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Sleep spindles –

Where they come from, what they do

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Abstract

Sleep spindles are extensively studied electroencephalographical rhythms that recur periodically during non-rapid-eye-movement sleep and that are associated with rhythmic discharges of neurons throughout the thalamocortical system. Their occurrence thus constrains many aspects of the communication between thalamus and cortex, ranging from sensory transmission, to cortical plasticity and learning, to development and disease. I review these functional aspects in conjunction with novel findings on the cellular and molecular make-up of spindle-pacemaking circuits. A highlight in the search of roles for sleep spindles is the repeated finding that spindles correlate with memory consolidation in humans and animals. By illustrating that spindles are at the forefront understanding on how the brain might benefit from sleep rhythms, I hope to stimulate further experimentation.

A What are sleep spindle waves?

Sleep spindles are brief oscillatory events that appear in the human electroencephalogram (EEG) during periods of drowsiness and sleep. These brain-generated electrical rhythms are prominent EEG hallmarks of non-rapid-eye-movement (NREM) sleep, which makes up for ~80% of a normal night's sleep. Per night, 4-5 epochs of ~90 min are spent in NREM sleep. Stage N1 of NREM sleep refers to drowsiness, stage N2 to light sleep, and stage N3 to deep and restorative sleep. The remaining 20% consist of rapid-eye-movement (REM) sleep that is well-known for the rapid movements of the eyes and for vivid dreaming periods.

During NREM sleep, the brain generates electrical rhythms that are synchronized throughout large cortical areas. The NREM sleep EEG shows a continuous power spectrum of

frequencies, from 0.75 Hz up to tens of Hz, with a broad peak centered ~0.5-4 Hz, indicating a dominance of low-frequency rhythms. Three rhythms have a distinct graphical signature on a background of on-going oscillations and serve as EEG hallmarks of NREM sleep: the K-complexes (0.5-1 Hz), delta (δ) rhythms (1-4 Hz) and the sleep spindles, also referred to as the sleep sigma (σ) band (10-15 Hz). A single sleep spindle lasts ~0.5-3 s and is recognized based on ~5-20 successive deflections of the human EEG signal (Fig. 1A). These deflections reach a maximum at the middle of the spindle event, gradually increasing before ("waxing") and decreasing thereafter ("waning"), thence the name "spindles" (Fig. 1A). Within the consolidated NREM sleep epochs lasting ~90 min in humans, spindles are but brief oscillatory events that recur maximally a few times per minute.

The neural mechanisms of spindle generation have been studied for decades in different mammalian species, initially via electrophysiological and computational methods, later in combination with molecular and imaging approaches. A surge of interest in spindles sparked in the past years in the context of sleep's implication in learning and memory formation (Fogel and Smith 2011; Rasch and Born 2013). In humans, spindles are now divided into two types that have different frequencies, topographic distributions and putative functional roles, as indicated by non-invasive scalp EEG recordings and functional imaging techniques (Fogel and Smith 2011; Rasch and Born 2013).

B Where do spindles come from?

The earliest reports on spindles go along with the very first attempts to monitor brain electricity across the skull using galvanometers (Box 1). Since then, the thalamus has moved to center stage as the site of origin of spindle wave generation. The electrical events underlying spindle rhythmicity, and their spread throughout the thalamocortical (TC) system,

are now documented at the level of EEG and of local field potential recordings in the anesthetized and sleeping brain, from rodent, to carnivore, to primate, to human (Fig. 1A, B). Moreover, spindle-related rhythms can be reproduced in brain slices from ferrets and rodents and have been followed from multiunit activity, to single cell discharge, to the flux of specific ions across the membrane (Fig. 1C-E).

There are three successive phases of spindles that are comparable between species and that I review sequentially here: 1) the initiation of spindle generation, 2) spindle pacemaking and synchronization, and 3) spindle termination. Many important mechanistic aspects of spindle generation have been previously reviewed in detail (Beenhakker and Huguenard 2009; McCormick and Bal 1997; Timofeev and others 2012). I will present well-established knowledge briefly referencing key review articles, while concentrating on recent insights in more detail. I will highlight in particular that spindles occur in temporal coordination with other sleep rhythms, and are increasingly understood as just one element in what appears to be a large-scale grouping of brain rhythms throughout the TC and the hippocampal system.

B1. The thalamic reticular nucleus

The first electrophysiological recordings of TC neurons, the principal excitatory neurons of thalamic nuclei projecting to cortex, indicated that rhythmic inhibitory synaptic potentials always occurred in phase with spindle wave generation (Purpura 1968). Andersen and Andersson (Andersen and Andersson 1968) postulated a set of widely disseminated inhibitory distributor neurons in thalamus to explain this coordinated inhibition. Based on lesion experiments, Steriade and colleagues realized that, rather than a diffusely distributed circuit, a well-defined nucleus encircling the thalamus, the thalamic reticular nucleus (TRN),

generates this inhibition and thereby acts as a spindle pacemaker (reviewed in (Fuentealba and Steriade 2005)). The TRN forms a thin envelope, ~1 mm in diameter in humans, around most of the principal thalamic nuclei and covers these like an umbrella along their frontal-caudal extent. TRN cells throughout mammalian species appear uniform in that they all express the neurotransmitter γ-aminobutyric acid (GABA), they possess long and thin dendrites running in parallel to the TRN borders, and most of them express the Ca^{2+}-binding protein parvalbumin; nevertheless, cells in TRN probably form functionally heterogeneous classes that go along with variable soma size, discharge properties, dendritic appendages and expression of other Ca^{2+}-binding proteins (Fig. 2)(Pinault 2004; Scheibel and Scheibel 1966). The TRN is subdivided into several, partially overlapping sensory sectors that receive topographically organized sensory information from visual, auditory, somatosensory, gustatory, visceral, motor and limbic circuits (Pinault 2004; Zikopoulos and Barbas 2007). The TRN does not project to cortex, whereas it is strongly innervated by descending cortical input arising in deep layer 6 from corresponding first-order sensory areas, and, at least in monkey, from prefrontal cortex that may overlap several sensory domains. The TRN cells target most principal thalamic nuclei in all species and synapse onto excitatory TC cells (reviewed in (Fuentealba and Steriade 2005; Pinault 2004)). Axonal projections arborize extensively and penetrate deeply into principal thalamus, crossing several nuclei in horizontal dorsal-caudal projections (Scheibel and Scheibel 1966) and innervating numerous TC neurons through multiple, closely spaced (< 10 μm) presynaptic boutons (Wanaverbecq and others 2008). By providing GABAergic inhibitory feedback to specific and non-specific thalamus, the TRN controls communication between thalamic nuclei and across TC loops. By being highly responsive to cortical feedback from sensory and higher cortical areas, the TRN shapes multiple aspects of sensory transmission, ranging from receptive field organization

up to anticipation and attentional modulation of sensory perception (reviewed in (Pinault 2004; Vukadinovic 2011)).

What is the current understanding of how the TRN network acts as a pacemaker for spindles? Since the first unit recordings from TRN cells in sleeping cats, it became clear that the TRN is a very active brain nucleus that discharges at typically at least ~20 Hz throughout all vigilance states, but the exact temporal pattern of discharge varies (Mukhametov and others 1970). Thalamic neurons, and TRN cells in particular, undergo substantial changes in resting membrane potentials according to the state of vigilance of the brain (reviewed in (Bal and McCormick 1997)). When the activity in wake-promoting structures ceases, as is the case at sleep onset (Saper and others 2010), depolarizing effects of monoaminergic neurotransmitters on TRN are withdrawn and the resting membrane potentials falls by 10-12 mV from levels ~-50 mV to <-60 mV. Whereas regularly timed action potential discharge is elicited from depolarized resting membrane potentials (>-55 mV), TRN cells switch to a drastically different discharge mode once they are resting at ~-65 mV or at more hyperpolarized levels. This is because membrane hyperpolarization overlaps with the activation range of a voltage-gated Ca^{2+} channel, also called low-threshold Ca^{2+} channel or T-channel (Huguenard 1996). T-channels are encoded by a family of 3 genes, *$Ca_V3.1$*, *$Ca_V3.2$* and *$Ca_V3.3$*, of which the latter two are expressed in TRN (Astori and others 2011). As their name says, these channels possess a low activation threshold (~-60 mV) and provoke triangular-shaped Ca^{2+} spikes that rapidly, yet transiently, depolarize the neuron to threshold for Na^+ action potentials (Huguenard 1996). Action potentials riding on top of these Ca^{2+} spikes are then grouped in high-frequency bursts of several hundred Hz that are key to TRN's capacities to pacemake spindles.

B2. The initiation of sleep spindles

Cells in TRN undoubtedly belong to the most dedicated burst-generating neurons in the brain, as exemplified by the combination of powerful cellular and synaptic specifications (Fig. 2). The most prevalent T-channel subtype of the TRN cells is the $Ca_V3.3$ channel that is expressed at high densities along TRN dendrites and that makes up for ~70% of somatically recorded current, with the remaining current being generated by $Ca_V3.2$ channels (Astori and others 2011). T-channel expression occurs in a gradient that increases with increasing somatodendritic distance and that reaches maximum levels ~100 μm from the soma, as indicated through Ca^{2+} imaging (Crandall and others 2010). At more distal sites (>125 μm), the large amount of T-channels boosts the burst propensity of these dendritic compartments. A burst generated therein propagates to the soma and ensures a massive depolarizing drive, explaining the robust Ca^{2+} spike generated in TRN somas that initiates bursts of action potential discharge in TRN axons (Destexhe and others 1996). Voltage-gated Ca^{2+} channels of the R-type (Zaman and others 2011) and rapid K^+ channels of the K_V3 gene family (Espinosa and others 2008) further sustain the rhythmic burst discharge mechanism.

What are the synaptic mechanisms triggering burst discharge? Low-threshold spikes are easily initiated in distal dendritic compartments through glutamatergic stimuli, independently of the somatic membrane polarization (Crandall and others 2010). At these sites, corticothalamic projections predominate and provide one of the most important initial triggers for TRN bursting through the generation of excitatory postsynaptic potentials (EPSPs) (Fig. 3A)(reviewed in (Fuentealba and Steriade 2005)). Cortico-TRN synapses are the most abundant excitatory afferents onto TRN cells; these synapses have high levels of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type ionotropic glutamate receptors at their postsynaptic sites and the pronounced frequency facilitation of the

synapses imposes a powerful excitatory drive (reviewed in (Fuentealba and Steriade 2005)). The dominant role of cortico-TRN projections in triggering burst discharge is important for the temporal coordination of spindles with other sleep rhythms (see also Chapter B6). Additionally, several modulatory synaptic afferents arising in wake-promoting brain stem impinge onto TRN cells that could regulate spindle occurrence at periods of NREM sleep termination (Fig. 2)(see e.g. (Sun and others 2013)).

Recently, light-induced activation of channelrhodopsin-2 expressed in mouse TRN cells was used to induce putative burst discharge, and such stimuli were occasionally followed by spindle-like rhythms in the EEG during NREM sleep states (Halassa and others 2011; Kim and others 2012). This shows directly that TRN bursts can drive EEG sleep spindles.

TRN cells provide a strong inhibitory output to TC cells via the generation of burst inhibitory postsynaptic potentials (burst IPSPs, Fig. 3B) that involve synaptic $GABA_A$ receptors (reviewed in (Beenhakker and Huguenard 2009; McCormick and Bal 1997)) and, through spillover of GABA, extrasynaptically located $GABA_A$ receptors (Fig. 3B)((Rovó and others, FENS Abstract 2012). Additionally, extrasynaptic $GABA_B$ receptors activated during bursts contribute a sizeable, slow component of the burst-mediated hyperpolarization (reviewed in (Beenhakker and Huguenard 2009)). Widely used sedative-hypnotic agents for insomnia allosterically strengthen $GABA_A$ receptor function and typically boost σ power in the NREM sleep EEG, consistent with the important role of these receptors in spindle-generating networks (reviewed in (Winsky-Sommerer 2009)).

B3. The pacemaking and synchronization of spindles

Through their divergent projections, single TRN cells inhibit multiple TC cells, thereby driving these to rebound burst discharge (Fig. 3B). In return, excitatory postsynaptic

potentials (EPSPs) generated at TC-TRN synapses re-excite TRN cells. In a back-and-forth excitation and inhibition cycle, TRN and TC neuron pools interact like two ping-pong players and increasingly recruit cells into the synchronized discharge (reviewed in (Beenhakker and Huguenard 2009; McCormick and Bal 1997)).

In TRN dendrites, T-channels are integrated in cooperative ionic assemblies that render burst generation repetitive. T-channel activation causes a rapid and homogeneous accumulation of Ca^{2+} in the interior of TRN dendrites to levels of at least a few micromolar (Fig. 1E)(Crandall and others 2010; Cueni and others 2008). These Ca^{2+} levels are high enough to activate Ca^{2+}-dependent small-conductance type 2 (SK2) K^+ channels that are found throughout large portions of proximal TRN dendrites. Low-threshold Ca^{2+} spikes are thus invariably followed by SK2-channel-mediated afterhyperpolarizations (Fig. 3B)(Cueni and others 2008). Through T-channel - SK2 channel interplay, TRN cells generate rhythmic sequences of bursts (Fig. 3B), further sustaining rhythmic inhibition of TC cells.

When portions of the cortex are lesioned, the occurrence and synchrony of spindles in thalamus is greatly reduced in the remaining areas (reviewed in (Steriade 2006)). To conceptualize this cortical control of spindles, it is important to remember that once excited, TRN cells will produce widespread recruitment of TC cells not only in the topographically corresponding section, but also in non-specific thalamic areas. As also demonstrated in computational models (Bonjean and others 2011), these will in turn excite broad cortical areas.

Very important for the controlled build-up of spindle synchronization are the reciprocal inhibitory interactions between TRN cells that are mediated through slowly deactivating $GABA_A$ receptors. These limit the participation of neighboring TRN cells in synchronized discharge through shunting and burst inhibition (reviewed in (Beenhakker and

Huguenard 2009)). Additionally, TRN cells are assembled in clusters with juxtaposed cell bodies that are connected through electrical synapses built by connexin36-containing gap junctions (reviewed in (Cruikshank and others 2005)). Their low-pass filter characteristics allow the low-threshold spikes to spread well and augment synchronized burst propensity in coupled cells.

B4. The propagation of spindles to cortical areas

EEG spindle waves are scalp reflections of synaptic currents generated by cortical neurons. How do intrathalamic network reverberations produce EEG spindle waves? Thalamocortical cell axons from specific and non-specific nuclei propagate to cortical recipient areas, making the majority of their synapses in layer 4 and 5 of sensory cortices, respectively, with additional projections to supra- and infragranular layers (Meyer and others 2010). When many TC cells burst simultaneously, strong and rhythmic feedforward excitatory volleys are observed in both sensory and prefrontal cortices that entrain ~30% of pyramidal and ~80% of fast-spiking interneurons into rhythmic discharges (Peyrache and others 2011). Surprisingly, rhythmic activity is seen with only millisecond-delays in upper cortical layers, indicating that intracortical circuitry is entrained, and further amplifies, the thalamically imposed rhythm (Kandel and Buzsáki 1997). The initial waxing phase of EEG spindles is likely related to this accruing participation of larger cortical cell populations into the rhythms.

B5. The termination of spindles

Several intrinsic and synaptic mechanisms exist in TRN cells that dampen rhythmic burst discharge. Great importance has been attributed to lateral inhibition between TRN

cells that is mediated by GABAergic inhibition, but probably also by peptidergic modulation, which ensure that bursting TRN cells limit the participation of their neighbors in synchronized discharge. Inhibitory inputs also arise from basal ganglia, substantia nigra and pretectal areas (reviewed in (Zikopoulos and Barbas 2007)). Dendrites of TRN cells intertwine into small bundles and form dendrodendritic synapses that are thought to act as a brake, inhibiting neighboring neurons and destabilizing network synchrony (Pinault 2004). Other mechanisms of termination are found in intrinsic ionic mechanisms that reduce burst propensity in both TRN and TC cells as a result of repetitive burst discharge and accumulated Ca^{2+} entry through T-channels (Fig. 3C). In TC cells, Ca^{2+}-sensitive adenylate cyclases activate gradually once repetitive rebound spikes occur, leading to the synthesis of cAMP and the activation of the hyperpolarization-activated cation-nonselective (HCN) channels (Lüthi and McCormick 1999). This depolarization reduces the excitability of TC neurons, contributing to the breakdown of TC-TRN reverberations. In TRN cells, Ca^{2+}-induced Ca^{2+} release and associated cation channel activation at somatic levels, and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)-mediated sequestration of Ca^{2+} (Cueni and others 2008) and recruitment of Na^{+}-dependent K^{+} channels (reviewed in (Coulon and others 2012)) in dendrites, attenuate TRN burst firing. Finally, asynchronous cortical synaptic activity also contributes to spindle termination (Bonjean and others 2011).

B6. The temporal organization of spindles

Spindles appear during NREM sleep in temporal coherence with several sleep rhythms that emerge from distinct brain areas (reviewed in (Steriade 2006)). A dominant role in the temporal organization of sleep spindles is played by the cortical “slow waves” that underlie the low-frequency power (<1 Hz) of the NREM sleep EEG. Slow waves arise from substantial

(>10 mV), rapid and highly synchronized fluctuations of the membrane potential of cortical neurons between two states, a depolarized “up” state (>-60 mV) and a hyperpolarized “down” (<-70 mV) state (reviewed in (Steriade 2006; Timofeev and others 2012)). The “up” state is accompanied by wake-like repetitive action potential firing in cortical neurons across all layers and cell types, creating a strong excitatory feedback via cortico-TRN synapses (Sanchez-Vives and McCormick 2000). Cortical waves thus impose periodically recurring excitation onto TRN and TC cells, thereby triggering intrathalamic network reverberations. Through prolonged excitation during “up” states, slow rhythms matching those of cortex are also induced in TC and TRN neurons (Crunelli and Hughes 2010). The strong feedback control, combined with widespread TC projections, ensures spatially coherent spreading of spindling over cortical territories (reviewed in (Steriade 2006)). The cortical control of spindles explains their link to EEG slow waves, resulting in spike-like waveforms called K-complexes in humans (Fig. 1A). However, spindles also occur independently of slow rhythms, occasionally in localized cortical regions (Nir and others 2011). In rodents, spindle waves appear throughout NREM sleep (Fig. 1B)(Vyazovskiy and others 2004), predominantly at periods of NREM sleep termination when low-frequency δ power weakens. Well-documented is the surge of σ power (10-15 Hz) ~30 s before NREM-REM sleep transitions that depends on burst discharge in TRN (Astori and others 2011; Wimmer and others 2012).

Interestingly, spindles are also temporally associated with hippocampal activity. High-frequency (100-400 Hz) sharp-wave ripples (SPWRs) are found in hippocampus during quiet wakefulness and NREM sleep and represent a re-activation of firing patterns found during exploration, probably contributing to the formation of memory traces (reviewed in (Girardeau and Zugaro 2011)). Close temporal associations exist between hippocampal SPWRs and cortical spindles mostly in prefrontal, but also in sensory areas in rats and

humans (reviewed in (Rasch and Born 2013)). Such observations point to a widely coordinated synchronization of major brain memory systems and TC rhythms, and embed spindles in the transfer of newly acquired hippocampal memory traces to cortex for long-term storage.

B7. Human sleep spindles

Two different types of spindles were already reported in early stages of EEG recordings from humans (reviewed in (Rasch and Born 2013)). The slower spindle with a frequency ~12 Hz predominates in frontal cortical areas, whereas the faster ones ~14 Hz is found in parietal zones. Presurgical depth EEG recordings from epileptic patients suggest a sharp border between slow and fast spindles in the supplementary motor area (Andrillon and others 2011), while other experimenters observed more gradual frequency changes along the rostro-caudal axis (Peter-Derex and others 2012). The origin of fast and slow spindles is not clarified, but is relevant as fast and slow spindles differentially engaged in learning-related brain activity (Mölle and others 2011). Functional magnetic resonance imaging (fMRI) showed that both spindle types largely, but not completely, shared the thalamic areas activated (Schabus and others 2007). There were also common activation patterns in cortex, including cingulate cortex, anterior insula and superior temporal gyrus. However, fast spindles overall showed a broader cortical activation pattern, covering also supplementary motor areas and, notably, a greater recruitment of hippocampal areas. Interestingly, spindle activity during stage N2 of NREM sleep correlated with neuronal activation in hippocampus, suggesting that TC oscillations drive hippocampus (Andrade and others 2011; Peter-Derex and others 2012).

C What do spindles do?

C1. Sensory transmission

The definition of sleep states involves a reduced responsiveness to sensory inputs. As the TC system is the major recipient of sensory afferents, changes in its electrical activity will affect the throughput of sensory stimuli. Early *in vivo* studies, some of which carried out by the Nobel Prize winner David Hubel, demonstrated that the fidelity of retinogeniculate information transfer varied with the state of arousal, and is in particular degraded during periods of drowsiness and sleep (Livingstone and Hubel 1981), when TC neurons are hyperpolarized and preferentially discharge in bursts rather than in linearly coding tonic trains of action potentials (reviewed in (Bal and McCormick 1997)).

Spindles are particularly efficient in vetoing sensory activation of the cortex and are key elements in the reduced sensory responsiveness during sleep. For example, it is harder to wake-up someobody while his brain generates spindles than when it does not do so (Yamadori 1971). Event-related potentials, which are EEG deflections in response to sensory stimuli, are altered in shape during NREM sleep, but do so more extensively when elicited during a spindle (Cote and others 2000). A recent imaging study looking at auditory cortex activation during sounds showed that activation was essentially nil during spindles, but remained substantial during spindle-free NREM sleep (Fig. 4) (Dang-Vu and others 2011; Schabus and others 2012). The dominant recruitment of inhibition throughout cortical layers plausibly contributes to the lack of cortical response (Peyrache and others 2011). A protective role of sleep spindles was directly demonstrated in mice in which spindle power at periods of exit from NREM sleep was intensified through genetic modification of TRN bursting (Wimmer and others 2012). These animals showed a higher acoustic arousal

threshold when exposed to incremental white noise, suggesting that sleep spindles retard the transition from NREM sleep to waking.

Why would the brain use short spindle events to protect itself from arousal? Sensory disconnection during sleep varies constantly, with recurring periods of strong sensory decline and of weakened, but still significant responsiveness closer to the waking state. It seems that NREM sleep repeatedly opens time windows for sensory stimuli to penetrate into cortex. Considering that spindles often occur during cortical up states, it is possible that they represent cortically triggered efforts to enhance sensory shut-down after a brief period of heightened responsiveness. Conversely, properly timed auditory stimuli can enhance slow fluctuations in the cortex as they probably boost cortical activity during up states (Ngo and others 2013).

C2. Synaptic plasticity and memory

Via the excitation imposed by TC afferents onto cortex, spindles induce coordinated firing in cortical circuits. Across cortical layers, single action potentials occur time-locked to the spindle rhythm, as monitored in local field potential recordings (Contreras and others 1997), priming connected neurons for spike-timing-dependent forms of plasticity. Additionally, groups of action potentials discharge at >100 Hz in cortical neurons may modify synaptic strength in a non-Hebbian form of plasticity, i.e. independently of the temporal correlation between pre- and postsynaptic activity. For example, burst-like discharge in cortical layer 5 neurons induces a long-term depression of excitatory synapses formed by supragranular afferents and a strengthening of somatic inhibitory inputs (Birtoli and Ulrich 2004; Kurotani and others 2008).

Spindle-related spike trains recorded in anesthetized cat were applied in cellular studies in rat cortical brain slices to mimic temporally correlated pre- and postsynaptic activity thought to occur during spindles (Rosanova and Ulrich 2005). As a result, a long-term potentiation (LTP) at excitatory connections between layer 2/3 and layer 5 was observed (Fig. 5A). Interestingly, the detailed sequence of interspike interval in the spindle train mattered, as a random shuffling of these or using mean frequency rates (30 Hz) were inferior in inducing LTP. Aside from cortex, recent studies have unraveled that rhythmic bursting in TRN cells induces LTP at TC-TRN synapses (Astori and Lüthi 2013). Spindle rhythms thus drive cortical synchronized discharge and associated long-term synaptic strengthening and override a global downregulation of synapses during low-frequency rhythms in cortex.

The synaptic potentiation observed by spindles links to repeated observations that spindle correlate with memory formation and learning capacity in humans. Declarative memory tasks involving cued recall of word pairs went along with increased spindle density (in number of events per 30 s) during the first period of N2 NREM sleep, without any changes in sleep structure or other frequency bands (Gais and others 2002; Schabus and others 2004). Spindle density increased locally within brain areas implicated in learning, as documented in other training tasks (Fig. 5B) (Johnson and others 2012). The increase in spindle density correlated linearly with the performance improvement. Spindle density also correlates positively with learning capacity and intelligence measures (Fig. 5C). Links between spindles and memory formation are now documented for rodents exposed to associative learning (Eschenko and others 2006; Mölle and others 2009) and for more sophisticated aspects of the learning process in humans (reviewed in (Rasch and Born 2013)).

How could changes in spindle parameters, such as their intensity/amplitude or their frequency/occurrence, facilitate learning? A combined EEG and fMRI study on associative visual memory found that, during post-learning sleep, the amplitude of individual spindles correlated with neuronal activation in both hippocampus and in higher visual areas selective for the visual objects (Bergmann and others 2012). This finding is consistent with observations on enhanced temporal coupling between cortical slow oscillations, spindles and hippocampal ripples after learning (Mölle and others 2009). However, in naturally sleeping rats, prefrontal cortex responded more weakly to hippocampal SPWRs during spindle epochs than during spindle-free periods (Peyrache and others 2011), leaving open whether spindle-related proplastic effects are related to spindle-ripple coupling. It is also important to note that not only spindles, but slow wave intensity also correlates with declarative learning (Rasch and Born 2013), indicating that sleep-related learning is a multi-component process with several sleep rhythms involved.

C3. Neuronal development

Spindles are accompanied by rhythmic fluctuations of intracellular signaling systems. In TRN cells, Ca^{2+} increases during every burst discharge in large portions of dendrites and is sequestered through SERCAS into the endoplasmic reticulum (ER) (Fig. 1E, Fig. 3C). Luminal Ca^{2+} levels regulate major ER functions in cell physiology, including gene transcription and protein synthesis. T-channel-mediated Ca^{2+} accumulation in the ER may hence drive transcriptional activity specifically during spindle rhythmicity. Furthermore, in TC cells, rhythmic low-threshold bursts lead to waves of cAMP synthesis through activation of Ca^{2+}-sensitive adenylate cyclases (Fig. 3C)(Lüthi and McCormick 1999), known to be important for synapse specification (Gorbunova and Spitzer 2002). Interestingly, first signs of organized

electrical activity in the developing brain of rodents and humans occur as spindle-like, 10 Hz-rhythms in association with spontaneous muscle twitches in limbs (reviewed in (Hanganu-Opatz 2010)). It is proposed that these early brain rhythms act as a template for cortical map organization, but whether spindle-associated Ca^{2+} and cAMP signals are relevant for sensory circuit formation remains open. Spindle-mediated fluctuations in intracellular signaling perhaps play a role throughout adulthood by maintaining and shaping TC connectivity in the mature brain in response to on-going sensory activity and learning.

D Conclusions and Perspectives

Spindles were the first EEG rhythm reported and the first network oscillation for which a cellular mechanism was elucidated. These oscillations continue to exemplify the neuronal structure and significance of rhythms for the brain, as indicated by the make-up of oscillatory units, the design of circuits engaging in successive synchronization and desynchronization and their conditioned recruitment by other brain areas. Spindle rhythmogenesis is based on an elaborated cooperation between ionic and synaptic mechanisms in the pacemaker circuits. Knowledge about these is relevant in the continued search of specific drug targets that could improve treatment of sleep disorders. Spindles separate sleep into distinct phases of sensory accessibility, enabling periods of almost complete annihilation of sensory throughput with periods in which sensory information does reach the cortex. At the same time, spindles enable epochs of heightened plasticity that fall together with the emergence of hippocampal rhythms. Therefore, do spindles promote sensory protection to ensure the internal processing of information? Do spindles facilitate the association between hippocampal and cortical activity that allows the purported transfer from short-term to long-term memory sites? How does such a function correlate with the

dominant recruitment of inhibition in cortex by spindles? From all we can see today, spindles seem designed to unify the brain's diverse structures into a single major processing center, yet more analysis is required to link their impact on cortical units and on hippocampal-cortical coordination.

First efforts to modify spindles specifically are underway. Chronic manipulations, such as those possible with genetic interference (Astori and others 2011; Wimmer and others 2012), will determine their role in sleep architecture and TC connectivity. Acute modification (Halassa and others 2011) could be envisaged as a way to test their implication in learning processes, for example by combining associative learning in rodents with optogenetic manipulation of spindle occurrence during post-learning sleep. These possibilities currently remain overflown with questions to be resolved experimentally: Do optogenetic stimuli come close to cortically mediated initiation of thalamic pacemakers? How should optogenetically elicited spindles be timed relative to the occurrence of other rhythms? Is it sufficient to modify spindle occurrence independently of other rhythms to induce learning? What will be the impact of global vs. modality-specific TRN stimulation?

Decreased spindle activity has been presented as a hallmark in schizophrenia (Ferrarelli and others 2010), and developmental models of schizophrenia in rat revealed aberrant timing of spindles relative to cortical and hippocampal rhythms (Phillips and others 2012). Such sleep disturbances fit well with the idea that abnormal perceptions in schizophrenia are due to impaired corticothalamic communication (Vukadinovic 2011). Such novel links between spindles and neurological disease may ultimately prompt therapeutic approaches involving directed manipulation of sleep rhythms.

Box 1.

The historical origins of sleep spindle research. The German medical doctor Hans Berger (1873-1941), working as a psychiatrist in Jena, was driven to identify measurable parameters about the brain's psychical energy and about mental telepathy. He initially experimented on human patients with skull defects to measure brain volume, pulsations of intracranial blood flow and intracranial temperature. Around 1900, he turned to his first recording to record the electrical activity of the brain, but then suspended these efforts due to the poor data quality, clinical and academic obligations, and medical service during the First World War. He picked up his scientific work in the 1920's, and, through a decade-long painstaking improvement of galvanometric recordings from the human brain, he came up with the first clear recordings of rhythmic brain activities. He noted in particular the rhythmic appearance of brief oscillatory events in the 10 Hz range, which he called alpha waves, and which he thought were representing some form of mental activity, while the faster beta waves (15-20 Hz) represented the brain's metabolic state (Berger 1933). In the last decade of his life, great skepticism was brought against his work and the significance of Berger's "Elektrenphalogram" was not recognized until researchers such as Sir Adrian, Jaspers and Bremer reproduced his findings and further developed techniques and animal preparations to record brain electrical activity. Their studies sparked discussions about the electrical signals generating and the cellular mechanisms underlying the observed rhythms. Spindle waves soon became the focus of these discussions when Adrian noted that rhythmic activity observed in cortex during anesthesia could be recorded in the white matter, from the cut end of fibers from thalamus to cortex (Adrian 1941). The 50's and 60's brought landmark developments within the field of thalamic function. Moruzzi and Magoun identified the brainstem reticular activating system as a main wake-promoting structure (Moruzzi and

Magoun 1949). Moreover, intracellular recordings from thalamic neurons revealed that these neurons experience prolonged rhythmic inhibitory synaptic events in phase with the oscillatory periods (Purpura 1968), originating from an intrathalamic source (Andersen and Andersson 1968). The leading researcher at the end of 1900's was Mircea Steriade, who, from the 1980s on, demonstrated that TRN was the pacemaker for sleep spindles and responsible for generating rhythmic inhibitory synaptic potentials throughout principal thalamic nuclei (reviewed in (Fuentealba and Steriade 2005)). His work on the intact brain found even greater acclaim when spindle rhythms could be reproduced in brain slice preparations containing the TRN, further confirming that they arose from a localized pacemaker element within the brain (reviewed in (Steriade and others 1993)).

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Figure legends

Figure 1. Sleep spindles and spindle-like rhythms are documented at multiple levels of neuronal circuitry analysis. A, EEG recording of spindles from a sleeping human subject from two corresponding sites on primary somatosensory cortex (C3-A2, C4-A1). Note the regular appearance of sleep spindles, evident as grouped EEG deflections occurring at higher frequencies than background (*). K-complexes intertwined with sleep spindles are marked by #. The spindle labeled with ** is expanded on the right to illustrate the waxing and waning waveform. Traces were kindly provided by Dr. R. Heinzer, Cantonal Hospital of the University of Lausanne, Switzerland. **B**, Local field potential recordings from layer 5 of primary somatosensory (S1) and auditory (A1) areas in an adult C57Bl/6J mouse during NREM sleep. Same labeling for *,** and # as in A. Spindles were detected based on band-pass filtering of the traces between 10-15 Hz (bottom trace on the right). Traces were kindly provided by Dr. L. Fernandez, University of Lausanne, Switzerland. **C**, Spontaneous rhythmic multiunit discharges in a mouse brain slice containing TRN and adjacent ventrobasal thalamic complex. Waxing and waning waveform of each grouped discharge is apparent. Expanded portion is taken from the area labeled with a small black bar and shown to the right. **D**, Intracellular recording of a TC neuron engaged in spindle-like rhythms in a ferret slice containing the dorsal lateral geniculate nucleus of the thalamus and the adjacent perigeniculate nucleus, a carnivore equivalent of the visual TRN (for further information, see Steriade and others, 1993). Note rhythmic occurrence of inhibitory postsynaptic potentials

that are occasionally eliciting rebound burst discharge. Black bar illustrates portion of the trace that is expanded to the right. Time scale in D also applies for A-C. **E**, Ca^{2+} imaging of proximal dendrites of a TRN cell as it generates repeated burst discharge elicited through somatic current injection. Images were acquired at the time of discharge indicated by vertical lines. Imaging was carried out as described in Cueni and others (2008). Note the marked increase in Ca^{2+} levels throughout the imaged dendritic portion and its almost complete decay during the interburst phase.

Figure 2. Morphological, ionic and synaptic properties of TRN cells that contribute to vigorous burst discharge. Reconstructed rat TRN cell labeled with neurobiotin during a juxtacellular recording (for details, see Pinault, 2004 and references therein). Note the multiple thin dendrites emerging from the soma, some of which branch extensively and form hairy appendages. The subcellular distribution is indicated for T-channels (orange-brown gradient, with darker colors indicating greater expression levels) and for SK2 channels (green). SK2 channel expression was demonstrated up to ~100 μm from the soma. Single dendrites were chosen to illustrate T- and SK2 channel distribution in color code. Mechanisms of burst control involving high-voltage-activated Ca^{2+} channels, Ca^{2+}-induced Ca^{2+} release and Ca^{2+}-activated cation channels were described at the somatic level. The axon, emerging from a primary dendrite, expresses high levels of Na^{+} channels (red). Synaptic afferents for which their subcellular distribution is known are positioned accordingly. Excitatory afferents are labeled with a circle, inhibitory afferents with a line. Cholinergic afferents mediate both excitation and inhibition. Insets show a single burst and repetitive burst discharge (for details, see Pinault, 2004 and references therein). CT,

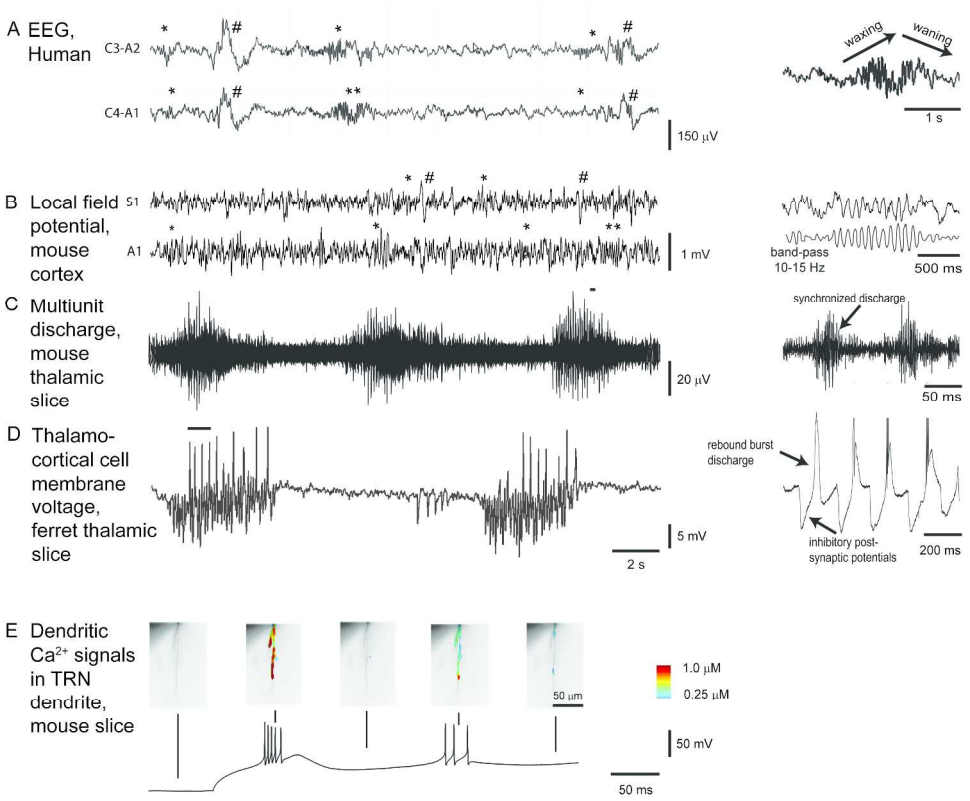
corticothalamic; ACh, acetylcholine. Reconstructed cell and electrophysiological traces were kindly provided by Dr. D. Pinault, INSERM, University of Strasbourg, France.

Figure 3. T-channel-dependent mechanisms of spindle initiation, synchronization and termination. A-C, Schematic drawings of membrane portions from TRN and TC cells with color-coded Ca_V3 channels (yellow), SK2 channels (green), HCN channels (blue) and cation channels (violet) and associated mechanisms of Ca^{2+} signaling. Na^+ channels are not represented. Colored circles and associated color clouds indicate ion flow through channels. Yellow, Ca^{2+}; Green K^+; Blue Na^+. Membrane voltage traces indicate the electrophysiological events associated to these schemes. Traces are from recordings in mouse thalamic slices and were kindly provided by Dr. S. Astori, University of Lausanne, Switzerland. **A**, Initiation of spindles occurs through recruitment of burst discharge via cortical EPSPs. **B**, Spindle synchronization is mediated via reciprocal synaptic interactions between TRN and TC cells through burst IPSPs, rebound burst generation in TC cells, and TC-TRN EPSPs. Repetitive burst discharge in TRN cells occurs through $Ca_V3.3$-SK2 channel interplay. **C**, Termination of spindles occurs through multiple mechanisms involving Ca^{2+}-mediated alterations of TRN and TC cell excitability. *Top*, TRN cells undergo a dampening of burst discharge through (*left*) somatic activation of cation channels (via activation of Ca^{2+} channels and Ca^{2+}-induced Ca^{2+} release (CICR) through ryanodine receptors) and through (*right*) dendritic sequestration of Ca^{2+} via SERCAs, limiting SK2 channel activation. *Bottom*, TC cells generate a membrane afterdepolarization (arrow) through repetitive burst IPSPs, Ca^{2+}-mediated activation of adenylate cyclases and consequent upregulation of HCN channels.

Figure 4. Annihilation of auditory cortex activation during spindle-containing, but not during spindle-free epochs of NREM sleep. A, Schematic of experimental design. Human EEG trace of NREM sleep with spindle-free and spindle-containing episodes. Brief auditory stimuli (300 ms, 400 Hz) were applied at time points indicated by blue squares. EEG trace was provided by Dr. R. Heinzer, Cantonal University Hospital of Lausanne, Switzerland. **B**, Functional magnetic resonance imaging of responses to auditory stimuli during three EEG-defined states (indicated above the images). Yellow voxels indicate brain areas activated in response to the stimulus. During waking, clear responses are evoked in auditory cortex (top) and in brainstem and collicular areas (bottom) involved in sound detection. During spindle-free NREM sleep, similar brain areas are activated; see Dang-Vu and others (2011) for explanations as to the difference in activation patterns between waking and spindle-free NREM sleep. During spindle-containing NREM sleep epochs, no fMRI signals are detectable in cortex and brainstem. Red arrows highlight small activation of inferior collicular areas (enlarged in inset). X and y refer to brain coordinates used for fMRI. Picture adapted, with permission, from Dang-Vu and others (2011).

Figure 5. **Sleep spindles and their relation to synaptic plasticity, memory and intelligence. A**, Application of spindle-like rhythmic action potential trains in rat somatosensory cortical brain slices induces long-term potentiation. *Left*, Stimulation train applied to presynaptic afferents (Pre, upper electrode) and to postsynaptic cells (Post, lower electrode) to induce joint pre- and postsynaptic activity. The stimulation protocol was repeated 30 times at 0.6 Hz. Right, sample EPSPs before (dotted line) and > 30 min after (solid line) conditioning protocols. Adapted, with permission, from Rosanova and Ulrich 2005. **B**, High-resolution EEG monitoring of post-training spindle rates in human subjects exposed to a brain-computer

interface task. Warm and cold colors indicate percentage increases and decreases in spindle rates, respectively, relative to the day preceding training. White circle indicates the position of the task electrode used for brain-computer interfacing. Note local increases in spindle density consistent with the site of learning. Grey colors indicate no change. Adapted from (Johnson and others 2012). **C**, Linear correlation between total number of spindles measured at sites C3 and C4. Spindles were included in the count if the intraspindle frequency was 12.0 –16.0 Hz, if the duration was > 0.5 s, and the amplitude reached 10 mV. Every data point represents one participant. Correlation coefficient was 0.75. Adapted from Fogel and others ((Fogel and others 2007)).



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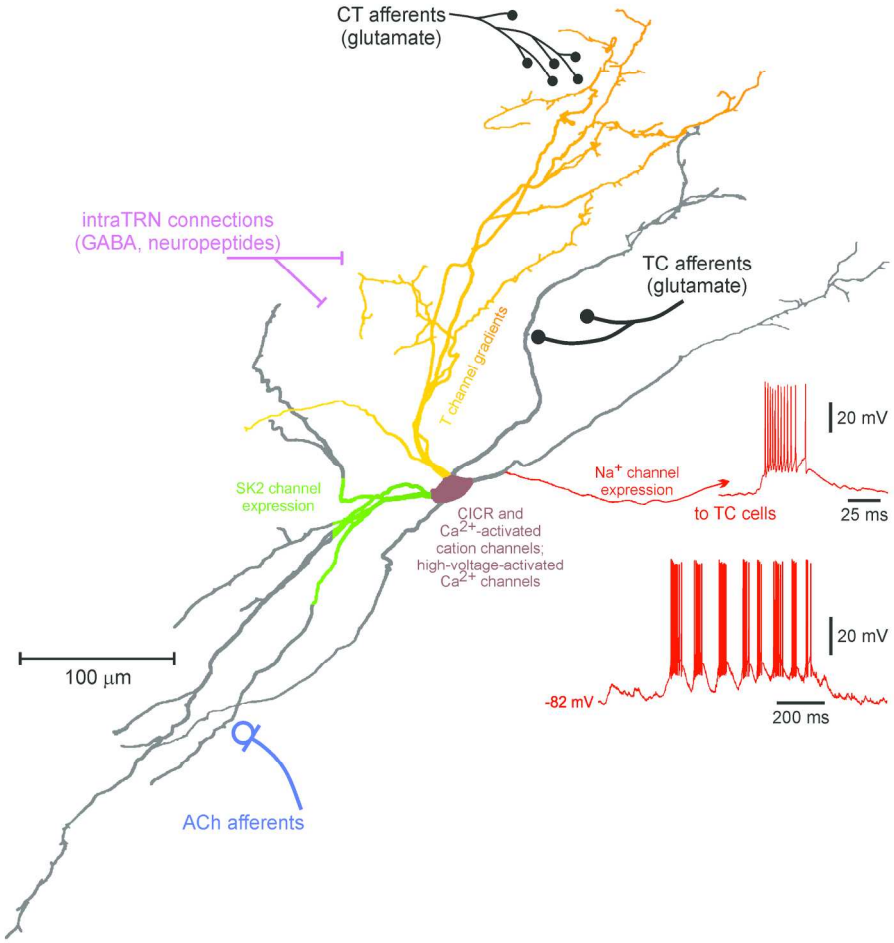

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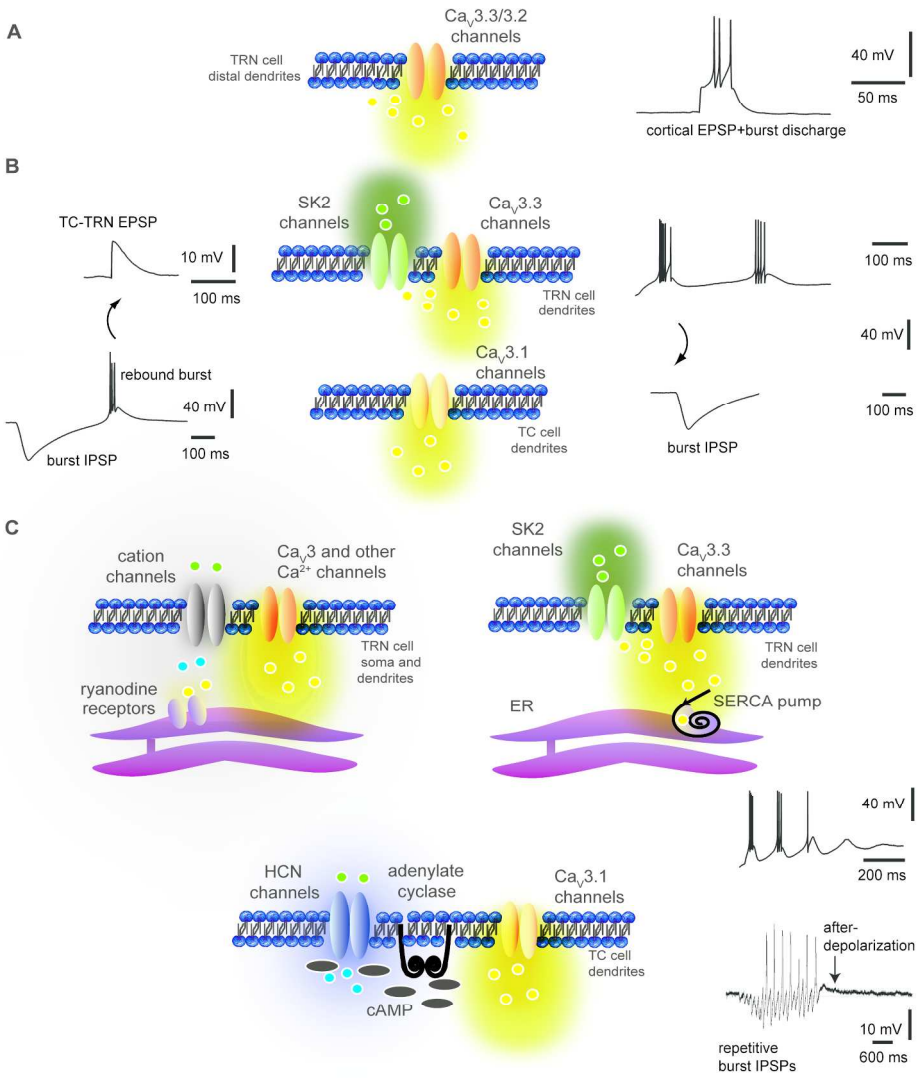

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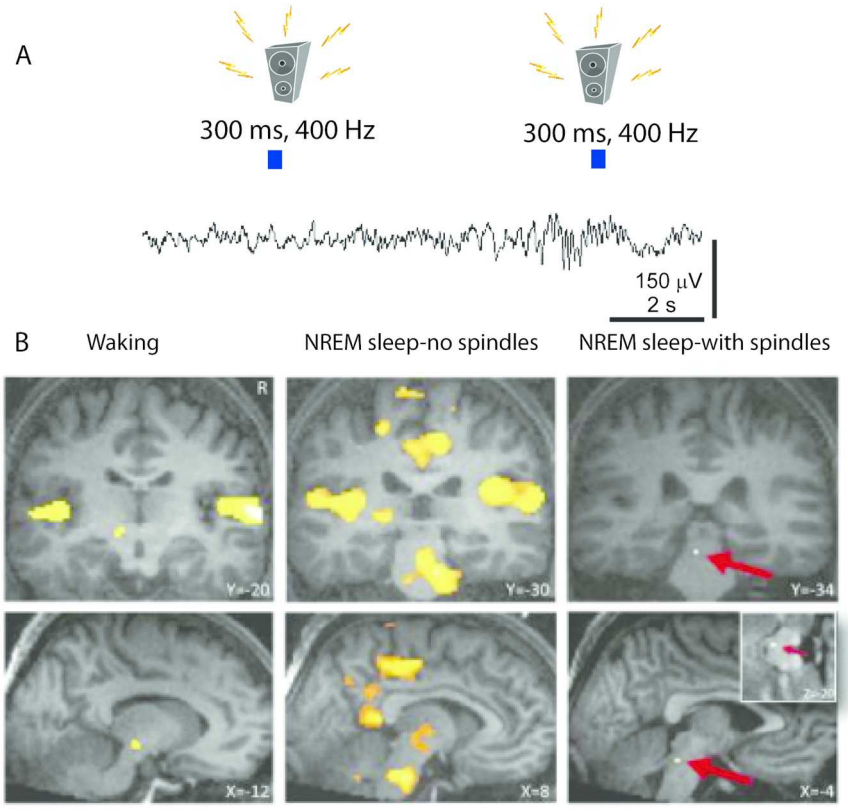

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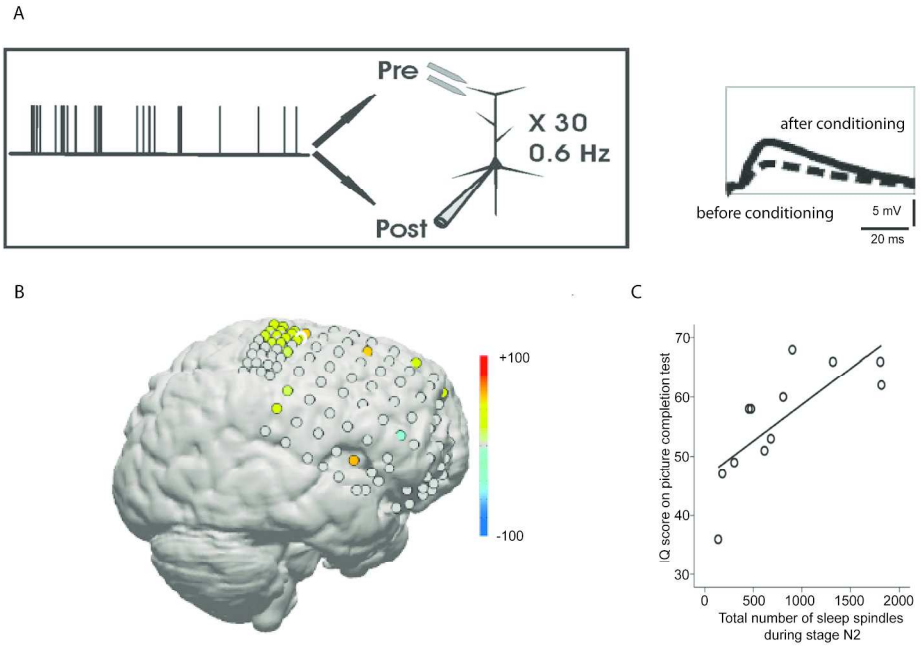

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