et al.*: **Prolonged sleep deprivation induces a cytokine-storm-like syndrome in mammals**. *Cell* 2023, **186**:5500-5516 e21.
12. Borbely AA: **A two process model of sleep regulation**. *Hum Neurobiol* 1982, **1**:195-204.
13. Loomis AL, Harvey EN, Hobart G: **Potential rhythms of the cerebral cortex during Sleep**. *Science* 1935, **81**:597-598.
14. Loomis AL, Harvey EN, Hobart GA: **Cerebral states during sleep, as studied by human brain potentials**. *J Exp Psychol* 1937, **21**:127-144.
15. Flores AE, Flores JE, Deshpande H, Picazo JA, Xie XS, Franken P, *et al.*: **Pattern recognition of sleep in rodents using piezoelectric signals generated by gross body movements**. *IEEE Trans Biomed Eng* 2007, **54**:225-233.
16. Yaghouby F, Donohue KD, O'Hara BF, Sunderam S: **Noninvasive dissection of mouse sleep using a piezoelectric motion sensor**. *J Neurosci Methods* 2016, **259**:90-100.
17. Joshi SS, Sethi M, Striz M, Cole N, Denegre JM, Ryan J, *et al.*: **Noninvasive sleep monitoring in large-scale screening of knock-out mice reveals novel sleep-related genes**. *bioRxiv* 2019, https://doi.org/10.1101/517680
18. Grizzard S, Lord JS, Ravens AL, Hao Y, Tang W, Lee SH, *et al.*: **Unbiased genetics identifies a glutamatergic signaling network as a mediator of daily sleep patterns**. *bioRxiv* 2025, https://doi.org/10.1101/2025.05.08.652901

19. Pack AI, Galante RJ, Maislin G, Cater J, Metaxas D, Lu S, *et al.*: **Novel method for high-throughput phenotyping of sleep in mice**. *Physiol Genomics* 2007, **28**:232-238.

20. Fisher SP, Godinho SI, Pothecary CA, Hankins MW, Foster RG, Peirson SN: **Rapid assessment of sleep-wake behavior in mice**. *J Biol Rhythms* 2012, **27**:48-58.

21. Banks GT, Guillaumin MCC, Heise I, Lau P, Yin MH, Bourbia N, *et al.*: **Forward genetics identifies a novel sleep mutant with sleep state inertia and REM sleep deficits**. *Sci Adv* 2020, **6**:eabb3567.

22. Wang G, Li Q, Xu J, Zhao S, Zhou R, Chen Z, *et al.*: **Somatic genetics analysis of sleep in adult mice**. *J Neurosci* 2022, **42**:5617-5640.

23. Sunagawa GA, Sumiyama K, Ukai-Tadenuma M, Perrin D, Fujishima H, Ukai H, *et al.*: **Mammalian reverse genetics without crossing reveals Nr3a as a short-sleeper gene**. *Cell Rep* 2016, **14**:662-677.

24. Zou X, Ptacek LJ, Fu YH: **The genetics of human sleep and sleep disorders**. *Annu Rev Genomics Hum Genet* 2024, **25**:259-285.

25. Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, *et al.*: **Forward-genetics analysis of sleep in randomly mutagenized mice**. *Nature* 2016, **539**:378-383.

26. Hsu PD, Lander ES, Zhang F: **Development and applications of CRISPR-Cas9 for genome engineering**. *Cell* 2014, **157**:1262-1278.

27. Xie K, Minkenberg B, Yang Y: **Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system**. *Proc Natl Acad Sci USA* 2015, **112**:3570-3575.

28. Yin LL, Maddison LA, Li MY, Kara N, LaFave MC, Varshney GK, *et al.*: **Multiplex conditional mutagenesis using transgenic expression of Cas9 and sgRNAs**. *Genetics* 2015, **200**:431-441.

29. Tatsuki F, Sunagawa GA, Shi S, Susaki EA, Yukinaga H, Perrin D, *et al.*: **Involvement of Ca(2+)-dependent hyperpolarization in sleep duration in mammals**. *Neuron* 2016, **90**:70-85.

30. Susaki EA, Ukai H, Ueda HR: **Next-generation mammalian genetics toward organism-level systems biology**. *NPJ Syst Biol Appl* 2017, **3**:15.

31. Niwa Y, Kanda GN, Yamada RG, Shi S, Sunagawa GA, Ukai-Tadenuma M, *et al.*: **Muscarinic acetylcholine receptors Chrm1 and Chrm3 are essential for REM sleep**. *Cell Rep* 2018, **24**:2231-2247 e7.

32. •• Wang Y, Cao S, Tone D, Fujishima H, Yamada RG, Ohno RI, *et al.*: **Postsynaptic competition between calcineurin and PKA regulates mammalian sleep-wake cycles**. *Nature* 2024, **636**:412-421.

A large set of prenatal/postnatal KO mice revealed that PKA promotes wakefulness, while protein phosphatases, PP1 and calcineurin, promote sleep. It was also shown that wake-promoting PKA and sleep-promoting PP1/calcineurin have competing actions at excitatory postsynapses.

33. Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, *et al.*: **Subregion- and cell type-restricted gene knockout in mouse brain**. *Cell* 1996, **87**:1317-1326.

34. Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P, *et al.*: **Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases**. *Nucleic Acids Res* 1999, **27**:4324-4327.

35. Muheim CM, Spinnler A, Sartorius T, Durr R, Huber R, Kabagema C, *et al.*: **Dynamic- and frequency-specific regulation of sleep oscillations by cortical potassium channels**. *Curr Biol* 2019, **29**:2983-2992 e3.

36. Chan KY, Jang MJ, Yoo BB, Greenbaum A, Ravi N, Wu WL, *et al.*: **Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems**. *Nat Neurosci* 2017, **20**:1172-1179.

37. •• Yu J, Liu H, Gao R, Wang TV, Li C, Liu Y, *et al.*: **Calcineurin: an essential regulator of sleep revealed by biochemical, chemical biological, and genetic approaches**. *Cell Chem Biol* 2025, **32**:157-173 e7.

This study reported that calcineurin regulates sleep by dephosphorylating SIK3.

38. •• Yin X, Zhang Z, Zhou R, Zuo P, Sang D, Zhou S, *et al.*: **Calcineurin governs baseline and homeostatic regulations of non-rapid eye movement sleep in mice**. *Proc Natl Acad Sci USA* 2025, **122**:e2418317122.

This study showed that calcineurin is a crucial regulator of sleep.

39. Zhou S, Shi B, Chen L, Yin X, Liu M, Wu C, *et al.*: **PP2Acalpha regulates sleep amount and sleep homeostasis in mice**. *Commun Biol* 2025, **8**:1018.

40. Kim SJ, Hotta-Hirashima N, Asano F, Kitazono T, Iwasaki K, Nakata S, *et al.*: **Kinase signalling in excitatory neurons regulates sleep quantity and depth**. *Nature* 2022, **612**:512-518.

41. Zhou R, Wang G, Li Q, Meng F, Liu C, Gan R, *et al.*: **A signalling pathway for transcriptional regulation of sleep amount in mice**. *Nature* 2022, **612**:519-527.

42. 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**:eaai9219.

43. 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.

44. Wang ZQ, Ma J, Miyoshi C, Lie YX, Sato M, Ogawa Y, *et al.*: **Quantitative phosphoproteomic analysis of the molecular substrates of sleep need**. *Nature* 2018, **558**:435-439.

45. Bruning F, Noya SB, Bange T, Koutsouli S, Rudolph JD, Tyagarajan SK, *et al.*: **Sleep-wake cycles drive daily dynamics of synaptic phosphorylation**. *Science* 2019, **366**:eaav3617.

46. Ode KL, Ueda HR: **Phosphorylation hypothesis of sleep**. *Front Psychol* 2020, **11**:575328.

47. Sulaman BA, Wang S, Tyan J, Eban-Rothschild A: **Neuro-orchestration of sleep and wakefulness**. *Nat Neurosci* 2023, **26**:196-212.

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

49. Scammell TE, Arrigoni E, Lipton JO: **Neural circuitry of wakefulness and sleep**. *Neuron* 2017, **93**:747-765.

50. Krone LB, Yamagata T, Blanco-Duque C, Guillaumin MCC, Kahn MC, van der Vinne V, *et al.*: **A role for the cortex in sleep-wake regulation**. *Nat Neurosci* 2021, **24**:1210-1215.

51. Gerashchenko D, Wisor JP, Burns D, Reh RK, Shiromani PJ, Sakurai T, *et al.*: **Identification of a population of sleep-active cerebral cortex neurons**. *Proc Natl Acad Sci USA* 2008, **105**:10227-10232.

52. Morairty SR, Dittrich L, Pasumarthi RK, Valladao D, Heiss JE, Gerashchenko D, *et al.*: **A role for cortical nNOS/NK1 neurons in coupling homeostatic sleep drive to EEG slow wave activity**. *Proc Natl Acad Sci USA* 2013, **110**:20272-20277.

53. Funk CM, Peelman K, Bellesi M, Marshall W, Cirelli C, Tononi G: **Role of somatostatin-positive cortical interneurons in the generation of sleep slow waves**. *J Neurosci* 2017, **37**:9132-9148.

54. •• Tossell K, Yu X, Giannos P, Anuncibay Soto B, Nollet M, Yustos R, *et al.*: **Somatostatin neurons in prefrontal cortex initiate sleep-preparatory behavior and sleep via the preoptic and lateral hypothalamus**. *Nat Neurosci* 2023, **26**:1805-1819.

Activity-tagging showed that SST-expressing GABAergic neurons in the prefrontal cortex respond to increased sleep need. These cells project to the lateral preoptic hypothalamus and the lateral hypothalamus and mediates sleep-preparatory behavior and recovery sleep.

55. Susaki EA, Tainaka K, Perrin D, Kishino F, Tawara T, Watanabe TM, *et al.*: **Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis**. *Cell* 2014, **157**:726-739.

56. Susaki EA, Shimizu C, Kuno A, Tainaka K, Li X, Nishi K, *et al.*: **Versatile whole-organ/body staining and imaging based on**

electrolyte-gel properties of biological tissues. *Nat Commun* 2020, **11**:1982.

57. •• Kon K, Ode KL, Mano T, Fujishima H, Takahashi RR, Tone D, *et al*.: **Cortical parvalbumin neurons are responsible for homeostatic sleep rebound through CaMKII activation**. *Nat Commun* 2024, **15**:6054.
This study identified cortical PV-expressing neurons as crucial regulators of sleep homeostasis. Moreover, the CaMKII activity within the neurons mediates the homeostatic mechanism.

58. • Ben-Simon Y, Hooper M, Narayan S, Daigle TL, Dwivedi D, Way SW, *et al*.: **A suite of enhancer AAVs and transgenic mouse lines for genetic access to cortical cell types**. *Cell* 2025, **188** 3045-3064, e23.
This study identified a large collection of enhancer AAVs that target diverse neocortical cells.

59. Hunker AC, Wirthlin ME, Gill G, Johansen NJ, Hooper M, Omstead V, *et al*.: **Enhancer AAV toolbox for accessing and perturbing striatal cell types and circuits**. *Neuron* 2025, **113**:1507-1524 e17.

60. Kussick E, Johansen N, Taskin N, Chowdhury A, Quinlan MA, Fraser A, *et al*.: **Enhancer AAVs for targeting spinal motor neurons and descending motor pathways in rodents and macaque**. *Cell Rep* 2025, **44**:115730.

61. 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.

62. Tone D, Ode KL, Zhang Q, Fujishima H, Yamada RG, Nagashima Y, *et al*.: **Distinct phosphorylation states of mammalian CaMKIIbeta control the induction and maintenance of sleep**. *PLoS Biol* 2022, **20**:e3001813.

63. •• Lee SS, Liu Q, Cheng AHR, Kim DW, Boudreau DM, Mehta A, *et al*.: **Sleep need-dependent plasticity of a thalamic circuit promotes homeostatic recovery sleep**. *Science* 2025, **388**:eadm8203.
This study identified a thalamic circuit of sleep homeostasis that undergoes plasticity depending on sleep need changes.

64. Kempf A, Song SM, Talbot CB, Miesenböck G: **A potassium channel β-subunit couples mitochondrial electron transport to sleep**. *Nature* 2019, **568**:230-234.

65. Tian Y, Kang L, Ha NT, Deng J, Liu D: **Hydrogen peroxide in midbrain sleep neurons regulates sleep homeostasis**. *Cell Metab* 2025, **37**:1442-1452 e7.

66. Osorio-Forero A, Foustoukos G, Cardis R, Cherrad N, Devenoges C, Fernandez LMJ, *et al*.: **Infraslow noradrenergic locus coeruleus activity fluctuations are gatekeepers of the NREM-REM sleep cycle**. *Nat Neurosci* 2025, **28**:84-96.

67. • Silverman D, Chen C, Chang S, Bui L, Zhang Y, Raghavan R, *et al*.: **Activation of locus coeruleus noradrenergic neurons rapidly drives homeostatic sleep pressure**. *Sci Adv* 2025, **11**:eadq0651.
This study showed that prolonged activation of LC noradrenergic neurons leads to a reduction in NE release and neuronal activity through autoinhibitory α2A adrenergic receptors. This auto-suppression of NE release is suggested to contribute to sleep drive by diminishing the wake-promoting capacity of the LC.

68. Chung S, Weber F, Zhong P, Tan CL, Nguyen TN, Beier KT, *et al*.: **Identification of preoptic sleep neurons using retrograde labelling and gene profiling**. *Nature* 2017, **545**:477-481.

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

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

71. 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.

72. Pal D, Lipinski WJ, Walker AJ, Turner AM, Mashour GA: **State-specific effects of sevoflurane anesthesia on sleep homeostasis**. *Anesthesiology* 2011, **114**:302-310.

73. Clement EA, Richard A, Thwaites M, Ailon J, Peters S, Dickson CT: **Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia**. *Plos One* 2008, **3**:e2004.

74. Brankack J, Yanovsky Y, Tort ABL, Draguhn A: **Similarities and differences between natural sleep and urethane anesthesia**. *Sci Rep* 2025, **15**:18270.

75. Franks NP: **General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal**. *Nat Rev Neurosci* 2008, **9**:370-386.

76. Jiang-Xie LF, Yin L, Zhao S, Prevosto V, Han BX, Dzirasa K, *et al*.: **A common neuroendocrine substrate for diverse general anesthetics and sleep**. *Neuron* 2019, **102**:1053-1065 e4.

77. Zhang Z, Ferretti V, Guntan I, Moro A, Steinberg EA, Ye Z, *et al*.: **Neuronal ensembles sufficient for recovery sleep and the sedative actions of alpha2 adrenergic agonists**. *Nat Neurosci* 2015, **18**:553-561.

78. Kanaya HJ, Kuwajima K, Ito Y, Shinohara Y, Okubo Y, Shiono S, *et al*.: **Isoflurane activates the type 1 ryanodine receptor to induce anesthesia in mice**. *PLoS Biol* 2025, **23**:e3003172.