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Trends in Neurosciences

Manipulating Sleep Spindles - Expanding Views on Sleep, Memory and Disease --Manuscript Draft--

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Abstract:	<p>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.</p>

Astori et al.

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.

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4 **Review**
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7 **Manipulating Sleep Spindles –**
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9 **Expanding Views on Sleep, Memory and Disease**
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48 **Keywords:** synaptic plasticity, optogenetics, calcium channels, SK channels,
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50 electroencephalography, thalamocortical system, sleep waves.
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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.

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

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26 Sleep spindles emerge from a limited set of cellular participants: the resonating core of
27 these waxing-and-waning oscillations resides in the nRt-TC loop, which sustains repetitive burst
28 discharges of its cellular components under the control of cortical inputs (Figure 1). Neurons in
29 the nRt, the main spindle pacemaker, possess a specialized assembly of ion channels, synaptic
30 receptors and mechanisms for intracellular Ca^{2+} handling to sustain the vigorous rhythmic burst
31 discharges necessary for spindle generation. Foremost amongst the ionic mechanisms underlying
32 rhythmic nRt bursting are the low-voltage gated T-type Ca^{2+} channels (T channels) and the
33 small-conductance Ca^{2+} -activated type-2 K^+ channel (SK2 or Kcnn2 channel). Bursting is
34 additionally shaped by voltage-dependent K^+ channels [8], R-type channels [9],
35 sarco/endoplasmic reticulum Ca^{2+} -ATPases [10], and Ca^{2+} -induced Ca^{2+} release via ryanodine
36 receptors [11]. However, genetic manipulations of T channels and SK2 channels have proven
37 particularly useful in selectively modifying sleep spindles [12, 13].
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55 At NREMS onset, a progressive hyperpolarization of nRt neurons, caused by altered
56 activity in modulatory afferents and in glutamatergic input, favors the activation of T channels
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4 that rapidly and transiently depolarize the membrane voltage and elicits bursts of action
5 potentials [14]. Reticular cells express two T channel subtypes encoded by the *Ca_v3.2*
6 (*CACNA1h*) and the *Ca_v3.3* (*CACNA1i*) genes [15], which show strong dendritic expression with
7 a somatofugally increasing gradient [16]. This organization enables amplification of distal
8 synaptic inputs via low-threshold Ca²⁺ spikes and enhances dendritic responsiveness to somatic
9 voltage fluctuations, as predicted by computational models of reconstructed nRt cells [17]
10 (Box2). In addition, large [Ca²⁺]_i accumulations are generated upon low-threshold bursting
11 triggered by synaptic stimulation or somatic current injections [10, 16]. Genetic deletion of
12 Ca_v3.3 channels strongly reduces cellular T currents and prevents low-threshold bursting elicited
13 through somatic hyperpolarizations [12]. Although the role of Ca_v3.2 channels in burst discharge
14 still remains to be defined, these findings show that a single Ca²⁺ channel subtype, which has
15 comparatively restricted expression throughout the TC system [18], dominates the unique
16 discharge pattern important for spindle pacemaking.
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36 Another important factor in the generation and maintenance of reticular intrinsic
37 oscillations is the aforementioned SK2 or *Kcnn2* channel. The vigorous Ca²⁺ influx in nRt
38 dendrites originating from Ca_v3.3 channels gates SK2 channels, thereby creating a burst-
39 afterhyperpolarization (bAHP) [12, 19]. As T channels recover partially from inactivation during
40 bAHPs, nRt cells typically generate series of low-threshold bursts, with a rhythmicity also seen
41 in nRt of sleeping animals [20, 21]. In SK2^{-/-} mice, this oscillatory discharge is replaced by a
42 single burst followed by a slowly decaying plateau potential [10], demonstrating that the cyclical
43 Ca_v3.3-SK2 channel interaction is necessary for nRt rhythmicity. Conversely, genetic
44 overexpression of SK2 channels resulted in increased bAHPs and prolonged cycles of repetitive
45 bursting [13].
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4 In sum, Ca_v3.3 and SK2 channels represent promising targets for manipulating the nRt
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6 intrinsic oscillations that underlie spindle generation in mouse (Figure 2). Indeed, knock-out of
7
8 the Ca_v3.3 gene has led to selective impairment of spindles, as indicated by the reduction of σ
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10 power (10-15 Hz) at transitions between NREMS and REMS [12]. Conversely, SK2
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12 overexpression resulted in prolongation of these transitory periods [13].
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16 Deficits in EEG σ power were also detected in a mouse lacking the Ca_v3.1 isoform [22],
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18 the only T channel subtype expressed in relay neurons [15]. However, this phenotype was
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20 accompanied by a dramatic loss of spectral power in the δ frequency range (1-4 Hz), suggesting
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22 that TC cell burst discharge contributes to several sleep rhythms.
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26 Neurons in nRt express a set of synaptic receptors that control initiation and
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28 synchronization of spindle oscillations. However, none of the genetic removals of these receptors
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30 has led to alterations selective for sleep spindles. For example, deletion of the β 3 subunit of
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32 GABA_A receptors, which in thalamus is specifically expressed in nRt, caused network
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34 hypersynchrony and generalized epilepsies [23]. Similarly, deletion of the α 3 subunit of GABA_A
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36 receptors, which in thalamus is also uniquely expressed in nRt, impaired evoked intrathalamic
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38 spindle-like oscillations by producing a compensatory gain in inhibitory inputs onto reticular
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40 cells [24], but led to a non-specific EEG phenotype [25]. Similar compensatory mechanisms
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42 underlie the thalamic hyperexcitability in stargazer mice, in which the deficient AMPAR
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44 trafficking was accompanied by an increased NMDAR component in nRt cells [26]. Finally,
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46 deletion of the GluA4 subunit in the *Gria4*^{-/-} mouse specifically reduced cortico-nRt inputs, but,
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48 as a result, provoked global network hyperexcitability due to disinhibition of TC cells [27].
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56 Another access point into spindle pacemaking is offered by the strong electrical coupling
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58 of reticular cells via Connexin36-dependent gap junctions [28], which promotes synchronization
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4 and propagation of activity across nRt regions [29]. Recent work has highlighted unexpected
5 features of this electrical coupling, such as a modified strength upon repetitive burst firing [30].
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7 Local genetic or pharmacological manipulation of Connexin36 might thus represent a further
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9 tool to explore how subsets of nRt cells are engaged in reverberatory activity.
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16 **Temporal and spatial organization of sleep spindles**

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18 The coalescence of spindles with other sleep rhythms is being examined in both humans
19 and rodents. A role in determining the temporal organization of sleep spindles was attributed to
20 the cortical slow oscillations underlying the low-frequency power (<1 Hz) of the NREMS EEG
21 (Box1)[31]. Through excitatory feedback via cortico-nRt synapses [32], cortical waves impose
22 periodically recurring excitation onto nRt cells, thereby entraining intrathalamic spindles [31].
23
24 This cortical control could explain the frequently observed link of spindles to EEG slow-waves,
25 and to K-complexes in humans [6]. This link is less evident in rodent EEG: spindles are hardly
26 detectable as discrete events, but their spectral frequency (σ , primarily 10-15 Hz) appears
27 throughout NREMS [33], with a predominance at periods when low-frequency power weakens.
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29 Accordingly, spindle activity in mice is estimated from the surge in σ power that starts ~30 s
30 before NREMS-REMS transitions [12, 34, 35].
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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

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4 succeeding troughs of spindles, generating so-called “spindle-ripple events”. Human studies
5 confirmed these findings [40], and revealed a fine-tuned coupling dependent on spindle
6 topography, as discussed below [41]. The coalescence of TC and hippocampal rhythms provides
7 strong experimental support for theories of sleep-promoted information transfer for long-term
8 memory storage [5, 42]. Notably, during spindle epochs, cortical pyramidal neurons do not
9 respond to ripples in deep layers, where hippocampal inputs arrive [37]. This suggests that the
10 cortex is functionally deafferented from its hippocampal inputs during spindles, which is likely
11 to have important implications for sensory processing and memory consolidation.
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23 Spindle discharges have long been considered as global events occurring in synchrony
24 across widespread cortical territories [43]. Accumulating data challenge this view by reporting a
25 more complex topology. A first distinction involves a segregation of spindles according to their
26 spectral frequency, as indicated by early observations [44] and as confirmed by recent studies
27 using high resolution EEG or fMRI techniques. There is consistent evidence in humans for
28 slower spindles (~9-13 Hz) occurring over frontal cortex, whereas faster spindles (~13-15 Hz)
29 dominate in parietal and central sites and typically precede slow spindles by hundreds of ms [45-
30 47]. *In vivo* work in rats and cats also hinted at the existence of two categories of spindles,
31 characterized by distinct average frequency and amplitude: slow events (7-8 Hz) of high
32 amplitude and fast events (primarily 10-20 Hz) of lower amplitude [48, 49]. These slow spindles
33 display the typical waxing-and-waning pattern, whereas fast spindles are mainly waning [50].
34 Interestingly, fast spindles preferentially occur at transitions from hyperpolarized-to-depolarized
35 states in the cortex [48], and appear to be correlated with reactivation of memory traces in rats
36 [51]. Recent observations confirmed that, in humans, fast spindles also tend to be associated with
37 depolarizing phases of slow oscillations, followed by slow spindles riding on hyperpolarizing
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4 phases [41]. Moreover, the coupling between fast spindles and slow-waves is intensified after
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6 declarative learning [52, 53]. This suggests a scenario whereby fast spindles are implicated in
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8 hippocampal-cortical transfer of memory-related information, whereas slow spindles, in a second
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10 step, act to recruit frontal areas for memory storage [5].
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14 A second (related) distinction involves the existence local spindles: evidence from EEG
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16 and MEG recordings in humans indicates that the majority of spindles are local events occurring
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18 in restricted brain regions [54, 55]. Local spindles can be either slow or fast, have a spatial extent
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20 that correlates with their amplitude, and, importantly, occur also in isolation from local slow
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22 oscillations. In addition, spindles can also be detected in the parahippocampal gyrus and
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24 hippocampus, and, to a minor extent, in entorhinal cortex and amygdala [45, 46].
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28 The reasons for this complex topographic distribution are currently debated and include
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30 aspects of neocortical propagation and resonance, different contribution of thalamic nuclei and
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32 focal versus distributed TC projections from first- and higher-order thalamic nuclei [56] and the
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34 possibility of several spindle-generating loci [5]. Importantly, the local character of spindles is
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36 consistent with the observation of learning-induced local regulation of sleep waves [57], and,
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38 thus, represents a strong argument for a role of spindles in sleep-dependent memory
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40 consolidation.
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47 **Spindle function I: sleep quality and arousal threshold**

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50 One of the behavioral criteria defining sleep is an increased threshold to respond to
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52 external stimuli. The thalamus is the major source of input to the cortex and therefore represents
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54 a first site where sensory throughput can be vetoed. Historically, TC cells are thought to gate
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56 sensory transmission by switching from tonic to burst discharge mode [31]. Recent views
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58 propose that both modes can relay stimuli to the cortex, but, while tonic spikes reliably transmit
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4 information, the stereotyped discharge profile of bursts leads to non-linear distortion of sensory
5 inputs [58]. Burst firing of TC cells during spindles would thus filter external stimuli. Indeed,
6 spindle activity has long been hypothesized to protect sleep against environmental disturbances:
7 stronger acoustic stimulation is necessary to awaken human subjects during NREMS episodes
8 containing spindles [59], and the natural variability in spontaneous spindle number during human
9 sleep positively correlates with tolerance for environmental noise [60]. Furthermore, auditory
10 cortex activation detected through fMRI is observed when subjects are exposed to noise during
11 NREMS, but is virtually absent during spindle events [61].
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23 Manipulations producing altered or inducible spindle activity in rodents offer new tools
24 to address directly the role of spindles in gating sensory transmission, and to verify their impact
25 on sleep quality. First evidence for a link between spindle and arousal threshold was recently
26 provided by a mouse overexpressing SK2 channels and showing enhanced nRt bursting together
27 with prolonged spindle activity at transitional periods out of NREMS [13]. These mice displayed
28 higher arousal thresholds in response to white noise exposure, suggesting that nRt burst
29 discharge contributes to the efficiency of sensory throughput in thalamus during sleep. In
30 addition, NREMS episodes were less fragmented in these animals, indicating that sustaining
31 spindle oscillations represents an approach to consolidate sleep. Increased sleep spindle activity
32 was also reported when nRt was stimulated pharmacologically with a MT₂ melatonin receptor
33 agonist [62]. This model provides an additional tool to assess the link between spindles and sleep
34 quality by pharmacological means.
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52 Two reports suggest that optogenetics will be helpful in functional investigations of sleep
53 spindles. In the first case, nRt neurons were optically driven by expressing light-activated
54 Channelrhodopsin-2 (ChR2) under the control of the vesicular GABA transporter (VGAT2). A
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4 single, brief (20 ms) nRt activation intermittently resulted in cortical spindles during NREMS
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6 [63]. In transgenic mice expressing ChR2 under the Thy-1 promoter, photo-activation of the nRt
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8 at 8 Hz enhanced EEG power between 7-15 Hz [64]. In these animals, photostimulation
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10 increased NREMS duration, and the number of NREMS-REMS transitions correlated with the
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12 density of induced rhythmic activity. These first reports await further elaboration (Box3),
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14 nevertheless, photostimulated spindles will soon become exploitable to explore spindle function.
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21 **Spindle function II: cellular plasticity and memory consolidation**

22 *Novel forms of synaptic plasticity*

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25 Burst discharges constitute a powerful way to convey reliable synaptic inputs and often
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27 trigger synaptic plasticity [65]. Sleep spindles, being composed of discrete recurring packages of
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29 bursts, hence represent a potential substrate for synaptic plasticity. Data from simulations have
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31 indeed indicated that repetitive thalamic bursts generate robust Ca^{2+} entry in cortical dendrites
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33 [66], which might produce the favorable conditions to prime synapses for plastic changes, e.g. by
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35 activating proplastic signaling molecules such as protein kinase A and CaMKII [67].
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41 The first described spindle-related plasticity was a form of short-term potentiation of
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43 electrical potentials in cortex referred to as “augmenting responses” [68], which could be reliably
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45 elicited by 10 Hz stimuli in different conditions *in vitro* and *in vivo* [69, 70]. Augmenting
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47 responses could be accompanied by medium-term cortical plasticity [71], but both potentiation
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49 and depression were possible outcomes depending on the background level of neuronal activity
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51 *in vivo* [72].
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56 Long-term synaptic changes induced by spindles were first isolated by Rosanova and
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58 Ulrich (2005), who showed that *in vitro* reproduction of a natural firing pattern recorded in
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4 cortex *in vivo* during sleep spindles induced hebbian long-term potentiation in rat somatosensory
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6 layer V pyramidal cells [73].
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9 The synaptic potentiation induced by spindles seems to support the hypothesis of active
10 system consolidation [5], which proposes that sleep, through specific rhythms such as spindles
11 and ripples, potentiates memory traces by reactivating selected neuronal circuits. By contrast,
12 slow oscillations, in which spindles are embedded, appear to also support depotentiation and to
13 satisfy the principles of the synaptic homeostasis hypothesis (SHY) [74]. Opposite to the active
14 system consolidation theory, this hypothesis posits that sleep promotes a global synaptic
15 downscaling, ensuring an energetically sustainable redistribution of synaptic weights. Cellular
16 work on slow oscillations has provided evidence for synaptic depotentiation. Pairing synaptic
17 inputs to postsynaptic bursts at frequencies typical for slow-wave sleep in layer V pyramidal
18 cells of somatosensory cortex induced long-term depression of glutamatergic transmission
19 through removal of Ca²⁺-permeable AMPARs [75]. In addition, AMPAR subunit content at these
20 synapses displayed a time-of-day dependence consistent with a regulation through sleep-related
21 plasticity [76]. Slow oscillations, mimicked through repetitive low-frequency discharge, have
22 also been shown to potentiate inhibitory inputs in layer V in rat visual cortex [77]. Thus,
23 according to these studies, slow oscillations promote an overall reduction of cortical excitation,
24 which is consistent with the global downregulation proposed by SHY [74].
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48 Two recent studies in naturally sleeping animals offered further insight on sleep-
49 dependent plasticity. Chauvette et al. (2012) showed that somatosensory cortical-evoked local
50 field potentials are upregulated after the first epochs of NREMS [78]. Grosmark et al. (2012)
51 measured hippocampal firing rates during sequences of sleep stages [79]. Cell discharge was
52 enhanced during NREMS and decreased during subsequent REMS. Across sleep, global
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4 hippocampal spiking was reduced, but, opposite to this trend, the mean firing rate during ripples
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6 was augmented, due to increased synchrony of pyramidal cell firing. Taken together, the data
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8 from naturally sleeping animals suggests that, across the whole sequence of sleep stages, global
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10 synaptic downscaling develops in parallel with local reinforcement of specific networks.
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12 Furthermore, they highlight the importance of NREMS and REMS sequences in mnemonic
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14 processes and help to reconcile the opposing views of active system consolidation and SHY, as
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16 recently proposed [80].
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23 *Declarative and implicit memory*

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26 Accumulating evidence from studies in humans and animal models supports the view that
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28 learning and memory benefit from sleep. A fundamental open question concerns the role of the
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30 different sleep stages and of the underlying brain rhythms in relation to specific types of learning
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32 [81]. Thus, do spindles support memory consolidation? Similarly, does the capability of spindles
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34 in mediating synaptic plasticity represent the cellular basis of spindle-dependent learning?
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39 Spindle activity during diurnal or nocturnal sleep following learning has been linked to
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41 recall performance of both explicit and procedural memories [5, 82, 83]. The main results come
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43 from studies on declarative memory in humans and in rodents; namely, increased spindling
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45 positively correlates with learning in declarative memory tasks [84, 85], with retention of a
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47 verbal task [86], and with recall of remote memories [87]. More recent studies indicate that
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49 spindles associate with subtle aspects of mnemonic processes, such as the integration of newly
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51 acquired information with the existing knowledge [88], or the preferential enhancement of
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53 memories expected to be recalled [89]. Although only a few reports have differentiated between
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55 slow and fast spindles [84, 90], the greater association between 13-15 Hz spindles, ripples and
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4 slow oscillations ([41, 52, 91] but see [92]) hints to a specialized role of fast spindles in the
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6 transfer of hippocampal-dependent declarative memories to the cortex.
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9 In addition to the link between sleep spindles and declarative memory reviewed above,
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11 there is some evidence indicating that spindles are also important for implicit, procedural
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13 memory, in particular for consolidation of simple motor skills. Human studies have shown a
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15 positive correlation between performance in motor tasks and the density of spindles [82, 93] or
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17 the duration of stage 2 NREMS (see Box1) after a training session [94-96]. Moreover, spindle
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19 activity appears to be locally modulated after training in restricted cortical areas involved in
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21 motor performance [94, 97], which corroborates the observation that learning induces local
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23 enhancement of sleep oscillations [57].
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28 In sum, the evidence for a proplastic potential of spindles at cortical synapses [73] and for
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30 their close association with other sleep rhythms [37-41, 91] are substantiated by data reporting a
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32 positive correlation with learning performance. However, a direct non-correlative proof is still
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34 lacking. Assuming the case that spindles contribute to memory formation, it also remains to be
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36 assessed whether their function is active or only permissive: consolidation could be directly
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38 triggered by spindles, or merely benefit from their protective role on sleep architecture. With
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40 reduced interference by external stimuli and brief awakening episodes, the sequence of sleep
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42 stages becomes more consolidated, which could favor the process of long-term storage. In the
43
44 absence of a causal link, it also remains a matter of debate whether spindles *per se* are important
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46 for learning, or whether spindling propensity merely reflects the efficiency and the connectivity
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48 of the TC system. A recent report, for example, states that the subjective spindle profile only
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50 provides an indirect measure of the cognitive and encoding performance during the learning
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52 phase, but is not decisive for sleep-dependent consolidation [98]. This interpretation is consistent
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4 with two related observations. First, a correlation between spindle profile and intellectual ability,
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6 as assessed with IQ tests, has been reported by several studies (reviewed in [82]). This has led to
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8 the view that spindles, at least in non-pathological conditions, constitute a biophysical measure
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10 of intelligence. Second, spindle density in humans shows high inter-individual variability, but is
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12 very stable within subjects across different nights [99], which seems incompatible with a
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14 significant learning-dependent regulation.
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19 In conclusion, although sleep spindles are increasingly recognized in the field of learning,
20
21 there is no doubt that their exact role necessitates further investigation. An ideal approach to
22
23 address this issue requires selective manipulation of this electrical rhythm during sleep after the
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25 training session. For example, in sleeping rats, specific suppression of hippocampal ripples
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27 successfully perturbed the acquisition of spatial memory [100], and presentation of a task-
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29 associated sensory cue biased hippocampal replay during sleep [101]. Similar attempts to acutely
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31 manipulate spindle activity without interfering with other sleep rhythms have not been
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33 implemented so far, but new tools, such as the optogenetic approaches mentioned, will certainly
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35 open up such possibilities.
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43 **Spindle function III: neuronal development**

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45 Neuronal activity emerges in the developing TC system before sensory perception
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47 matures and probably contributes to the early steps of cortical map organization [102]. In human
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49 brain, such primary electrical events, called δ -brushes, are observed from around 24 weeks of
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51 gestation. These occur as a slow wave superimposed with events at >8 Hz on a largely silent
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53 background and are sometimes accompanied by muscle twitches or limb movements. The rodent
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55 equivalent of δ -brushes are spindle bursts, which occur predominantly at σ frequencies, are
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4 observed in the first postnatal week, and lead to transient synchronization of small cortical areas.
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6 Albeit shorter and highly localized, their waxing-and-waning waveform, the depth profile of
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8 cortical layer activity, and their association with thalamic burst discharge suggests a relation to
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10 sleep spindles [102, 103]. In somatosensory cortex of P0-P8 rats, spatially confined and
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12 somatotopically organized spindle bursts were triggered by limb movement in association with
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14 rhythmic thalamic burst discharge [103]. In visual cortex, spontaneous retinal waves occurring
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16 before eye opening elicited rhythmic spindle-like activity in a manner that is at least partially
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18 dependent on thalamic activity [102]. This indicates that spindle-like activity might be a common
19
20 factor supporting cortical development across mammalian species [102]. Whether spindle bursts
21
22 and classical sleep spindles share the same cellular and network mechanisms is an important
23
24 issue that remains to be clarified. Interestingly, sleep spindles, and perhaps spindle bursts as well,
25
26 may be accompanied by rhythmic fluctuations of intracellular Ca^{2+} and cAMP levels, which,
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28 when occurring phasically, can be important for synaptic development [104, 105]. For example,
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30 Ca^{2+} transients in cultured thalamic neurons drive the speed of TC axon growth through
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32 regulated expression of guidance receptors [106].
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43 **Sleep spindles in pathology**

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45 Aberrant spindle-like oscillations were first documented in an early study describing the
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47 presence of recurrent high-voltage events in mentally retarded children, referred to as “extreme
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49 spindles” [107]. Abnormal sleep spindles also characterize patients with a developmental
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51 disorder called Costello syndrome [108] and are found in subjects affected by Huntington's
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53 disease [109].
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57 An extensively studied pathological perversion of spindle-generating circuits are the
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59 spike-wave discharges (SWDs) found in some types of idiopathic generalized epilepsies,
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4 particularly in absence epilepsies [3]. During SWDs, nRt-TC interactions are abnormally
5
6 powerful and lead to hypersynchronous discharges of both cell populations. Studies in animal
7
8 models have implicated cortical hyperexcitability and unbalanced excitation/inhibition in the
9
10 thalamic network, which is consistent with the activity observed in these regions during seizure
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12 attacks in humans [110]. Several genetic mouse lines with alterations of nRt excitability
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14 represent a good model to test therapeutic interventions for absence epilepsy [9, 24, 26, 27].
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19 While the aforementioned cases involve thalamic hypersynchronization, a number of
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21 neuropathological conditions appear to be associated with a reduction in spindle activity, e.g.
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23 Alzheimer's disease [111], sporadic Creutzfeldt-Jakob disease [112], autism and Asperger's
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25 syndrome [113, 114], and affective disorders [115]. In most of these cases, spindle reduction is
26
27 just one of several sleep abnormalities associated with the pathology. However, a singular case is
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29 represented by schizophrenia: recent reports using high-density EEG consistently indicate that
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31 the alterations in the spindle profile of schizophrenic subjects occur in dissociation from other
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33 sleep rhythms [116]. Ferrarelli et al. (2010) compared schizophrenic patients receiving
34
35 pharmacological treatment with non-schizophrenic psychiatric patients receiving similar
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37 medications and with healthy control subjects. Aside from minor changes in global sleep
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39 architecture, schizophrenic patients showed major decreases in spindles. Interestingly, spindle
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41 number inversely correlated with the severity of clinical symptoms, but not with general
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43 cognitive ability [117]. Wamsley et al. (2011) reported that reduced spindle activity correlates
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45 with impaired consolidation of procedural memory and with increased severity of positive
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47 symptoms of schizophrenia [118]. In contrast, control participants showed no correlation
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49 between memory improvement and spindle number. Two other studies confirmed that, in
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51 schizophrenic patients, implicit memory does not benefit from sleep [119, 120].
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4 The strong link between spindles and schizophrenia supports the view that this disorder
5 originates from neurodevelopmental defects. Thus, impaired spindle activity could compromise
6 the establishment of cortical sensory representations and give rise to the alterations in sensory
7 processing that contribute to aberrant perception and distorted self-recognition in patients [121].
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14 In sum, alterations in spindling profile have been linked to different neuropsychiatric
15 disorders, suggesting that spindle hyper- and hypofunction can both contribute to the generation
16 of clinical symptoms and, in some cases, could also play a decisive role in the etiology of the
17 disease.
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23 24 25 26 **Concluding remarks**

27
28 Recent studies in humans and rodents have generated a broader view of spindle functions and
29 motivated novel approaches to selectively manipulate sleep spindles. Many new questions and
30 hypotheses have arisen from these studies and should now be tested directly (Box3). One crucial
31 area in which there is still debate is sleep's contribution to memory consolidation. Whether
32 spindles actively promote learning or passively participate in mnemonic processes by preserving
33 sleep quality is still an open question requiring spatiotemporally controlled manipulation of
34 spindles during post-learning sleep. Whether slow and fast spindles represent separated
35 phenomena with specific functions is another important question that awaits further anatomical
36 and cellular analyses. Finally, it is striking that an altered spindle profile is associated with
37 several disorders. Whether aberrant spindling is a source of pathology or merely reflects global
38 TC deficits is a fundamental question that could be approached in animal models with altered
39 spindle density to understand the significance of spindles in the development and maintenance of
40 the healthy brain.
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5 **Box 1. Sleep stages in humans and rodents**
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8 Human sleep is composed of alternating periods of non-rapid-eye-movement sleep (NREMS)
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10 and rapid-eye-movement sleep (REMS, also called paradoxical sleep), with NREMS subdivided
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12 in further 3 stages (N1, N2 and N3). As NREMS deepens, electrical potentials measured in
13
14 cortical EEG recordings display rhythmic patterns of progressively lower frequencies with larger
15
16 amplitude. Sleep spindles are brief (0.5-3 s) waxing-and-waning oscillations in the σ frequency
17
18 range (~9-15 Hz) that are most prevalent during N2 and, thus, a defining feature of this stage. In
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20 rodents, sleep is more fragmented and composed of alternating bouts of NREMS, REMS and
21
22 short awakenings. NREMS is not subdivided in further stages.
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30 **Figure I.** Comparison of sleep stages in humans and rodents. **(a)** Schematic representation of a
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32 human and a rodent hypnogram during the first hours of sleep. In humans, NREMS is subdivided
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34 in stages of progressive depth (N1, N2 and N3). Of note, rodent sleep is more fragmented and is
35
36 composed of brief episodes of NREMS and REMS alternated by waking. **(b)** Portions of EEG
37
38 (color-coded) and EMG (gray) recordings in a human subject and a mouse, representing the
39
40 distinct rhythms characterizing wake, NREMS and REMS. While muscle activity can occur
41
42 during NREMS, REMS is characterized by complete atonia. In humans, sleep spindles become
43
44 evident during N2 (highlighted in green and enlarged in the inset), and are often associated with
45
46 K-complexes. In rodents, sleep spindles are typically not apparent in EEG traces from rodents,
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48 although they accumulate at periods before NREMS is terminated (see text for further details).
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50 Instead, local field potentials (LFP) recordings in deep cortical areas reveal comparable a profile
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52 of spindle event in humans and rodents **(c)**. For further details on human and mouse EEG/EMG
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4 recordings see ref. [122] and [13]. Traces in (c) are modified with permission from ref. [45] and
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6 [37].
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10 **Box 2. Exploring the mechanisms of spindle generation with computational models**

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12 An essential role in the exploration of cellular and circuit mechanisms underlying spindle
13
14 generation has been played by the computational approach, initially developed by Destexhe and
15
16 colleagues (reviewed in [17]). Models of incremental complexity, from single cells to TC
17
18 assemblies, were generated based mainly on experimental data from cats and ferrets. Notably,
19
20 several mechanisms predicted *in computo* inspired subsequent experimental investigation and
21
22 found validation *in vitro* and *in vivo*. For example, the dendritic localization of Ca^{2+}
23
24 conductances in nRt cells was confirmed in a functional study demonstrating a somatofugal
25
26 increase of T currents [16]. While the capability of the isolated nRt to generate spindles [123]
27
28 could be reproduced in a network of reticular cells connected via GABAergic synapses,
29
30 additional features became evident in models that included thalamic relay and cortical cells. A
31
32 key example is the predicted role of Ca^{2+} -induced up-regulation of I_h in TC cells in governing
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34 spindle termination, which was subsequently confirmed experimentally [124]. The inclusion of
35
36 cortical elements unraveled the important role of corticoreticular inputs in both triggering
37
38 spindles and in synchronizing them across cortical regions. Interestingly, the model also
39
40 predicted the perversion of spindles into hypersynchronous discharges of 3 Hz, typical for
41
42 absence epilepsy and dependent of GABA_B -mediated currents. The involvement of increased
43
44 corticothalamic feedback in provoking these paroxysmal oscillations was confirmed in ferrets
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46 [125, 126]. Subsequent computational work suggested a cortical contribution to spindle
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48 termination: asynchronous cortical firing during the waning phase depolarizes TC cells, thereby
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4 inactivating T currents [127, 128]. A very recent modeling study implements TC matrix and core
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6 pathways, equivalent to distributed and focal projections, respectively, to show that global and
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8 local spindle detection may result from preferential monitoring of superficial and deeper cortical
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10 layer activity, respectively [56].
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16 **Box 3. Outstanding questions**

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19 • Many functional studies will be carried out through spindle manipulation in rodents, but
20
21 how can spindles be optimally detected in these species? How does σ power, currently
22
23 used as a measure of spindle activity, reflect discrete events? Are there differences in
24
25 cortical areas with respect to spindle occurrence and frequency? Which measures
26
27 optimally reflect learning-related changes in spindle profile?
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- 30
31 • What is the origin of slow and fast spindles? Can the two types be directly and selectively
32
33 manipulated based on their different pharmacological profile, as indicated by studies in
34
35 humans [129] and animal models [50]? Do the similarities between slow spindles and α
36
37 rhythms (~8-13 Hz) in humans and primates, typical for quiet wakefulness [130], reflect
38
39 common generating mechanisms and functions?
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43 • At what level do spindles “protect” the brain from sensory throughput? What is the role
44
45 played by thalamic bursting? How is thalamic information carried by spindles integrated
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47 in cortex? Can we define layer- and neuron-specific roles? Cellular and EEG recording
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49 techniques in the naturally sleeping animal will need to be combined to address this
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51 question.
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- 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]?

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- Is the molecular make-up of nRt cells (including ion channels 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?

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4 **Figure 1.** Spindle generating circuit and overview of proposed spindle function.
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7 Spindle oscillations arise in the thalamocortical (TC) network from a combination of intrinsic
8
9 cellular and network properties. At the heart of the spindle rhythm lies the *nucleus Reticularis*
10
11 *thalami* (nRt), a thin layer of GABAergic neurons that envelopes the dorsal thalamus (green).
12
13 Rhythmic inhibition provided by nRt cells entrains rebound burst activity of glutamatergic TC
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15 projection neurons (red). Excitatory TC-nRt connections allow the oscillation to resonate in an
16
17 intra-thalamic loop. Spindles are generated autonomously in the thalamus, resist complete
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19 decortication, as originally observed in cats by Morison and Bassett [133], and even persist in the
20
21 deafferented nRt [123]. Yet, the cortical (blue) feedback is essential to provide synchronization
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23 and spatial coherence of spindling over widespread thalamic regions [48]. More recently, a
24
25 cortical control of intra- and inter-spindle periods has been postulated [128]. Three distinct
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27 phases can be distinguished in sleep spindles, as consistently shown by *in vivo* intracellular
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29 recordings. In the initial waxing phase, the barrage of inhibitory postsynaptic potentials provided
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31 by nRt is not sufficient to induce rebound bursting in TC cells. In the middle phase, rebound
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33 bursts accompanied by action potentials recur in many TC cells, which excite both nRt and
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35 cortical neurons. In the final waning phase, both thalamic and cortical firing become less regular
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37 and the oscillation terminates. *In vitro* work has explained spindle termination with the Ca^{2+} -
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39 dependent upregulation of a depolarizing I_h current in TC cells [124, 134], which induces a
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41 refractory inter-spindle period. However, recent data based on a computational model of the TC
42
43 system have highlighted a contribution of corticothalamic inputs [128]; during the waning phase,
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45 cortical firing is no longer phase-locked with inhibitory postsynaptic potentials and drives an
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47 overall depolarization in TC cells. This prevents deinactivation of T channels, thus impeding
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49 rebound bursting. Traces on the left are modified with permission from ref. [128].
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5 **Figure 2.** Cellular and sleep phenotype of mice with genetic manipulations of ion channels
6 involved in nRt low-threshold bursting. **(a)** Schematic representation of voltage-gated T-type
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8 Ca^{2+} channels and Ca^{2+} -activated K^+ channels expressed in the membrane of nRt neurons in
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10 wild-type, in $\text{Ca}_v3.3^{-/-}$, $\text{SK2}^{-/-}$ and SK2-overexpressing (SK2-OE) mice. Note the additional
11
12 expression of $\text{Ca}_v3.2$ channels [15]. **(b)** T channel activation produces biphasic currents (black
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14 line) resulting from an inward (orange) and an outward (blue) component, mediated by T
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16 channels and by SK2 channels, respectively. Suppression of Ca^{2+} influx in $\text{Ca}_v3.3^{-/-}$ mice impairs
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18 SK2 activation [12]. Manipulation of SK2 expression in $\text{SK2}^{-/-}$ and SK2-OE mice does not
19
20 induce compensatory effects in T channel expression [10, 13]. **(c)** Repetitive burst discharge
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22 elicited at the offset of somatic hyperpolarizations is impaired in $\text{Ca}_v3.3^{-/-}$ mice. In $\text{SK2}^{-/-}$ mice,
23
24 the initial burst discharge is followed by a slow-decay plateau potential, whereas SK2
25
26 overexpression prolongs repetitive discharge. **(d)** At transition between epochs of NREMS and
27
28 REMS, the EEG power spectrum displays a characteristic surge in the σ frequency range, which
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30 is strongly decreased by genetic deletion of $\text{Ca}_v3.3$ and SK2 channels. In SK2-OE mice, peak of
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32 σ power is reduced compared to wild-type mice, but the surge is prolonged. Gray traces represent
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34 single EEG recordings in naturally sleeping mice filtered in the σ frequency range, whereas blue
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36 traces represent average σ power profile normalized to the period between 1-3 min prior to
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38 transition (for details on EEG analysis see [12, 13]).
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Figure 1

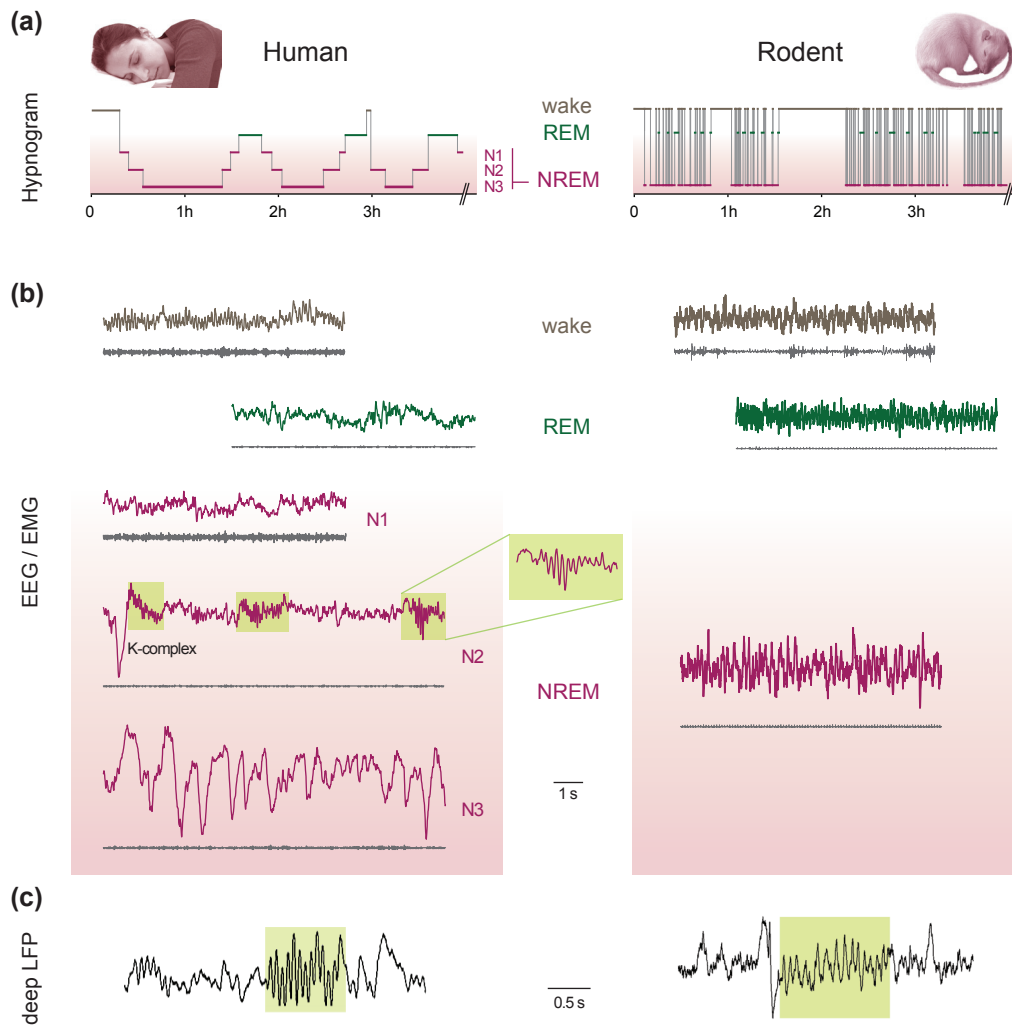


Figure 1

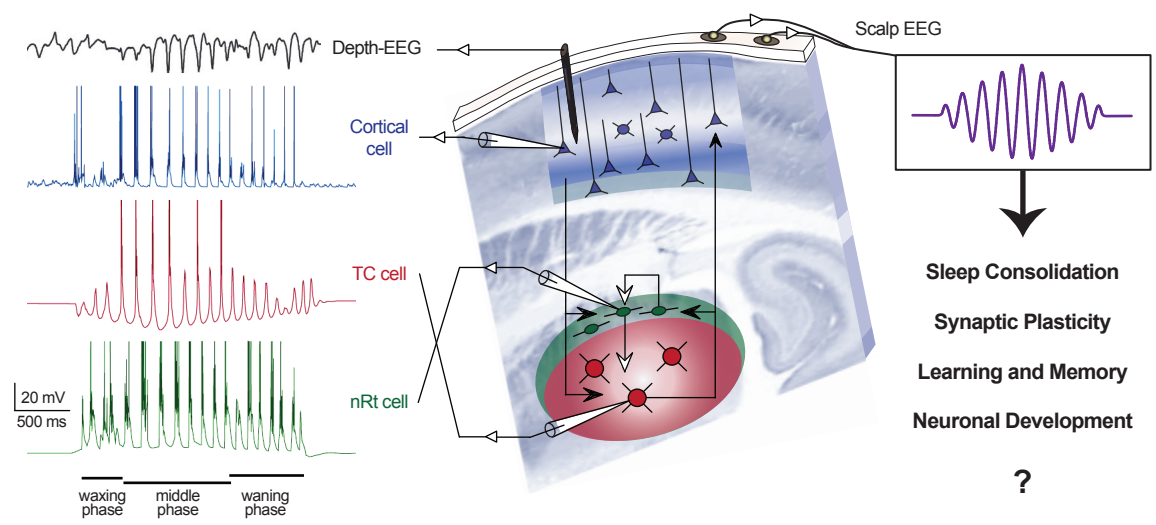


Figure 2

