Published in final edited form as:
Nat Neurosci. 2010 January ; 13(1): 9–17. doi:10.1038/nn.2445.

The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators

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Abstract

The slow (<1 Hz) rhythm, the most significant EEG signature of non-rapid eye movement (NREM) sleep, is generally viewed as originating exclusively from neocortical networks. Here we argue that the full manifestation of this fundamental sleep oscillation within a corticothalamic module requires the dynamic interaction of three cardinal oscillators: a predominantly synaptically-based cortical oscillator and two intrinsic, conditional thalamic oscillators. The functional implications of this hypothesis are discussed in relation to other key EEG features of NREM sleep, with respect to coordinating activities in local and distant neuronal assemblies and in the context of facilitating cellular and network plasticity during slow wave sleep.

Although membrane potential fluctuations at a low frequency had already been observed in neurons of the rat cortex *in vivo*[1], the discovery of the slow (<1 Hz) rhythm in the EEG, and of its cellular counterpart, the slow (<1 Hz) oscillation, rests with the pioneering work of Mircea Steriade and his coworkers[2-4]. In 1993, using intracellular microelectrode recordings from morphologically identified neurons in different layers of the sensory, motor and association cortex of anesthetized cats (Fig. 1a), these authors described the presence of a slow oscillation of the membrane potential, consisting of regularly repeating sequences of depolarizations (most often with firing) and hyperpolarizations (with no firing) at a low (0.2 - 0.9 Hz) frequency[2,3], which are nowadays commonly referred to as UP and DOWN states, respectively (Figs. 1b and 2a) (Supplementary Note A). The slow oscillation was also present in the glutamatergic thalamocortical (TC) neurons of various thalamic nuclei and in the GABAergic neurons of the nucleus reticularis thalami (NRT), with the respective UP and DOWN states showing good temporal correlation with the corresponding cortical states and with the respective negative and positive depth-EEG waves[4] (Figs. 1b and 2b,c). Other key findings from that original series of studies were that the slow oscillation could group together periods of sleep spindles and delta waves during its UP and DOWN states[2,3], respectively, and that it was present in a *cerveau isolé* preparation[3]. Moreover, the slow oscillation in cortex was shown to survive electrolytic lesions of extensive thalamic territories or destruction of TC neurons by kainic acid[3], leading to the conclusion that this rhythm is generated in the neocortex and then imposed on recipient thalamic territories[4].

In the intervening 16 years, extensive and ground-breaking investigations of the slow (<1 Hz) rhythm/oscillation, both in humans and in experimental animals, have now have provided us with a remarkably detailed picture of the salient features of this brain activity[5-14], powerful insights into its intricate mechanisms[15-25] and a clear window into its potential physiological significance[26-33]. However, despite the presence of compelling evidence to the contrary[4,15,18,20,34-37], the opinion has prevailed that the slow (<1 Hz)

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rhythm/oscillation is generated exclusively in the neocortex[38-43]. Here we propose an alternative view that balances the cortical and thalamic contributions to the EEG expression of the slow oscillation, and discuss the functional implications that this novel framework brings about for sleep as well as for intrinsic and synaptic plasticity in neocortex, thalamus and other brain areas.

The slow (<1 Hz) oscillation of natural sleep and during anesthesia

The slow (<1 Hz) oscillation permeates all stages of natural NREM sleep in humans[5,6,13,44]. Thus, the classical sleep K-complex (of stage 2) is the EEG manifestation of a single cycle of the slow oscillation, and its surface EEG-negative and -positive phases (corresponding to depth EEG-positive and -negative) simply reflect the DOWN state and the start of the UP state, respectively, of the oscillation[6,38,45]. Sleep spindles, which are prevalent in the early stages of NREM sleep[39,46], most often occur immediately after, or are superimposed on, the depth-negative peak of the EEG wave, i.e. on the early part of the UP state of the slow oscillation[47] (Figs. 2c and 3a). As sleep deepens, the frequency of the slow oscillation, and thus that of K-complexes, increases until it develops into the slow waves of deep NREM sleep[38,47] (Fig. 3a). From stage 2 to 4, therefore, the slow oscillation shows an increase in frequency (from around 0.03 to almost 1 Hz) and occupies an increasingly larger component of the EEG signal with clear periods of delta waves becoming more frequent and longer.

EEG waves with extremely similar features to those in humans also characterize natural NREM sleep in many animal species[48-51] (Fig. 3). It is also well documented that the slow oscillation heavily permeates the EEG of these species during light and deep anesthesia[48,49,52] (Figs. 2 and 3a), thus validating investigations of the slow oscillation under such experimental conditions (Supplementary Note B). Various anesthetics, however, lead to slightly different manifestations of this brain rhythm. For example, the ketamine-xylazine combination results in a slow oscillation with a higher frequency (0.6-1 Hz) than that observed under urethane anesthesia (0.3-0.4 Hz)[2], and in a shorter latency of the UP state firing of cortical interneurons compared to pyramidal cells in layer 5 (ref [9]). Isoflurane drastically decreases the frequency of the slow oscillation[35], and barbiturate anesthetics fundamentally alter its cellular activity resulting in the disappearance of the characteristic UP and DOWN state fluctuations[2,53]. Therefore, although the use of certain anesthetics, and especially ketamine-xylazine and urethane, has greatly facilitated investigations of the cellular correlates of the slow oscillation in intact animals, these drugs essentially ‘clamp’ the slow oscillation at particular frequencies and bring about an overall rhythm which lacks the dynamic complexity of the EEG waves of natural sleep in humans and animals[5,44,48,49,50]. Thus, there is a need to extend the limited number of studies of the cellular correlates of the slow oscillation during natural sleep[48,49].

Features of the slow oscillation in neocortex and thalamus in vivo

Single cell investigations in anesthetized cats have shown that virtually all cortical neurons participate in the slow oscillation[33,34,53-55] (Figs. 1b and 2a), and that the UP and DOWN states occur in a quasi-synchronous manner amongst different types of cortical neurons, even between relatively distant cortical territories (e.g. 10-15 mm in the cat)[14] (Supplementary Note C). The active participation of a substantial number of cortical cells in the slow oscillation is also supported by investigations in layers 2/3 and 5 of anesthetized rats and mice[9,11] (but see ref. [8]). Analysis of large neuronal ensembles in rat layer 5 has indicated that the slow oscillation behaves as a traveling wave[9], as during natural sleep in humans[10], and that the UP state firing of individual cortical neurons is highly stereotypical, both in the spatial and temporal domains, and independent from the direction of the wave, suggesting interactions with local intrinsic processes. Interestingly, the presence of two populations of morphologically and electrophysiologically identified fast-spiking

interneurons that preferentially fire either at the start or towards the end of each UP state during the slow oscillation, but which are both strongly phase-locked to gamma waves, has also been highlighted in anesthetized rats[12].

As with cortical neurons, single cell investigations in anesthetized preparations have demonstrated that almost all thalamic neurons, i.e. TC and NRT cells, actively participate in the slow oscillation[5,35,61,62] (Figs. 1b and 2b,c) and that their UP and DOWN state dynamics occur quasi-synchronously, both locally and with cortical neurons[2,34,54-56] (Fig. 4a). Regrettably, no study of large thalamic neuronal ensembles has so far been carried out in naturally sleeping preparations. The slow oscillation in thalamic neurons *in vivo* shows a considerably stereotypical appearance and a conserved waveform from cycle to cycle[4,34,57] (Figs. 1b and 2b,c). In particular, there is a large (15-20 mV) and constant voltage difference between the UP and DOWN states, and the evolution of the membrane potential during the slow oscillation is characterized by two unmistakable signatures at the transition between states. Specifically, the transition from DOWN to UP state is punctuated by a low threshold Ca^{2+} potential (LTCP) and associated high frequency (150-300 Hz) burst of action potentials (Figs. 1b and 2b,c), whereas the transition between the UP and DOWN state is marked by a clear inflection point in the membrane potential[34,53,57] (arrows in Fig. 2b,c). Following the initial LTCP-mediated burst, the UP state of any TC neuron either shows additional sustained firing (Fig. 2b, left) or does not[34,53,57] (Figs. 1b and Fig. 2b, middle and right). NRT neurons, on the other hand, always exhibit sustained firing on the UP state following the initial LTCP burst, with this firing being of a substantially higher frequency than in TC neurons[4,34,53,57], (Figs.1b and 2c).

Corticothalamic relationships during the slow oscillation in vivo

The LTCP-mediated burst of action potentials that invariably marks the beginning of each UP state in TC neurons *in vivo* often precedes by 20-50 msec the start of the UP state in simultaneously recorded cortical neurons and the peak negativity of the depth-EEG wave[57] (Fig. 4a) (see Figs. 8-10 in ref. [34]). This finding led these authors to suggest that the "… spike bursts of TC cells…….are good candidates to trigger the depolarizing phases [i.e. UP states] at every cycle of the slow oscillation"[34]. Unfortunately, this key experimental observation and its intuitive interpretation have been somewhat overlooked in the intervening years, as the emphasis on a solely cortical generator of the slow rhythm later grew increasingly stronger, mainly supported by two *in vivo* results and one *in vitro* finding. Firstly, that the slow oscillation is abolished in the majority of TC and NRT neurons *in vivo* following decortication or transection of their cortical afferents[3,58], and secondly, that the slow oscillation persists in a de-afferented cortical slab preparation *in vivo*[59]. In these lesion-based studies, however, no systematic and quantitative comparison of the properties of the slow oscillation in cortex before and after lesioning was undertaken. Indeed, the illustrated examples of cortical UP and DOWN states from the cat *in vivo* appear less regular and rhythmic in the absence of a thalamic input[3,59], and a localized pharmacological block of intracortical connectivity has little effect on the long-range cortical coherence of the slow oscillation[60]. Moreover, disfacilitation of thalamic activity by intrathalamic application of muscimol almost completely abolishes slow oscillations in individual rat cortical neurons *in vivo*[35]. Thus, whereas a slow oscillation does persist in an isolated cortex *in vivo*, its properties are certainly not identical to those observed in the presence of intact intracortical, corticothalamic and thalamocortical connections. Consistent with these *in vivo* findings, in slices with viable corticothalamic and thalamocortical connections, sectioning both afferents leads to a reduced incidence of cortical UP states (up to 60% for those occurring at <6 sec interval)[20].

The third (*in vitro*) evidence in support of a purely cortical generator of the slow oscillation is the widely acknowledged ability to record UP and DOWN state dynamics in cortical, but apparently not thalamic, slices. This issue is re-appraised below.

Mechanism of the slow (<1 Hz) oscillation

Neocortex

Although spontaneous UP and DOWN state dynamics can be observed in the cortex *in vitro* under control conditions[16], modifications of the Ca^{2+} concentration of the perfusing solution[19-23,61] or addition of the cholinergic agonist, carbachol (Lőrincz, Bao, Crunelli & Hughes *Soc. Neurosci. Abstr.* 881.19, 2007; 41.6, 2008), are required to elicit an activity that closely reproduces the slow oscillation of NREM sleep and anesthesia (Supplementary Note D). *In vitro* studies that have used these approaches, as well as analysis of *in vivo* data[62], demonstrate that the slow oscillation mainly results from the regular recurrence of intense, but balanced, intracortical excitatory and inhibitory synaptic barrages, which generate the UP state, and their absence, that constitutes the DOWN state (Fig. 2a). However, for an UP state to be reliably initiated in an isolated cortical network where all constituent elements are simultaneously in a state of prolonged neuronal silence (i.e. the DOWN state), at some point a net inward current must occur in a cell or group of cells within that network. Computer simulations indicate that such a depolarization can be achieved by the '…spontaneously occurring coincidence of miniature EPSPs…'[63], the action of some neurons that have a '…slightly lower spiking threshold…[and]…fire spontaneously…'[64], or a combination of both[65]. Indeed, sparsely distributed neocortical cells that intrinsically generate UP and DOWN states have been recently identified[66]: a subset of pyramidal neurons in layers 2/3 and 5, and a group of Martinotti cells which is exclusively located in layer 5. In both pacemaker cell types, a persistent Na^+ current (I_{NaP}) plays a key role in the generation of their UP states[66]. Other intrinsic currents believed to be important for the cortical slow oscillation *in vitro* include $I_{K(Ca)}$[63-65] and $I_{K(Na)}$[64] (Ca^{2+}- and Na^+-activated, K^+ currents, respectively) as well as $I_{K(ATP)}$[67].

In summary, cortical circuits have the innate and autonomous ability to generate oscillatory UP and DOWN states that can be reproduced *in vitro* through different manipulations that enhance the basic level of excitability in a slice preparation. Furthermore, neocortical UP states are predominantly synaptically based, though they are potentially aided by intrinsically oscillating neurons.

The preponderance of intrinsically oscillating neurons in layer 5 (ref. [66]) underscores the original observation that, at least in isolated cortical slices, the slow oscillation preferentially originates spontaneously within or near this cortical layer[22]. Another *in vitro* study, however, has identified 'core neurons' in layer 4 that consistently contribute to successive, either spontaneous or thalamically-elicited, UP states[19]. Indeed, in thalamocortical slices with intact thalamocortical and corticothalamic afferents, electrical or chemical stimulation of a restricted thalamic area reliably elicits UP states in layer 4, which are almost indistinguishable from those occurring spontaneously[20]. In this respect, it is worth stressing that *in vivo* i) augmenting neocortical responses can be induced by thalamic electrical stimulation[68], ii) whisker stimulation delivered during the DOWN state is highly effective in triggering UP states in layer 2/3 (ref. [11]), and iii) LTCP-mediated thalamic bursts potently activate cortical circuits[69]. In contrast to this facilitating effect of thalamocortical inputs on neocortical UP and DOWN state dynamics, however, *in vitro* studies report that localized electrical stimulation close to the recording site or in layer 2/3 blocks existing UP states in layer 4/5 neurons, and is unable to evoke new ones except at low stimulation intensities[16,23]. Indeed, whereas cortical stimulation at low intensity enhances the ability of thalamic stimuli to evoke UP states, it reduces it at high intensity[16,23]. Note that the so-called 'burst firing' or

'high frequency' (≤40 Hz) stimuli employed in some *in vitro* studies[16,19,,20,23] reviewed above is of a far lower frequency, and might therefore be less effective, than the 150-300 Hz LTCP-mediated burst that characterizes the TC neuron output at the start of each UP state *in vivo*[4,34,57] and *in vitro*[15,18,37]. Future experiments, therefore, should compare the effects on the UP and DOWN state dynamics of layer 4 cells between an LTCP-like burst stimulus-protocol delivered through the thalamic afferents and a similarly physiological stimulus-protocol applied to their cortical afferents.

Thus, although complex interactions among intracortical neuronal ensembles within a localized cortical territory (i.e. the cortical slice) allow potential multiple foci of initiation for the slow oscillation, thalamic inputs *in vitro* and *in vivo* appear to be a more efficient and reliable way of triggering UP and DOWN state dynamics in cortical networks than intracortical stimulation.

Thalamus

Although not as widely acknowledged as for the neocortex, a slow oscillation with characteristics identical to those observed *in vivo* can be reliably recorded *in vitro* under control conditions in a small number (5%) of TC and NRT neurons[36,70], and in almost all mouse, rat and cat TC and NRT neurons when the metabotropic glutamate receptors (mGluRs), that are located postsynaptically to their cortical afferents[71-74] (i.e. mGluR1a), are activated either pharmacologically (by exogenous application of an mGluR agonist) or physiologically (by the glutamate released following electrical stimulation of the corticofugal afferents that are present in the thalamic slice) (Fig. 2b,c)[15,18,37]. In particular, a single train of stimuli applied to the corticothalamic fibers can elicit a couple of slow oscillation cycles[15,18], while the continuous pharmacological activation of mGluR1a evokes a slow oscillation that can last for many hours[15,37]. As *in vivo*, both the pharmacologically- and the synaptically-evoked slow oscillation in TC and NRT neurons *in vitro* is characterized by a large (15-20 mV) and constant membrane potential difference between UP and DOWN states, and consistently displays an inflection point at the transition between the UP and DOWN state and a robust LTCP-mediated burst of action potentials at the DOWN to UP state transition[15,18] (Fig. 2b,c). Moreover, the slow oscillation recorded *in vitro* can intrinsically group together periods of sleep spindles during the UP state of NRT neurons (Fig. 2c), and delta waves in the DOWN state of both TC and NRT neurons[15,18] (Fig. 2b), as observed in the intact brain[34,53-56,75] (Fig. 2b,c). Importantly, in the thalamic slice a slow oscillation can be recorded not only from single TC neurons but also as a large local field potential[76] (Fig. 5), indicating the ability of a thalamic nucleus to produce a synchronized population output even when deprived of the activity of cortical and NRT afferents. Whether these field potentials and associated neural synchrony depend on the activity of intranuclear synapses of TC neuron axon collaterals onto other TC neurons or interneurons[77,78,79,80] and/or on gap junction-based electrical synapses among TC neurons[76,81] remains to be determined.

The slow oscillation recorded in single thalamic neurons *in vitro* is not blocked by tetrodotoxin and its frequency is strictly governed by the amount of steady intracellular current injection[15,18,37] (Fig. 3b), indicating that it is intrinsically generated as a pacemaker activity. In fact, it results from the membrane potential bistability[82] that is generated by the interplay of I_{Leak} and the window component of the low threshold Ca^{2+} current I_T ($I_{Twindow}$), such that the UP state essentially corresponds to the condition when $I_{Twindow}$ is active and the DOWN state to when $I_{Twindow}$ is inactive[83,84]. The key role for mGluR1a activation in promoting this bistability is in reducing I_{Leak}[85,86], since in order for bistability to occur I_{Lead} must be below a specific threshold[18,83,84]. Other membrane currents that are essential for the expression of the slow oscillation in TC and NRT neurons include I_{CAN} (Ca^{2+}-activated non-selective cation current) and I_h[15,18,83,84], which in the absence of

synaptic inputs are the main determinants of the duration of the UP and DOWN states, respectively. In NRT neurons, the slow oscillation is also shaped by $I_{K(Ca)}$ and $I_{K(Na)}$ (for a detailed description of bistability in thalamic neurons, see refs. [83] and [84]).

None of these *in vitro* data are in contradiction with the results of the lesion-based experiments *in vivo*[4],[58],[59]. Rather, they indicate that the inability to observe the slow (<1 Hz) oscillation in thalamic neurons *in vivo* following decortication or transection of the corticofugal afferents can be simply explained by the absence of the cortical-mediated activation of thalamic mGluR1a and subsequent lack of a reduction in I_{Leak} below the threshold required to bring about membrane potential bistability and intrinsic slow oscillations (see Fig. 1 in ref. [83]). This framework also likely explains why a slow oscillation is apparently not detected in TC neurons of thalamocortical slices despite the presence of UP and DOWN state dynamics in neocortex[19] because i) the use of an 'unmodified' perfusing solution led to infrequent UP states that occurred in only 40% of cortical neurons, and ii) the activity of corticothalamic fibers (and thus the activation of thalamic mGluR1a) was probably small or absent, as indicated by the lack of firing or of increased synaptic noise in TC neurons following spontaneous, or thalamically-evoked, cortical UP states. Unfortunately, no firm conclusion can be drawn from the other significant *in vitro* study on UP and DOWN state dynamics in thalamocortical slices since no intracellular recordings were performed in thalamus[20].

In summary, in the same way that an isolated neocortex can, under conditions of increased excitability, elicit 'bona fide' UP and DOWN state dynamics that resemble those occurring during the slow oscillation of NREM sleep and anesthesia, so can the isolated thalamus produce a synchronized slow (<1 Hz) oscillation when the function of its postsynaptic mGluRs is re-instated. Therefore, a <1 Hz cortical rhythm is not necessary for the expression of the slow oscillation in either TC or NRT neurons. Instead, the slow oscillation in thalamus can be generated by each TC and NRT neuron operating as a 'conditional oscillator', i.e. by the dynamic interplay of their intrinsic voltage-dependent membrane currents, with sustained mGluR1a activation providing the necessary 'condition' for these oscillators to work.

The three oscillator hypothesis of the slow (<1 Hz) sleep rhythm

We would therefore argue that, contrary to the pervading cortico-centric view, the EEG slow (<1 Hz) rhythm of NREM sleep is an emergent property of cortico-thalamo-cortical networks. In particular, within a corticothalamic module it originates from the dynamic interplay of three cardinal oscillators: the mainly, but not necessarily exclusively, synaptically-based cortical oscillator (with a layer 4 thalamofugal input and a layer 5/6 corticofugal output), and two intrinsic, conditional thalamic oscillators, i.e. TC and NRT neurons (Fig. 6). Although each of these three oscillators is capable of producing its own slow oscillation, the full EEG manifestation of the slow rhythm requires the essential dynamic tuning provided by their complex synaptic interactions. Far from opening a meaningless controversy about a thalamic versus cortical and intrinsic versus synaptic genesis, our view is one that promotes strong mutual interactions between the intrinsically and synaptically based oscillators of these brain regions as the underlying mechanism of the slow rhythm.

Since two of the oscillators (i.e. the neocortical network and the TC neuron population) are capable of an independent synchronized output (Fig. 6), the start of a new UP state within any thalamocortical module will depend on the relative strength and timing of both the TC neuron and the cortical network oscillator, with the latter being in turn determined by the short- and long-distance intracortical inputs, the dynamic conditions of its layer 4 thalamo-

recipient excitatory and inhibitory neurons, and potentially by its intrinsic pacemaker neurons. Unfortunately, no systematic *in vivo* analysis of this issue, i.e. recording of synaptically connected TC neurons and layer 4 (or 6) cortical cells during the slow oscillation, has been carried out. Nor are there suitable data on large ensemble recordings in topographically-linked thalamic and cortical territories during natural NREM sleep. Nevertheless, the available evidence strongly points to the TC neuron output (i.e. the LTCP-mediated burst of action potentials that is invariably present at the onset of the TC neuron UP states) as being at least as frequent and effective a signal for eliciting the start of a new cortical UP state in a given thalamocortical module as an intracortical input. In fact, i) in the cat *in vivo* the LTCP-mediated burst of TC neurons often precedes the start of simultaneously recorded cortical UP states[34,57] (Fig. 4a), ii) in rats and mice, thalamic volleys, even at frequencies lower than that of an LTCP-mediated burst, are the most effective way of eliciting cortical UP states *in vitro* and *in vivo*[11,16,19,23,87], iii) spontaneous LTCP-mediated thalamic bursts powerfully activate neurons in the rabbit primary somatosensory cortex *in vivo*[69], iv) in cortico-thalamo-cortical slices, a brief train of stimuli that apparently produce an LTCP-mediated burst reliably triggers a cortical UP state whereas a single stimulus does not[19], and v) also in cortico-thalamo-cortical slices, the TC neuron firing precedes the onset of cortical UP states in a large number of cases despite these measurements being biased against the thalamus because the start of the cortical UP state was defined as the onset of the local field potential and not at the cellular level[20] (Fig. 4b) (Supplementary Note E).

Two additional, and in our view stronger, pieces of evidence support the TC neuron oscillator as being the most fundamental signal driving the start of cortical UP states. Firstly, that in response to increasing hyperpolarization the intrinsic slow oscillation recorded *in vitro* from TC neurons of various thalamic nuclei in different species shows a characteristic increase in frequency (i.e. from 0.03 to 1 Hz) (Fig. 3b)[15,18,37] that closely matches that observed during both the natural progression from light to deep NREM sleep and the deepening of anesthesia (Fig. 3a). In contrast, modeling studies based on experimental data from isolated cortical networks[59,63,64] show that a simulated increase in I_{Leak}, which is used to mimic the physiological changes that occur during the deepening of NREM sleep[88], actually decreases the frequency of the cortical slow oscillation, suggesting that the operation of an isolated cortex is at odds with the temporal evolution of slow sleep waves as it occurs in the intact brain. Secondly, within a particular stage of NREM sleep or state of anesthesia, and as sleep and anesthesia are deepened, individual slow wave events, i.e. K-complexes, exhibit a remarkably conserved waveform[38,45,47] (Fig. 3a). This is difficult to satisfactorily reconcile with *in vitro* recordings of the neocortical slow oscillation because when examined in slices the key cellular counterparts of the K-complex, i.e. mainly the DOWN state and DOWN to UP state transition, show considerable variability between oscillation cycles[4,47,53] (Fig. 1b and 2a). In stark contrast, in thalamic slices the components of the intrinsic TC neuron slow oscillation that correspond to the K-complex in the intact brain, i.e. the DOWN state waveform and duration, and the LTCP-punctuated DOWN to UP state transition, are not only virtually identical from cycle to cycle for any given level of membrane polarization, but are also essentially unchanged by hyperpolarization[18,37] (Figs. 1b, 2b,c and 3b). Put simply, as individual TC neurons in slices are hyperpolarized, the changes that occur in the intrinsic slow oscillation exactly mirror those that occur in the EEG slow rhythm as sleep or anesthesia deepens, whereas in the isolated neocortex this is not the case. Indeed, even from a cursory comparison of the slow oscillation observed in neocortical slices with that exhibited by individual TC neurons *in vitro*, it is clear that the latter corresponds much more convincingly to the waveform of individual EEG slow waves/ K-complexes in the intact brain, a conclusion that was also reached by the original investigators of the slow (<1 Hz) rhythm[38,45,47].

Functional implications of the three oscillator hypothesis

The three oscillator hypothesis endows the slow (<1 Hz) rhythm with three key attributes. Firstly, *rhythmicity*, i.e. UP and DOWN states in both isolated cortical networks[43] and individual thalamic neurons[84] exhibit strong periodicity. Secondly, *robustness*, i.e. individual cortical circuits[22], some types of individual cortical neurons[66], and individual TC and NRT neurons[84] have the innate ability to generate robust UP and DOWN states. Thirdly, *reciprocity*, i.e. mutually supporting actions among its main constituent elements. In particular, the LTCP-mediated burst at the start of TC neuron UP states provides the ideal signal for entraining the natural dynamics of cortical circuits. In return, action potential firing during the cortical UP state, which is of similar intensity as during wakefulness[48,89], ensures the requisite sustained activation of postsynaptic mGluRs on thalamic neurons and thus the generation of the long-lasting component of the corticothalamic EPSPs[18,74,85,86], which is essential for these neurons to behave as conditional oscillators[83,84]. Finally, the inhibitory NRT neurons guarantee an effective moderation of the slow oscillation in TC neurons since they exhibit i) a more prolonged LTCP-burst at the beginning of the UP state, ii) more intense firing during the subsequent part of the UP state, and iii) a longer intrinsic UP state to ensure inhibitory input throughout the TC neuron UP state[15,18,53].

A clear advantage of the three oscillator system as the underlying neuronal basis of the slow (<1 Hz) rhythm is that the full independence of the conditional thalamic oscillators from rhythmic synaptic inputs and their almost complete reliance on intrinsic voltage-dependent currents ensure the robustness of the basic temporal framework of the slow oscillation UP and DOWN states. This, in turn, guarantees that the regularity of the slow oscillation is unaffected by, and occurs independently from, concomitant data-processing streams in neocortex and other higher brain regions (e.g. hippocampus, entorhinal cortex), which can then 'focus' on other potentially more sophisticated tasks (e.g. replay/consolidation of previous wake-related activities[29,33,90-93]) without being 'distracted' by the need to maintain the basic rhythm of the slow oscillation.

Another potential advantage afforded by the three oscillator hypothesis is that the spread of the slow oscillation across different thalamocortical modules would rely not only on the short- and long-distance intracortical connections but also on thalamofugal fiber activity, with the synchronized output of sensory thalamic nuclei providing a timing signal to their respective primary cortical areas and the divergent efferents of intralaminar thalamic nuclei supplying a more diffuse input to both superficial and deep layers of non-primary cortical areas[94]. In this respect, future studies in freely waking-sleeping animals should investigate the degree of synchronization across sensory, motor and intralaminar thalamic nuclei and how this contributes to the traveling of slow waves across the cortical mantle.

The LTCP-mediated burst firing at the start of each TC neuron UP state[18,34] may carry further computational significance in addition to being a simple restart signal for cortical UP states. The large and dendritically widespread Ca^{2+} entry associated with the LTCP-mediated bursts of the slow oscillation in TC neurons (Errington, Lörincz, Hughes & Crunelli *Soc. Neurosci. Abstr.* 44.1, 2008) may provide a 'biochemical' window for modifications of intrinsic excitability and synaptic strength (see also ref. [95]), whereas the temporal dynamics of this firing pattern might instate an 'electrophysiological' window for spike-timing dependent synaptic plasticity when coupled with the immediately following, top-down information arriving via corticothalamic fibers. Indeed, it is at the distant dendritic sites where these afferents make synapses on thalamic neurons[71,73] that there exists the key combination of T-type Ca^{2+} channels and mGluRs which may be essential for establishing burst firing-dependent synaptic plasticity[96]. The presence of these 'windows' for plasticity at the start of the UP state would thus offer a physiological explanation as to why thalamic

neurons require these types of oscillation dynamics during NREM sleep and why all the important EEG signatures of NREM sleep (e.g. ripples, spindle waves) that have been associated with memory processes invariably occur during the UP states[38,39,46,90]. An LTCP-mediated burst at the start of the UP states could also be important for synaptic plasticity in neocortical neurons, which shows a brief firing elevation at this very phase of the slow oscillation[48,89]. Indeed, it would be important to establish whether synaptic plasticity is specifically expressed at layer 4 thalamocortical synapses when the paired protocol includes an LTCP-like high-frequency burst similar to that elicited at the start of each TC neuron UP state (Supplementary Note F).

Recently, a potential key role for the slow (<1 Hz) oscillation of natural NREM sleep in memory processes has been highlighted by sophisticated experiments in humans[28,30,33] and by the observation in experimental animals that firing sequences generated during previous active wakefulness are re-played, albeit in a temporally compressed manner, and potentially consolidated during synchronized cortical UP states[29]. Interestingly, as the firing of TC neurons at the start of an UP state often precedes that of cortical neurons by a few tens of milliseconds[34,75] (Fig. 4a), so does the cortical firing typically precede, by about 50 msec, the activity of hippocampal neurons in freely behaving animals[29]. Where in this temporal sequence the firing of other brain areas that express UP and DOWN states and which are important for motor and limbic plasticity, e.g. striatum[97], or for behavioral state-related shifts, e.g. parabrachial nucleus[98], locus coeruleus (Sara, S.J. & Eschenko, O. *Soc. Neurosci. Abstr.* 688.22, 2008) and various hypothalamic areas[99], precisely fits in is of importance but at present unknown.

Conclusions

On the basis of a re-appraisal of the available *in vivo* and *in vitro* evidence, we have argued here that the slow (<1 Hz) rhythm of NREM sleep should no longer be viewed as originating solely from cortical territories, but from the intricate dynamic interactions of three cardinal oscillators: a predominantly synaptically-based cortical oscillator and two intrinsic, conditional thalamic oscillators. In particular, the prominent LTCP-mediated bursts that initiate the UP states in TC neurons provide an ideal signal for initiating large-scale UP states in cortical networks. Moreover, the gradual increase in slow wave frequency that occurs as sleep or anesthesia are deepened, and the conserved waveform of individual slow waves, or K-complexes, can only be satisfactorily explained by considering the intrinsic oscillatory properties of individual thalamic neurons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Our work in this area is supported by The Wellcome Trust (grants 71436, 78311 and 78403). Additional information regarding this and other published work is available at http://www.thalamus.org.uk.

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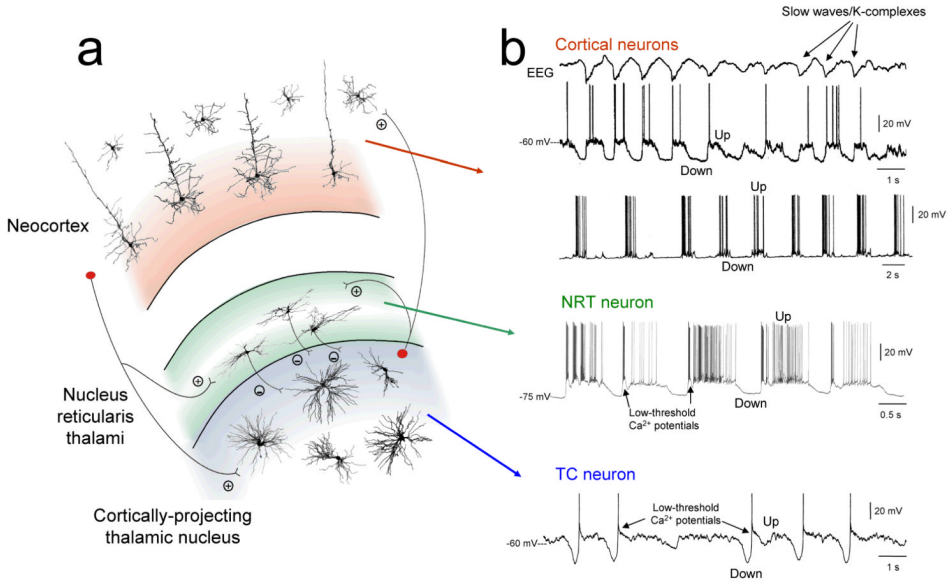

Figure 1. The EEG slow (<1 Hz) rhythm and its cellular counterpart in cortical and thalamic neurons

(**a**) Schematic diagram of a cortico-thalamo-cortical module with its most relevant cellular components and synaptic connections (thalamic interneurons and neocortical neurons other than those in layer 4 and 5/6 have been omitted for clarity). (+) and (−) indicate excitatory and inhibitory synapses, respectively. (**b**) The slow (<1 Hz) rhythm in the EEG (top trace), and its cellular counterpart, the slow (<1 Hz) oscillation, recorded in cortical and thalamic neurons of anaesthetized cats. TC: thalamocortical; NRT: nucleus reticular thalami. Traces in **b** are reproduced with permission from refs. [47,3,100,57] (from top to bottom).

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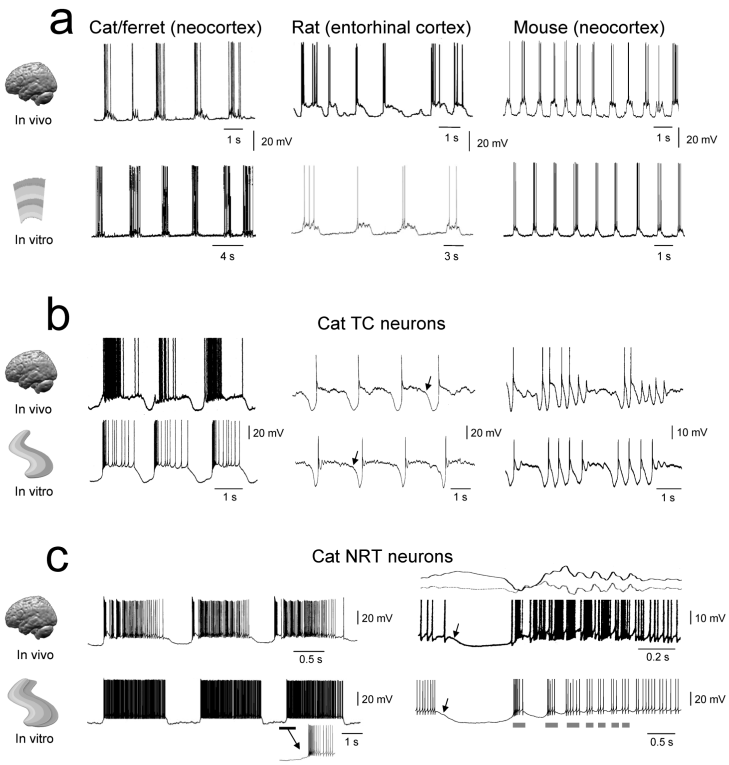

Figure 2. The slow (<1 Hz) oscillation in cortical and thalamic neurons *in vivo* and its reproduction *in vitro*

(**a**) Comparison of the slow oscillation recorded in cat area 5, rat entorhinal and mouse auditory cortex *in vivo* during anaesthesia (top traces, from left to right, respectively) with that recorded *in vitro* in slices of ferret prefrontal, rat entorhinal and mouse auditory cortex (bottom traces from left to right, respectively). *In vitro* recordings were obtained in the presence of a modified medium containing either a reduced Ca^{2+} concentration (left and middle) or the cholinergic agonist carbachol (right). (**b**) Comparison of the slow oscillation recorded *in vivo* in three TC neurons of anaesthetized cats (top traces) and with that observed in slices of the cat dorsal lateral geniculate nucleus (LGN) *in vitro* in the presence of an mGluR agonist (bottom traces). Arrows mark inflection points in the membrane potential at the transition from the UP to the DOWN state. (**c**) Comparison of the slow oscillation recorded *in vivo* in two nucleus reticularis thalami (NRT) neurons of anaesthetized cats (top traces) with that observed in NRT neurons from cat LGN-perigeniculate nucleus slices *in vitro* in the presence of an mGluR agonist (bottom traces). Note the presence of sleep spindle activity in the top two traces on the right (surface and depth EEG records). Traces in **a** are reproduced with permission from refs. [2,18,22,67] (from top left to bottom right). Traces in **b** are reproduced with permission from refs. [100,57,4,18] (from top left to bottom right). Traces in **c** are reproduced with permission from refs. [34,53,15] (from top left to bottom right).

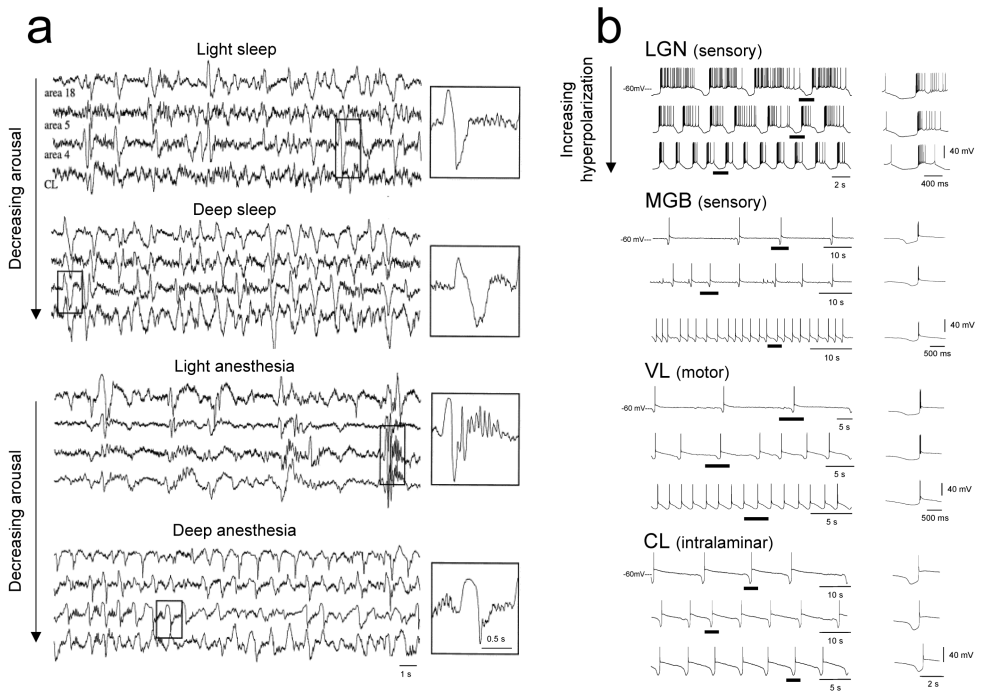

Figure 3. The frequency of the EEG slow rhythm at different depths of sleep and anesthesia matches the voltage-dependence of the slow oscillation frequency in TC neurons

(**a**) EEG recordings during light and deep natural sleep in freely moving cats are compared to those during light and deep anaesthesia in the same species. The frequency of the slow oscillation increases with the deepening of natural sleep and anaesthesia. Note the presence of sleep spindles occurring in combination with some of the individual K complexes/slow waves that are enlarged on the right. (**b**) Voltage-dependence of the slow oscillation in TC neurons of sensory, motor and intralaminar thalamic nuclei of the cat *in vitro*. For each nucleus, three traces recorded from the same TC neuron in the presence of an mGluR agonist are shown at increasingly negative values of steady current injection (from top to bottom). Note how the increase in frequency with increasingly negative steady current is due to the shortening of the UP state duration, whereas the length of the DOWN state remains constant. (LGN: dorsal lateral geniculate nucleus; MGB: medial geniculate body; VL: ventrolateral nucleus; CL: central lateral nucleus). **a** and **b** are reproduced with permission from refs. [47] and [37], respectively. CL traces in **b** are unpublished observations (Watson J. & Crunelli, V.).

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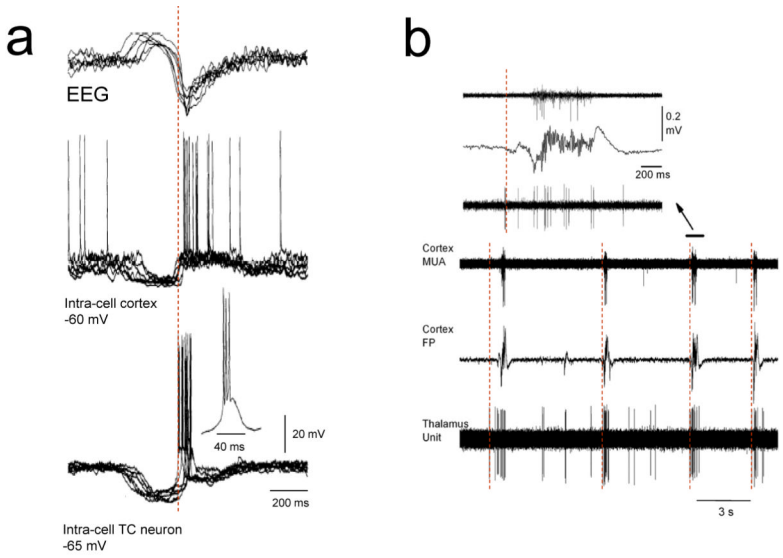

Figure 4. The start of the TC neuron UP state firing precedes that of the cortical UP states

(**a**) Simultaneous EEG and intracellular recordings from a cortical and a TC neuron from an anaesthetized cat show that the LTCP-mediated burst of action potentials that is present at the start of the TC neuron UP state precedes the firing of the cortical neuron and the depth-negative peak of the EEG wave. The seven events superimposed on each panel are aligned with respect to the peak-negativity of the EEG wave. The dashed red bar marks the earliest LTCP-burst of the illustrated TC neuron records. One LTCP burst is enlarged. (**b**) Simultaneous cortical local field potential (FP) and multi-unit activity (MUA), together with a thalamic single unit recording obtained in a slice with functionally viable thalamocortical and corticothalamic connections show the TC neuron firing to precede the cortical population firing. The dashed red bars mark the start of the thalamic unit firing. **a** and **b** are reproduced with permission from refs. [34] and [20], respectively.

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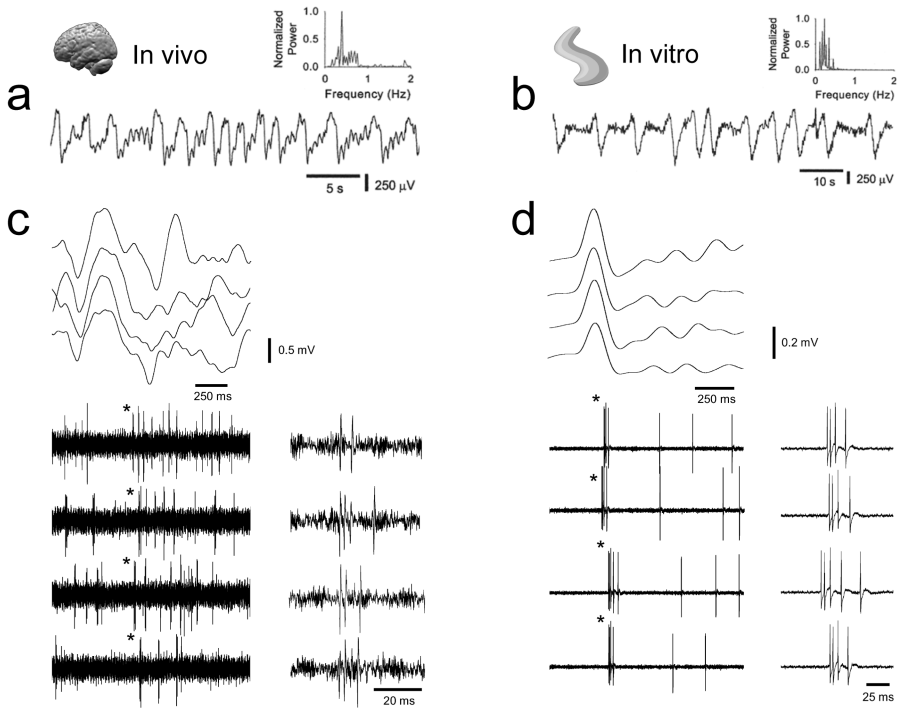

Figure 5. Synchronized thalamic slow oscillation during natural sleep and in a brain slice

(**a**) Slow (<1 Hz) oscillation recorded in the local field potential from the dorsal lateral geniculate nucleus of a naturally sleeping cat and (**b**) in a slice of the same thalamic nucleus in the presence of an mGluR agonist, with the respective power spectra illustrated at the top right. (**c** and **d**) Four consecutive slow waves (top traces) recorded under the same experimental conditions as in **a** and **b**, respectively, and the corresponding single unit activity (bottom left traces). In both cases, during the large positive wave the neuron is silent, whereas at the end of the wave it generates a characteristic LTCP-mediated high-frequency burst of action potentials with interspike intervals that increase as the burst progresses (enlarged in the bottom right traces). All traces are reproduced with permission from ref. [76].

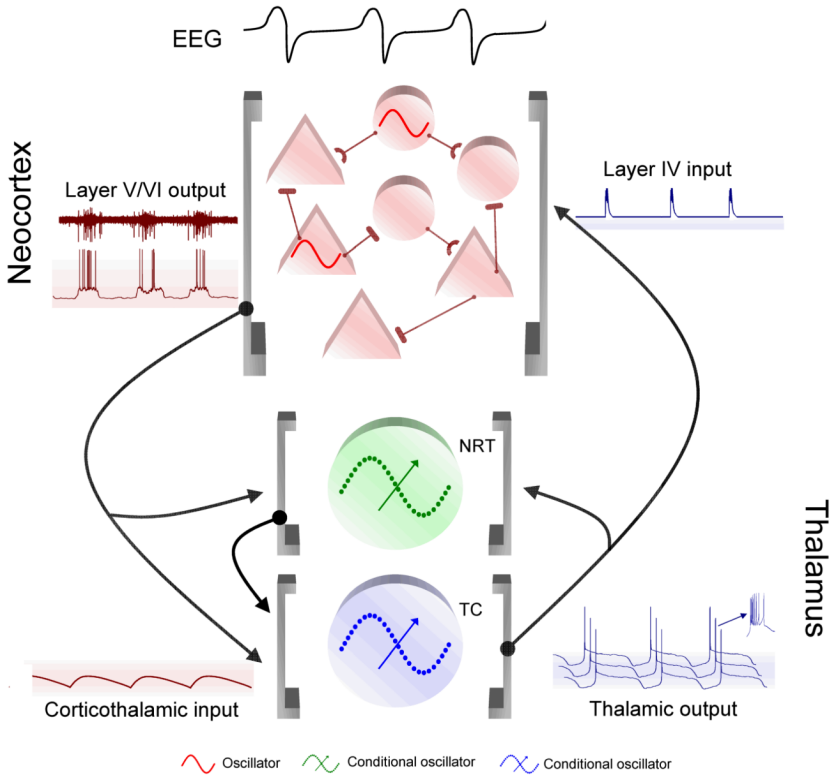

Figure 6. Schematic flow diagram of the dialogue between cortical and thalamic oscillators that underlies the slow (<1 Hz) rhythm within a thalamocortical module

The prolonged UP states of the slow oscillation in layer 5/6 cortical neurons lead to long-lasting corticothalamic EPSPs in TC and NRT neurons. These slow EPSPs represent an mGluR-induced reduction in I_{Leak} which is the necessary 'condition' that must be met in order for thalamic neurons to exhibit the slow oscillation. The LTCP-mediated high-frequency burst that is invariably present at the start of each UP state of the TC neuron slow oscillation leads to highly effective bursts of thalamocortical EPSPs that initiate a new UP state in NRT and layer 4 neurons. The overall UP and DOWN state dynamics of a cortical region, however, are maintained by synaptically-generated barrages of excitation and inhibition from other cortical neurons as well as being potentially fine tuned by additional intracortical inputs from intrinsically oscillating neurons in layer 2/3 and 5. Across different thalamocortical modules (including both primary and association cortices), additional synchronizing inputs are provided by short- and long-distance intracortical connections and by intralaminar thalamic afferents which are not restricted to layer 4 (not shown).