

Ca²⁺-Dependent Hyperpolarization Pathways in Sleep Homeostasis and Mental Disorders

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Although we are beginning to understand the neuronal and biochemical nature of sleep regulation, questions remain about how sleep is homeostatically regulated. Beyond its importance in basic physiology, understanding sleep may also shed light on psychiatric and neurodevelopmental disorders. Recent genetic studies in mammals revealed several non-secretory proteins that determine sleep duration. Interestingly, genes identified in these studies are closely related to psychiatric and neurodevelopmental disorders, suggesting that the sleep-wake cycle shares some common mechanisms with these disorders. Here we review recent sleep studies, including reverse and forward genetic studies, from the perspectives of sleep duration and homeostasis. We then introduce a recent hypothesis for mammalian sleep in which the fast and slow Ca²⁺-dependent hyperpolarization pathways are pivotal in generating the SWS firing pattern and regulating sleep homeostasis, respectively. Finally, we propose that these intracellular pathways are potential therapeutic targets for achieving depolarization/hyperpolarization (D/H) balance in psychiatric and neurodevelopmental disorders.

awake to sleep. In this sense, Process S is a negative feedback system for maintaining a constant total wake duration (or quality) (Figure 1B), whereas Process C is a feed-forward system for synchronizing the internal states of the brain (and body) with the external environment. A major focus of modern sleep studies is to identify the molecular mechanisms involved in Process S.

According to basic control theory, robust control can be achieved by an integrated negative feedback loop, in which the integral of the time difference between the current state (e.g., the current wake duration) and a set point (e.g., a target wake duration) is negatively fed back into the system^[2] (Figure 1C). Therefore, Process S might be represented as an integrated negative feedback system that includes an accumulation process, although it is still unclear what is actually accumulated (or recorded) during wakefulness and how and where this accumulation occurs. One of the simplest

realizations of Process S is an integrated system within cells during the awake state (Figure 1D). In this case, activity-dependent changes in intracellular processes, including ion concentration, gene expression, post-translational modification, or the degradation of ion channels or pumps are possible candidates for Process S. Alternatively, Process S might consist of intercellular mechanisms involving different brain regions,^[3,4] in which neurotransmitters or other extracellular substances might accumulate outside cells during an awake state (Figure 1E). In this case, one possible realization of Process S is the accumulation of extracellular substances such as sleep-promoting substances (SPSs), whose concentration increases during prolonged wakefulness and decreases during the subsequent recovery sleep.

1. How Do Animals Implement a Sleep-Wake Cycle?

To describe the mechanisms of sleep, Alexander Borbély postulated the two-process model,^[1] in which sleep is regulated by “Process C” and “Process S” (Figure 1A). Process C is determined by circadian clocks, and regulates the daily pattern of sleep (e.g., rodents usually sleep during the day whereas humans sleep at night), and Process S regulates the sleep duration per day. This homeostatic Process S represents the sleep pressure, which increases and decreases during wakefulness and sleep, respectively, and promotes the transition from

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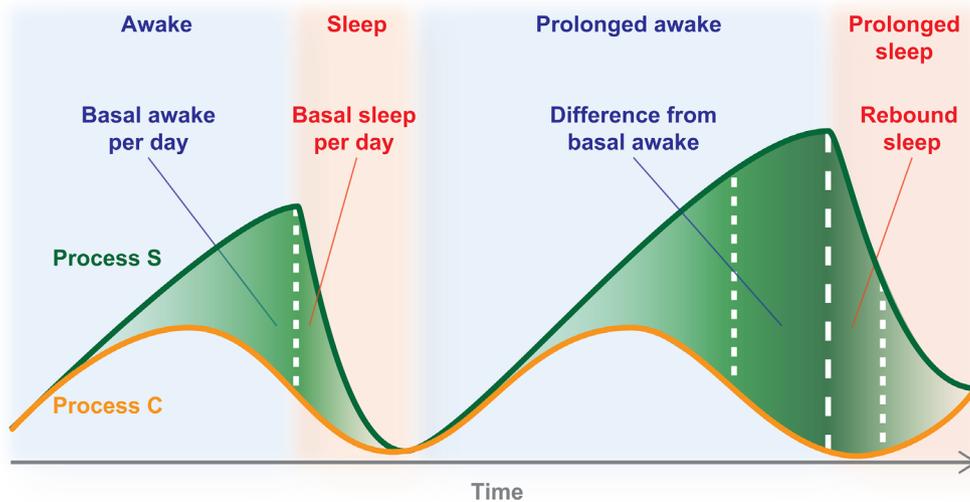
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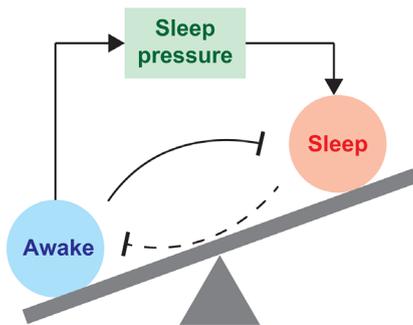
1.1. Is Sleep Homeostasis Regulated by Sleep-Promoting Substances?

SPSs were first discovered over 100 years ago in the cerebrospinal fluid (CSF) of sleep-deprived dogs.^[5] This finding was confirmed by Pappenheimer and colleagues in 1967, who observed that the CSF from a sleep-deprived goat induced sleep when injected intraventricularly into cats and rats. These experiments suggested that endogenous humoral substances accumulate during prolonged wakefulness and are important drivers of homeostatic sleep pressure.^[6] In later studies, several SPSs were identified, including cytokines,

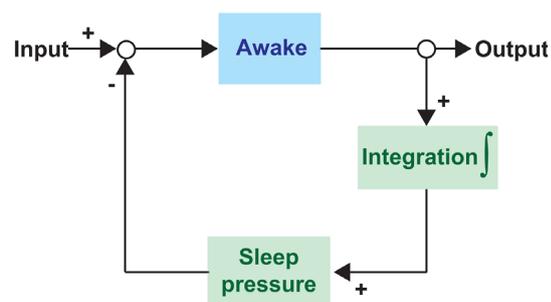
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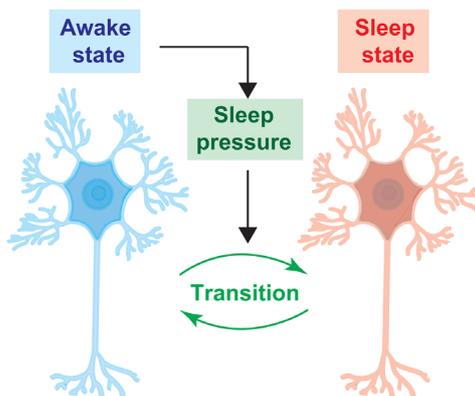
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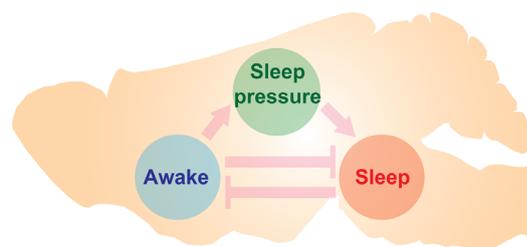


Figure 1. Models for the regulation of sleep-wake cycles. A) The two-process model. The sleep-wake cycle is regulated by Process C and Process S. Process C regulates the daily pattern of sleep, and Process S regulates the amount of basal sleep/awake duration per day. A prolonged awake state results in prolonged sleep, called rebound sleep. B) Negative feedback system in the sleep-wake cycle. The sleep-wake cycle can be achieved by a negative feedback system, in which the awake and sleep states are mutually inhibited by each other. Sleep pressure increases during the awake state, which promotes sleep. C) Integrated feedback system in the sleep-wake cycle. The sleep-wake cycle can be achieved by an integrated feedback system, in which the time integration of the awake state negatively feeds back on itself by increasing the sleep pressure. D) The simplest realization of the sleep-wake cycle. A neuron can hold each state, awake and sleep. The sleep pressure is represented as intracellular properties, which trigger the transition between the two states. E) Another proposed realization of the sleep-wake cycle. Awake and sleep states are maintained by different populations of neurons. Sleep pressure occurs by the increased concentration of extracellular substances.

prostaglandin D2 (PGD2), and adenosine.^[7,8] Cytokines regulate the immune system. In addition, several cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF α), whose receptors are present in the brain, can promote sleep. The administration of each of these cytokines increases the duration of non-rapid eye movement (NREM) sleep in mice, and the levels of these cytokines in the brain increase with prolonged wakefulness, fulfilling criteria for Process S.^[9] However, knockout (KO) mice of the receptors of these cytokines show significantly but only slightly decreased sleep duration,^[10] suggesting that the sleep-promoting role of cytokines is not essential for daily sleep-wake cycles.

The injection of either PGD2 or adenosine into the brain induces sleep,^[8,11] and prolonged wakefulness induces elevated concentrations of both molecules in CSF.^[12] The adenosine level also increases in response to glutamatergic stimulation of the basal forebrain (BF).^[13] Consistent with this finding, a lesion of cholinergic neurons in the BF attenuates the sleep deprivation-induced increase in adenosine, suggesting that adenosine's main source is localized to the BF.^[14] However, adenosine A2A receptor KO mice do not exhibit significant changes in basal sleep duration, but do show a decrease in rebound sleep,^[15] indicating that the regulation of sleep duration involves mechanisms other than the adenosine pathway.

1.2. Genetics in Sleep Research

Recent forward and reverse genetic experiments in fly and mouse identified various molecules involved in the regulation of sleep duration, which may shed light on Process S. Forward and reverse genetics are complementary approaches; the former starts with a phenotype (e.g., a specific sleep phenotype) and seeks to identify the genotype that causes the phenotype, and the latter starts with a genotype (e.g., the KO of a specific gene) and examines the phenotype caused by the genotype. A successful example of forward genetics in animal sleep behavior was the isolation of clock mutants: Konopka and Benzer used ethyl methanesulfonate (EMS) to induce point mutations in the *Drosophila melanogaster* genome, and isolated three clock mutants (independent mutations of *per*) that showed abnormal rhythms in eclosion.^[16]

Forward genetic experiments using EMS in flies also revealed the first sleep mutant with strikingly short sleep duration, *minisleep*.^[17] The responsible mutation is a loss-of-function mutation in the voltage-dependent potassium channel alpha subunit (*Shaker*), which mediates a voltage-activated fast-inactivation I_A current. The mammalian homologs of *Shaker* based on sequence similarity are the alpha subunits of the K_{v1} , K_{v2} , K_{v3} , and K_{v4} potassium channels. Although $K_{v1,2}$ KO mice show a short sleep phenotype, which is not as severe as the *Shaker* phenotype in *Drosophila melanogaster*,^[18] $K_{v3,1}$ and $K_{v3,3}$ double KO mice exhibit a significant short-sleep phenotype, and no significant rebound response to 6 h of sleep deprivation.^[19] These observations suggested that potassium channels might have an important role in regulating sleep duration and Process S. This possibility is supported by another striking short-sleep mutant in the fly, *sleepless*.^[20] The responsible mutation for *sleepless* is in a glycosylphosphatidylinositol-anchored protein, which has a similar expression pattern to *Shaker*. In the absence

of *Sleepless*, *Shaker* shows an altered localization, and a decreased current density and kinetics, suggesting that *Sleepless* regulates *Shaker*.^[21] Although the detailed molecular mechanism underlying the interaction between *shaker* and *sleepless* is still unknown, recent studies showed that neural excitability is increased in both mutants and that a neuron-glia interaction via GABA transaminase is involved in the *sleepless* phenotype.^[21,22] Thus, these genes may be important for regulating the sleep duration and Process S.

Reverse genetic experiments have also contributed to sleep research, especially for validating proposed hypotheses. In the ascending reticular activation system (ARAS), a central mechanism of arousal maintenance, reverse genetic studies showed that the noradrenergic and histaminergic systems are important for maintaining arousal; *Hdc* KO (histamine-deficient) or *Dbh* KO (noradrenaline/adrenaline-deficient) mice have an increased sleep phenotype.^[23] Moreover, adenosine receptor (A2AR) KO mice are insensitive to the wake-promoting effects of caffeine.^[24] This result is consistent with a study showing that a genetic variant in A2AR contributes to individual sensitivity to the wake-promoting effects of caffeine.^[25] These studies illustrate that reverse genetics is a powerful tool for validating the roles of molecules, especially when used to test a hypothesis. In this respect, reverse genetic experiments can be profoundly powerful when combined with computational biology, which can provide testable hypotheses for complex systems such as the sleep-wake cycle.

1.3. What Have Computational Studies Brought to Sleep Research? – Recent Findings via the Averaged-Neuron Model

1.3.1. Recapitulating Oscillatory Patterns During Sleep

Computational biology is playing an increasing role in biology studies. For example, a computational model can be used to reveal the mechanism for a complex biological phenomenon by presenting it as a system with a simple set of components. One of the most elegant examples is Hodgkin and Huxley's model for the action potential in neurons: a model combining simple nonlinear differential equations for the membrane potential and ion currents successfully described how action potentials in neurons are initiated and propagated.^[26]

Computational models have also contributed to sleep studies. During sleep, EEG scans exhibit typical oscillatory patterns (spindle oscillation, delta oscillation, and slow-wave oscillation), which result from the integration of various dynamic neuronal membrane potentials in the cortex (Figure 2A and B). Intracellular and local field potential recordings in vivo and in vitro together with computational models have begun to elucidate the molecular and cellular mechanisms underlying these oscillations. A spindle oscillation of the EEG consists of 7–14 Hz waves that recur periodically with a rhythm slower than 0.1–0.2 Hz, and are typically observed during the early stage of NREM sleep. Intracellular and local field potential recordings in vivo and in vitro indicated that the minimal unit for generating a spindle

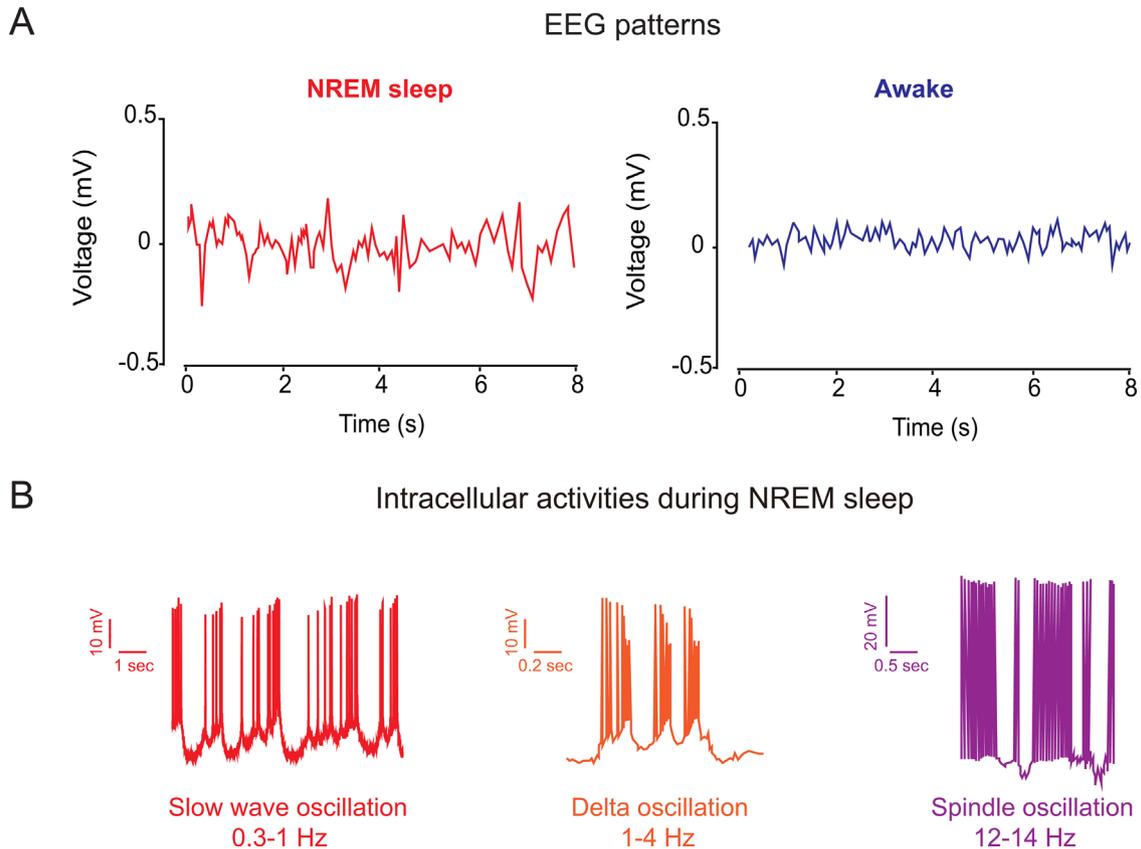


Figure 2. Typical neuronal activities during the sleep-wake cycle. A) Typical EEG patterns during the NREM sleep and the awake state in mice. The NREM sleep state is characterized by high-amplitude and low-frequency fluctuations on EEG scans (left). The awake state is characterized by low-amplitude and high-frequency fluctuations on EEG scans (right). B) Typical intracellular firing patterns in a cortical neuron during slow wave oscillation (left) and delta oscillation (middle). A typical intracellular firing pattern in a reticular thalamic neuron during spindle oscillation (right).

oscillation is the reticular thalamic (RT) neuron itself^[27] and/or the interactions between RT neurons and thalamocortical (TC) neurons in the thalamus.^[28] In particular, the latter hypothesis has been computationally modeled,^[29] and this model shows that a burst firing of RT neurons decreases the membrane potential of TC neurons via GABA_A and GABA_B, which activates a low-threshold T-type calcium current, resulting in the burst firing of TC neurons, and finally, TC neurons induce the burst firing of RT neurons via AMPA.

Another well-known EEG feature during sleep is the delta oscillation. Delta oscillations (1–4 Hz) are recorded in human and animal models during sleep, and have a thalamic origin.^[30] A thalamic delta oscillation appears to be generated by a cell-intrinsic oscillator in TC neurons involving a low-threshold T-type calcium current (I_t) and a hyperpolarization-activated cation current (I_h).^[31] According to a computational model proposed for a thalamic delta oscillation,^[32] the after-hyperpolarization of I_t induces I_h , which depolarizes the membrane potential and in turn re-activates I_t to generate a periodic membrane potential.

Compared to the spindle and thalamic delta oscillations, less is known about the molecular and cellular mechanisms underlying a slow-wave oscillation in the EEG (<1 Hz), a dominant oscillation seen during slow-wave sleep but not during

REM sleep and awake state. During slow-wave oscillations, cortical neurons exhibit a typical membrane-potential pattern (slow-wave-sleep firing pattern, SWS firing pattern) characterized by alternating depolarized bursting and hyperpolarized silent phases.^[33,34] Several computational models have been constructed to elucidate the molecular or cellular mechanism underlying the SWS firing pattern.^[35–38] These neural-network models are based on the TC neural network, and successfully reconstruct the SWS firing pattern and recapitulate the membrane-potential changes of cortical and thalamic neurons during slow-wave oscillations. In short, these studies indicate that the transition from bursting phase to silent phase is facilitated by 1) the enhancement of Ca²⁺- and Na⁺-dependent K⁺ channels in pyramidal neurons^[36–38], or 2) inhibition of the excitatory synaptic current between pyramidal neurons,^[37] or 3) the enhancement of feedback inhibition among pyramidal neurons and interneurons.^[38] However, these simulated neural-network models usually involve a huge parameter space, so the number and type of parameter sets that can generate slow-wave oscillation or the SWS firing pattern cannot be identified comprehensively. Therefore, a simple computational model is required to elucidate the mechanisms underlying the SWS firing pattern.

1.3.2. The Averaged-Neuron Model

To simplify these neural-network models, Ueda and colleagues performed mean-field approximations of a homogeneous population of neurons to construct an “averaged-neuron” (AN) model.^[39] The ion fluxes regulating the averaged neuron are as follows: Na^+ currents are mediated by AMPA receptors (I_{AMPA}), and Ca^{2+} currents are mediated by NMDA receptors (I_{NMDA}). The neuron also contains Cl^- currents mediated by GABA_A receptors, I_{GABA} as extrinsic currents. Depolarizing Na^+ currents are mediated by voltage-gated (I_{Na}) or persistent (I_{NaP}) Na^+ channels, Ca^{2+} currents and/or flux are mediated by voltage-gated Ca^{2+} channels (I_{Ca}) and Ca^{2+} pumps/exchangers with a time constant (τ_{Ca}), and K^+ currents are mediated by several types of K^+ channels, including voltage-gated (I_{K}), leak (I_{L}), fast A-type (I_{A}), inwardly rectifying (I_{AR}), slowly inactivating (I_{KS}), and Ca^{2+} -dependent (I_{KCa}) K^+ channels as intrinsic currents. By searching nearly 20,000,000 randomly generated parameter sets, they identified 1113 parameter sets that generate SWS firing patterns with alternating bursting and silent phases, suggesting that SWS firing patterns can be generated by a homogeneous population of neurons. Using these parameter sets, they also conducted the bifurcation analysis (i.e., asking whether a gradual change in each parameter value induces a sleep to wakefulness transition) of the conductance of each intrinsic and extrinsic current and of the Ca^{2+} efflux rate, based on the idea that prolonged sleep changes the quality or quantity of these channels or pumps, leading to the transition from sleep to wakefulness. This analysis revealed that decreasing the conductance through the NMDAR (g_{NMDA}), Ca_v channels (g_{Ca}), K_{Ca} channels (g_{KCa}), and/or reducing the time constant (τ_{Ca}) of Ca^{2+} efflux mediated by PMCA caused the transition from SWS to awake firing patterns. In addition, parameter searches under the KO of individual or multiple channels (by assuming the conductance equals 0) revealed that the Ca^{2+} influx is redundantly mediated by Ca_v channels and that the NMDAR activates K_{Ca} channels to induce the SWS firing patterns.

According to Tatsuki et al., the putative mechanism of generating the SWS firing pattern is as follows (**Figure 3A**): 1) The bursting phase of the SWS firing pattern is initiated by Ca^{2+} entry mainly through the NMDA receptor (NMDAR) and voltage-gated Ca^{2+} channels (Ca_v channels). 2) The intracellular Ca^{2+} concentration is determined by the ratio of the Ca^{2+} influx and efflux through the NMDAR and Ca_v channels or the plasma-membrane Ca^{2+} ATPases (PMCA), respectively. 3) The transition to the silent phase occurs when the Ca^{2+} -dependent K^+ channels (K_{Ca} channels) is activated, which requires a certain level of intracellular Ca^{2+} concentration. 4) The activation of K_{Ca} channels induces a K^+ efflux-mediated depolarization of the membrane potential and generates the silent state. Because the major difference between the cortical firing patterns during NREM sleep and other states,^[34] awake and REM sleep, is whether the silent phase exists or not, this Ca^{2+} -dependent K^+ efflux-mediated depolarization might underlie the mechanism of generating the SWS firing pattern. Building upon this putative mechanism, the hypothesis that Ca^{2+} -dependent hyperpolarization pathway plays a role in generating the SWS firing pattern was proposed.

Recent studies showed that extracellular environment (e.g., extracellular ion concentration or astroglial networks) might contribute to the regulation of the sleep-wake cycle.^[40,41] According to Ding et al., the extracellular ion concentrations alter in response to sleep or awake state change in vivo and the extracellular K^+ concentration is relatively low during sleep and high during awake state. Interestingly, this extracellular K^+ bias between sleep and awake state might be consistent with the hypothesis that the Ca^{2+} -dependent hyperpolarization pathway plays a role in generating the SWS firing pattern. Because the ratio between the extracellular and intracellular K^+ concentrations affects the relative tendency of K^+ flux, the relatively low extracellular K^+ concentration during sleep could support K^+ efflux through K_{Ca} channels, and hence generate the silent phase. Consistently, the extracellular Ca^{2+} concentration is relatively high during sleep,^[41] which could support Ca^{2+} influx through Ca_v channel and NMDAR and hence generate the silent phase via activating K_{Ca} channels during sleep. However, there is no obvious evidence to validate that the intracellular Ca^{2+} concentration during the SWS firing pattern is higher than the awake one. Further Ca^{2+} imaging studies with high time resolution, which is enough to capture the dynamics of the SWS firing pattern, will clarify the relationship between the intracellular Ca^{2+} concentration and the SWS firing pattern.

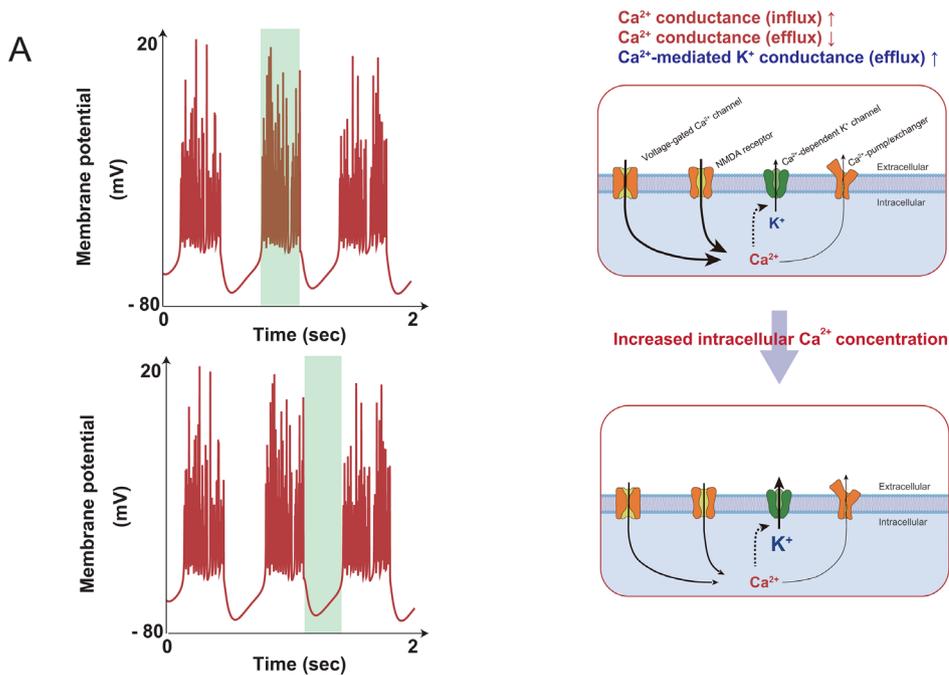
2. Role of Fast and Slow Ca^{2+} -Dependent Hyperpolarization Pathways in the Regulation of Sleep Duration

The AN model and its comprehensive bifurcation analyses predicted that a fast Ca^{2+} -dependent hyperpolarization pathway consisting of ion channels (NMDAR, Ca_v , and K_{Ca} channels) and pumps (PMCA) that directly generate relatively fast dynamics like the SWS firing pattern, plays a central role in the regulation of sleep duration (probably NREM sleep duration), where 1) impairment of ion channels involved in the Ca^{2+} -dependent hyperpolarization pathway will increase the awake duration and 2) impairment of Ca^{2+} -pumps/exchangers will increase the sleep duration (probably NREM sleep duration).^[39] On the other hand, the AN model also predicted that higher hyperpolarization activity (i.e., sleep state) can be triggered via Ca^{2+} influx by slowly activating a slow Ca^{2+} -dependent pathway, which consists of molecules, such as calcium/calmodulin-dependent kinase II (CaMKII) family members, that might be able to modulate the components in the fast pathway and hence indirectly modulate the firing patterns.

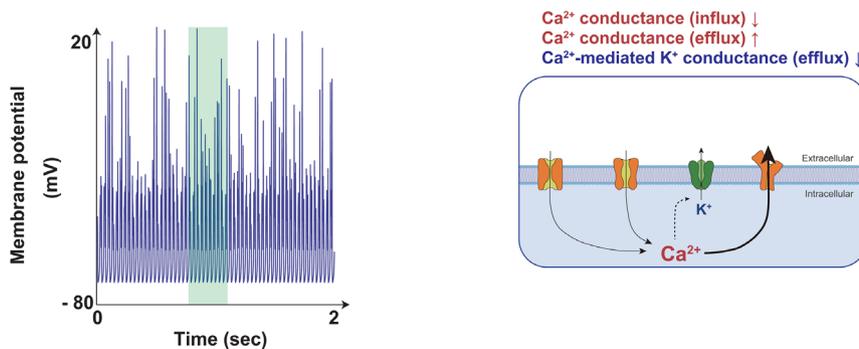
2.1. Fast Ca^{2+} -Dependent Hyperpolarization Pathway in the Regulation of Sleep Duration

To test their predictions about the fast Ca^{2+} -dependent hyperpolarization pathway, Ueda and colleagues conducted a comprehensive in vivo KO study in which they produced and analyzed the phenotypes of 26 KO mice of K_{Ca} channels, Ca_v channels, NMDARs, and PMCA. Their results revealed that the

SWS firing pattern



Awake firing pattern



B

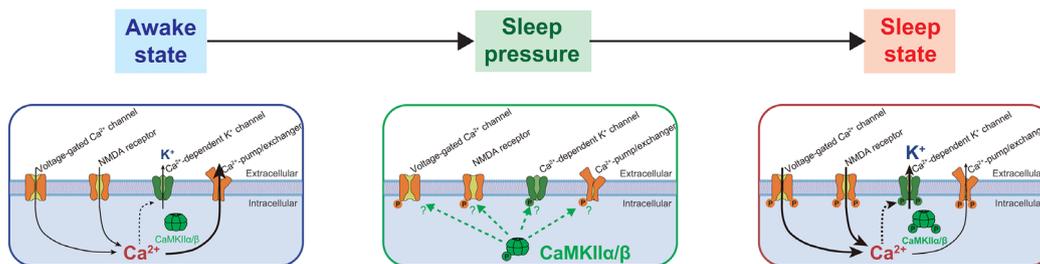


Figure 3. The role of Ca²⁺-dependent hyperpolarization pathway in fast and slow dynamics. A) Schematic diagram of the role of Ca²⁺-dependent hyperpolarization pathway in generating the SWS firing pattern. Predominant Ca²⁺ influx via NMDARs and Ca_v channels generates the bursting state of the SWS firing pattern (top). K⁺ efflux via activated K_{Ca} channels generates the down state of the SWS firing pattern (middle). Predominant Ca²⁺ efflux generates the awake firing patterns in sleep homeostasis. B) Hypothetical mechanism for the possible interactions between the slow and fast Ca²⁺-dependent hyperpolarization pathways in sleep homeostasis. During awake state, Ca²⁺ influx activates CaMKIIα/β (a component of the slow Ca²⁺-dependent hyperpolarization pathway) quantitatively and/or qualitatively via increasing phosphorylated CaMKIIα/β (left). These phosphorylated CaMKIIα/β modify molecules involved in the fast Ca²⁺-dependent hyperpolarization pathway via phosphorylation (middle), which alters the properties (e.g., conductance) of the molecules and elicit the transition from awake state to sleep state (right).

KO mice of Ca²⁺-dependent K⁺ channels [*SK2* (*Kcnn2*) and *SK3* (*Kcnn3*)], voltage-gated Ca²⁺ channels [*Ca_v3.1* (*Cacna1g*) and *Ca_v3.2* (*Cacna1h*)], or NMDAR subunit (*Nr3a*) exhibit a significantly decreased sleep duration, whereas the KO mouse of PMCA (*Atp2b3*) exhibits a significantly increased sleep duration.^[39,42] Furthermore, the acute or chronic pharmacological inhibition of NMDARs (possibly *Nr1/Nr2b*) in glutaminergic and GABAergic neurons in WT mice results in a short-sleep phenotype, confirming the contribution of *Nr1* or *Nr2b*, the KO of which is embryonically lethal, in sleep-duration regulation.^[39] In addition, a whole-brain imaging study of neural activity at single-cell resolution followed by an in-situ hybridization analysis of brain slices revealed that inhibiting NMDARs (possibly *Nr1/Nr2b*) in glutaminergic and GABAergic neurons directly induces neural excitability in the cortex.^[39] These results supported a model in which the sleep duration in mammals is regulated by a Ca²⁺-dependent hyperpolarization pathway, in which Ca²⁺ influx through voltage-dependent Ca²⁺ channels and NMDARs activates Ca²⁺-dependent potassium channels to induce the SWS firing pattern.

2.2. Slow Ca²⁺- Dependent Hyperpolarization Pathway in the Regulation of Sleep Duration

The bifurcation analysis of the AN model predicted that the fast Ca²⁺-dependent hyperpolarization pathway can be targeted by the homeostatic Process S. The molecular mechanism of Process S also presumably senses the awake and/or slow-wave-sleep states (e.g., through the concentration or temporal patterns of Ca²⁺) to allow reversible transitions between the SWS and awake firing patterns. For this Ca²⁺-sensing pathway, Ueda and colleagues focused on the major protein kinase in the brain, calcium/calmodulin-dependent protein kinase type II (CaMKII),^[39] which can sense Ca²⁺. To test their prediction about the slow Ca²⁺-dependent hyperpolarization pathway, Ueda and colleagues conducted an in vivo KO study of the CaMKII family. The production and phenotype analysis of four KO mice of the CaMKII family revealed that CaMKII α (*Camk2a*) and CaMKII β (*Camk2b*) KO mice exhibit a significantly decreased sleep duration.^[39] These results indicated that higher hyperpolarization activity, such as the NREM sleep state, can be induced by Ca²⁺ influx by activating a Ca²⁺-dependent pathway mediated by CaMKII α/β . On the other hand, it is reported that the acute pharmacological inhibition of CaMKII in the pedunculopontine tegmental nucleus (PPT) or the dorsal raphe nucleus (DRN), which consists of wake-promoting neurons,^[4] decrease awake. Thus, the brain-region-specific (e.g., cortex-specific) down/up-regulation of CaMKII as well as the slow Ca²⁺-dependent hyperpolarization pathway will be required to validate their role in sleep. The fast Ca²⁺-dependent hyperpolarization pathway is initiated by Ca²⁺ influx via Ca_v channels and/or NMDARs, followed by the activation of K_{Ca} channels and Ca²⁺ efflux through PMCAs. The slow Ca²⁺-dependent hyperpolarization pathway is initiated by the activation of CaMKII, which enhances the fast Ca²⁺-dependent hyperpolarization pathway to induce cell hyperpolarization. Thus, the fast and slow Ca²⁺-dependent

hyperpolarization pathways, the separate pathways that both use Ca²⁺, could underlie the mechanism of generating the SWS firing pattern and sleep homeostasis, respectively. However, detailed molecular interactions between the fast and slow Ca²⁺-dependent hyperpolarization pathways remain to be elucidated.

3. Role of Fast and Slow Ca²⁺- Dependent Hyperpolarization Pathways in Sleep Homeostasis

Although the above results suggested that fast and slow Ca²⁺-dependent hyperpolarization pathways are involved in the regulation of sleep duration, it remains unclear how the fast Ca²⁺-dependent hyperpolarization pathway, including K_{Ca} channels [*SK2* (*Kcnn2*) and *SK3* (*Kcnn3*)], Ca_v channels [*Ca_v3.1* (*Cacna1g*) and *Ca_v3.2* (*Cacna1h*)], MK-801-targeted NMDAR subunits (*Nr1* and *Nr2b*), and Ca²⁺ pumps (*Atp2b3*), or the slow Ca²⁺-dependent hyperpolarization pathway initiated by CaMKII [CaMKII α (*Camk2a*) and CaMKII β (*Camk2b*)], affects sleep homeostasis. One possibility is that Process S can be represented directly as the quality or quantity of molecules involved in the fast and slow Ca²⁺-dependent hyperpolarization pathways. In this case, Process S is identified as an activity-dependent effect (e.g., phosphorylation) and/or a mechanism affecting the quantity (e.g., translation) of target molecules (Figure 3B). Given the increasing evidence for the post-translational modification of K_{Ca} channels,^[43] Ca_v channels,^[44] and NMDARs,^[45] one of the plausible execution mechanisms of Process S is activity-dependent protein modifications (e.g., phosphorylation or dephosphorylation) of components of the fast and slow Ca²⁺-dependent hyperpolarization pathways. The finding that the impairment of CaMKII α (*Camk2a*) and CaMKII β (*Camk2b*), which are well-known to bind and modify Ca_v channels and NMDARs, decreases sleep duration may support this mechanism. Interestingly, a recent forward genetic study identified a gain-of-function mutation in the *Sik3* protein kinase gene that causes a profound increase in sleep duration^[46]; this finding also supports the idea that Process S is driven by activity-dependent protein modifications. Building on this idea, the intracellular model of sleep-wake-cycle regulation (Figure 1D) can be realized by interactions between fast and slow Ca²⁺-dependent hyperpolarization pathways.

The two-process model assumes that the mechanisms for regulating the basal amount of sleep and response to sleep deprivation are tightly coupled and difficult to separate. However, in more general homeostatic systems, a mechanism determining a basal set point can be independent from a responsive mechanism to perturbation from the set point. For example, a mutant with altered set point (i.e., a short/long sleeper mutant) could exhibit a normal response to the perturbation (i.e., a normal response to sleep deprivation) whereas a mutant with normal set point (i.e., a mutant with normal daily sleep duration) could exhibit an abnormal response to the perturbation (i.e., an abnormal response to sleep deprivation). In other words, the complementary Process S, complementary mechanisms to specifically control rebound sleep, could be added to the basic core mechanism to control

daily sleep duration. In fact, a recent neural circuit screen in *Drosophila* revealed that a subset of neurons generates sleep pressure; the neurons respond to prolonged wakefulness, and their inhibition eliminates the rebound sleep after sleep deprivation, while the basal sleep duration is unaffected.^[47] Therefore, one possible mechanism for the complementary Process S is that the amount of SPSs or the neural activity of specific neurons integrates prolonged wakefulness with the basal wake duration (or insufficient sleep amount with the basal sleep duration) and then promotes sleep (Figure 1E). An important remaining challenge is to discover how such extracellular signaling mechanisms can affect fast and slow Ca²⁺-dependent hyperpolarization pathways to transiently regulate sleep duration.

4. Role of Fast and Slow Ca²⁺- Dependent Hyperpolarization Pathways in Sleep-Wake Cycle

One interesting and remaining question is how the transition between sleep and awake state is regulated. As discussed, the transition from awake state to sleep might be driven by the regulation of fast Ca²⁺-dependent hyperpolarization pathway via the slow pathway; the increase in quality or quantity of the phosphorylated CaMKII activates the fast Ca²⁺-dependent hyperpolarization pathway, which drives the transition from awake firing pattern to the SWS firing pattern, and hence awake state to sleep. In this context, it might be reasonable to assume that the transition from sleep to awake state is regulated by down-regulating the fast Ca²⁺-dependent hyperpolarization pathway. In other words, the increased quality or quantity of the phosphorylated CaMKII and the activity of the fast Ca²⁺-dependent hyperpolarization pathway should be decreased during sleep via translational or post-translational mechanisms (e.g., dephosphorylation by phosphatase or natural turn-over of phosphorylated proteins). Since the sleep-related phosphatase has not been discovered in mammals, further studies will be needed to understand phosphorylation-mediated sleep-wake cycle regulation.

To summarize the above discussion, the simplest mechanism of the sleep-wake cycle may be as follows: During the awake state, the quality or quantity of phosphorylated CaMKII will increase in neurons (probably cortical neurons). CaMKII activates the components of the fast Ca²⁺-dependent hyperpolarization pathway via modifying the conductance of components by translational or post-translational mechanisms. This activation of the fast Ca²⁺-dependent hyperpolarization pathway induces the SWS firing pattern in cortical neurons, the slow-wave oscillation in the cortex, and hence sleep. On the other hand, during the sleep state, the quality or quantity of the phosphorylated CaMKII and the activity of the fast Ca²⁺-dependent hyperpolarization pathway will decrease via translational or post-translational mechanisms (e.g., dephosphorylation by phosphatase or natural turn-over of phosphorylated proteins), which prevents neurons to continue the SWS firing pattern as well as the cortex to continue the slow-wave oscillation and induces the transition from sleep to awake state. In this sense, the slow pathway (i.e., the change of quality or quantity of the

phosphorylated CaMKII) regulates the fast pathway to achieve sleep homeostasis.

5. Role of Ca²⁺- Dependent Hyperpolarization Pathways in Mental Disorders

Sleep disorders are major complications of psychiatric disorders such as schizophrenia,^[48] bipolar disorder,^[49] and major depressive disorders.^[50] It also accompanies neurodevelopmental disorders such as autism spectrum disorders (ASD).^[51] Indeed, patients with schizophrenia are reported to exhibit longer sleep latency and increased sleep duration during the daytime.^[52] Patients with bipolar disorder are also reported to exhibit longer sleep latency.^[53] It is unclear whether there is a causal relationship between sleep disorders and psychiatric or neurodevelopmental disorders. However, it is reasonable to assume that there is a common molecular mechanism among sleep disorders and psychiatric and neurodevelopmental disorders because of a high complication rate among these disorders. In fact, Ca²⁺-dependent hyperpolarization pathways appear to play important roles in psychiatric and neurodevelopmental disorders. For example, a genome-wide association study (GWAS) revealed a correlation between some key molecules in the Ca²⁺-dependent hyperpolarization pathways and psychiatric disorders, such as schizophrenia, bipolar disorder, and major depressive disorders, and neurodevelopmental disorders such as ASD.^[54–58] Thus, evidence suggests that Ca²⁺-dependent hyperpolarization pathways are involved in psychiatric and neurodevelopmental disorders, and therefore, Ca²⁺-dependent hyperpolarization pathways are potential drug targets for psychiatric and neurodevelopmental disorders (Figure 4A).

5.1. Ca²⁺ Influx via Voltage-Gated Calcium Channels in Mental Disorders

Ca_v channels are important conveyors of Ca²⁺ influx in neurons. A correlation between single nucleotide polymorphisms (SNPs) in calcium-channel genes and psychiatric disorders has been reported.^[56] In particular, Ca_v1.2 (*CACNA1C*) has been repeatedly reported to be associated with various psychiatric disorders, including schizophrenia,^[57,59,60] bipolar disorders,^[54,55,61] and major depressive disorders.^[60] In addition, a study comparing the whole genome sequences of healthy versus schizophrenic individuals revealed that rare (less than 1 in 10,000) alleles in several calcium channels [e.g., Ca_v2.2 (*CACNA1B*), Ca_v1.2 (*CACNA1C*), and Ca_v3.2 (*CACNA1H*)] were enriched in the schizophrenia group^[62] [e.g., two point mutations in Ca_v1.2 (*CACNA1C*) and one deletion in Ca_v3.2 (*CACNA1H*)]. In particular, one mutation in Ca_v1.2 (*CACNA1C*) converts a glutamine residue to a stop codon, indicating that it is a loss-of-function mutation. Interestingly, infants who have the *CACNA1C* variant rs4765913, rs4765914, or rs2239063 that is reported to have significant association with bipolar disorder or schizophrenia,^[55,56] exhibited longer sleep latency.^[63] Indeed, patients with schizophrenia or bipolar disorder both exhibited longer sleep latency, suggesting that *CACNA1C* might be

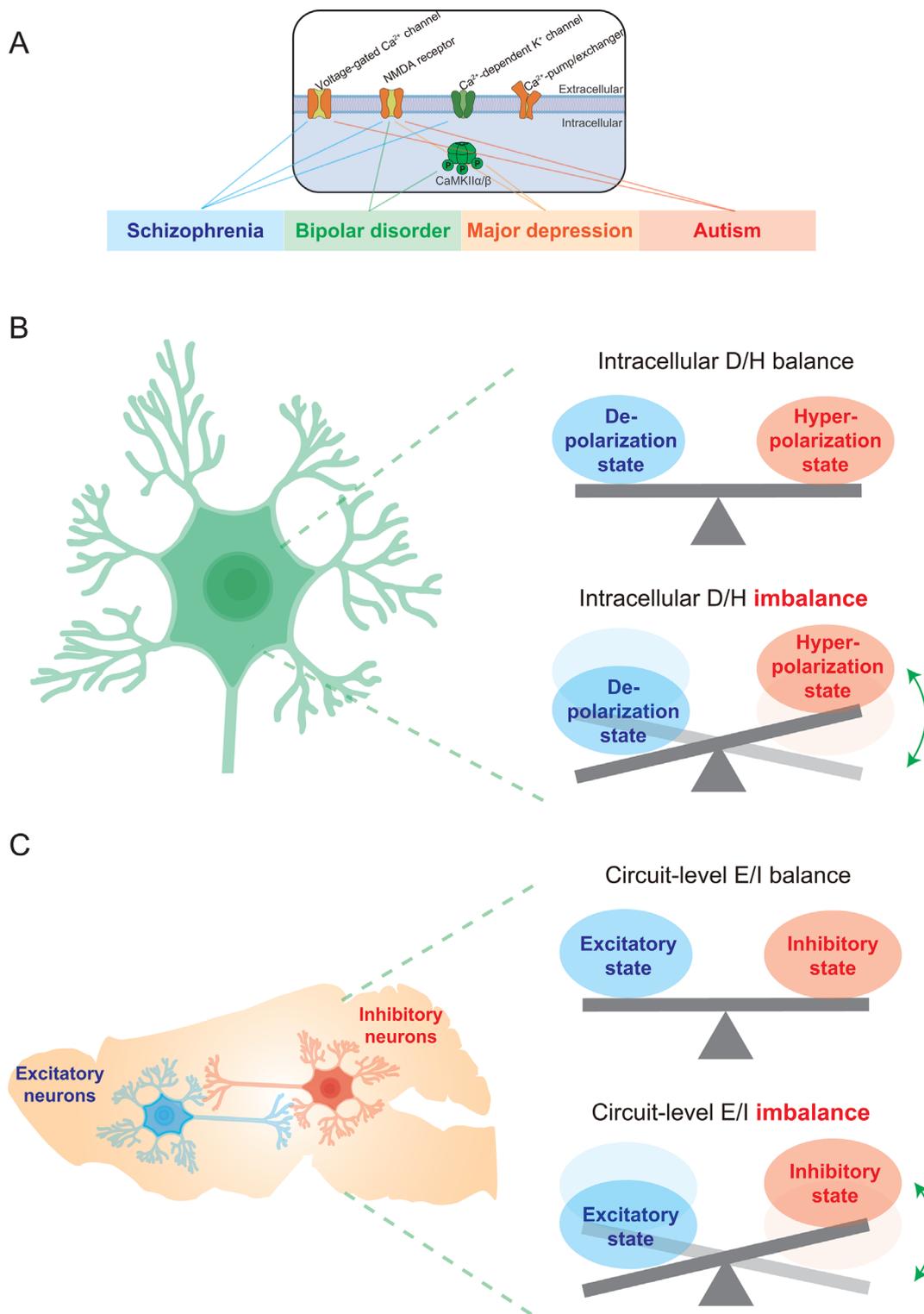


Figure 4. Slow and fast Ca²⁺-dependent hyperpolarization pathways in mental disorders. A) Schematic diagram of the role of Ca²⁺-dependent hyperpolarization pathways in psychiatric and neurodevelopmental disorders. Lines of different colors represent reported correlations between each molecule and each psychiatric and neurodevelopmental disorders. B) Schematic diagram of the intracellular depolarization/hyperpolarization (D/H) balance model. A single neuron holds each state, depolarization and hyperpolarization, whose balance is maintained appropriately in normal conditions, but impaired in psychiatric and neurodevelopmental disorders. C) Schematic diagram of the circuit-level excitatory/inhibitory (E/I) balance model. Excitatory and inhibitory neurons comprise neural circuits, whose balance is maintained appropriately in normal conditions, but impaired in psychiatric and neurodevelopmental disorders.

associated with both sleep and psychiatric disorders.^[52,53] These results suggest that an impairment of voltage-gated calcium channels and hence an impairment of Ca^{2+} -dependent hyperpolarization pathways may contribute to schizophrenia.

Several calcium channels are also associated with ASD [e.g., $Ca_v1.2$ (*CACNA1C*), $Ca_v1.3$ (*CACNA1D*), $Ca_v3.1$ (*CACNA1G*), $Ca_v3.2$ (*CACNA1H*), and $Ca_v3.3$ (*CACNA1I*)].^[64] Interestingly, a whole-genome sequencing (WGS) study in families with ASD detected a rare mutation in $Ca_v1.2$ (*CACNA1C*) (R1522Q).^[65] Another ASD-associated point mutation in $Ca_v1.2$ (*CACNA1C*) is G406R, which has also been identified as the causal mutation of Timothy syndrome,^[66] a disorder characterized by multiorgan dysfunction, including lethal arrhythmias, the webbing of fingers and toes, congenital heart disease, immune deficiency, intermittent hypoglycemia, cognitive abnormalities, and ASD. This mutation results in a prolonged inward Ca^{2+} current through $Ca_v1.2$ (*CACNA1C*), suggesting that the molecular properties of $Ca_v1.2$ (*CACNA1C*) play a role in ASD.^[66] Consistent with this possibility, a heterozygous knock-in (KI) of this human mutation into mice results in ASD-like behavior.^[67] These findings suggest that a dysfunction of voltage-gated calcium channels and hence Ca^{2+} -dependent hyperpolarization pathways may play a role in ASD.

5.2. Ca^{2+} Influx via NMDARs in Mental Disorders

NMDARs also contribute to Ca^{2+} influx in neurons and are associated with psychiatric disorders. Ketamine and phencyclidine (PCP), noncompetitive antagonists for NMDARs,^[68] can induce psychotogenic effects.^[69] PCP at plasma concentrations that block the NMDAR can induce positive, negative, and cognitive symptoms associated with schizophrenia.^[70] Similarly, ketamine can induce positive, negative, and cognitive schizophrenia symptoms when administered to healthy volunteers.^[71] Interestingly, these effects of PCP and ketamine could not be observed in the neonatal rat.^[72] Furthermore, patients with encephalitis associated with antibodies against NMDARs exhibit positive, negative, and cognitive schizophrenia symptoms.^[73] Interestingly, a GWAS also revealed that the NMDAR subunits *Nr3a* and *Nr3b*, whose KO mice exhibit a short-sleep phenotype, are associated with schizophrenia.^[74] On the other hand, the inhibitor of glycine transporter, which results in NMDAR activation, exhibits significant therapeutic effects on schizophrenia symptoms.^[75] Consistent with these results in humans, PCP and MK-801, a more specific inhibitor of NMDARs, can induce positive-, negative-, and cognitive-symptom-like phenotypes in animals.^[76] In addition, mice with reduced expression of the NMDAR subunit (*Nr1*) display the positive-, negative-, and cognitive-symptom-like phenotypes related to schizophrenia.^[77] These results indicate that NMDAR dysfunction and hence the impairment of Ca^{2+} -dependent hyperpolarization pathways may play a role in schizophrenia.

In addition to their relationship with schizophrenia, NMDARs have been implicated as a potential drug target for major depressive disorders. For example, ketamine, an NMDAR antagonist, can rapidly improve the symptoms of major depressive disorders.^[78] Consistent with these clinical results, ketamine and MK-801 can rapidly trigger anti-

depressant effects in animals.^[79] These findings suggest that an enhancement of Ca^{2+} -dependent hyperpolarization pathways, which can be treated by blocking NMDARs, may be associated with some major depressive disorders.

In addition to their involvement in psychiatric disorders, NMDARs are also associated with ASD. Genome sequencing of NMDARs in patients with ASD revealed de novo mutations in the *NR2B* gene.^[80] SNP analysis for NMDARs also revealed a significant association between ASD and haplotypes in the *NR2B* gene.^[81] Importantly, clinical studies suggested that the pharmacological enhancement of NMDA receptors can improve ASD symptoms; for example, D-cycloserin, an agonist for NMDARs, significantly improves symptoms, such as social withdrawal and repetitive behaviors in ASD patients,^[82] suggesting that NMDARs are impaired in some types of ASD. In addition, an enhancement of NMDARs may be associated with other types of ASD, given that memantine, an antagonist for NMDARs, and its analog amantadine can improve ASD symptoms in some cases.^[83]

Consistent with the results of clinical studies, a role of NMDARs in ASD-related symptoms has also been demonstrated in animals. For example, KO mice for *Neuregilin-1* exhibit decreased NMDAR function,^[84,85] because *Neuregilin-1* directly interacts with NMDARs and promotes their localization to synapses. Interestingly, *Neuregilin-1* homozygous mutants exhibit increased self-grooming behavior, which represents ASD-related symptoms in mice. Notably, this symptom is quickly normalized in about 30 min by injecting D-cycloserin.^[84] In addition, some ASD patients have a mutation in the *Shank2* gene, in which exons 6 and 7 are missing.^[86] *Shank2* homozygous mutant mice exhibit decreased NMDAR function and social deficits, which are quickly abolished by the systemic administration of D-cycloserin.^[87]

In contrast to *Neuregilin-1* or *Shank2* mutant mice, IPSP53 homozygous KO mice exhibit increased NMDAR function and impaired social interaction.^[88] Memantine, an antagonist of NMDARs, normalizes the NMDAR function and social interaction in these mice. Collectively, these results suggest that the dysfunction (both the enhancement and the impairment) of NMDARs^[89] and hence the dysfunction of Ca^{2+} -dependent hyperpolarization pathways may be associated with ASD.

5.3. K_{Ca} Channels in Mental Disorders

In Ca^{2+} -dependent hyperpolarization pathways, Ca^{2+} influx via Ca_v channels and/or NMDARs activates K_{Ca} channels, which are also associated with mental disorders. *SK3* (*Kcnn3*), a Ca^{2+} -dependent K^+ channel, contains a CAG repeat region in its first exon that is highly conserved among species. Several studies have shown that an increased length of this region is associated with psychiatric disorders (e.g., anorexia nervosa, migraine, ataxia, epilepsy, and schizophrenia).^[90,91] The longer CAG repeat decreases SK3 (*Kcnn3*) channel conductance.^[92] With respect to Ca^{2+} -dependent hyperpolarization pathways, this decreased channel conductance of SK3 (*Kcnn3*) could increase neuronal excitability, as observed in mice treated with an NMDAR inhibitor.^[39] Although the

correlation between CAG-repeat length and the development of schizophrenia is still controversial,^[90,92,93] it will be interesting to determine whether the impairment of Ca²⁺-dependent hyperpolarization pathways caused by a decreased channel conductance of SK3 (Kcnn3) due to longer CAG repeats can be associated with the development and/or symptom variations of mental disorders such as schizophrenia.

5.4. Ca²⁺/ Calmodulin-Dependent Protein Kinase II in Mental Disorders

In the Ca²⁺-dependent hyperpolarization pathways, the Ca²⁺ influx via Ca_v channels and/or NMDARs also activates downstream Ca²⁺-dependent intracellular signaling pathways, such as those involving CaMKII protein kinases. Interestingly, the potential involvement of *CAMK2* in psychiatric disorders has been shown in human clinical studies, in which changes in *CAMK2* gene expression were found in bipolar disorders,^[94] schizophrenia,^[95,96] and major depressive disorders.^[95,97] In addition, the potential involvement of *CAMK2* in neurodevelopmental disorders was indicated by the identification of a de novo *CAMK2A* missense mutation in an ASD proband in the Simons Simplex Collection.^[98] In animal studies, roles of CaMKII in major depressive disorders and schizophrenia have been well documented.^[99] In a rat model for major depressive disorders, CaMKIIβ is significantly increased in the habenula,^[100] and this increase is abolished by imipramine, an antidepressant drug. CaMKIIβ overexpression in the habenula results in depression-like behaviors in rats and mice,^[100] whereas *Camk2b* KO mice have an antidepressant phenotype, suggesting that CaMKIIβ is involved in major depressive disorders and that CaMKIIβ might serve as a potential drug target for major depressive disorders. Interestingly, CaMKIIα also plays a role in major depressive disorders in a different brain region. The antidepressant effects of fluoxetine in mice are abolished by overexpressing CaMKII in the nucleus accumbens (NAc), whereas the inhibition of NAc CaMKII activity mimics the fluoxetine-treatment phenotype.^[99] Based on the short-sleep phenotype of *Camk2a* and *Camk2b* KO mice, CaMKII might be involved in decreasing neuronal excitability. With respect to the Ca²⁺-dependent hyperpolarization pathways, CaMKII inhibition might enhance neural excitability, which could provide resistance to major depressive disorders. In animal studies of schizophrenia, *Camk2a* heterozygous KO mice exhibited behavioral phenotypes, such as working memory deficits, social withdrawal, and hyperactivity, which are observed in schizophrenia patients.^[101] In addition, *Camk2a* heterozygous KO mice have an immature dentate gyrus (DG), which is also reported in schizophrenia patients.^[102] Interestingly, forebrain-specific KO mice of *Ppp3r1*, a regulatory subunit of the Ca²⁺-dependent phosphatase Calcineurin, exhibit hyperactivity and have an immature dentate gyrus.^[103] With respect to the Ca²⁺-dependent hyperpolarization pathways, impairments of CaMKII and other molecules, such as Calcineurin, might inhibit the Ca²⁺-dependent hyperpolarization via phosphorylated/dephosphorylated regulations and lead to enhanced neural excitability, which could explain the

hyperactivity and other clinical phenotypes observed in schizophrenia patients.

Animal studies for neurodevelopmental disorders have indirectly suggested the roles for CaMKII.^[99] For example, fragile X syndrome, which is caused by a lack of the fragile X mental retardation protein (FMRP),^[104] leads to an autistic phenotype with a severe cognitive disability. FMRP is an mRNA-binding protein^[105] that is important for translational control in dendritic spines.^[106] In dendritic spines, the translation of CaMKIIα mRNA is controlled by neural activities,^[107] and FMRP targets many mRNAs, including the CaMKIIα mRNA, and suppresses their translation.^[108] Therefore, the synapses of FMRP KO mice exhibit upregulated CaMKIIα protein^[108]. Another example is spinocerebellar ataxia type 2, caused by a mutation of the *ataxin-2* gene,^[109] which codes for another mRNA-binding protein.^[110] Interestingly, a fly study demonstrated that Ataxin-2 can also bind to CaMKIIα mRNA, and negatively regulates its translation together with FMRP.^[111] Therefore, FMRP KO flies exhibited upregulated CaMKII translational reporter activity.^[111] On the other hand, a reduced CaMKIIα level and/or activity is also associated with neurodevelopmental disorders.^[99] For example, Rett syndrome is partly caused by a mutation of methyl-CpG binding protein 2 (MeCP2),^[112] which is important for the epigenetic control of genes. A mutation of MeCP2 results in a decrease in CaMKIIα protein.^[113] Another example is Angelman syndrome, which is caused by a mutation of the ubiquitin ligase E3A gene (*Ube3a*).^[114] *Ube3a* KO mice exhibit increased phosphorylation of T305/T306 of CaMKIIα, which results in decreased CaMKII activity.^[115] Crossing *Ube3a* KO mice with T305V/T306A CaMKIIα heterozygous mutant mice rescues the Angelman-syndrome-like phenotypes of *Ube3a* KO mice.^[116] Collectively, these results suggest that a dysfunction (either increase or decrease) of CaMKIIα may be associated with the clinical phenotypes observed in patients with neurodevelopmental disorders.

6. Conclusions and Perspectives: Ca²⁺-Dependent Hyperpolarization Pathways as Potential Therapeutic Targets to Achieve Depolarization/Hyperpolarization Balance (D/H Balance) in Mental Disorders

In the previous sections, we discussed the Ca²⁺-dependent hyperpolarization pathways as a plausible core mechanism for sleep homeostasis, and described the close association of these Ca²⁺-dependent hyperpolarization pathways with psychiatric and neurodevelopmental disorders. In this section, we will discuss the potential of these hyperpolarization pathways as drug targets for psychiatric and neurodevelopmental disorders. As discussed in the previous sections, the Ca²⁺-dependent hyperpolarization pathways appear to be impaired in some psychiatric disorders, such as schizophrenia, bipolar disorders, and some neurodevelopmental disorders, indicating that the appropriate balance between depolarization and hyperpolarization of neurons (termed here as “D/H balance”) is impaired in these disorders. In this context, the goal of the pharmacological regulation of Ca²⁺-dependent hyperpolarization pathways is to

restore the imbalanced D/H balance to treat psychiatric and neurodevelopmental disorders (Figure 4B).

A similar but different concept, an excitation/inhibition balance (E/I balance) of circuits, is a well-known hypothesis in neuroscience that emphasizes the balance of neural circuits composed of excitatory and inhibitory neurons^[117] (Figure 4C). According to the E/I balance hypothesis, psychiatric disorders, such as schizophrenia, and neurodevelopmental disorders, such as ASDs, are caused by an E/I imbalance of neural circuits.^[118] On the other hand, the D/H balance hypothesis is a cell-autonomous concept that originated in sleep studies,^[39,42] and emphasizes the balance in neural excitability achieved by the control of Ca²⁺-dependent hyperpolarization pathways. According to this hypothesis, psychiatric disorders, such as schizophrenia, bipolar and major depressive disorders, and neurodevelopmental disorders, such as ASDs, are caused by a D/H imbalance of neural excitability. Interestingly, the animal model of schizophrenia generated by NMDAR inhibitor administration exhibits the enhanced excitability of both excitatory and inhibitory neurons,^[39] suggesting that the D/H imbalance (i.e., increased depolarization) in both neurons might respectively underlie the very heterogeneous phenotype of this animal model and hence schizophrenia. Therefore, to comprehensively elucidate the pathology of psychiatric and neurodevelopmental disorders, both circuit-level abnormalities, such as E/I imbalance, and cell-autonomous abnormalities, such as D/H imbalance, should be investigated. These studies will lead to better strategies for treating and preventing these diseases in the future.

Abbreviations

ASD, autism spectrum disorders; AN model, averaged-neuron model; CSF, cerebrospinal fluid; K_{Ca} channel; Ca²⁺-dependent K⁺ channel; NMDAR, N-methyl-D-aspartic acid receptor; PMCA, plasma membrane Ca²⁺ ATPase; RT, reticular thalamic; SPSs sleep-promoting substances; SWS, slow-wave-sleep; TC, thalamocortical; D/H balance, depolarization/hyperpolarization balance; E/I balance, excitatory/inhibitory balance; Ca_v channel voltage-gated Ca²⁺ channel.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Ca²⁺-dependent hyperpolarization pathways, depolarization/hyperpolarization (D/H) balance, neurodevelopmental disorders, psychiatric disorders, sleep

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