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Abstract:	Sleep spindles are distinctive electroencephalographic (EEG) oscillations emerging during non-rapid-eye-movement sleep (NREMS) that have been implicated in multiple brain functions, including sleep quality, sensory gating, learning and memory. Despite considerable knowledge about the mechanisms underlying these neuronal rhythms, their function remains poorly understood and current views are largely based on correlational evidence. Here, we review recent studies in humans and rodents that have begun to broaden our understanding of the role of spindles in the normal and disordered brain. We show that newly identified molecular substrates of spindle oscillations, in combination with evolving technological progress, offer novel targets and tools to selectively manipulate spindles and dissect their role in sleep-dependent processes.

Highlights

- Newly recognized ion channel subtypes generating spindle rhythms are described.
- The contribution of spindles to arousal threshold and sleep quality is discussed.
- The proposed role of spindles in memory consolidation is examined.
- A function of thalamic spindles in neural development is suggested.

Review

Manipulating Sleep Spindles –

Expanding Views on Sleep, Memory and Disease

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Sleep spindles are distinctive electroencephalographic (EEG) oscillations emerging during non-rapid-eye-movement sleep (NREMS) that have been implicated in multiple brain functions, including sleep quality, sensory gating, learning and memory. Despite considerable knowledge about the mechanisms underlying these neuronal rhythms, their function remains poorly understood and current views are largely based on correlational evidence. Here, we review recent studies in humans and rodents that have begun to broaden our understanding of the role of spindles in the normal and disordered brain. We show that newly identified molecular substrates of spindle oscillations, in combination with evolving technological progress, offer novel targets and tools to selectively manipulate spindles and dissect their role in sleep-dependent processes.

Sleep spindles: from their first identification to their molecular substrates

Eighty years after the first description by the pioneers of EEG recordings [1, 2], sleep spindles have developed into a topical subject lying at the intersection of major areas of research in the neurosciences. The cellular and circuit bases of these unique EEG rhythms have been studied for decades *in vitro*, *in vivo* and *in computo* [3, 4], whereas, more recently, the search for their neurobiological functions has gained considerable attention. Research on sleep spindles has not only pioneered approaches to unravel novel functions of sleep, but has also extended to the pathophysiology of neuropsychiatric disorders.

In the sleeping human brain (Box1), spindle oscillations appear as brief (0.5-3 s) episodes of waxing-and-waning field potentials within a frequency range of ~9-15 Hz [5, 6]. Spindles are a hallmark for light stages of NREMS, during which they recur prominently once every 3-10 s in conjunction with other EEG rhythms between 0.5-16 Hz, but they are also found during deeper sleep stages [5]. Spindle-generating neuronal circuits reside in the intrathalamic network of *nucleus Reticularis thalami* (nRt) cells and thalamocortical (TC) cells (Figure 1).

What is the contribution of these discrete brief oscillatory events to sleep and its reportedly beneficial effects on brain function? Several correlational studies implicate spindles in memory consolidation and neuronal development, but there is little direct causal evidence. However, with recent technological progress, spindles now appear accessible as targets for selective manipulations that spare other sleep rhythms. For example, mutations inducing loss- or gain-of-function in nRt discharge have offered evidence for previously unrecognized roles of spindles in sleep quality and arousal threshold. With the upcoming optogenetic control of nRt, a battery of tools is currently being developed to unravel spindle function in the normal and diseased state.

Previous excellent reviews have thoroughly described cellular and network bases of spindle generation [3, 6, 7]. Here, we first review recent work on genetic models that has expanded the mechanistic understanding of this EEG rhythm through the modification of novel molecular substrates. We then discuss the current views about the functional aspects of spindles in brain physiology and pathology, as obtained from studies in naturally sleeping animals and humans. We highlight studies indicating that spindles are accessible for selective interventions, and that, with emerging technologies, will open avenues to decipher spindle function.

Novel molecular aspects of spindle generation

Sleep spindles emerge from a limited set of cellular participants: the resonating core of these waxing-and-waning oscillations resides in the nRt-TC loop, which sustains repetitive burst discharges of its cellular components under the control of cortical inputs (Figure 1). Neurons in the nRt, the main spindle pacemaker, possess a specialized assembly of ion channels, synaptic receptors and mechanisms for intracellular Ca^{2+} handling to sustain the vigorous rhythmic burst discharges necessary for spindle generation. Foremost amongst the ionic mechanisms underlying rhythmic nRt bursting are the low-voltage gated T-type Ca^{2+} channels (T channels) and the small-conductance Ca^{2+}-activated type-2 K^+ channel (SK2 or Kcnn2 channel). Bursting is additionally shaped by voltage-dependent K^+ channels [8], R-type channels [9], sarco/endoplasmic reticulum Ca^{2+}-ATPases [10], and Ca^{2+}-induced Ca^{2+} release via ryanodine receptors [11]. However, genetic manipulations of T channels and SK2 channels have proven particularly useful in selectively modifying sleep spindles [12, 13].

At NREMS onset, a progressive hyperpolarization of nRt neurons, caused by altered activity in modulatory afferents and in glutamatergic input, favors the activation of T channels

that rapidly and transiently depolarize the membrane voltage and elicits bursts of action potentials [14]. Reticular cells express two T channel subtypes encoded by the *$Ca_V3.2$* (*CACNA1h*) and the *$Ca_V3.3$* (*CACNA1i*) genes [15], which show strong dendritic expression with a somatofugally increasing gradient [16]. This organization enables amplification of distal synaptic inputs via low-threshold Ca^{2+} spikes and enhances dendritic responsiveness to somatic voltage fluctuations, as predicted by computational models of reconstructed nRt cells [17] (Box2). In addition, large $[Ca^{2+}]_i$ accumulations are generated upon low-threshold bursting triggered by synaptic stimulation or somatic current injections [10, 16]. Genetic deletion of $Ca_V3.3$ channels strongly reduces cellular T currents and prevents low-threshold bursting elicited through somatic hyperpolarizations [12]. Although the role of $Ca_V3.2$ channels in burst discharge still remains to be defined, these findings show that a single Ca^{2+} channel subtype, which has comparatively restricted expression throughout the TC system [18], dominates the unique discharge pattern important for spindle pacemaking.

Another important factor in the generation and maintenance of reticular intrinsic oscillations is the aforementioned SK2 or Kcnn2 channel. The vigorous Ca^{2+} influx in nRt dendrites originating from $Ca_V3.3$ channels gates SK2 channels, thereby creating a burst-afterhyperpolarization (bAHP) [12, 19]. As T channels recover partially from inactivation during bAHPs, nRt cells typically generate series of low-threshold bursts, with a rhythmicity also seen in nRt of sleeping animals [20, 21]. In $SK2^{-/-}$ mice, this oscillatory discharge is replaced by a single burst followed by a slowly decaying plateau potential [10], demonstrating that the cyclical $Ca_V3.3$-SK2 channel interaction is necessary for nRt rhythmicity. Conversely, genetic overexpression of SK2 channels resulted in increased bAHPs and prolonged cycles of repetitive bursting [13].

In sum, $Ca_V3.3$ and SK2 channels represent promising targets for manipulating the nRt intrinsic oscillations that underlie spindle generation in mouse (Figure 2). Indeed, knock-out of the $Ca_V3.3$ gene has led to selective impairment of spindles, as indicated by the reduction of σ power (10-15 Hz) at transitions between NREMS and REMS [12]. Conversely, SK2 overexpression resulted in prolongation of these transitory periods [13].

Deficits in EEG σ power were also detected in a mouse lacking the $Ca_V3.1$ isoform [22], the only T channel subtype expressed in relay neurons [15]. However, this phenotype was accompanied by a dramatic loss of spectral power in the δ frequency range (1-4 Hz), suggesting that TC cell burst discharge contributes to several sleep rhythms.

Neurons in nRt express a set of synaptic receptors that control initiation and synchronization of spindle oscillations. However, none of the genetic removals of these receptors has led to alterations selective for sleep spindles. For example, deletion of the β3 subunit of $GABA_A$ receptors, which in thalamus is specifically expressed in nRt, caused network hypersynchrony and generalized epilepsies [23]. Similarly, deletion of the α3 subunit of $GABA_A$ receptors, which in thalamus is also uniquely expressed in nRt, impaired evoked intrathalamic spindle-like oscillations by producing a compensatory gain in inhibitory inputs onto reticular cells [24], but led to a non-specific EEG phenotype [25]. Similar compensatory mechanisms underlie the thalamic hyperexcitability in stargazer mice, in which the deficient AMPAR trafficking was accompanied by an increased NMDAR component in nRt cells [26]. Finally, deletion of the GluA4 subunit in the $Gria4^{-/-}$ mouse specifically reduced cortico-nRt inputs, but, as a result, provoked global network hyperexcitability due to disinhibition of TC cells [27].

Another access point into spindle pacemaking is offered by the strong electrical coupling of reticular cells via Connexin36-dependent gap junctions [28], which promotes synchronization

and propagation of activity across nRt regions [29]. Recent work has highlighted unexpected features of this electrical coupling, such as a modified strength upon repetitive burst firing [30]. Local genetic or pharmacological manipulation of Connexin36 might thus represent a further tool to explore how subsets of nRt cells are engaged in reverberatory activity.

Temporal and spatial organization of sleep spindles

The coalescence of spindles with other sleep rhythms is being examined in both humans and rodents. A role in determining the temporal organization of sleep spindles was attributed to the cortical slow oscillations underlying the low-frequency power (<1 Hz) of the NREMS EEG (Box1)[31]. Through excitatory feedback via cortico-nRt synapses [32], cortical waves impose periodically recurring excitation onto nRt cells, thereby entraining intrathalamic spindles [31]. This cortical control could explain the frequently observed link of spindles to EEG slow-waves, and to K-complexes in humans [6]. This link is less evident in rodent EEG: spindles are hardly detectable as discrete events, but their spectral frequency (σ, primarily 10-15 Hz) appears throughout NREMS [33], with a predominance at periods when low-frequency power weakens. Accordingly, spindle activity in mice is estimated from the surge in σ power that starts ~30 s before NREMS-REMS transitions [12, 34, 35].

Cortical top-down control extends to hippocampal ripples, which are brief high-frequency oscillations (~100-250 Hz) detectable via local field potential recordings in hippocampal areas during NREMS [36]. Recordings in freely moving rodents revealed a close temporal association of ripples with spindles occurring mostly in prefrontal, but also in sensory cortical areas [37-39]. At low time scale, ripple power density increases before peaks of spindle activity within a 1-2 s window, and both oscillations are entrained by cortical inputs generated in depolarizing phases of slow rhythms. Fine-grained analyses revealed that ripples are nested into

succeeding troughs of spindles, generating so-called "spindle-ripple events". Human studies confirmed these findings [40], and revealed a fine-tuned coupling dependent on spindle topography, as discussed below [41]. The coalescence of TC and hippocampal rhythms provides strong experimental support for theories of sleep-promoted information transfer for long-term memory storage [5, 42]. Notably, during spindle epochs, cortical pyramidal neurons do not respond to ripples in deep layers, where hippocampal inputs arrive [37]. This suggests that the cortex is functionally deafferented from its hippocampal inputs during spindles, which is likely to have important implications for sensory processing and memory consolidation.

Spindle discharges have long been considered as global events occurring in synchrony across widespread cortical territories [43]. Accumulating data challenge this view by reporting a more complex topology. A first distinction involves a segregation of spindles according to their spectral frequency, as indicated by early observations [44] and as confirmed by recent studies using high resolution EEG or fMRI techniques. There is consistent evidence in humans for slower spindles (~9-13 Hz) occurring over frontal cortex, whereas faster spindles (~13-15 Hz) dominate in parietal and central sites and typically precede slow spindles by hundreds of ms [45-47]. *In vivo* work in rats and cats also hinted at the existence of two categories of spindles, characterized by distinct average frequency and amplitude: slow events (7-8 Hz) of high amplitude and fast events (primarily 10-20 Hz) of lower amplitude [48, 49]. These slow spindles display the typical waxing-and-waning pattern, whereas fast spindles are mainly waning [50]. Interestingly, fast spindles preferentially occur at transitions from hyperpolarized-to-depolarized states in the cortex [48], and appear to be correlated with reactivation of memory traces in rats [51]. Recent observations confirmed that, in humans, fast spindles also tend to be associated with depolarizing phases of slow oscillations, followed by slow spindles riding on hyperpolarizing

phases [41]. Moreover, the coupling between fast spindles and slow-waves is intensified after declarative learning [52, 53]. This suggests a scenario whereby fast spindles are implicated in hippocampal-cortical transfer of memory-related information, whereas slow spindles, in a second step, act to recruit frontal areas for memory storage [5].

A second (related) distinction involves the existence local spindles: evidence from EEG and MEG recordings in humans indicates that the majority of spindles are local events occurring in restricted brain regions [54, 55]. Local spindles can be either slow or fast, have a spatial extent that correlates with their amplitude, and, importantly, occur also in isolation from local slow oscillations. In addition, spindles can also be detected in the parahippocampal gyrus and hippocampus, and, to a minor extent, in entorhinal cortex and amygdala [45, 46].

The reasons for this complex topographic distribution are currently debated and include aspects of neocortical propagation and resonance, different contribution of thalamic nuclei and focal versus distributed TC projections from first- and higher-order thalamic nuclei [56] and the possibility of several spindle-generating loci [5]. Importantly, the local character of spindles is consistent with the observation of learning-induced local regulation of sleep waves [57], and, thus, represents a strong argument for a role of spindles in sleep-dependent memory consolidation.

Spindle function I: sleep quality and arousal threshold

One of the behavioral criteria defining sleep is an increased threshold to respond to external stimuli. The thalamus is the major source of input to the cortex and therefore represents a first site where sensory throughput can be vetoed. Historically, TC cells are thought to gate sensory transmission by switching from tonic to burst discharge mode [31]. Recent views propose that both modes can relay stimuli to the cortex, but, while tonic spikes reliably transmit

information, the stereotyped discharge profile of bursts leads to non-linear distortion of sensory inputs [58]. Burst firing of TC cells during spindles would thus filter external stimuli. Indeed, spindle activity has long been hypothesized to protect sleep against environmental disturbances: stronger acoustic stimulation is necessary to awaken human subjects during NREMS episodes containing spindles [59], and the natural variability in spontaneous spindle number during human sleep positively correlates with tolerance for environmental noise [60]. Furthermore, auditory cortex activation detected through fMRI is observed when subjects are exposed to noise during NREMS, but is virtually absent during spindle events [61].

Manipulations producing altered or inducible spindle activity in rodents offer new tools to address directly the role of spindles in gating sensory transmission, and to verify their impact on sleep quality. First evidence for a link between spindle and arousal threshold was recently provided by a mouse overexpressing SK2 channels and showing enhanced nRt bursting together with prolonged spindle activity at transitional periods out of NREMS [13]. These mice displayed higher arousal thresholds in response to white noise exposure, suggesting that nRt burst discharge contributes to the efficiency of sensory throughput in thalamus during sleep. In addition, NREMS episodes were less fragmented in these animals, indicating that sustaining spindle oscillations represents an approach to consolidate sleep. Increased sleep spindle activity was also reported when nRt was stimulated pharmacologically with a MT_2 melatonin receptor agonist [62]. This model provides an additional tool to assess the link between spindles and sleep quality by pharmacological means.

Two reports suggest that optogenetics will be helpful in functional investigations of sleep spindles. In the first case, nRt neurons were optically driven by expressing light-activated Channelrhodopsin-2 (ChR2) under the control of the vesicular GABA transporter (VGAT2). A

single, brief (20 ms) nRt activation intermittently resulted in cortical spindles during NREMS [63]. In transgenic mice expressing ChR2 under the Thy-1 promoter, photo-activation of the nRt at 8 Hz enhanced EEG power between 7-15 Hz [64]. In these animals, photostimulation increased NREMS duration, and the number of NREMS-REMS transitions correlated with the density of induced rhythmic activity. These first reports await further elaboration (Box3), nevertheless, photostimulated spindles will soon become exploitable to explore spindle function.

Spindle function II: cellular plasticity and memory consolidation

Novel forms of synaptic plasticity

Burst discharges constitute a powerful way to convey reliable synaptic inputs and often trigger synaptic plasticity [65]. Sleep spindles, being composed of discrete recurring packages of bursts, hence represent a potential substrate for synaptic plasticity. Data from simulations have indeed indicated that repetitive thalamic bursts generate robust Ca^{2+} entry in cortical dendrites [66], which might produce the favorable conditions to prime synapses for plastic changes, e.g. by activating proplastic signaling molecules such as protein kinase A and CaMKII [67].

The first described spindle-related plasticity was a form of short-term potentiation of electrical potentials in cortex referred to as "augmenting responses" [68], which could be reliably elicited by 10 Hz stimuli in different conditions *in vitro* and *in vivo* [69, 70]. Augmenting responses could be accompanied by medium-term cortical plasticity [71], but both potentiation and depression were possible outcomes depending on the background level of neuronal activity *in vivo* [72].

Long-term synaptic changes induced by spindles were first isolated by Rosanova and Ulrich (2005), who showed that *in vitro* reproduction of a natural firing pattern recorded in

cortex *in vivo* during sleep spindles induced hebbian long-term potentiation in rat somatosensory layer V pyramidal cells [73].

The synaptic potentiation induced by spindles seems to support the hypothesis of active system consolidation [5], which proposes that sleep, through specific rhythms such as spindles and ripples, potentiates memory traces by reactivating selected neuronal circuits. By contrast, slow oscillations, in which spindles are embedded, appear to also support depotentiation and to satisfy the principles of the synaptic homeostasis hypothesis (SHY) [74]. Opposite to the active system consolidation theory, this hypothesis posits that sleep promotes a global synaptic downscaling, ensuring an energetically sustainable redistribution of synaptic weights. Cellular work on slow oscillations has provided evidence for synaptic depotentiation. Pairing synaptic inputs to postsynaptic bursts at frequencies typical for slow-wave sleep in layer V pyramidal cells of somatosensory cortex induced long-term depression of glutamatergic transmission through removal of Ca^{2+}-permeable AMPARs [75]. In addition, AMPAR subunit content at these synapses displayed a time-of-day dependence consistent with a regulation through sleep-related plasticity [76]. Slow oscillations, mimicked through repetitive low-frequency discharge, have also been shown to potentiate inhibitory inputs in layer V in rat visual cortex [77]. Thus, according to these studies, slow oscillations promote an overall reduction of cortical excitation, which is consistent with the global downregulation proposed by SHY [74].

Two recent studies in naturally sleeping animals offered further insight on sleep-dependent plasticity. Chauvette et al. (2012) showed that somatosensory cortical-evoked local field potentials are upregulated after the first epochs of NREMS [78]. Grosmark et al. (2012) measured hippocampal firing rates during sequences of sleep stages [79]. Cell discharge was enhanced during NREMS and decreased during subsequent REMS. Across sleep, global

hippocampal spiking was reduced, but, opposite to this trend, the mean firing rate during ripples was augmented, due to increased synchrony of pyramidal cell firing. Taken together, the data from naturally sleeping animals suggests that, across the whole sequence of sleep stages, global synaptic downscaling develops in parallel with local reinforcement of specific networks. Furthermore, they highlight the importance of NREMS and REMS sequences in mnemonic processes and help to reconcile the opposing views of active system consolidation and SHY, as recently proposed [80].

Declarative and implicit memory

Accumulating evidence from studies in humans and animal models supports the view that learning and memory benefit from sleep. A fundamental open question concerns the role of the different sleep stages and of the underlying brain rhythms in relation to specific types of learning [81]. Thus, do spindles support memory consolidation? Similarly, does the capability of spindles in mediating synaptic plasticity represent the cellular basis of spindle-dependent learning?

Spindle activity during diurnal or nocturnal sleep following learning has been linked to recall performance of both explicit and procedural memories [5, 82, 83]. The main results come from studies on declarative memory in humans and in rodents; namely, increased spindling positively correlates with learning in declarative memory tasks [84, 85], with retention of a verbal task [86], and with recall of remote memories [87]. More recent studies indicate that spindles associate with subtle aspects of mnemonic processes, such as the integration of newly acquired information with the existing knowledge [88], or the preferential enhancement of memories expected to be recalled [89]. Although only a few reports have differentiated between slow and fast spindles [84, 90], the greater association between 13-15 Hz spindles, ripples and

slow oscillations ([41, 52, 91] but see [92]) hints to a specialized role of fast spindles in the transfer of hippocampal-dependent declarative memories to the cortex.

In addition to the link between sleep spindles and declarative memory reviewed above, there is some evidence indicating that spindles are also important for implicit, procedural memory, in particular for consolidation of simple motor skills. Human studies have shown a positive correlation between performance in motor tasks and the density of spindles [82, 93] or the duration of stage 2 NREMS (see Box1) after a training session [94-96]. Moreover, spindle activity appears to be locally modulated after training in restricted cortical areas involved in motor performance [94, 97], which corroborates the observation that learning induces local enhancement of sleep oscillations [57].

In sum, the evidence for a proplastic potential of spindles at cortical synapses [73] and for their close association with other sleep rhythms [37-41, 91] are substantiated by data reporting a positive correlation with learning performance. However, a direct non-correlative proof is still lacking. Assuming the case that spindles contribute to memory formation, it also remains to be assessed whether their function is active or only permissive: consolidation could be directly triggered by spindles, or merely benefit from their protective role on sleep architecture. With reduced interference by external stimuli and brief awakening episodes, the sequence of sleep stages becomes more consolidated, which could favor the process of long-term storage. In the absence of a causal link, it also remains a matter of debate whether spindles *per se* are important for learning, or whether spindling propensity merely reflects the efficiency and the connectivity of the TC system. A recent report, for example, states that the subjective spindle profile only provides an indirect measure of the cognitive and encoding performance during the learning phase, but is not decisive for sleep-dependent consolidation [98]. This interpretation is consistent

with two related observations. First, a correlation between spindle profile and intellectual ability, as assessed with IQ tests, has been reported by several studies (reviewed in [82]). This has led to the view that spindles, at least in non-pathological conditions, constitute a biophysical measure of intelligence. Second, spindle density in humans shows high inter-individual variability, but is very stable within subjects across different nights [99], which seems incompatible with a significant learning-dependent regulation.

In conclusion, although sleep spindles are increasingly recognized in the field of learning, there is no doubt that their exact role necessitates further investigation. An ideal approach to address this issue requires selective manipulation of this electrical rhythm during sleep after the training session. For example, in sleeping rats, specific suppression of hippocampal ripples successfully perturbed the acquisition of spatial memory [100], and presentation of a task-associated sensory cue biased hippocampal replay during sleep [101]. Similar attempts to acutely manipulate spindle activity without interfering with other sleep rhythms have not been implemented so far, but new tools, such as the optogenetic approaches mentioned, will certainly open up such possibilities.

Spindle function III: neuronal development

Neuronal activity emerges in the developing TC system before sensory perception matures and probably contributes to the early steps of cortical map organization [102]. In human brain, such primary electrical events, called δ-brushes, are observed from around 24 weeks of gestation. These occur as a slow wave superimposed with events at >8 Hz on a largely silent background and are sometimes accompanied by muscle twitches or limb movements. The rodent equivalent of δ-brushes are spindle bursts, which occur predominantly at σ frequencies, are

observed in the first postnatal week, and lead to transient synchronization of small cortical areas. Albeit shorter and highly localized, their waxing-and-waning waveform, the depth profile of cortical layer activity, and their association with thalamic burst discharge suggests a relation to sleep spindles [102, 103]. In somatosensory cortex of P0-P8 rats, spatially confined and somatotopically organized spindle bursts were triggered by limb movement in association with rhythmic thalamic burst discharge [103]. In visual cortex, spontaneous retinal waves occurring before eye opening elicited rhythmic spindle-like activity in a manner that is at least partially dependent on thalamic activity [102]. This indicates that spindle-like activity might be a common factor supporting cortical development across mammalian species [102]. Whether spindle bursts and classical sleep spindles share the same cellular and network mechanisms is an important issue that remains to be clarified. Interestingly, sleep spindles, and perhaps spindle bursts as well, may be accompanied by rhythmic fluctuations of intracellular Ca^{2+} and cAMP levels, which, when occurring phasically, can be important for synaptic development [104, 105]. For example, Ca^{2+} transients in cultured thalamic neurons drive the speed of TC axon growth through regulated expression of guidance receptors [106].

Sleep spindles in pathology

Aberrant spindle-like oscillations were first documented in an early study describing the presence of recurrent high-voltage events in mentally retarded children, referred to as “extreme spindles” [107]. Abnormal sleep spindles also characterize patients with a developmental disorder called Costello syndrome [108] and are found in subjects affected by Huntington's disease [109].

An extensively studied pathological perversion of spindle-generating circuits are the spike-wave discharges (SWDs) found in some types of idiopathic generalized epilepsies,

particularly in absence epilepsies [3]. During SWDs, nRt-TC interactions are abnormally powerful and lead to hypersynchronous discharges of both cell populations. Studies in animal models have implicated cortical hyperexcitability and unbalanced excitation/inhibition in the thalamic network, which is consistent with the activity observed in these regions during seizure attacks in humans [110]. Several genetic mouse lines with alterations of nRt excitability represent a good model to test therapeutic interventions for absence epilepsy [9, 24, 26, 27].

While the aforementioned cases involve thalamic hypersynchronization, a number of neuropathological conditions appear to be associated with a reduction in spindle activity, e.g. Alzheimer's disease [111], sporadic Creutzfeldt-Jakob disease [112], autism and Asperger's syndrome [113, 114], and affective disorders [115]. In most of these cases, spindle reduction is just one of several sleep abnormalities associated with the pathology. However, a singular case is represented by schizophrenia: recent reports using high-density EEG consistently indicate that the alterations in the spindle profile of schizophrenic subjects occur in dissociation from other sleep rhythms [116]. Ferrarelli et al. (2010) compared schizophrenic patients receiving pharmacological treatment with non-schizophrenic psychiatric patients receiving similar medications and with healthy control subjects. Aside from minor changes in global sleep architecture, schizophrenic patients showed major decreases in spindles. Interestingly, spindle number inversely correlated with the severity of clinical symptoms, but not with general cognitive ability [117]. Wamsley et al. (2011) reported that reduced spindle activity correlates with impaired consolidation of procedural memory and with increased severity of positive symptoms of schizophrenia [118]. In contrast, control participants showed no correlation between memory improvement and spindle number. Two other studies confirmed that, in schizophrenic patients, implicit memory does not benefit from sleep [119, 120].

The strong link between spindles and schizophrenia supports the view that this disorder originates from neurodevelopmental defects. Thus, impaired spindle activity could compromise the establishment of cortical sensory representations and give rise to the alterations in sensory processing that contribute to aberrant perception and distorted self-recognition in patients [121].

In sum, alterations in spindling profile have been linked to different neuropsychiatric disorders, suggesting that spindle hyper- and hypofunction can both contribute to the generation of clinical symptoms and, in some cases, could also play a decisive role in the etiology of the disease.

Concluding remarks

Recent studies in humans and rodents have generated a broader view of spindle functions and motivated novel approaches to selectively manipulate sleep spindles. Many new questions and hypotheses have arisen from these studies and should now be tested directly (Box3). One crucial area in which there is still debate is sleep's contribution to memory consolidation. Whether spindles actively promote learning or passively participate in mnemonic processes by preserving sleep quality is still an open question requiring spatiotemporally controlled manipulation of spindles during post-learning sleep. Whether slow and fast spindles represent separated phenomena with specific functions is another important question that awaits further anatomical and cellular analyses. Finally, it is striking that an altered spindle profile is associated with several disorders. Whether aberrant spindling is a source of pathology or merely reflects global TC deficits is a fundamental question that could be approached in animal models with altered spindle density to understand the significance of spindles in the development and maintenance of the healthy brain.

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Box 1. Sleep stages in humans and rodents

Human sleep is composed of alternating periods of non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS, also called paradoxical sleep), with NREMS subdivided in further 3 stages (N1, N2 and N3). As NREMS deepens, electrical potentials measured in cortical EEG recordings display rhythmic patterns of progressively lower frequencies with larger amplitude. Sleep spindles are brief (0.5-3 s) waxing-and-waning oscillations in the σ frequency range (~9-15 Hz) that are most prevalent during N2 and, thus, a defining feature of this stage. In rodents, sleep is more fragmented and composed of alternating bouts of NREMS, REMS and short awakenings. NREMS is not subdivided in further stages.

Figure I. Comparison of sleep stages in humans and rodents. **(a)** Schematic representation of a human and a rodent hypnogram during the first hours of sleep. In humans, NREMS is subdivided in stages of progressive depth (N1, N2 and N3). Of note, rodent sleep is more fragmented and is composed of brief episodes of NREMS and REMS alternated by waking. **(b)** Portions of EEG (color-coded) and EMG (gray) recordings in a human subject and a mouse, representing the distinct rhythms characterizing wake, NREMS and REMS. While muscle activity can occur during NREMS, REMS is characterized by complete atonia. In humans, sleep spindles become evident during N2 (highlighted in green and enlarged in the inset), and are often associated with K-complexes. In rodents, sleep spindles are typically not apparent in EEG traces from rodents, although they accumulate at periods before NREMS is terminated (see text for further details). Instead, local field potentials (LFP) recordings in deep cortical areas reveal comparable a profile of spindle event in humans and rodents **(c)**. For further details on human and mouse EEG/EMG

recordings see ref. [122] and [13]. Traces in **(c)** are modified with permission from ref. [45] and [37].

Box 2. Exploring the mechanisms of spindle generation with computational models

An essential role in the exploration of cellular and circuit mechanisms underlying spindle generation has been played by the computational approach, initially developed by Destexhe and colleagues (reviewed in [17]). Models of incremental complexity, from single cells to TC assemblies, were generated based mainly on experimental data from cats and ferrets. Notably, several mechanisms predicted *in computo* inspired subsequent experimental investigation and found validation *in vitro* and *in vivo*. For example, the dendritic localization of Ca^{2+} conductances in nRt cells was confirmed in a functional study demonstrating a somatofugal increase of T currents [16]. While the capability of the isolated nRt to generate spindles [123] could be reproduced in a network of reticular cells connected via GABAergic synapses, additional features became evident in models that included thalamic relay and cortical cells. A key example is the predicted role of Ca^{2+}-induced up-regulation of I_h in TC cells in governing spindle termination, which was subsequently confirmed experimentally [124]. The inclusion of cortical elements unraveled the important role of corticoreticular inputs in both triggering spindles and in synchronizing them across cortical regions. Interestingly, the model also predicted the perversion of spindles into hypersynchronous discharges of 3 Hz, typical for absence epilepsy and dependent of $GABA_B$-mediated currents. The involvement of increased corticothalamic feedback in provoking these paroxysmal oscillations was confirmed in ferrets [125, 126]. Subsequent computational work suggested a cortical contribution to spindle termination: asynchronous cortical firing during the waning phase depolarizes TC cells, thereby

inactivating T currents [127, 128]. A very recent modeling study implements TC matrix and core pathways, equivalent to distributed and focal projections, respectively, to show that global and local spindle detection may result from preferential monitoring of superficial and deeper cortical layer activity, respectively [56].

Box 3. Outstanding questions

- Many functional studies will be carried out through spindle manipulation in rodents, but how can spindles be optimally detected in these species? How does σ power, currently used as a measure of spindle activity, reflect discrete events? Are there differences in cortical areas with respect to spindle occurrence and frequency? Which measures optimally reflect learning-related changes in spindle profile?
- What it the origin of slow and fast spindles? Can the two types be directly and selectively manipulated based on their different pharmacological profile, as indicated by studies in humans [129] and animal models [50]? Do the similarities between slow spindles and α rhythms (~8-13 Hz) in humans and primates, typical for quiet wakefulness [130], reflect common generating mechanisms and functions?
- At what level do spindles "protect" the brain from sensory throughput? What is the role played by thalamic bursting? How is thalamic information carried by spindles integrated in cortex? Can we define layer- and neuron-specific roles? Cellular and EEG recording techniques in the naturally sleeping animal will need to be combined to address this question.

- What are the mechanisms and function of intrathalamic plasticity and to what extent do they contribute to the regulation of spindle intensity following learning or through sleep regulatory systems (e.g. circadian clock system)?
- The first implementations of optogenetics reported successful generation of photostimulated spindles, but to what extent do these reproduce genuine spindles or interfere with the endogenous spindle profile? Can the reverse approach be implemented, i.e. optogenetic inhibition of spindles? While optogenetics represents a promising tool, improvements are needed with respect to the efficiency and the spatial confinement of the photostimulation.
- Which tools and strategies can we derive from rodent studies to improve learning performance in humans?
- How do sleep spindles contribute to development of the TC circuits and to the establishment of cortical sensory maps? With mouse models showing chronically altered spindle activity, such questions can now be addressed.
- Which are the cellular mechanisms underlying spindle bursts? To what extent are these developmental rhythms premature sleep spindles? Will optogenetics be a helpful tool to dissect the role of these early brain rhythms in TC development?
- Do sleep spindles and associated $[Ca^{2+}]_i$ transients trigger biochemical signaling cascades that are important for sleep? cAMP waves are observed in TC cells during spindle-related burst discharges, but it is not clear how such signals are decoded in these cells. Is there a link between such cascades and a recent report showing that nuclear β-catenin is present in thalamus through adulthood and drives expression of genes involved in the particular excitability patterns of these cells [131]?

- Is the molecular make-up of nRt cells (including ion cannels such as $Ca_V3.3$, SK2 and receptors such as the MT_2 melatonin receptor) suitable to design novel drugs for pharmacotherapy to alleviate sleep disturbances and symptoms of neuropsychiatric diseases? Are pharmacosynthetic tools such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)[132] suitable for bi-directional modulation of overall excitability of nRt?

Figure 1. Spindle generating circuit and overview of proposed spindle function.

Spindle oscillations arise in the thalamocortical (TC) network from a combination of intrinsic cellular and network properties. At the heart of the spindle rhythm lies the *nucleus Reticularis thalami* (nRt), a thin layer of GABAergic neurons that envelopes the dorsal thalamus (green). Rhythmic inhibition provided by nRt cells entrains rebound burst activity of glutamatergic TC projection neurons (red). Excitatory TC-nRt connections allow the oscillation to resonate in an intra-thalamic loop. Spindles are generated autonomously in the thalamus, resist complete decortication, as originally observed in cats by Morison and Bassett [133], and even persist in the deafferented nRt [123]. Yet, the cortical (blue) feedback is essential to provide synchronization and spatial coherence of spindling over widespread thalamic regions [48]. More recently, a cortical control of intra- and inter-spindle periods has been postulated [128]. Three distinct phases can be distinguished in sleep spindles, as consistently shown by *in vivo* intracellular recordings. In the initial waxing phase, the barrage of inhibitory postsynaptic potentials provided by nRt is not sufficient to induce rebound bursting in TC cells. In the middle phase, rebound bursts accompanied by action potentials recur in many TC cells, which excite both nRt and cortical neurons. In the final waning phase, both thalamic and cortical firing become less regular and the oscillation terminates. *In vitro* work has explained spindle termination with the Ca^{2+}-dependent upregulation of a depolarizing I_h current in TC cells [124, 134], which induces a refractory inter-spindle period. However, recent data based on a computational model of the TC system have highlighted a contribution of corticothalamic inputs [128]; during the waning phase, cortical firing is no longer phase-locked with inhibitory postsynaptic potentials and drives an overall depolarization in TC cells. This prevents deinactivation of T channels, thus impeding rebound bursting. Traces on the left are modified with permission from ref. [128].

Figure 2. Cellular and sleep phenotype of mice with genetic manipulations of ion channels involved in nRt low-threshold bursting. **(a)** Schematic representation of voltage-gated T-type Ca^{2+} channels and Ca^{2+}-activated K^+ channels expressed in the membrane of nRt neurons in wild-type, in $Ca_V3.3^{-/-}$, $SK2^{-/-}$ and SK2-overexpressing (SK2-OE) mice. Note the additional expression of $Ca_V3.2$ channels [15]. **(b)** T channel activation produces biphasic currents (black line) resulting from an inward (orange) and an outward (blue) component, mediated by T channels and by SK2 channels, respectively. Suppression of Ca^{2+} influx in $Ca_V3.3^{-/-}$ mice impairs SK2 activation [12]. Manipulation of SK2 expression in $SK2^{-/-}$ and SK2-OE mice does not induce compensatory effects in T channel expression [10, 13]. **(c)** Repetitive burst discharge elicited at the offset of somatic hyperpolarizations is impaired in $Ca_V3.3^{-/-}$ mice. In $SK2^{-/-}$ mice, the initial burst discharge is followed by a slow-decay plateau potential, whereas SK2 overexpression prolongs repetitive discharge. **(d)** At transition between epochs of NREMS and REMS, the EEG power spectrum displays a characteristic surge in the σ frequency range, which is strongly decreased by genetic deletion of $Ca_V3.3$ and SK2 channels. In SK2-OE mice, peak of σ power is reduced compared to wild-type mice, but the surge is prolonged. Gray traces represent single EEG recordings in naturally sleeping mice filtered in the σ frequency range, whereas blue traces represent average σ power profile normalized to the period between 1-3 min prior to transition (for details on EEG analysis see [12, 13]).

Figurel

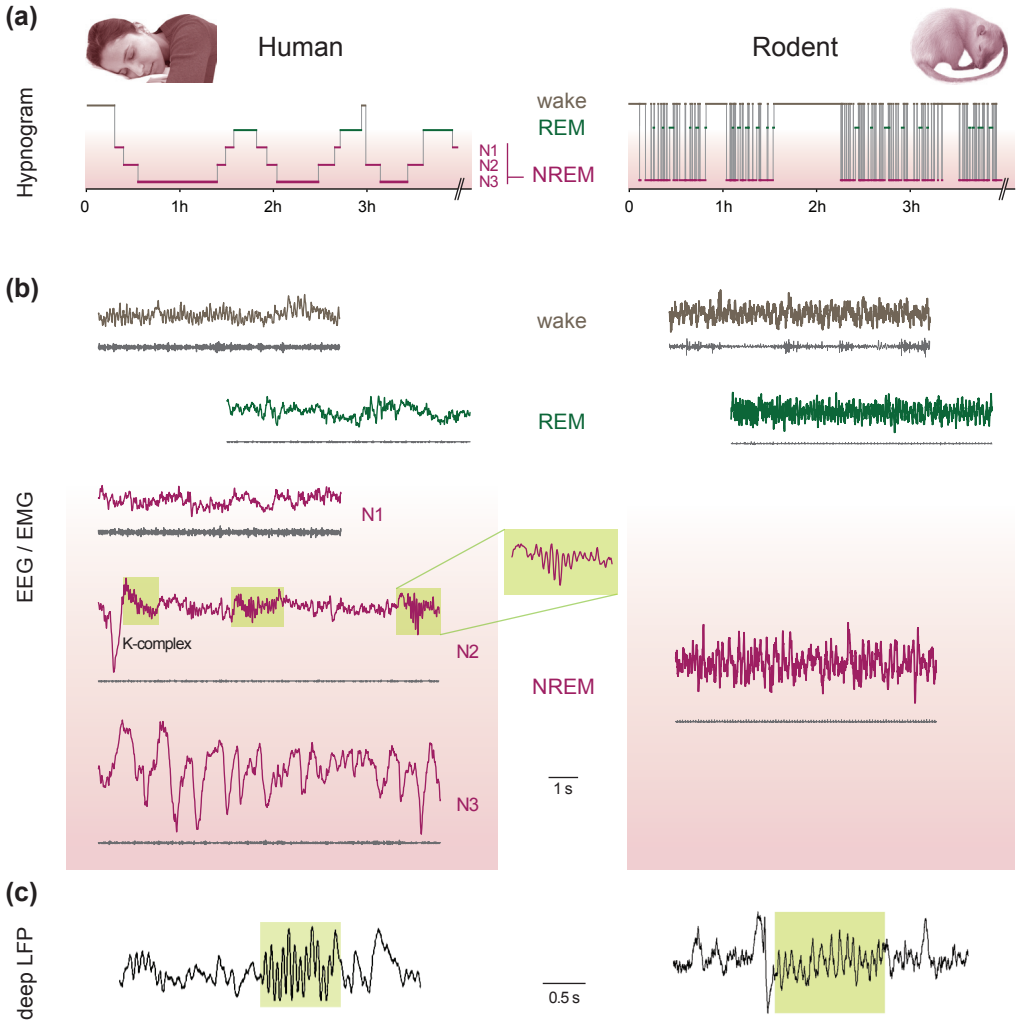

(a)
Human
Rodent
Hypnogram
wake
REM
N1
N2
N3
NREM
0
1h
2h
3h
(b)
wake
REM
EEG / EMG
N1
K-complex
N2
NREM
N3
1 s
(c)
deep LFP
0.5 s

Figure1

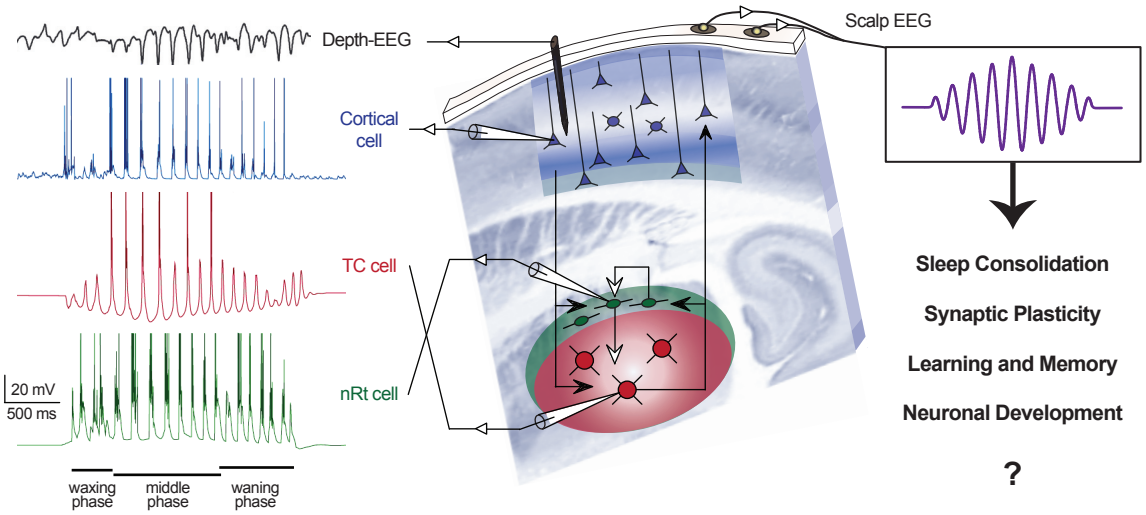
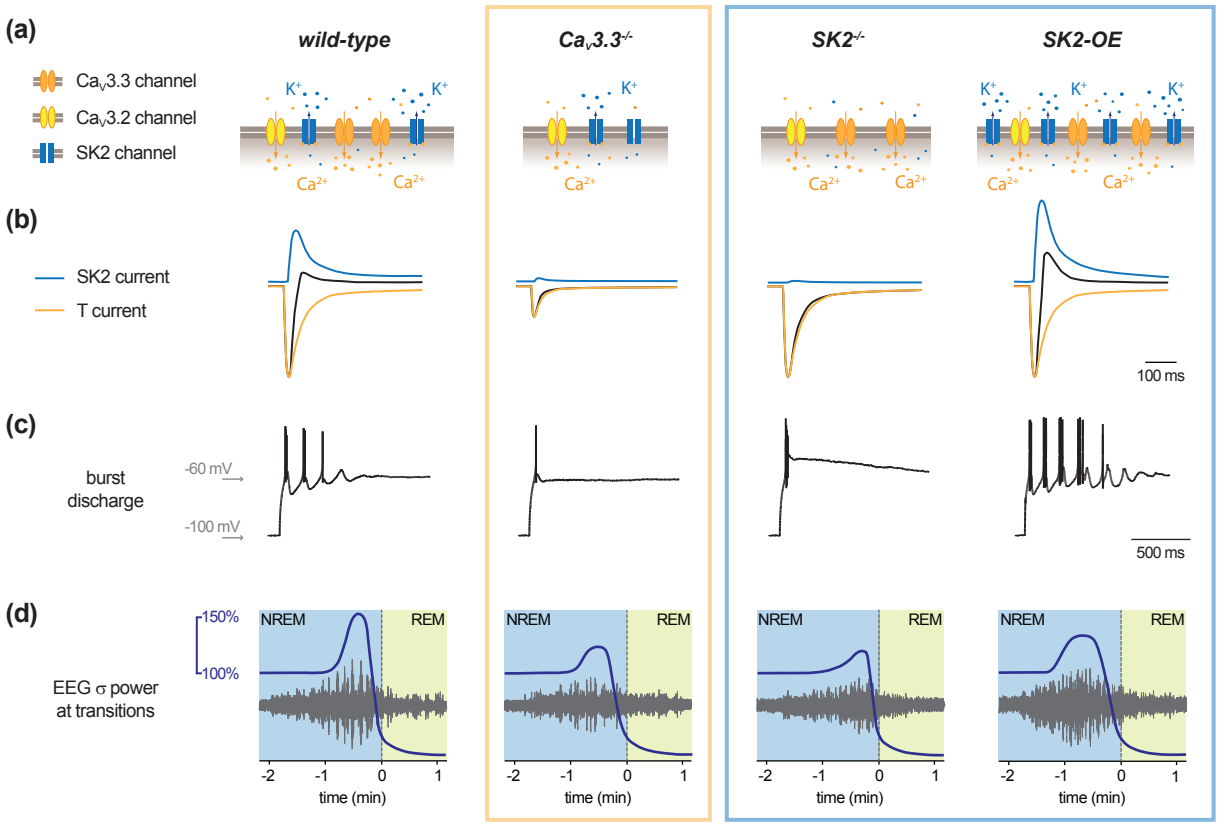

Depth-EEG
Cortical cell
TC cell
nRt cell
20 mV
500 ms
waxing phase
middle phase
waning phase
Scalp EEG
Sleep Consolidation
Synaptic Plasticity
Learning and Memory
Neuronal Development
?

Figure2


(a)
wild-type
Cav3.3-/-
SK2-/-
SK2-OE
Cav3.3 channel
Cav3.2 channel
SK2 channel
K+
Ca2+
(b)
SK2 current
T current
100 ms
(c)
burst discharge
-60 mV
-100 mV
500 ms
(d)
EEG σ power at transitions
150%
100%
NREM
REM
-2
-1
0
1
time (min)