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Role for T-type Ca^{2+} channels in sleep waves

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Abstract Since their discovery more than 30 years ago, low-threshold T-type Ca^{2+} channels (T channels) have been suggested to play a key role in many EEG waves of non-REM sleep, which has remained exclusively linked to the ability of these channels to generate low-threshold Ca^{2+} potentials and associated high-frequency bursts of action potentials. Our present understanding of the biophysics and physiology of T channels, however, highlights a much more diverse and complex picture of the pivotal contributions that they make to different sleep rhythms. In particular, recent experimental evidence has conclusively demonstrated the essential contribution of thalamic T channels to the expression of slow waves of natural sleep and the key role played by Ca^{2+} entry through these channels in the activation or modulation of other voltage-dependent channels that are important for the generation of both slow waves and sleep spindles. However, the precise contribution to sleep rhythms of T channels in cortical neurons and other sleep-controlling neuronal networks remains unknown, and a full understanding of the cellular and network mechanisms of sleep delta waves is still lacking.

Keywords Cortex · Thalamus · Sleep slow oscillation · Sleep spindles · Delta waves · Slow waves · Theta waves · I_{Twindow} · I_h · I_{CAN} · Sleep waves · Ca^{2+} channels · Neural networks

Introduction

As this review is part of a special issue on T-type Ca^{2+} channels (T channels), it feels appropriate to firstly provide the reader with an overview of the stereotyped sequences of electrical waves that are recorded in the EEG during natural non-REM sleep. Importantly, while the source(s) of the electrical waves observed in scalp EEG recordings are located in the upper layers of the neocortex, their generator(s) are the dynamical interactions between the different neuronal network activities that are expressed by various component neurons of the corticothalamic loop. In humans, the occurrence of theta waves (3–7 Hz) over a generally desynchronized EEG characterizes the first stage of non-REM sleep, while in stage 2 occasional K-complexes and slow waves start to appear. Sleep spindles are also present in stage 2, either in isolation or associated with a K-complex. The EEG in stage 3 sleep still presents spindle episodes but also shows clearly defined periods of delta waves (0.5–4 Hz) that together with slow waves (<1–2 Hz) become the predominant activity as sleep deepens into stage 4. This smooth and progressive transition from stage 1 to stage 4 non-REM sleep is invariably accompanied at the neuronal level by a reduction in the depolarizing tone exerted by cholinergic, monoaminergic, and histaminergic afferents from brainstem and mammillary body onto both cortical and thalamic neurons [60] (with some exceptions, see ref. [60]), leading to a progressive hyperpolarization of the majority of cortical and thalamic neurons [42, 78, 89].

The first insight into the role of T channels in sleep waves came from their discovery in thalamic neurons, and in particular the finding that activation of these channels, following a

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period of membrane hyperpolarization, leads to a voltage waveform known as the low-threshold Ca^{2+} potential (LTCP) or low-threshold spike [30, 32, 46, 47, 55]. Since then, the main and only widely recognized function of thalamic T channels in sleep waves has been that of providing the rhythmic LTCP-mediated sequences of high-frequency bursts of action potentials that characterize the cellular activity of these neurons during sleep spindles and delta waves as well as at the start of an up state of sleep slow waves [7, 8, 45, 53, 54, 61, 76, 81, 82, 84, 92]. However, the role of the T channels in sleep and non-REM EEG oscillations can no longer be restricted to the stereotypical LTCPs of thalamic neurons, since (1) it involves other physiological voltage waveforms that are dependent on the “window current” of these channels (i.e., I_{Twindow}) [10, 43, 44, 91, 93], and (2) because non-thalamic neuronal populations, e.g. those in the neocortex and in sleep-controlling brain regions, show a marked expression of T channels [28, 35, 38, 40, 41, 48, 71, 87]. This short review will address these issues after presenting a brief overview on the biophysics of T channels, and in particular on I_{Twindow} and its physiological consequences for neuronal excitability (for detailed descriptions of the molecular genetics, biophysics, and neuronal cell type distribution of T channels, see other contributions to this special issue). In addition, as few experiments have so far analyzed the role of T currents in naturally sleeping animals, we will also discuss how the current view of T channel function in sleep may be clouded by the speculative extrapolations of data obtained either in brain slices or in anesthetized preparations where EEG waves similar, though not identical, to those observed during the various stages of natural non-REM sleep can be recorded (see [21]). Finally, we will highlight the current difficulty in correctly identifying the cellular and network mechanisms of delta waves because of the partial overlap of their frequency band with that of slow waves.

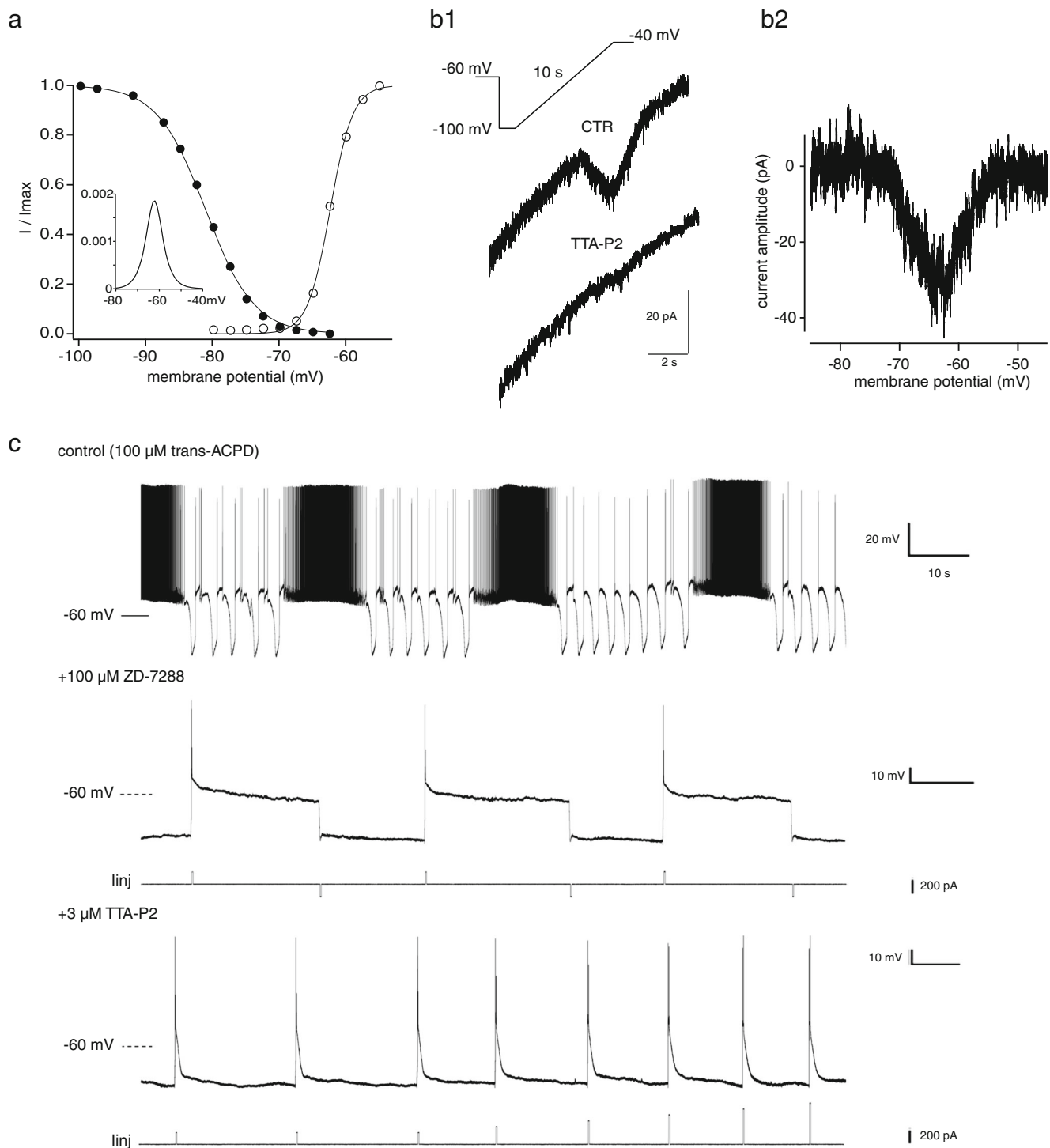
Biophysics and physiological impact of the “window current” generated by T channels

Since their original characterization in primary sensory neurons [11, 13, 36, 65], two main types of native T type Ca^{2+} currents that display either “fast” or “slow” activation and inactivation kinetics were reported [45, 68]. Cloning of the three low-threshold Ca^{2+} channel genes (Cav3) further confirmed this crude categorization. Cav3.1 and Cav3.2 ($\alpha 1\text{G}$ and $\alpha 1\text{H}$, respectively) generate low-threshold Ca^{2+} currents displaying fast activation and inactivation mechanisms [18, 69], while Cav3.3 ($\alpha 1\text{I}$) shows much slower kinetics [52]. Regardless of this difference in gating kinetics, all native and recombinant channels share the same basic voltage dependence with an activation threshold and a nearly complete steady-state inactivation around -60 mV [68].

However, a closer look at the gating properties of the T channels reveals that the steady-state activation and inactivation curves overlap (Fig. 1(a)). Therefore, in this voltage region that corresponds to neuronal resting membrane potentials, a few T channels are not inactivated and their open probability is close but not equal to zero, hence a tonic T current (i.e., I_{Twindow}) is generated. Since the activation and inactivation curves are obtained by fitting currents that in this voltage region are obviously very small, a precise estimation of I_{Twindow} , particularly for native channels, is difficult to achieve, and thus great caution should be used in interpreting these data. Nevertheless, investigations on recombinant channels have suggested that Cav3.3 channels may generate a larger and more depolarized I_{Twindow} (see Fig 7 in [68]; [15]) than that elicited by Cav3.1 and Cav3.2 channels. These differences in I_{Twindow} should be carefully considered when assessing the precise role of this tonic current in the excitability of neurons that possess different complements (and different subcellular distributions) of the three isoforms of T channels. In particular, the glutamatergic thalamocortical (TC) neurons only express Cav3.1 channels while the GABAergic neurons of the nucleus reticularis thalami (NRT) possess Cav3.3 channels in addition to a small component of Cav3.2 channels [45, 87].

Although I_{Twindow} is an inherent biophysical property of all T channels, its amplitude in some neurons may be too small,

Fig. 1 The window T current tightly controls thalamic neuron excitability. **a** Normalized activation and steady-state inactivation curves of T current recorded in a TC neuron from the rat ventrobasal thalamic nucleus. The activation curve was constructed by successive step depolarizations from -80 to -45 mV (2.5 mV increments) preceded by a 1-s hyperpolarizing pre-pulse to -100 mV. Inactivation of the T channels was induced using a 1-s pre-pulse of increasing potential (from -100 to -60 mV with 2.5 mV increments) and the resulting channel availability was estimated from the normalized current amplitude measured at -50 mV. Data were fitted by Boltzmann equations. *Inset* illustrates the voltage dependence of the steady-state channel activation (window current) estimated from the product of the Boltzmann fits of the normalized activation and inactivation curves. **b** The window T current evoked by a 10-s-long depolarizing voltage ramp from -100 to -40 mV preceded by a 1-s hyperpolarizing prepulse to -100 mV is fully blocked by the selective T channel blocker TTA-P2 (1 μM). **B2** Voltage dependence of the window T current shown in B1. **c** In the continuous presence of trans-ACPD, recording from a cat TC neuron in an thalamic intralaminar nucleus reveals a slow oscillation (*top trace*) consisting of regularly recurring up and down states intermixed with much longer up states with continuous tonic action potentials firing. Each down state starts with a clear inflection point leading to a stereotypical large hyperpolarizing potential that, upon I_h activation, slowly repolarizes the neuron up to the LTCP threshold (see also Fig. 2c). Following the block of I_h with ZD 7288 (*middle trace*), the neuron exhibits two stable resting membrane potentials. Transitions between stable equilibrium potentials are evoked by short steps of positive or negative injected currents (I_{inj}) that trigger an LTCP and switch off I_{Twindow} , respectively. Upon application of TTA-P2 that progressively blocks the T channel population, the bistable behavior quickly disappears due to the decrease in I_{Twindow} while enough T channels remain to evoke LTCPs (lower trace). **B1–B2**: reproduced with permission from [34]



thus precluding a significant physiological role in cellular excitability. However, both TC and NRT neurons express especially large T currents [6, 9, 31, 34] and a significant number of T channels are still de-inactivated around -60 mV. Thus, although the open probability of the channels is very low at these potentials, an I_{Twindow} of about 30 pA can be measured in TC neurons using voltage ramps that are slow

enough to achieve steady-state equilibrium between activation and inactivation of the T channels (Fig. 1(b)). Block of this tonic current with the specific T channel antagonist, TTA-P2, induces a 3-mV hyperpolarization of TC neurons held at -60 mV but has no effect when the neuron is held at a membrane potential outside the voltage range of I_{Twindow} activation (see Fig. 3 in [34]). As predicted from the

biophysics of recombinant Cav3.3 channels, an even larger hyperpolarization (5 mV) is observed upon application of TTA-P2 in NRT neurons [34], demonstrating that $I_{T\text{window}}$ play a crucial role in setting the resting membrane potential of both TC and NRT neurons. Moreover, when metabotropic glutamate or muscarinic receptors are activated, the interplay between the characteristic bell-shaped voltage dependence of $I_{T\text{window}}$ (see inset in Fig. 1(a)) and the leak current creates a marked (up to 20 mV) bistability of the resting membrane potential of TC and NRT neurons (Fig. 1(c), middle trace) [20, 44]. The shift between these two stable membrane potentials can occur spontaneously as an intrinsic mechanism (leading to the appearance of repetitive up and down states of sleep slow waves, see next section) (Fig. 1(c), top trace) [43] or can amplify small-amplitude subthreshold synaptic potentials leading to the generation of a rebound LTCP (see Fig. 6 in [93]).

Importantly, it is possible to block membrane bistability with the T channel blocker TTA-P2 while leaving the LTCP and its associate firing almost intact (Fig. 1(c), bottom trace). Indeed, up and down states quickly disappear upon a short period of TTA-P2 application that slightly reduces the functional T channel population whereas the full block of LTCPs requires much longer antagonist application (see Fig. 7 in [34]). These two experiments clearly demonstrated that the high density of T channels expressed in thalamic neurons far exceeds that required to generate a LTCP [9, 34]. Therefore, it is highly probable that such high density of T channels provides the significant number of de-inactivated channels at depolarized potentials that are required for the full repertoire of the physiological responses of thalamic neurons. Finally, it is important to point out that these T channels available at depolarized membrane potentials not only generate $I_{T\text{window}}$ in low-open probability conditions but are also recruited by synaptic activities and intrinsic noise that, by mediating a drastic increase in open probability, generate additional transient T currents that boost post-synaptic potentials (see Fig. 1 in [29]).

Thalamic and cortical T channel contribution to sleep slow waves

Sleep slow waves are one of the fundamental EEG wave of non-REM sleep (Fig. 2a). They are present in almost all non-REM sleep stages [1–3, 37, 58, 75, 77], underlie sleep K-complexes [2, 3], and group together periods of sleep spindles [3, 62] and delta waves [2, 77]. The cellular counterpart of the sleep slow rhythm recorded in the EEG is the regular recurrence of a depolarized (up) state and a hyperpolarized (down) state of the membrane potential, that occurs synchronously in all cortical [77, 85, 86] and thalamic neurons [3, 16, 37, 58, 62, 75, 77, 79].

In cortical neurones, sleep slow waves result from intense excitatory and inhibitory synaptic barrages that generate the up state and their absence that causes the down state [73, 85, 86, 89]. Although T currents are not considered to play a major role in this process, such powerful synaptic activity and the resulting changes in membrane potential do of course engage a variety of voltage-dependent channels, including T channels. Indeed clear examples of LTCPs can be seen in recordings from cortical neurons during slow waves in anesthetized animals [16], as one would expect from the presence of (1) all three T channel isoforms in the neocortex [87] and (2) LTCPs in layers V–VI pyramidal neurons [28, 38, 41], as well as in somatostatin [48] and VIP [71] interneurons recorded in slices. It is surprising, therefore, that no study has directly investigated so far the role of T channels in the activity of different cortical neurons during slow waves of non-REM sleep.

Because thalamic lesions do not suppress slow waves in anesthetized cats [85] and up and down states can be recorded in neocortical slices [17, 73] and in an isolated cortical gyrus in vivo during anesthesia [88], these EEG slow waves were originally viewed as a cortically generated rhythm [12, 14, 88]. However, up and down states and slow waves similar to those observed in vivo can be recorded in thalamic slices [10, 21, 43], and a recent study has conclusively shown that selective block of thalamic firing by tetrodotoxin markedly reduces the frequency of EEG slow waves both in anesthetized and naturally sleeping rats [26]. Thus, a dynamic interplay between the synaptically driven neocortical oscillator and the thalamic oscillators of slow waves is necessary for the full expression of these waves of natural non-REM sleep.

As far as the thalamic oscillators are concerned, many studies have clearly demonstrated that the T channels of TC and NRT neurons contribute to the expression of sleep slow waves in three ways. Firstly, by evoking the LTCP (with high-frequency burst of action potentials) that almost invariably marks the start of every up state (Fig. 2c). Secondly (as mentioned in the previous section), by providing the membrane potential bistability that underlies the up and down state transitions, i.e., up state = $I_{T\text{window}}$ “on” and down state = $I_{T\text{window}}$ “off” (Fig. 2c) [19, 22]. Thirdly, by providing the selective Ca^{2+} entry that is required to activate (1) the Ca^{2+} -activated, non-selective cation current (I_{CAN}), which tightly controls the durations of the up states [43] (Fig. 2c), and (2) the Ca^{2+} -activated, K^{+} channels (SK type) that contribute to the hyperpolarization that follows an LTCP [23, 24] (for a comprehensive biophysical description of these mechanisms and a list of the other currents contributing to sleep slow waves in thalamic neurons, see ref [22]).

On the basis of all these data, one would expect slow waves of natural sleep to be compromised in the absence of thalamic T channels. Surprisingly, the original study in mice with global knockout of the Cav3.1 isoform of the T channels

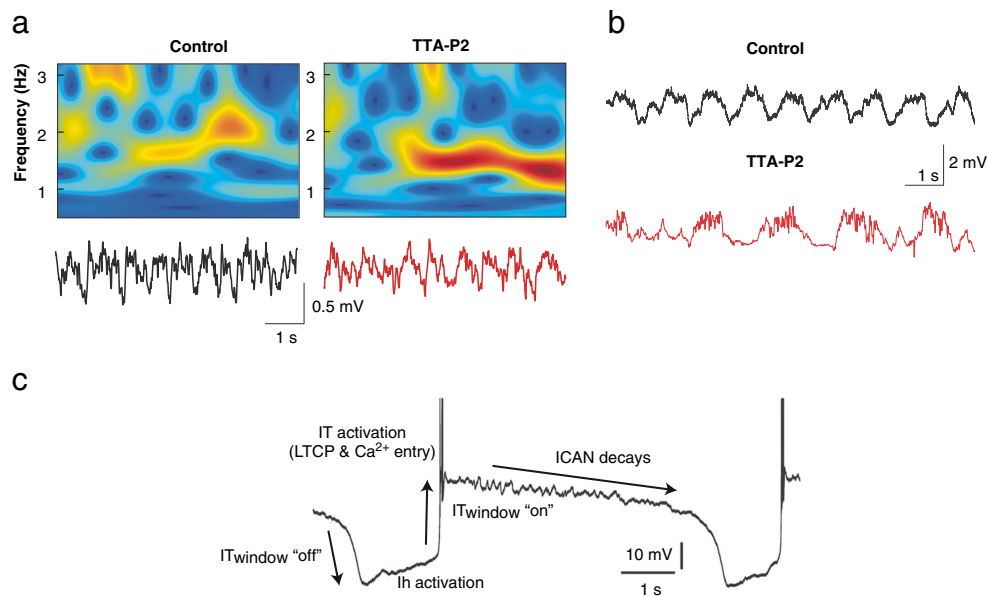


Fig. 2 Contribution of thalamic T channels to sleep slow waves. **a** EEG slow wave and corresponding wavelet scalograms recorded in a rat during natural sleep. Continuous thalamic microdialysis injection of a solution containing the T channel antagonist TTA-P2 (3 mM) reduces the frequency of slow waves compared to the injection of artificial cerebrospinal fluid (Control). **b** EEG slow wave recorded in a rat during ketamine/

xylazine anesthesia. Continuous thalamic microdialysis injection of a solution containing 3 mM of TTA-P2 decreases the frequency of slow waves compared to the injection of artificial cerebrospinal fluid (Control). **c** Schematic diagram of two cycles of slow waves in a TC neuron shows the various contributions of T channels to this oscillation. **a–c**: reproduced with permission from [26] and [20]

reported no change in EEG slow wave power [50]. Similar results were observed in mice with a “putative thalamic-selective” knockout of the same T channel isoform, though recombination was also present in some cortical regions and hypothalamic nuclei [4]. However, the negative results of these two studies cannot be simply interpreted as indicating a lack of involvement of the Cav3.1 isoform in slow waves since (1) compensation by other T channel isoforms or other voltage- and transmitter-gated channels might have occurred in the thalamus of these two types of KO mice, and (2) Cav3.1 T channels that are strongly expressed in brain areas other than the thalamus (see section below) were definitively knocked-out in these mice, with unpredictable consequences on slow waves and other sleep rhythms. Confirmation of an essential role for thalamic T channels in sleep slow waves has finally been provided by experiments where optogenetics and neuronal ensemble recordings were combined with localized thalamic microdialysis injections of the selective T channel antagonist TTA-P2 [26]. Thalamic dialysis concentration of TTA-P2 that fully blocks T channel mediated burst firing produces a consistent reduction in the frequency of slow waves during anesthesia and natural non-REM sleep (Fig. 2a, b). In addition, block of thalamic T channels suppresses the ability of selective optogenetic activation of TC neurons to entrain EEG slow waves [26]. These data, therefore, provide conclusive evidence on the essential role played by thalamic T channels in slow wave of non-REM sleep.

Thalamic and cortical T channel contribution to sleep spindles

A typical sleep spindle is a waxing and waning wave that lasts for a few seconds, has a frequency of 12–15 Hz in humans (but 8–12 Hz in rodents), and can occur in isolation from other sleep waves though it is mostly observed in close association with a K-complex (Fig. 3(A1, A2)) [3, 27, 62]. LTCPs are present at almost every cycle of the spindle wave in NRT neurons, and occasionally in TC neurones (Fig. 3(A3, A4)). The firing associated with the LTCPs generated by NRT neurons evokes GABA_A IPSPs in TC neurons, some of which provide enough time- and voltage-dependent removal of T channel inactivation so that an LTCP (with or without the associated high-frequency burst) can then be generated. In turn, the LTCP-evoked firing of TC neurons elicits EPSPs in NRT neurons which help to trigger LTCPs at spindle frequency (Fig. 3(A3, A4)).

In addition to the LTCPs, another key contribution of T channels to the electrical activity of TC neurons during sleep spindles is to provide the Ca²⁺ entry that regulates the cAMP-mediated upregulation of *I_h* [56, 57]. It has been postulated that the potentiation of this inward current leads to a progressively larger depolarization of TC neurones during a spindle wave and ultimately to their inability to generate LTCPs, thus contributing to the spindle wave termination. A similar mechanism may occur in NRT neurons where the presence of HCN

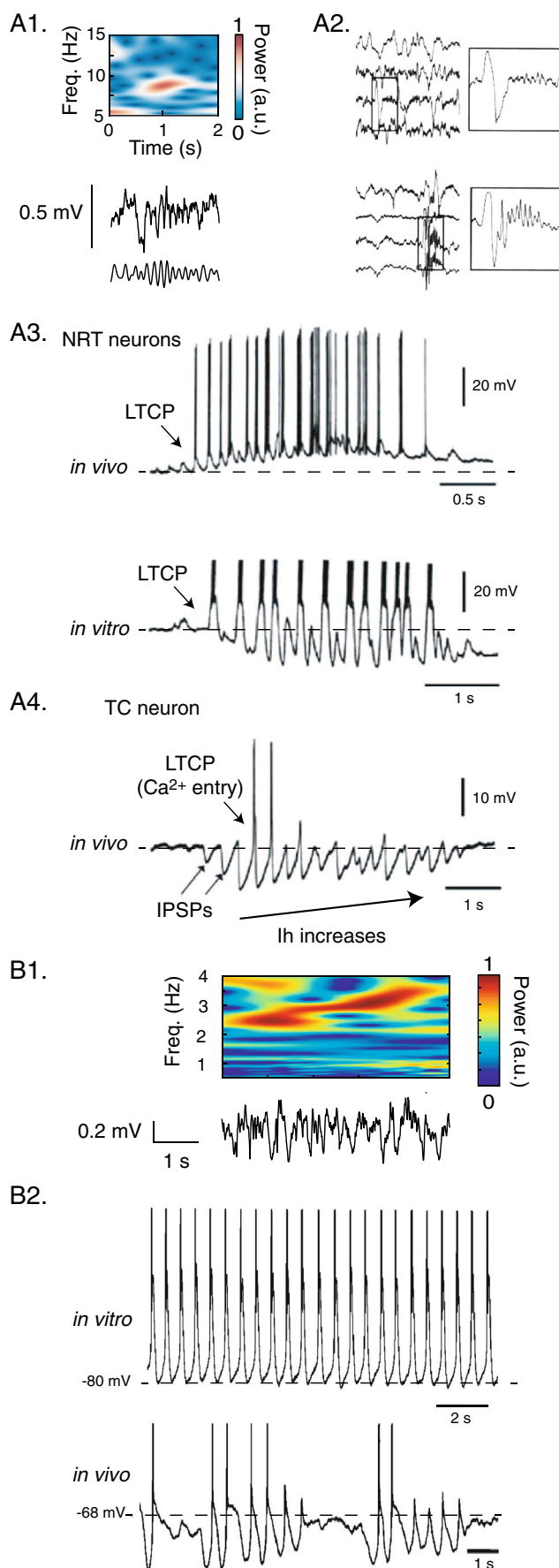


Fig. 3 Contribution of thalamic T channels to sleep spindles and comparison of delta waves recorded in vivo and in vitro. **A1** Filtered (5–15 Hz) (bottom trace) and unfiltered (upper trace) examples of a spindle recorded in a rat during natural sleep (graph shows the corresponding wavelet scalogram). Note the occurrence of the spindle immediately after a down state. **A2** Spindles recorded in a cat during natural light sleep and anesthesia (top and bottom set of traces, respectively). Note the association with a K-complex in the boxed spindles. **A3** Top trace: intracellular recording from a cat NRT neuron under barbiturate anesthesia shows rhythmic LTCPs superimposed on a depolarizing envelope during a spindle wave. Bottom trace: in contrast, in vitro intracellular activity from a ferret neuron during spindle-like oscillations recorded in a slice preparation revealed LTCPs of increasing amplitude followed by a progressively larger afterhyperpolarization. **A4** In vivo intracellular recording from a cat TC neurone under barbiturate anesthesia shows that the NRT-evoked IPSPs occasionally give rise to LTCPs. The resulting increase in intracellular Ca²⁺ concentration can progressively activate I_h, producing an increasing depolarizing drive that contributes to spindle wave termination. **B1** EEG trace showing delta waves at 2.5–3.5 Hz in a naturally sleeping rat and corresponding wavelet scalogram. **B2** In vitro recording of a TC neuron in an adult cat slice displays continuous oscillations at delta frequency upon hyperpolarizing DC current injection (top trace). In contrast, in vivo recordings in a cat TC neuron under ketamine/xylazine anesthesia clearly showed that delta waves occur as transient episodes during the down state of slow waves (bottom trace). **A1–B2**: reproduced with permission from [92], [70], [79], [26], [80]

isoforms [63, 64] and I_h [10, 72] has now been demonstrated. Other roles of T channels in NRT neurons during sleep spindles include the Ca²⁺ entry necessary to activate SK channels [24] and potentially I_{CAN} [10].

This well-accepted mechanism of spindle wave generation based on the recruitment of both TC and NRT T channels has emerged from intracellular recordings in anesthetized animals (often after systemic injection of barbiturates to increase the occurrence of spindle waves) and in in vitro slice preparations. Therefore, caution should be used when interpreting these results obtained in such different experimental conditions and extrapolating this mechanism to natural sleep spindles. Indeed, spindle waves tend not to occur in association with a K-complex under barbiturate anesthesia, contrary to what is observed during ketamine/xylazine anesthesia and natural sleep. In addition, the LTCPs of NRT neurons in vivo during spindle waves emerge from a depolarizing envelope, whereas spindle-like activity in vitro consists of LTCPs of increasing amplitude, each followed by a progressively larger afterhyperpolarization (Fig. 3(A3)).

Unfortunately, data obtained so far in naturally sleeping T channel KO mice do not help to clarify the role of the different T channel isoforms in the sleep spindle. Thus, genetic knock-out of Cav3.1 channels (which abolishes T current in all TC neurons) was originally reported to significantly decrease EEG power in the 8–10 Hz frequency band [50], although some remaining spindles showing a reduced amplitude were still present, whereas a later study by the same group reported no effect when spindle events were filtered at 6–15 Hz [51]. In

Cav3.3 KO mice, no difference in the 10–12 Hz EEG power was observed when compared to wild-type animals [6]. Only when the analysis was restricted to periods of transitions from non-NREM to REM sleep (i.e., when sleep spindles are more prominent), a reduction of the EEG power in the 10–12-Hz frequency band measured about 30 s before REM sleep onset was observed [6]. Since in NRT neurons, LTCPs are observed at every cycle of spindle waves both in vivo [30, 39] and in vitro [92] (see Fig. 3(A3)), the absence of a clear effect of genetically deleting Cav3.3 channels (the main isoform present in NRT neurons) on this sleep rhythm is highly surprising. These contradictory results may once again be in part explained by compensatory mechanisms that occur in these T channel isoform KO mice but may also result from difficulties in clearly identifying spindle episodes in the EEG of naturally sleeping mice. Indeed, although local field potential recordings in deep cortical areas reveal a comparable profile of spindles in humans and mice, spindles are not clearly apparent in EEG traces from mice and their identification require sophisticated analysis to assess EEG spectral changes at the level of individual sleep stage transitions [5, 94]. Notwithstanding these contrasting results in transgenic mice, recent experiments in rats, using microdialysis of TTA-P2 in the somatosensory thalamus and NRT, provide conclusive evidence of a drastic decrease of spindle waves both during anesthesia and natural sleep [26], confirming the key role of thalamic T channels in the generation of this sleep rhythm.

Finally, since many cortical neurones possess a vast repertoire of T channels (see previous section) and the waveform of

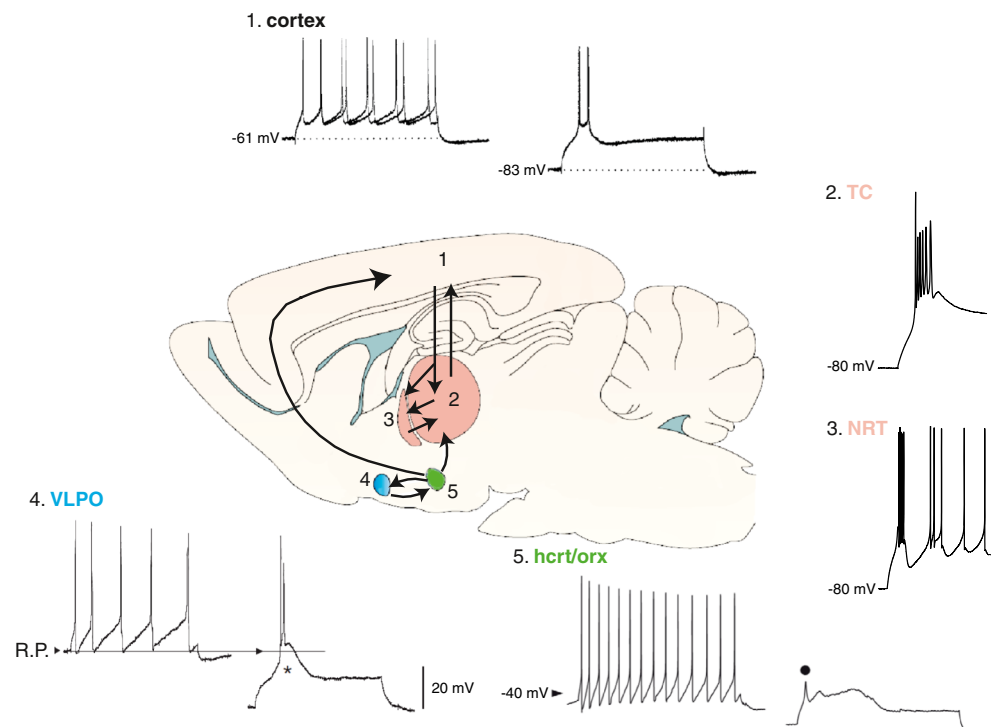
cortical spindle waves spans the voltage region of T channel activation/inactivation, a contribution of these cortical channels to the fine tuning of EEG sleep spindles would be expected, although to the best of our knowledge, this has not so far been rigorously investigated.

Thalamic and cortical T channel contribution to delta waves

In TC neurons, membrane potential oscillations at delta frequency (0.5–4 Hz) that consist of rhythmically occurring LTCPs were the first T channel-dependent activity whose mechanism was fully elucidated in vitro [53, 54, 61, 76]. This work, together with in vivo studies in anesthetized animals [33, 66, 67, 83], strongly suggested that delta oscillations in TC neurones are fully determined in a pacemaker fashion by the time and voltage dependencies of the h, T, and K^+ channels, in both TC and NRT neurons [10, 54, 61] stressing the key role of thalamic T channels in the generation of these waves.

One important issue that has often been overlooked, however, is that the majority of EEG delta waves of natural sleep, and its thalamic counterpart the delta oscillation, do not occur in very long periods, as a somewhat inaccurate interpretation of the initial in vitro studies (Fig. 3(B2), top trace) might led to conclude. Indeed, thalamic delta oscillations appear to occur mostly in discrete groups during the down state of slow waves in both TC and NRT neurons (Fig. 3(B2), bottom trace) [43,

Fig. 4 Interconnected brain areas involved in sleep control and EEG sleep waves generation that present T-type dependent activity. Example of LTCPs evoked from hyperpolarized membrane potentials in response to depolarizing current injections in layer V/VI cortical neuron (1), TC (2) and NRT (3) neurons, ventrolateral preoptic neuron (VLPO, 4) and hypocretin-orexin expressing neurons (*hcrt/orx*, 5) of the lateral hypothalamus. Both tonic firing and LTCP are presented in 1, 4, and 5. Traces reproduce with permission from [28], [34], [40], [25]



79, 90]. We are unaware of any evidence supporting the presence of long period of delta oscillations in TC neurons in vivo during natural sleep, raising the question of whether these long sequences of repetitive LTCPs are only observed in thalamic slices. An additional point of concern when interpreting EEG data is the ambiguity in the definition of delta waves of natural sleep and their potential overlap with slow waves. In other words, it is possible that EEG delta waves at the lower end of their frequency range (i.e., 0.5–2 Hz) may correspond to a thalamic (and cortical) cellular activity characterized by up and down states (i.e., slow waves) and not by repetitive LTCPs (compare Figs. 2(a) and 3(B1)).

Together with compensatory mechanisms (as highlighted in previous section), the above two issues might explain the contradictory results on delta waves obtained in mice with genetic ablation of T channels. Thus, total KO of the Cav3.1 isoform was shown to induce a marked decrease in the power density of delta frequency band (selected as 2–6.5 Hz) [50] whereas in the “putative thalamic-selective” Cav3.1 KO mouse, there is a moderate increase in EEG spectral power within the delta frequency range (selected as 1–4 Hz) [4]. Moreover, no change in delta frequency power was observed in global Cav3.3 KO mice [6], while the impact of deleting the Cav3.2 isoform on sleep rhythms has not yet been analyzed. To complicate this picture further, systemic injection of selective antagonists of all T channel isoforms has been shown to dose-dependently increase delta waves and reduce slow waves in wild-type mice and rats, respectively [26, 49].

As far as cortical T channels are concerned, the firing input at delta frequency from TC to cortical neurones might clearly play an important contribution to the expression of delta waves of natural sleep in the EEG. However, membrane potential oscillations at delta frequency have been observed in cortical neurones [77] and the full mechanisms of the delta waves (i.e., the relative contribution of synaptic and intrinsic conductances, including a precise role for T channels) in cortical neurons remains to be fully elucidated.

Contribution of T channels in other brain regions to the expression of sleep waves

The difficulty in the interpretation of sleep studies using global, genetic, or pharmacological T channel block that we have outlined in the previous sections clearly stems from our lack of knowledge of the contribution of T channels not only in cortical neurons but also in other brain networks that control and/or modulate natural sleep. In fact, neurons expressing LTCPs are present in the ventrolateral preoptic nucleus [40] and in the lateral hypothalamus (i.e., hypocretin-orexin-expressing neurons) [35], two strongly interacting regions that belong, respectively, to the sleep-promoting and ascending arousal pathways [74] (Fig. 4). The potential presence of T

channels mediated electrical events (e.g. LTCPs, $I_{T\text{window}}$, T channel-dependent Ca^{2+} modulation or activation of various voltage-gated channels, etc.) in neurons of these pathways should therefore be investigated to gain a full understanding of the role of T channels in sleep. In this respect, it is clear that manipulating T channels not only has an impact on various sleep rhythms but also on sleep architecture and the transitions between wake and sleep as well as between non-REM and REM sleep. Both global and “putative thalamic-selective” Cav3.1 KO mice (but not cortical KO mice) show an increased number of frequent brief awakenings that interrupt non-REM sleep and a delayed sleep onset [4, 50]. Moreover, systemic injection of TTA-A2/P2 in wild-type mice acutely reduces the mean time spent in active wake [49] and induces dose-dependent behavioral and EEG changes indicative of sedation/sleep in rats [59].

Conclusions

After 30 years since the discovery of T channels in thalamic neurons, we have a very comprehensive view of their precise contribution to slow waves and spindles of natural sleep, including the role of LTCPs and $I_{T\text{window}}$. A similar understanding of delta waves is still missing, in part because of lack of appropriate studies and uncertainties on an appropriate classification of these waves. Moreover, we still know very little about the contribution of T channels in neurons of the neocortex and other neuronal networks involved in sleep. Undoubtedly, the development of selective blockers for different T channel isoforms and of conditional and area-selective KO animals will contribute to unravel the full role played by these widely expressed voltage-gated channels in sleep waves and architecture.

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