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Thalamic T-type Ca^{2+} channels and NREM sleep

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Abstract

T-type Ca^{2+} channels play a number of different and pivotal roles in almost every type of neuronal oscillation expressed by thalamic neurones during non-Rapid Eye Movement (NREM) sleep, including those underlying sleep theta waves, the K-complex and the slow (<1 Hz) sleep rhythm, sleep spindles and delta waves. In particular, the transient opening of T channels not only gives rise to the ‘classical’ low threshold Ca^{2+} potentials, and associated high frequency burst of action potentials, that are characteristically present during sleep spindles and delta waves, but also contributes to the high threshold bursts that underlie the thalamic generation of sleep theta rhythms. The persistent opening of a small fraction of T channels, i.e. I_{Twindow} , is responsible for the large amplitude and long lasting depolarization, or UP state, of the slow (<1 Hz) sleep oscillation in thalamic neurones. These cellular findings are in part matched by the wake-sleep phenotype of global and thalamic-selective $\text{Ca}_v3.1$ knockout mice that show a decreased amount of total NREM sleep time.

T-type Ca^{2+} channels, therefore, constitute the single most crucial voltage-dependent conductance that permeates all activities of thalamic neurones during NREM sleep. Since I_{Twindow} and high threshold bursts are not restricted to thalamic neurones, the cellular neurophysiology of T channels should now move away from the simplistic, though historically significant, view of these channels as being responsible only for low threshold Ca^{2+} potentials.

Keywords

cortex; thalamus; slow sleep oscillations; sleep spindles; delta waves; alpha rhythm; theta rhythm; I_{Twindow} ; I_h ; I_{CAN}

Introduction

The first clear evidence of a role for T-type Ca^{2+} channels in a sleep related neuronal activity was the discovery in 1981 of the low threshold Ca^{2+} potential (LTCP) in thalamocortical (TC) neurones by Jahnsen and Llinas [1]. This and follow-up studies *in vitro* clarified how ‘post-anodal exaltation’ [2], i.e the transient depolarization following a period of hyperpolarization in TC neurones, was due to Ca^{2+} entry via channels that had a relatively low voltage-threshold of activation, and allowed the authors to speculate on the potential involvement of LTCPs in firing patterns at theta frequency [3,4]. At the same time, pioneering work by Steriade’s group using extracellular and intracellular recordings *in vivo* from TC and nucleus reticularis thalami (NRT) neurones started to directly link LTCPs to different types of sleep waves [5,6]. The next twenty years led these and many other groups to unravel the intricate contribution of T channels to the membrane potential oscillations underlying sleep spindles and delta waves [7-17]. More recently, important findings have

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identified the essential role played by the window component of the T-type Ca^{2+} current (I_{Twindow}) in the slow (<1 Hz) sleep oscillation of TC and NRT neurones [18-22], the contribution of T channels to the theta waves of stage 1 non-Rapid Eye Movement (NREM) sleep in TC neurones [23,24], and the decreased total NREM sleep time of global and thalamic-selective $\text{Ca}_v3.1$ knockout (KO) mice [25,26]. A quarter of a century after Jahnsen and Llinas' discovery, therefore, the emerging scenario identifies thalamic T channels as having a fundamental role in many EEG waves of natural NREM sleep, beyond the historically important, but now simplistic, description of these channels as being responsible *only* for the generation of LTCPs.

In this review, we firstly provide an outline of the different EEG waves of NREM sleep, briefly discuss some important issues of cellular sleep physiology, and summarize the thalamic T channel properties that are essential for understanding their involvement in sleep rhythms (for comprehensive descriptions of the molecular genetics, biophysics, CNS distribution and pathophysiology of T channels, see other reviews in this issue). We will then present a detailed analysis of the contribution of T channels to the membrane potential oscillations of thalamic cells underlying each EEG wave of NREM sleep. Although the emphasis will clearly be on cellular mechanisms operating in single thalamic neurones, wherever possible data will be put into the wider context of thalamic, cortical and thalamocortical network activities, in an attempt to show the links between T channel-based activities in single thalamic neurones and EEG waves of natural sleep.

EEG waves of NREM sleep: source and generator(s)

A natural night's sleep is characterized by the regular appearance of stereotyped sequences of EEG waves [27,28] which are essentially generated by the thalamo-cortico-thalamic network (see Fig. 1A for the main afferent and efferent connections among the principal neuronal elements of this loop). From an EEG of small amplitude waves mainly at beta (10-20 Hz) and gamma (20-80 Hz) frequencies characteristic of a behaviourally awake and fully attentive state, periods of waxing and waning waves at alpha frequency (8-13 Hz) appear predominantly in occipital and somatosensory cortices when relaxed wakefulness starts to set in (Fig. 1B). These are followed by the emergence of theta waves (3-7 Hz) during stage 1 NREM sleep, and in stage 2 by the presence of K-complexes and the slow (<1 Hz) sleep rhythm (Fig. 1B). Sleep spindles are also present in stages 2 and 3, either in isolation or often grouped together by a K-complex (Fig. 1B). The EEG in stage 3 shows clearly defined periods of delta waves (1-4 Hz) that together with slow (<1 Hz) waves become the predominant activity as sleep deepens into stage 4 (Fig. 1B). This patterned sequence of EEG waves and the associated changes in physiological parameters then proceed in the reverse direction back to stage 1, from where a natural night's sleep progresses into REM sleep.

Two important aspects of cellular sleep physiology that are essential to understand the crucial role of T channels in the generation of the EEG waves of NREM sleep must be addressed here. Firstly, whereas the 'source' of the EEG waves is within the upper cortical layers, long lasting controversies have arisen as to whether the 'generator(s)' of the various rhythms are located either in the cortex or the thalamus. Many of the membrane potential oscillations that contribute to the expression of EEG waves of natural NREM sleep can be generated by the pacemaker activities of individual neurones or small neurone networks. Each EEG wave, however, is the result of an essential and fine synaptic tuning of these independent thalamic and/or cortical oscillators by all neuronal elements of the thalamo-cortico-thalamic loop. A more appropriate approach to this issue, therefore, is to think in terms of the *extent* to which each single neuronal element of this loop contributes to the final product, i.e. the EEG wave. In this respect, the emphasis given to thalamic T channels in the

following sections should not be viewed as a suggestion of a less critical involvement of these channels in the NREM sleep-related oscillations of cortical neurones, but simply reflects the larger number of studies that have *directly* addressed this question in thalamic neurones.

Secondly, the behavioural, physiological and EEG changes observed during the transition from fully attentive wakefulness to stage 4 NREM sleep are invariably accompanied by a reduction in the depolarizing tone exerted by cholinergic, monoaminergic and histaminergic afferents from brain stem and mammillary body onto both cortical and thalamic recipient neurones [29] (with some notable exceptions, see ref. [29]). This process gives rise to a progressive hyperpolarization of the majority of the recipient cortical and thalamic neurones [30] into membrane potential regions where activation and inactivation of T channels becomes possible.

Tonic vs burst firing: the historical view of T channel neurophysiology

The majority of T-type Ca^{2+} channels in TC neurones have a somatic location [31-33], though there is now evidence for their presence at proximal and distal dendritic sites [34-36]. The T channels present in GABAergic NRT neurones have a highly preferential dendritic location [37], and those in interneurones, the other major neuronal type present in some thalamic nuclei, also appear to have a high dendritic density of T-type Ca^{2+} channels [38-40]. In terms of T channel isoforms, TC neurones mainly express the $\alpha 1\text{G}$ subunit (i.e. $\text{Ca}_v3.1$ channels) with a small amount of channels probably being formed by the $\alpha 1\text{H}$ subunit ($\text{Ca}_v3.2$), while those in NRT neurones are made up almost exclusively by the $\alpha 1\text{I}$ subunit ($\text{Ca}_v3.3$), with very few $\text{Ca}_v3.2$ channels [41,42]. It is unfortunate, however, that no detailed investigation exists so far on the fine somato-dendritic distribution of Ca_v3 isoforms either in TC and NRT neurones or in interneurones.

When the membrane potential of TC neurones is more hyperpolarised than -65 mV, the transient opening of T channels leads to the generation of a slowly rising and falling depolarization called an LTCP (or low threshold spike), usually accompanied by a high frequency burst of action potentials. The same occurs in NRT neurones, though due to the more positive activation and inactivation properties of their prevalent $\text{Ca}_v3.3$ channels LTCPs can also occur from potentials < -60 mV. The classical view of T channel neurophysiology [15,42-47], therefore, has identified them as being solely responsible for generating this LTCP-mediated 'burst firing' at relatively hyperpolarised membrane potentials, compared to depolarised potentials where these channels are inactivated and repetitive single action potential or 'tonic firing' occurs (Fig. 2A). Recent studies, however, have demonstrated that T channel function in thalamic neurones is much more complex than this 'dual firing mode' pattern, and that this simplistic view has in part resulted from the limitations of thalamic slices that generally lack 'active' cortical inputs. Thus, following synaptic or pharmacological activation of the 1α -type metabotropic glutamate receptors (mGluR1a), that are postsynaptic to the cortical afferents [48], two novel aspects of T channel function are unmasked. Firstly, the *transient* opening of probably dendritic T channels contributes to the generation of a high threshold burst (HTB) of action potentials that is elicited from membrane potentials more depolarised than -55 mV (Fig. 2B, top trace) [23,24]. Secondly, the *persistent* opening of a small proportion of T channels generates a tonic inward current (I_{Twindow}) and a characteristic tonic depolarization manifest as the UP state of the slow (< 1 Hz) oscillation (Fig. 2B, third and fourth traces) [18-22]. Thus, the repertoire of T channel-mediated activities that are present in a single TC neurone is enlarged following cortical afferent stimulation without changes either in the tonic (Fig. 2, second trace) or the LTCP-evoked burst firing (Fig. 2B, bottom trace). Note that a similar

pattern of activities can be evoked in single NRT neurones under identical experimental conditions (see Figs. 1 and 2 in ref. [22]).

In the following sections we will discuss each of the cellular activity patterns that are generated by TC and NRT neurones from a perspective of EEG waves of NREM sleep, and present the supporting evidence for the underlying T channel involvement.

Alpha and theta waves

Although an EEG rhythm of relaxed wakefulness, alpha waves are discussed here because recent studies suggest that they share an identical thalamic mechanism with the theta waves of stage 1 NREM sleep, and that this mechanism partly involves T channels.

Extracellular single unit recordings in freely moving animals undergoing natural wake-sleep cycles have shown that thalamic alpha/theta waves are elicited by a subset (20-25%) of TC neurones that fire repetitive, relatively short bursts of action potentials in synchrony with EEG waves at these frequencies (Fig. 3A) [23,24,49]. These alpha/theta bursts exhibit comparatively large interspike intervals (~10 ms) which do not greatly change as the burst progresses (Fig. 3A, 3D), and therefore are different from those generated by LTCPs which display considerably smaller inter-spike intervals (2-5 ms) that gradually and markedly increase during the burst (Figs. 2B and 3D). Intracellular recordings from TC neurones *in vitro* during synaptic or agonist-induced activation of the mGluR1a that are postsynaptic to the cortical afferents have confirmed the firing characteristics of the alpha/theta rhythm-generating bursts and also shown that their underlying waveform is very different from that of LTCPs since it is contained within potentials >-55 mV (Fig. 3B, see also Fig. 2B) [23]. Thus, to clearly distinguish them from the LTCPs, the bursts underlying alpha/theta waves have been named high threshold bursts (HTBs). Although originally described in the dorsal lateral geniculate of adult cats [23,24], HTBs can also be observed in every thalamic nucleus so far investigated, including the cat ventrobasal complex and ventrolateral nucleus, as well as the rat and mouse visual and somatosensory thalamus [24,50]. Indeed, HTBs are not a peculiarity of the small proportion of alpha/theta rhythm generating TC neurones but are present in every TC neurone provided large enough depolarizing levels are reached, for instance by a stronger mGluR1a activation or intense activation of muscarinic receptors [51].

The ability of HTBs to give rise to oscillations either at alpha or theta frequency solely depends on the membrane potential of the TC neurone, as the inter-HTB frequency decreases with increasing tonic hyperpolarization (Fig. 3B) [23,24]. It is therefore easy to envisage how the hyperpolarization of TC neurones, resulting from the decreasing brain stem activity that accompanies the shift from relaxed wakefulness to early sleep [28-30] leads to a correspondingly progressive slowing of HTBs from the alpha to theta frequency (Fig. 3B). Interestingly, the occurrence of theta waves in humans in the awake state is indicative of several pathological conditions, collectively termed 'thalamic dysrhythmias' [52], that may result from a shortfall in the actions of certain key neurotransmitters associated with these neurological disorders [53,54].

T channels contribute to the HTBs that underlie alpha/theta rhythms

HTBs are intrinsically generated in a pacemaker manner by single TC neurones since following the block of action potentials with tetrodotoxin an underlying oscillation remains which has the same alpha/theta frequency as the HTBs and is composed of high threshold Ca^{2+} 'spikes'. Original observations on the high threshold spikes of TC neurones suggested that they were mediated by high threshold Ca^{2+} channels [3,4,33,55]. However, the spikes that underlie HTBs are more effectively blocked by relatively small concentrations of Ni^{2+}

than Cd^{2+} (Fig. 3E1) [23], a result that on the basis of previous voltage clamp experiments in adult TC neurones of the same species [31] is strongly indicative of T channel involvement. Indeed, although there is a modest effect of the L channel blocker nifedipine, HTBs themselves show the same preferential selectivity of Ni^{2+} over Cd^{2+} (Fig. 3E2), so that in the presence of Ni^{2+} repetitive HTBs at theta frequency are transformed into tonic firing (Fig. 3C) [23].

The T channel contribution to the generation of HTBs is not in conflict with their expression at relatively depolarized membrane potentials. Since HTBs are evoked following synaptic or pharmacological activation of mGluR1a [23,24,49] that are preferentially located on distal TC neurone dendrites [48], it is feasible that the apparent depolarized voltage of activation of the HTBs is due to a membrane potential mismatch between the somatic recording site and the dendritic T channel location. Alternatively, dendrites of TC neurones may contain $\text{Ca}_v3.3$ channels which have more positive voltage-regions of activation and inactivation than $\text{Ca}_v3.1$ and $\text{Ca}_v3.2$ channels [41,42]. Indeed, direct measurement of dendritic T channels have indicated that they indeed exhibit steady-state activation and inactivation curves which are shifted by about 10 mV more depolarised in comparison to those at the soma and more proximal dendrites [35]. In addition, in many other neuronal types including hippocampal CA1 pyramidal neurones [56,57], cortical [58,59] and thalamic interneurones [39,40], and NRT neurones [60,61], activities similar to HTBs have been shown, or implied, to involve a contribution by T channels.

Since HTBs are also moderately suppressed by nifedipine (Fig. 3E2), it is unlikely that T channels are solely responsible for their generation. Moreover, the kinetics of both native [31] and cloned [42] T channels are too slow to account for their fast time-course. Thus, HTBs are either due to a combination of T and other Ca^{2+} channel subtypes (see ref. [56]), or to a set of Ca^{2+} channels that exhibit unusual properties that lie somewhere between classical T channels and high-voltage-activated channels [62,63]. Interestingly, TC neurones in the rat laterodorsal nucleus possess two pharmacologically distinct populations of low voltage-activated Ca^{2+} channels, one of which exhibits relatively fast kinetics, is susceptible to nifedipine and exhibits a steady-state inactivation curve that is about 15 mV more depolarized than the nifedipine-insensitive channels [64,65] (see also ref. [66]).

The presence of field potentials at alpha/theta frequencies in isolated dorsal thalamic nuclei is due to gap junction-based electrical synapses [23,24,49] that are present between TC neurones [67]. The synchronized thalamic wave is then transmitted to the cortex, but the extent to which cortical neurones are simply recruited by this thalamically generated volley, or actively shape it by intracortical synaptic connections and intrinsic activities, is not fully understood (but see refs. [68-70]). Notwithstanding the absence of detailed cortical data, however, the available information can be used to clarify the relationship between individual oscillators and the global thalamo-cortico-thalamic activity seen in the EEG. Although alpha/theta HTBs are intrinsically generated in a pacemaker manner by individual TC neurones, this process requires a functional corticothalamic mGluR1a pathway [23]. Therefore, suggestions of either a thalamic or cortical 'generator' of alpha/theta waves are inappropriate and misleading.

Sleep K-complexes and the slow (<1 Hz) sleep rhythm

The slow (<1 Hz) sleep rhythm is one of the most fundamental EEG waves of natural NREM sleep in humans because it is present in almost all of its stages [28,71-78], underlies the sleep K-complex (Fig. 4A1) [71,73], and exquisitely groups together periods of sleep spindles (Fig. 5B) [73,79] and delta waves [71,76]. The cellular counterpart of the EEG slow (<1 Hz) rhythm is the cyclic oscillation of the membrane potential between a depolarized

(UP) state and a hyperpolarized (DOWN) state, that has been observed in every type of cortical neurone so far investigated [76,80,81] as well as in TC and NRT neurones (Fig. 4A) [76,82,83]. Simultaneous recordings have shown that the UP and DOWN states of the slow (<1 Hz) oscillation are highly synchronized across widespread cortical and thalamic territories and across different neuronal types within these brain areas [73,74-79]. Moreover, extensive *in vivo* work by Steriade's group has demonstrated that the K-complex is the EEG manifestation of a single cycle of this slow oscillation, with its depth-negative and depth-positive phases reflecting the UP and DOWN states, respectively (Fig. 4A) [71,73].

In cortical neurones, the slow (<1 Hz) oscillation is believed to result from intense intracortical synaptic barrages which generate the UP state and synaptic silence causing the DOWN state, although there is great variation among the waveforms of the different published examples of this oscillation recorded *in vivo* and *in vitro* [80,81,84,85]. In contrast, the waveform of the slow (<1 Hz) oscillation recorded *in vivo* in NRT neurones and TC neurones of different dorsal thalamic nuclei is often highly stereotyped, suggesting a predominant contribution by intrinsic mechanisms [82,83]. In particular, the transition from the UP to the DOWN state is commonly marked by a clear inflection point, whereas the transition from the DOWN to the UP state is mediated by an LTCP-elicited burst of action potentials (see enlargements of the start of the UP state on the far right of Fig. 4A2, A3). In contrast to previous suggestions [84,86], however, the slow (<1 Hz) sleep oscillation is not an exclusive characteristic of cortical networks that is simply imposed onto thalamic networks, since it can be recorded *in vitro* in a few TC and NRT neurones in control conditions [18,20,22], and in every TC and NRT neurone provided certain conditions are met (see below) [20-22]. In addition, *in vitro* and dynamic clamp experiments as well as computer simulations [18-22], have now conclusively demonstrated that the UP and DOWN states of the slow (<1 Hz) sleep oscillation in these two thalamic neuronal types are due to the presence and absence, respectively, of $I_{Twindow}$ (Fig. 4B, C), which rhythmically switches 'on' and 'off' [87,88].

$I_{Twindow}$ underlies the UP state of the slow (< 1Hz) oscillation in thalamic neurones

The manner by which $I_{Twindow}$ instates the slow (<1 Hz) oscillation is by the creation of a bistable membrane potential due to its interaction with the leak K^+ current (I_{Leak}). In Fig. 4B2, the absolute amplitude of $I_{Twindow}$ (bell-shaped curve) has been plotted against the membrane potential, while two I_{Leak} s are represented by the blue and green lines. When the slope of I_{Leak} (i.e. g_{Leak}) is relatively small (Fig 4B2, green line), I_{Leak} crosses the bell-shaped $I_{Twindow}$ curve at three points, at which both currents have equal values. Since $I_{Twindow}$ is an inward and I_{Leak} an outward current, these are three points of net zero current (Fig. 4B3): the leftmost and rightmost points are stable equilibrium points (Fig. 4B3, filled and empty circle on green line, respectively) whilst the middle point is unstable (Fig. 4B3, grey circle). Under this condition, the system generated by $I_{Twindow}$ and I_{Leak} is bistable and neurones show two stable resting membrane potentials: one depolarized (UP state) where $I_{Twindow}$ is 'on' (Fig. 4B3, empty circle on green line) and one hyperpolarized (DOWN state) where $I_{Twindow}$ is 'off' (Fig. 4B3, filled circle on green line). In contrast, when g_{Leak} is relatively large (Fig. 4B2, blue line) I_{Leak} crosses the $I_{Twindow}$ curve at only one point, which represents the (only) resting membrane potential (Fig. 4B3, filled circle on blue line). For a comprehensive biophysical description of these events, see ref [88].

In TC neurones, membrane potential bistability is observed only after appropriate block of the hyperpolarization-activated, mixed Na^+-K^+ current, I_h , that is the most prominent current present within the voltage region where $I_{Twindow}$ is expressed. As it had been predicted by computer simulations [19], appropriate voltage steps are able to switch the membrane

potential between the two stable states [18,20,21]. This bistability is unaffected by Ba^{2+} and blockers of high threshold Ca^{2+} currents, whereas its preferential block by relatively small concentrations of Ni^{2+} compared to Cd^{2+} indicates its dependence on T channels [18,20,21]. Additional and conclusive evidence for the involvement of I_{Twindow} in this membrane potential bistability is provided by appropriately manipulating I_{T} (and thus I_{Twindow}) using the dynamic clamp [20,21], a technique that allows a computer-generated current to be either added to, or subtracted from a living neurone [89,90] (see legend of Fig. 4C for further details).

The full expression of the slow (<1 Hz) oscillation in TC and NRT neurones requires a complex dynamic interplay between the I_{Twindow} -based membrane potential bistability and other voltage-dependent and Ca^{2+} -activated currents. In TC neurones, I_{h} and the Ca^{2+} -activated, non-selective cation current, I_{CAN} , control the duration of the DOWN and UP state, respectively [21] (for a detailed summary of the mechanism, see Fig. 3 in ref. [88]). Interestingly, I_{CAN} in TC neurones appears to be activated solely by Ca^{2+} entry via T channels [21], either during the LTCP that is present at the start of each UP state or progressively during the ‘groups of LTCPs’ that occur in the DOWN state (see Fig. 6A1). Whereas in the absence of I_{CAN} the slow oscillation can exist only for a very narrow range of tonic current input, I_{CAN} activation prolongs and enhances the UP state, and enlarges the range of tonic current input for which the oscillation is present (Fig. 6A2) (see also Fig. 8 in ref. [21]). So, in addition to LTCPs and I_{Twindow} an indirect, but essential, role of T channels in the slow (<1 Hz) sleep oscillation is to provide the selective and highly periodic entry of the Ca^{2+} required to activate I_{CAN} .

In NRT neurones, the mechanism of the slow (<1 Hz) oscillation also relies on I_{Twindow} - I_{Leak} based bistability [22], but it is even more complex than in TC neurones, since in addition to I_{h} and I_{CAN} , Na^{+} - and Ca^{2+} -activated K^{+} currents are involved in its fine tuning. In particular, the latter plays a critical role in controlling the duration both of the UP and DOWN states (for a detailed summary of the mechanism, see Supplementary Fig. 5 in ref. [22]).

Physiological implications of the thalamic slow (<1 Hz) sleep oscillation

The consistency in waveform of the slow (<1 Hz) sleep oscillation in NRT cells [22] and in TC neurones of various dorsal thalamic nuclei of different species [21,91], and its temporal robustness (up to 6 hours *in vitro*), seem highly appropriate in imposing the slow (<1 Hz) rhythm across the whole thalamocortical network: indeed the start of the synaptic barrage of the UP state in cortical neurones has been ascribed to a strong contribution by the powerful LTCP-mediated burst of action potentials that is invariably present at the start of each UP state in TC neurones [81,83]. In this respect, two points deserve particular attention.

Firstly, the biophysical properties of the slow (<1 Hz) oscillation in TC neurones might also explain the increase in frequency, and the reduction in the duration of the positive wave, of the K-complex as NREM sleep deepens [71,73]. In fact, mimicking the increasing hyperpolarizing tone experienced by TC and NRT neurones during the deepening of sleep by injecting steady d.c. hyperpolarizing current into these neurones *in vitro* results in an increase in frequency of the slow (<1 Hz) oscillation due to the shortening of the UP state while the duration of the DOWN state remains constant (see third and fourth traces in Fig. 2B) [21,22,91]. The progressive shortening of the UP state with increasing hyperpolarization ultimately leads to continuous delta oscillations (see bottom trace in Fig. 2B), as the membrane potential moves out of the voltage region where I_{Twindow} is active.

Secondly, a phosphorylation-dependent potentiation of the T current, which occurs at relatively depolarized potential (>−60 mV) and is removed by hyperpolarization, has

recently been described in TC neurones of sensory thalamic nuclei [92]. Thus, two mechanisms with opposing effect on T channels occur after hyperpolarization, i.e. de-phosphorylation and de-inactivation. Because the de-phosphorylation is much slower than the de-inactivation, there exists an optimal duration (0.7 - 1.5 sec) of transient hyperpolarizations to facilitate a subsequent maximal T current [92]. Thus, the occurrence of the UP state of the slow (<1 Hz) oscillations at potential ~ -60 mV and the constant duration (1 sec) of the DOWN state across different nuclei and species, and its independence from the level of tonic hyperpolarization [21,22,91] are optimally suited to elicit a maximal T current during NREM sleep stages where the slow oscillation is present. Whether this mechanism is required to ensure the robustness of the LTCP burst at the start of the UP state, or the recruitment of enough $I_{T\text{window}}$ to secure the subsequent UP state of the slow (<1 Hz) oscillation remains to be established. It may also be that a maximal Ca^{2+} entry through T channels is necessary to modulate/activate other currents, e.g. I_h and I_{CAN} , that are critical for the slow and other NREM sleep oscillations, or indeed to control cytoplasmic, nuclear or genetic processes in thalamic neurones during this behavioural state.

The inability to observe the slow (<1 Hz) oscillation in TC and NRT neurones *in vivo* following cortical disconnection [76,86] or in the majority of these neurones maintained *in vitro* [21,22] has now been demonstrated not to be due to the absence of rhythmic corticothalamic volleys imposing this rhythm on TC or NRT neurone but to the lack of an active mGluR1a corticothalamic input that sufficiently reduce I_{Leak} such that the $I_{T\text{window}}$ -based bistability can become operational [21,22,91]. Indeed, even in the absence of cortical mGluR1a activation, a decrease in I_{Leak} capable of allowing the expression of the slow oscillation in thalamic slices can be achieved by activation of muscarinic receptors in TC neurones (Hughes and Crunelli, unpublished observation) and of TRH receptors in NRT neurones [93]. In summary, both isolated cortex and thalamus can generate the slow (<1 Hz) sleep oscillation, the former as an emergent property of the cortical network dynamics and the latter in a pacemaker manner by individual TC and NRT neurones with active cortical afferents. Therefore, as for the HTBs of alpha/theta rhythm, suggestions of either a thalamic or cortical 'generator' of the slow (<1 Hz) sleep rhythm are inappropriate and misleading.

T channels and sleep spindle waves

Sleep spindles are undoubtedly one of the NREM sleep rhythms which has received the most attention in the last 60 years [2,7,8,28,94-97]. A typical spindle wave during stages 2 and 3 of natural NREM sleep in humans has a frequency of 12-15 Hz (7-14 Hz in the cat), lasts for a few seconds, and has a classical waxing and waning waveform [28]. Although sleep spindles can be seen in isolation from other EEG waves (Fig. 1B, third line from top), the vast majority of them in stages 2 and 3 of NREM sleep occur in close association with a K-complex (Fig. 1B, third line from top) [73,79,96]. From the early 1960s, many experimental investigations have made use of systemic injection of barbiturates to increase the occurrence of spindle waves in the EEG. Barbiturate-induced spindles [2,7,8,94], however, are different from the majority those of natural sleep spindles [73,79,96], as the former tend not to occur in association with a K-complex (Fig 5A). Under ketamine/xylazine anaesthesia, on the other hand, spindle waves always appear grouped as part of a K-complex (Fig. 5D) [95]. Since the K-complex is the EEG reflection of a single cycle of the slow (<1 Hz) oscillation [71,73,75] caution should be used when interpreting and comparing results on barbiturate-induced spindle waves and those recorded under ketamine/xylazine anaesthesia, since the mechanisms underlying their generation in these two conditions may be substantially different.

Spindle waves in naturally sleeping animals are generated at the thalamic level [7,8], although waves at spindle frequency can be observed during the UP state of the slow (<1

Hz) oscillation in cortical neurones [76], that may not depend on thalamic input. Since cortical neurones possess T channels and the waveform of cortical spindle waves spans the voltage region of T channel activation, a contribution of these channels to the fine tuning of EEG sleep spindles would not be unexpected, although this has not been rigorously tested. As far as the thalamic generation of spindle waves is concerned, *in vivo* data strongly suggests that they can be generated by the isolated NRT [7,8], whereas *in vitro* an intact TC-NRT network is required [14,16,17]. Interestingly, simulations with a biophysically realistic single column thalamo-cortico-thalamic network have recently shown that tonic depolarization of the NRT neurone pool allows it to produce spindle waves even in isolation from the TC neurone pool [98].

Intracellular recordings *in vivo* under both ketamine/xylazine [95] and barbiturate anaesthesia [7,8] in the cat and *in vitro* in the ferret [14,16,17], from NRT and TC neurones, have highlighted the key role of T channels in sleep spindles, since LTCPs are present at almost every cycle of the spindle wave in NRT neurones (Fig. 5B), and occasionally in TC neurones (Fig. 5C). In particular, the firing associated with the LTCPs generated by GABAergic NRT neurones elicits summing GABA_A IPSPs in the TC neurones, some of which provide enough time- and voltage-dependent removal of T channel inactivation so that an LTCP can then be generated (bottom trace in bottom right box of Fig. 5). In turn, the output firing generated by the LTCPs of an ensemble of the excitatory TC neurones provides EPSPs at spindle frequency back to NRT neurones (eliciting LTCPs, top trace in bottom right box of Fig. 5), as well as to cortical neurones.

Despite these overall similarities, major differences exist in the intracellularly recorded waveform of NRT spindle waves between different experimental conditions. Firstly, the almost regular LTCPs that are observed *in vivo* during spindle waves develop from what appears as a clear depolarizing envelope under barbiturate anaesthesia (Fig. 5B, top trace) but on the UP state of the slow oscillation during ketamine/xylazine anaesthesia (Fig. 5E, top trace). In contrast, the majority of the published examples of NRT spindle oscillations in the ferret *in vitro* never originate from a depolarizing envelope, but are essentially made up of LTCPs of increasing size, each followed by a progressively larger afterhyperpolarization (Fig. 5B, bottom trace). Interestingly, however, oscillations at spindle frequency, with identical properties to those observed *in vivo* under ketamine/xylazine anaesthesia, can be recorded *in vitro* on the UP state of the slow (<1 Hz) oscillation when a low conductance state is instated by mGluR1a activation (Fig. 5E) [22]. Secondly, the amplitude of the LTCPs recorded *in vivo*, both under barbiturate (Fig. 5B, top trace) or ketamine/xylazine anaesthesia (Fig. 5E, top trace) is similar to that of the LTCP recorded *in vitro* during the UP state of the slow oscillation (Fig. 5E, bottom trace), but is much smaller than the 20-30 mV amplitude of LTCPs recorded *in vitro* during spindle waves in ferret NRT neurones that do not show the slow (<1 Hz) oscillation (Fig. 5B, bottom trace). Also, the burst firing associated with the latter activity is much stronger than that observed *in vivo* or *in vitro* during the slow (<1 Hz) oscillation.

An additional, important role of the T channels in TC neurones is to provide the Ca²⁺ entry that regulates the cAMP-mediated up-regulation of I_h [99,100]. This up-regulation leads to a progressively increasing depolarization of the TC neurones during a spindle wave and their eventual inability to generate LTCPs, thus contributing to the spindle wave termination. Clearly, the T channel-dependent up-regulation of I_h may be involved in the termination of the DOWN state of the slow (<1 Hz) oscillation when it contains 'groups of LTCPs' (see Fig. 6A). Similarly, there is likely to be a contribution of I_{CAN} to the termination of spindle waves in TC neurones. Moreover, since biochemical and electrophysiological evidence have recently demonstrated the presence of I_h [22,101] and HCN isoforms [102,103] in NRT

neurones, a similar process may occur in these neurones both during spindle waves and slow (<1 Hz) oscillations (see Fig. 6B).

LTCPs in delta oscillations

The delta oscillation in TC neurones consists of regularly occurring LTCPs at 0.5-4 Hz (Fig. 2A, B), and was the first T channel-dependent physiological activity whose mechanism was fully elucidated [9-12]. Both extensive *in vivo* and *in vitro* experiments, supported by the results of computer simulations, demonstrated that the waveform, frequency and voltage-dependence of the delta oscillation in TC neurones are fully determined in a pacemaker fashion by the time- and voltage-dependencies of the h and T channels, and the level of tonic inputs to the TC neurones. One point that has often been overlooked, however, is that the majority of EEG delta waves, 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 might have suggested. Indeed, as shown *in vivo* and in recent *in vitro* investigations, thalamic delta oscillations can occur in discrete groups, almost always paced by, and during the DOWN state of, the slow (< 1Hz) oscillation (Fig. 6A) [21,82,88]. It is only during the deepest part of stage 4 NREM sleep that due to the progressive, tonic hyperpolarization of TC neurones the voltage region where $I_{T\text{window}}$ can be activated is no longer reached [21,88], resulting in the abolition of the slow (<1 Hz) oscillation and the observation of long periods of delta waves (bottom trace in Fig. 2B).

The ability of NRT neurones to generate oscillations at delta frequency is similar to that of TC neurones. Thus, together with continuous delta oscillations at the deepest stage of NREM sleep (see Fig. 2B1 in ref. [22]), groups of delta waves can be seen in the DOWN state of the slow oscillation, and their duration increases with enhancing tonic hyperpolarization (Fig. 6B). Nevertheless, except for the involvement of LTCPs, the detailed mechanism, and in particular the precise role of I_h , persistent Na^+ current, and Na^+ -activated and Ca^{2+} -activated K^+ currents, in delta wave generation in NRT neurones has not been investigated.

The strong input at delta frequency from TC to cortical neurones clearly plays an important contribution to the expression of EEG delta waves. Oscillations at delta frequency have been observed in cortical neurones [76], some of which possess T channels [41,42,44,104-106]. However, the relative contribution of intrinsic and synaptic activities to their expression, and thus the precise role of T channels in cortical delta oscillations, has still not been fully established.

T channels of thalamic interneurones

The absence of LTCPs in thalamic interneurones had been originally interpreted as indicating a lack of T channels [107]. Later, however, it was shown that a T current is present in interneurones but the expression of LTCPs is prevented by an I_A current whose voltage-dependence closely overlaps with that of the T channels [108]. Clearly, the LTCPs and associated action potential bursts of the thalamic interneurones differ markedly from those both of TC and NRT neurones [109,110]. Different types of oscillatory activity, some reported to be T-channel dependent are generated by thalamic interneurones [39,40], though regrettably direct investigations of their relationship to NREM sleep waves have so far been neglected.

Sleep phenotype of $\text{Ca}_v3.1$ KO mice

The continued lack of pharmacological tools to potently and specifically block T channels [42,44] means that T channel KO mice currently represent the most direct route to determine

T channel function in NREM sleep. Two recent studies [25,26] have generated conditional and unconditional $\text{Ca}_v3.1$ KO mice, and examined their wake-sleep patterns. In the first study [25], mice with an unconditional $\text{Ca}_v3.1$ null mutation show no LTCPs in TC neurones and a wake-sleep phenotype that includes no change in REM, but a 15% decrease in NREM sleep time and a concomitant 25% increase in wake time, both only during the light period (Fig. 7A). Frequency analysis of NREM EEG waves highlight an abolition of activity in the delta frequency band (1-4 Hz), a small decrease in power in the 8-10 Hz spindle frequency band and a higher incidence of brief awakenings longer than 16 sec, whereas the power of slow (<1 Hz) waves and the number of brief awakenings shorter than 16 sec are not affected. While these data directly confirm the involvement of $\text{Ca}_v3.1$ channels in the expression of delta waves, on the basis of the suggested cellular and network mechanisms for sleep spindle generation a stronger reduction in the power of sleep spindle frequency in the KO mice would have been anticipated. Indeed, whatever the precise site of sleep spindle generation (i.e. NRT alone or NRT-TC network), the ability to record sleep spindle waves in the EEG relies on signal transmission from thalamic nuclei to the cortex, which in turn depends on the LTCP-mediated action potential bursts of TC neurones. One can suggest that either the cortex has the ability to strongly generate or compensate for waves at sleep spindle frequency in the absence of the thalamic drive, and/or the 8-10 Hz EEG waves measured by Lee et al. [25] included non-sleep spindle activities, and/or processes involving other Ca^{2+} channels, or even other ion channels, compensate for the $\text{Ca}_v3.1$ null mutation. Clearly, similar compensatory processes might also affect the expression of slow (<1 Hz) waves, whose analysis would have benefited by a direct measurement of K-complexes.

In the second study, Anderson et al. [26] investigated the sleep profile in three types of KO mice, where either global, cortical- or thalamic-selective deletion of $\text{Ca}_v3.1$ channels was achieved. A *CAMKII* promoter was used to drive the cortical $\text{Ca}_v3.1$ deletion, though some $\alpha 1G$ mRNA seemed to remain in upper cortical layers. Thalamic KO mice were produced using a $\text{K}_v3.2$ promoter, which, starting from about postnatal day 12, resulted in strong deletion of $\text{Ca}_v3.1$ channels mainly in rostral and midline thalamic nuclei, with a moderate ablation in some antero and ventral thalamic nuclei and piriform cortex, and a weak removal in some dorsal thalamic nuclei. All three transgenic lines show no effect on REM sleep. Global and cortico-selective deletion results in a marked and a small reduction, respectively, in NREM sleep time, but only during the dark period. The thalamic-selective KOs have a marked decrease in the first half of NREM sleep only in the dark period, followed by a small overshoot in the later part of the second half (Fig. 7B). Interestingly, the reduction in NREM sleep seems stronger and more long-lasting in the thalamic-selective than in the global KOs, and the reduction in the cortical-selective KOs appears not to reach statistical significance even if a smaller change in the global KOs does. Moreover, it is interesting that the global KOs have a smaller and shorter decrease in NREM than the thalamic-selective KOs. This may indicate some compensatory cortical activity in the global KOs since the cortical-selective KOs have almost no change in this parameter. Indeed, problems of compensation probably exist even in thalamic-selective KOs as $\text{Ca}_v3.1$ deletion starts after postnatal day 12, and there is no indication at which age the sleep phenotype of the different KOs was assessed.

Whereas the importance of these two pioneering studies in directly assessing the role(s) of $\text{Ca}_v3.1$ channels in sleep physiology cannot be underestimated, some conflicting issues are difficult to reconcile. Both studies show a decreased NREM sleep time in unconditional $\text{Ca}_v3.1$ KOs, but it is intriguing to see that this occurs either in the dark or the light period, respectively, in the two studies. Also, Anderson et al. [26] report an increase in delta frequency power in global (and thalamic-selective KOs), which is clearly in contrast to the abolition of these EEG waves reported by Lee et al. [25].

Conclusions

T-type Ca^{2+} channels constitute the single, most crucial, voltage-dependent conductance that permeates all major NREM sleep oscillations in TC and NRT neurones, including those underlying sleep theta waves, the K-complex and the slow (<1 Hz) sleep rhythm, sleep spindles and delta waves. This T channel function is exerted via the transient opening of T channels, leading to HTBs and LTCPs, and the persistent opening of a small fraction of T channels leading to I_{Twindow} and membrane potential bistability, and is partly reflected by major abnormalities in the sleep phenotype of global and thalamic-selective $\text{Ca}_v3.1$ KO mice. Since LTCPs, I_{Twindow} and HTBs are not restricted to thalamic neurones, one important point emerging from research on the role of T channels in thalamic sleep oscillations is that the cellular neurophysiology of T-type Ca^{2+} channels should now move away from the simplistic, though historically significant, description of these channels as being responsible *only* for the generation of LTCPs and associated high frequency bursts of action potentials.

It is also intriguing to speculate whether the only functions of T channel activation during NREM sleep are to generate certain voltage waveforms and to activate/modulate other currents (e.g. I_{CAN} and I_{h}), or whether the selective and highly regular entry of Ca^{2+} through T channels that occurs in this behavioural state has other important cytoplasmic, nuclear and/or genetic consequences for thalamic neurones. In the light of current views on the critical function of NREM sleep in learning and memory [111–112], the latter proposal seems plausible and would, if true, confer even more physiological ‘responsibilities’ to thalamic T-type Ca^{2+} channels.

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Figure 1. The thalamo-cortico-thalamic loop and the EEG waves of NREM sleep

A. Schematic diagram of the afferent and efferent connections among the principal neuronal elements of the thalamo-cortico-thalamic loop. For clarity, intracortical networks are not included, though it is worth highlighting that thalamocortical afferents arrive in layer IV and corticothalamic efferents mainly originate from layer V/VI. (+): excitatory synapse, (–): inhibitory synapse. B. Human EEG showing characteristic waves of relaxed wakefulness and NREM sleep. B: reproduced from ref. [27].

Figure 2. Current view of T channel neurophysiology

A. The classical view of T-type Ca^{2+} channel neurophysiology highlights their role in the LTCPs that underlie the burst firing (and delta oscillation) generated by TC neurones when their membrane potential is more negative than -65 mV. B. The current view is that in addition to LTCPs T channels contribute to two additional cellular activities: 1) the high threshold bursts (HTBs), that are due to the transient opening of possibly dendritic T channels (see box in the top far right), and 2) the UP state of the slow (<1 Hz) oscillation, that is due to the opening of non-inactivating T channels in the voltage region around -60 mV (see box in the middle far right). The unmasking of these additional T channel-dependent activities is possible when the metabotropic glutamate receptors that are postsynaptic to the cortical afferents to TC neurones are activated either synaptically (see Fig. 5 in ref. [21] and Fig. 3B in ref. [22]) or exogenously (as shown here using $100 \mu\text{M}$ trans-ACPD). Note how this activation of the cortical afferents has enlarged the repertoire of firing patterns that involves T channel activation without affecting their ability to generate both tonic or burst firing/delta oscillations. A and B: reproduced from ref. [91].

Figure 3. Sleep theta waves and HTBs

A. Simultaneous field and single unit recordings *in vivo* from animals undergoing natural sleep-wake cycles show short bursts of action potentials (with almost constant interspike intervals, ISIs) during alpha and theta waves (marked portions of the traces are enlarged on the right). B. Intracellular recordings of HTBs in a TC neurone *in vitro*, showing how increasing tonic hyperpolarization shifts HTB firing from alpha to theta frequency. C. HTBs are abolished by Ni^{2+} , so that in this condition the TC neuron fires single action potentials. D. ISIs of HTBs are markedly different from those of LTCPs, recorded in the same TC neurone. E. Pharmacological profile of high threshold (HT)-spikes (E1) and high threshold (HT)-bursts (E2). All sections of this figure are reproduced from ref. [23].

Figure 4. Sleep K-complex and the role of $I_{Twindow}$ in slow (< 1Hz) oscillation

A. *In vivo* simultaneously recorded EEG K-complexes and cortical slow (<1 Hz) sleep oscillation (A1) highlight the synchrony between the K-complex and the slow oscillation in a single cortical neurone. A1 and A2. Slow (<1 Hz) sleep oscillations recorded *in vitro* from an NRT and a TC neurone highlight how the switching 'on' (green area) and 'off' (yellow area) of $I_{Twindow}$ underlies the generation of the UP and DOWN states, respectively, of the oscillation (marked portions of the traces are enlarged on the right). B1. The overlap (grey area) of the steady-state activation and inactivation curves of I_T indicate the very small proportion of T channels responsible for $I_{Twindow}$. B2. Plot of the absolute value of the normal and a reduced $I_{Twindow}$ (black and red bell-shaped curves) and a large and a small

I_{Leak} (blue and green line, respectively). B3. Net current-voltage plot for the colour coded conditions depicted in B2: bistability, i.e. two stable membrane potentials (filled and empty circle), is present for the green but not for the blue or red lines. C1. In an originally bistable TC neurone where I_T (and thus bistability) had been eliminated by Ni^{2+} , bistability can be re-instated by adding an amount of computer-generated I_T commensurate with the apparent I_{Leak} of that neurone (top traces), such that the net current-voltage plot for that neurone satisfies the theoretical prediction (green line in plot). Bistability, however, is lost (bottom traces), when artificial I_T is slightly decreased (red line in plot). C2. Even in the presence of a reduced $I_{Twindow}$ (red line in plot), bistability can be re-introduced by adding a steady current to the neurone, since this procedure will re-instate two stable equilibrium points in the net current-voltage plot (arrow in plot). See ref. [88] for a full description of this and other experiments related to the role of $I_{Twindow}$ in the slow (<1 Hz) sleep oscillation. A1 and C: reproduced from refs. [73] and [88], respectively.

Figure 5. EEG spindle waves and their thalamic counterparts

A. EEG spindle wave under barbiturate anaesthesia. B. Intracellular recording from an NRT neurone *in vivo* under barbiturate anaesthesia shows how the LTCPs are superimposed on a depolarizing envelope during a spindle wave (top trace), whereas this depolarizing envelope is absent in the intracellular recordings from a neurone in the PGN (the visual sector of the NRT) *in vitro* during a spindle wave (bottom trace) (enlarged in the top trace of the bottom right box). C. Intracellular recordings from a TC neurone *in vivo* (under barbiturate anaesthesia, top trace) and *in vitro* (bottom trace) show that the NRT-evoked IPSPs occasionally give rise to LTCPs (enlarged in the bottom trace of the bottom right box). D. EEG spindle wave occurring in close association with a K-complex under ketamine/xylazine

anaesthesia. E. Intracellular recordings during a spindle wave associated with a K-complex (top trace) in a ketamine/xylazine anaesthetized preparation show how the spindle wave occurs on the UP state of the slow (<1 Hz) sleep oscillation. The bottom trace shows intracellular recordings from an NRT neurone with activity at spindle frequency occurring on the UP state of the slow oscillation (during exogenous activation of mGluR1a with 100 μ M of trans-ACPD). A, B, C, D and E: reproduced from refs. [14], [15], and [95], respectively.

Figure 6. Delta waves are grouped by the slow (<1 Hz) sleep oscillation

A1. Intracellular recordings from TC neurones *in vivo* and *in vitro* show groups of LTCPs at delta frequency in the DOWN state of the slow (<1 Hz) sleep oscillation. One transition of the DOWN to the UP state is enlarged on the right to highlight the last LTCP occurring during the DOWN state and the LTCP at the start of the UP state. A2. Computer simulations indicate the essential role of I_{CAN} in generating periods of grouped delta oscillations during the slow oscillation, and its similarity to the experimental findings. Note also how increasing steady hyperpolarization leads from a slow oscillation with grouped LTCPs at delta frequency (middle traces) to continuous delta oscillations (bottom traces). B. Intracellular recording of the slow (<1 Hz) sleep oscillation in an NRT neurone *in vitro* and the presence

of grouped delta activity during the DOWN state of the slow (<1 Hz) sleep oscillation. A1(top trace), A1 (bottom trace), A2 and B: reproduced from refs. [82], [21] and [22], respectively.

Figure 7. Cav3.1 KO mice have a reduced NREM sleep

NREM sleep time in Cav3.1 KO mice generated by Lee et al. [25] (A), and in the global, thalamic-selective and cortical-selective Cav3.1 KO mice developed by Anderson et al. [26] (B). Note how a reduced amount of total NREM sleep time occurs in the light (A) and dark (B) period, respectively. A and B: reproduced from refs. [25] and [26], respectively.