Novel neuronal and astrocytic mechanisms in thalamocortical loop dynamics

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In this review, we summarize three sets of findings that have recently been observed in thalamic astrocytes and neurons, and discuss their significance for thalamocortical loop dynamics.

- (i) A physiologically relevant 'window' component of the low-voltage-activated, T-type Ca^{2+} current (I_{Twindow}) plays an essential part in the slow (less than 1 Hz) sleep oscillation in adult thalamocortical (TC) neurons, indicating that the expression of this fundamental sleep rhythm in these neurons is not a simple reflection of cortical network activity. It is also likely that I_{Twindow} underlies one of the cellular mechanisms enabling TC neurons to produce burst firing in response to novel sensory stimuli.
- (ii) Both electrophysiological and dye-injection experiments support the existence of gap junction-mediated coupling among young and adult TC neurons. This finding indicates that electrical coupling-mediated synchronization might be implicated in the high and low frequency oscillatory activities expressed by this type of thalamic neuron.
- (iii) Spontaneous intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) waves propagating among thalamic astrocytes are able to elicit large and long-lasting *N*-methyl-D-aspartate-mediated currents in TC neurons. The peculiar developmental profile within the first two postnatal weeks of these astrocytic $[\text{Ca}^{2+}]_i$ transients and the selective activation of these glutamate receptors point to a role for this astrocyte-to-neuron signalling mechanism in the topographic wiring of the thalamocortical loop.

As some of these novel cellular and intracellular properties are not restricted to thalamic astrocytes and neurons, their significance may well apply to (patho)physiological functions of glial and neuronal elements in other brain areas.

Keywords: T-type calcium current; electrical coupling; astrocytes; calcium waves; development

1. INTRODUCTION

In this review, three novel findings that we have highlighted in the thalamus over the past few years will be presented and discussed with emphasis to their established or potential significance for thalamic (patho)physiological functions as well as for the overall dynamics of the thalamocortical loop.

The first section will describe the presence of a physiologically relevant 'window' (i.e. steady-state) component of the low-voltage-activated, I_{Twindow} in adult rat and cat TC neurons. The essential role that I_{Twindow} and an I_{CAN} have in the generation of the slow (less than 1 Hz) sleep oscillation of TC neurons indicates that the expression of

this fundamental sleep rhythm in these neurons is not a simple reflection of cortical network activity. In addition, we will discuss how I_{Twindow} might underlie one of the cellular mechanisms enabling TC neurons to produce burst firing in response to novel stimuli.

The second section will summarize our evidence in favour of the existence of electrical coupling among young and adult rat and cat TC neurons, and discuss the implications of a gap junction-mediated synchronization of TC neuron firing in relation to the high and low frequency oscillatory activities expressed by the different neuronal elements of the thalamocortical loop.

The third section will focus on glial cells, and describe the occurrence of spontaneous $[\text{Ca}^{2+}]_i$ waves in thalamic astrocytes and their ability to elicit large and long-lasting NMDA-mediated currents in TC neurons from young rats. The peculiar developmental profile of the spontaneous astrocytic $[\text{Ca}^{2+}]_i$ transients, the selective activation of this type of glutamate receptors by the astrocytic $[\text{Ca}^{2+}]_i$ waves and the associated long-lasting increase of $[\text{Ca}^{2+}]_i$ in TC neurons point to a role for this astrocyte-to-neuron signalling mechanism in the topographic wiring of the thalamocortical loop.

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As these novel cellular and intracellular properties, together with their underlying biophysical and biochemical mechanisms, are not restricted to thalamic astrocytes and neurons, their significance may well apply to (patho)physiological functions of glial and neuronal elements in other brain regions, including thalamic non-relay nuclei and different cortical layers.

2. THE 'WINDOW' COMPONENT OF THE I_T

The original analysis of the low-voltage-activated I_T in TC neurons had shown this current to be characterized by steep steady-state activation and inactivation properties (figure 1a), and a relatively long time of recovery from inactivation (Coulter *et al.* 1989; Crunelli *et al.* 1989; Hernandez-Cruz & Pape 1989; Huguenard 1996). In line with these biophysical properties, a depolarizing input from membrane potentials less than -65 mV opens T-type Ca^{2+} channels, thus eliciting a transient depolarization, the so called LTCP. The LTCP, however, is not the only voltage expression of I_T activation. As it is evident in all biophysical studies on native T-type Ca^{2+} channels in both TC (Coulter *et al.* 1989; Crunelli *et al.* 1989; Hernandez-Cruz & Pape 1989) and NRT neurons (Huguenard & Prince 1992; Huguenard 1996), the steady-state activation and inactivation curves show a region of overlap (highlighted by a red circle in figure 1a) within a narrow voltage range that in TC neurons is centred on -60 mV. It follows that within this membrane potential region, a small proportion of T-type channels remain open, providing a steady influx of Ca^{2+} into the neuron, and thus a steady $I_{T\text{window}}$, and a resulting steady depolarization.

Recent investigations on cloned $\alpha 1G$, $\alpha 1H$ and $\alpha 1I$, the pore-forming subunits of T-type Ca^{2+} channels, while overall confirming the results of the original studies on native channels, have also highlighted differences in the biophysical characteristics of the current generated by each subunit (Klockner *et al.* 1999; Lee *et al.* 1999), and in their distribution in different neuronal types (Craig *et al.* 1999; Talley *et al.* 1999). Within the thalamocortical loop, TC neurons mostly contain $\alpha 1G$ and to a lesser extent $\alpha 1H$, NRT neurons are mainly endowed with $\alpha 1I$ and smaller amounts of $\alpha 1G$ and $\alpha 1H$, while different relative proportions of the three subunits are present throughout the cortical layers (Craig *et al.* 1999; Talley *et al.* 1999). Interestingly, the three cloned T-type Ca^{2+} channels show different sizes of $I_{T\text{window}}$, with the largest being that generated by $\alpha 1I$ (Klockner *et al.* 1999; Lee *et al.* 1999).

Thus, although the LTCP has been considered the main voltage expression of T-type Ca^{2+} channel activation, these channels will also elicit a steady depolarization that is maximal in a small voltage region centred on -60 mV (figure 1a,b): the LTCP is due to the full, transient activation of I_T , the steady depolarization to $I_{T\text{window}}$.

(a) $I_{T\text{window}}$ and the membrane bistability of TC neurons

In order to understand the possible significance of $I_{T\text{window}}$ in the electrical behaviour of TC neurons, let us consider the relationship between this current and the I_{Leak} . In figure 1b, the absolute value of $I_{T\text{window}}$ (the bell-

shaped red curve) has been plotted against the membrane potential, using data from voltage-clamped TC neurons, while I_{Leak} is represented by the straight black line. When the conductance of I_{Leak} (i.e. g_{Leak}) is smaller than $(d|I_{T\text{window}}|/dV)_{\text{max}}$ (yellow line), the line representing I_{Leak} crosses the bell-shaped curve representing $I_{T\text{window}}$ in three points, at which both currents have equal values (figure 1b). Thus, as $I_{T\text{window}}$ is an inward current and I_{Leak} an outward current, these three points are points of zero net current (the green line in figure 1c). This means that at steady state and in the absence of other currents within this voltage region, the membrane potential displays three equilibrium points: USP, LSP and UP in figure 1c (see caption of figure 1 for further details). So, the system generated by the interaction of $I_{T\text{window}}$ and I_{Leak} is bistable: in other words, TC neurons should show two stable (resting) membrane potentials in the absence of other substantial currents in this voltage region (Toth *et al.* 1998).

Indeed, in 15% of TC neurons recorded *in vitro* under control conditions (Williams *et al.* 1997; Hughes *et al.* 1999), or in 95% of TC neurons in the presence of a mGluR agonist (Hughes *et al.* 2002b), bistability can be unmasked when the mixed Na^+/K^+ I_h is abolished using the specific blocker ZD 7288 (figure 1d). In this condition, the switch from one to the other stable potential can be achieved by the use of intracellular current steps (figure 1d). This bistable behaviour is unaffected by Na^+ or high-voltage-activated Ca^{2+} channel blockers, while Ba^{2+} has either no effect or may itself unmask bistability in TC neurons with a low input resistance in control conditions (Williams *et al.* 1997). However, the selective low-voltage-activated Ca^{2+} channel blocker, Ni^{2+} , fully blocks TC neuron bistability, supporting the notion that $I_{T\text{window}}$ is responsible for this electrical behaviour (Williams *et al.* 1997). Indeed, application of Ni^{2+} , but not of high-voltage Ca^{2+} channel blockers, to TC neurons held at *ca.* -60 mV is always accompanied by a 15–20 mV hyperpolarization, confirming that a steady influx of Ca^{2+} ions through T-type channels (i.e. $I_{T\text{window}}$) contributes to a steady depolarization of these neurons (Williams *et al.* 1997).

Further support to the $I_{T\text{window}}-I_{\text{Leak}}$ bistability mechanism is provided by experiments using the dynamic clamp (Hughes *et al.* 1999, 2002b), a technique that allows voltage- or transmitter-gated current generated by a computer to be either added to, or subtracted from a living neuron (Sharp *et al.* 1993). Thus, in an originally bistable TC neuron where both I_h and I_T have been pharmacologically eliminated by ZD 7288 and Ni^{2+} , respectively, bistability can only be reinstated by adding an amount of computer-generated I_T commensurate with the apparent input resistance (i.e. I_{Leak}) of the neuron (figure 1e), such that the net current for that neuron satisfies the theoretical prediction (Hughes *et al.* 1999) (see the caption of figure 1e for further details). In similar experiments, it can also be demonstrated that TC neurons lose their bistable properties if the steady-state activation and inactivation curves of the computer-generated I_T are moved apart by as little as 5 mV (Hughes *et al.* 1999).

Clearly, when I_h is present, its slow kinetics and the transient properties of I_T can prevent the system from attaining stable equilibrium. Thus, once activated by the preceding hyperpolarization generated by the switching off of $I_{T\text{window}}$, I_h will depolarize the neuron up to the level

Figure 1. Biophysical mechanism of $I_{Twindow}$ -based bistability, and its experimental confirmation. (a) Steady-state activation (right) and inactivation (left) curves of I_T from a voltage-clamped cat TC neuron *in vitro*. The region of overlap (red circle) between the two curves represents $I_{Twindow}$. (b) Plot of absolute current versus membrane potential for $I_{Twindow}$ (the bell-shaped red curve) and I_{Leak} (the straight black line). The gradient of I_{Leak} (i.e. g_{Leak}) is smaller than $(d|I_{Twindow}|/dV)_{max}$ (the yellow line, tangential to $I_{Twindow}$). Therefore, the plot of $I_{Twindow} + I_{Leak}$ exhibits a pronounced 'S' shape (the green line in (c)). (c) This means that at steady state, in the absence of I_h , the membrane potential can display three equilibrium points. The upper and lower of these points are stable whilst the remaining point is unstable (USP, upper stable point; LSP, lower stable point; UP, unstable point), i.e. the system is bistable. (d) Application of ZD 7288 (100 μ M), a selective blocker of I_h , unmasks bistability in a cat TC neuron, such that switching between the two stable resting membrane potentials can be achieved by current pulses (bottom trace). Note the presence of a pronounced ADP following the LTCP that characterize the transition from the lower to the upper stable membrane potential. (e) This dynamical clamp experiment demonstrates the validity of the $I_{Twindow}$ - I_{Leak} based bistability mechanisms. In a cat TC neuron recorded *in vitro* in the presence of Ni^{2+} (500 μ M) and ZD 7288 (300 μ M), I_T is reintroduced using the dynamic clamp technique (the trace marked I_T). The two stable membrane potentials accurately match the points of current balance in the voltage versus net current plot, constructed using this neuron's g_{Leak} (calculated as $1/R_N$) and the indicated artificial I_T (continuous green line in plot). A decrease in artificial I_T from 160 to 140 ns fails to elicit bistability as only one equilibrium point (the blue broken line in plot) is present for this combination of g_{Leak} and artificial I_T . Membrane potential bistability is reinstated when I_T is increased back to 160 ns. ((a), (b), (c) and (e) reproduced with permission from Williams *et al.* (1997) and Hughes *et al.* (1999); copyright © 1997, 1999 The Physiological Society. (d) reproduced with permission from Hughes *et al.* (2002b); copyright © 2002 Cell Press.)

Figure 2. The slow (less than 1 Hz) sleep oscillation in TC neurons. (a) EEG and intracellular recordings from cat TC neurons (i) *in vivo* and (ii) *in vitro* + *trans*-ACPD show the slow (less than 1 Hz) sleep oscillation. Note how in these neurons the 'up' state of the slow oscillation shows no action potential (slow 'quiet'). (b) Intracellular recordings of the slow oscillation (i) *in vivo* and (ii) *in vitro* + *trans*-ACPD, in neurons where the 'up' state is characterized by continuous 'tonic' firing. Note the similar features of the slow oscillation *in vivo* and *in vitro* in (a) and (b). In particular, each 'down' state is initiated by a clear inflection point (thin arrow) leading to a stereotypical large hyperpolarizing potential that is terminated by an LTCP (filled arrow). (c) The frequency of the oscillation increases with hyperpolarizing DC (as indicated), with the oscillation eventually generating 'grouped' delta oscillations, before becoming a continuous delta oscillation (action potentials truncated for clarity). A movie showing intracellular recordings of the slow oscillation in a TC neuron *in vitro* can be seen at http://www.thalamus.org.uk/html/neuron33_6.html. (Reproduced with permission from Hughes *et al.* (2002b); copyright © 2002 Cell Press.)

where an LTCP and associated burst firing will always be generated and $I_{T\text{window}}$ reinstated. Thus, as shown in the enlargement on the right-hand side of figure 2a, the $I_{T\text{window}} - I_{\text{Leak}} - I_h$ -based bistable mechanism in TC neurons gives rise to a stereotypical large hyperpolarizing waveform that is characterized by (i) an inflection point punctuating

its beginning (the thin arrow in figure 2a), and (ii) an LTCP and associated burst firing punctuating its end (the filled arrow in figure 2a).

Having proved that TC neurons possess a physiologically relevant $I_{T\text{window}}$, we will next describe how the $I_{T\text{window}} - I_{\text{Leak}}$ -based bistability (i) is the essential biophys-

ical mechanism underlying the electrical activity generated by these neurons during the slow (less than 1 Hz) sleep rhythm, one of the basic sleep waves (§§ 2b–2e), and (ii) might represent one of the cellular processes responsible for the burst firing that characterizes the response of these neurons to novel stimuli (§ 2f).

(b) *The slow (less than 1 Hz) sleep oscillation*

A characteristic feature of the EEG of humans and animals during deep sleep is the presence of slow waves that recur every 1–10 s (Steriade *et al.* 1993*b,c,d*, 2001; Achermann & Borbely 1997; Amzica & Steriade 1997; Simon *et al.* 2000). This so-called slow (less than 1 Hz) sleep rhythm is generated in neocortical networks (Steriade *et al.* 1993*c,d*; Sanchez-Vives & McCormick 2000) and serves to organize other sleep rhythms, such as sleep spindle and delta waves, into coherent, repeating episodes (Steriade *et al.* 1993*a,b*). At the cellular level, the slow rhythm of cortical neurons is manifested as recurring sequences of synaptic barrages, giving rise to periods of sustained action potential firing, interspersed with periods of disfacilitation or quiescence (Steriade *et al.* 1993*d*), and thus leading to prominent ‘up’ and ‘down’ states of the membrane potential (Amzica & Steriade 1995; Sanchez-Vives & McCormick 2000).

In TC neurons *in vivo*, the intracellular correlate to the slow sleep rhythm is also characterized by repeating ‘up’ and ‘down’ states of the membrane potential, which are separated by 15–25 mV and demonstrate a stereotypical waveform (Steriade *et al.* 1993*a*, 1996; Contreras & Steriade 1995; figure 2*a(i)*). In particular, the transition from the ‘up’ to ‘down’ state shows a characteristic inflection point, whereas the transition from the ‘down’ to ‘up’ state is marked by an LTCP and the accompanying high-frequency burst of action potentials (figure 2*a(i)*). As the slow (less than 1 Hz) oscillation in TC neurons is abolished following decortication (Timofeev & Steriade 1996), it has been suggested that it is primarily a simple reflection of rhythmic cortical activity (Steriade *et al.* 1993*a*).

In vitro, a slow membrane potential oscillation with similar properties as those described *in vivo* for the slow sleep rhythm can be recorded during exogenous activation of mGluRs with *trans*-ACPD in almost every TC neuron of a number of thalamic relay nuclei, including the dorsal lateral geniculate, the medial geniculate nucleus, the ventrolateral and ventrobasal nuclei in both young and adult rats and cats (figure 2*a(ii)*). Indeed, the same oscillation can be elicited by electrical stimulation of the corticothalamic afferents, and using a number of selective agonists and antagonists, it can be demonstrated that the receptors involved in this intracellularly recorded slow rhythm in TC neurons are the mGluR1a type (Hughes *et al.* 2002*b*), which are located postsynaptically (Godwin *et al.* 1996; Turner & Salt 2000) to the corticothalamic axons.

In different TC neurons *in vitro*, the ‘up’ state of the slow oscillation may either show ‘tonic’ firing (figure 2*b,c(ii)*), or lack any action potentials (‘quiet’) (figure 2*a,c(i)*): indeed, the percentage of neurons *in vitro* that exhibit firing during the ‘up’ state is similar to that observed *in vivo*. In addition, in all TC neurons, increasing DC hyperpolarization results in the appearance of ‘grouped’ delta oscillations during the ‘down’ state of the slow oscillation, before a continuous delta oscillation

becomes visible (figure 2*c(i)(ii)*): a feature that is also present in *in vivo* recordings (for a full description of these and other similarities, see Hughes *et al.* 2002*a*). The fact that *in vivo* the slow oscillation and the ‘grouped’ delta oscillation are dependent on an intact cortex (Timofeev & Steriade 1996), whereas *in vitro* they are both unmasked when cortical input is mimicked through activation of mGluR1a (Hughes *et al.* 2002*b*) (which are postsynaptic to the corticothalamic afferents; Godwin *et al.* 1996), adds further support to the similarity of the slow activity observed in the two experimental conditions.

(c) *I_{Twindow} is essential for the slow (less than 1 Hz) sleep oscillation in TC neurons*

The slow oscillation in TC neurons *in vitro* is insensitive to TTX, and to antagonists of ionotropic glutamate receptors, GABA_A and GABA_B receptors. In addition, it is unaffected by Ba²⁺, 4-AP, and by the high-voltage-activated Ca²⁺ channel blockers, Cd²⁺, nifedipine, ω -conotoxin-MVIIC or ω -conotoxin-GVIA (Hughes *et al.* 2002*b*).

However, the slow oscillation is abolished by ZD 7288, a selective blocker of the mixed Na⁺/K⁺ inward rectifying current (I_h), and this procedure uncovers the membrane potential bistability that underlies the slow oscillation (Pirchio *et al.* 1997; Hughes *et al.* 1999, 2002*b*) (figure 1*b(i),d*). Perfusion of the slice with Ni²⁺ (350–500 μ M) not only reversibly abolishes the slow oscillation (Pirchio *et al.* 1997; Hughes *et al.* 1999, 2002*b*) (figure 3*a,b(ii)*), demonstrating its dependence on IT, but also blocks the bistability, as the neurons express only one membrane potential.

(d) *Essential role of I_{CAN} in the slow (less than 1 Hz) oscillation*

Although the basic features of the slow oscillation in TC neurons can be accounted for by the bistability generated by the interaction of I_{Twindow} and I_{Leak} , coupled with the depolarizing influence of I_h , some important details of this sleep rhythm cannot. In particular, dynamic clamp experiments (figure 2*b*), or computer simulations which only take into account these three currents (plus those of the action potentials) (figure 4*a*), cannot faithfully replicate: (i) the slow ADP that characterizes the ‘up’ state of the oscillation; (ii) the full range of frequencies of the slow oscillation (see below); and (iii) the presence of ‘grouped’ delta oscillations.

The experiments with Ni²⁺ show that, in addition to blocking membrane potential bistability, the slow oscillation and the LTCP, these ions always abolish the ADP (figure 3*b*). However, whereas reintroduction of I_T by the dynamic clamp is able to restore bistability and the LTCP following their block by Ni²⁺, the ADP cannot be recreated by this procedure (Hughes *et al.* 2002*b*) (figure 3*b*). As the computer-generated I_T fully replicates the biophysical properties of endogenous I_T except that it is not carried by Ca²⁺ ions (Hughes *et al.* 1999), it appears that the ADP must involve a Ca²⁺-dependent conductance, a hypothesis supported by the observation that the inclusion of either BAPTA or EGTA in the recording electrode abolishes the ADP (Hughes *et al.* 2002*b*). Interestingly, the Ca²⁺ entry linked to the ADP seems to occur selectively via T-type Ca²⁺ channels as high-threshold Ca²⁺

Figure 3. Effect of blocking I_T on the slow (less than 1 Hz) oscillation in TC neurons. (a) (i) Control (*trans*-ACPD/TTX); (ii) $+Ni^{2+}$ (350 μ M). Application of Ni^{2+} abolishes the slow oscillation and the underlying membrane potential bistability in a cat TC neuron. (b) (i) Control (*trans*-ACPD/ZD 7288/TTX); (ii) following Ni^{2+} application (500 μ M), this neuron loses bistability and ADP; (iii) re-introduction of I_T with the dynamical clamp reinstates the bistability but not the ADP; and (iv) a comparison between the control and Ni^{2+} plus the dynamic clamp. (Reproduced with permission from Hughes *et al.* (2002b); copyright © 2002 Cell Press.)

channels blockers have no effect on the ADP. In addition, Ba^{2+} and 4-AP either are ineffective on the ADP, or lead to its enhancement due to an increase in apparent input resistance (Hughes *et al.* 2002b), thus ruling out the possible involvement of a Ca^{2+} -inactivated K^+ current (Tokimasa 1985).

The ADP is also unchanged either by TTX or replacement of NaCl with choline chloride. However, substitution of Na^+ with *N*-methyl-D-glucamine reversibly inhibits the ADP (Hughes *et al.* 2002b), indicating that this potential is generated by an I_{CAN} (Partridge & Swandulla 1988), as is the case for similar behaviour in other thalamic neurons (Bal & McCormick 1993; Zhu *et al.* 1999). Indeed, using a hybrid current/voltage clamp protocol, the current underlying the ADP is found to exhibit: (i) an amplitude linearly related to membrane potential; (ii) an extrapolated reversal potential of 8.7 mV; and (iii) a time-course that decays mono-exponentially in a voltage-independent manner ($\tau_{decay} = 1.44$ s) (Hughes *et al.* 2002b). These properties are unaffected by TTX and Ba^{2+} (1–2 mM), and are consistent with the features of an I_{CAN} (Zhu *et al.* 1999).

The significance of I_{CAN} in the slow (less than 1 Hz) oscillation can be fully assessed using a biophysical model of TC neurons (Williams *et al.* 1997) (figure 4b). In the presence of I_{CAN} , it is now possible to observe a region where the oscillation frequency *gradually* decreases from 0.24 to 0.06 Hz over a relatively large range of injected DCs (region marked 'slow' in figure 4b(ii)), with a mean

rate of frequency decrease with respect to current ($0.0024 \text{ Hz pA}^{-1}$) similar to that observed in the experiments ($0.0023 \text{ Hz pA}^{-1}$ for the example shown in figure 4c(ii)). As shown in these simulations, I_{CAN} stabilizes the slow oscillation by transforming what would normally be a delta oscillation (see the region labelled 'δ' in figure 4b(ii)) into a slow oscillation by temporarily depolarizing the cell following an LTCP (i.e. top trace in figure 4b(ii)) (Hughes *et al.* 2002b). Furthermore, as a transition stage between a continuous delta oscillation (bottom trace in figure 4b(i)) and a 'pure' slow oscillation (top trace in figure 4b(i)), the model exhibits 'grouped' delta activity (middle trace in figure 4b(i)), which occurs when the effect of I_{CAN} following a single LTCP is insufficient to force a prolonged depolarization. However, as a second LTCP occurs before I_{CAN} has significantly decayed, the conductance of I_{CAN} accumulates, eventually becoming large enough to instate a prolonged depolarization (figure 4b(i), middle trace) (Hughes *et al.* 2002b).

A similar ADP has recently been shown to occur in TC neurons of the ferret dorsal lateral geniculate nucleus (Luthi & McCormick 1998). In contrast to the results presented here, the ADP studied by these authors is thought to be generated by a Ca^{2+} -mediated upregulation of I_h (Luthi & McCormick 1998) and is believed to be, at least in part, responsible for the waxing and waning nature of thalamic spindle oscillations (Bal & McCormick 1996), in a similar manner to the way that the ADP described here controls the duration of 'grouped' episodes of delta

Figure 4. Effect of I_{CAN} on the slow oscillation simulated using a simplified model of a TC neuron. (a) In the absence of I_{CAN} (i.e. $g_{CAN} = 0$ ns), a model TC neuron with $g_{Leak} = 1.5$ ns exhibits a slow oscillation (i) but only for a very narrow range of DC inputs ('slow' in (ii)). (b) With $g_{CAN} = 10$ ns, the model exhibits a slow oscillation over a much greater range of DC inputs ((i) and (ii)). Furthermore, the model is able to exhibit 'grouped' delta oscillations as a transition stage between a continuous delta oscillation and a 'pure' slow oscillation. The 'grouped' delta activity occurs whilst the conductance of I_{CAN} is accumulating to a point where it is able to facilitate the generation of a prolonged 'up' state. (c) Experimental traces obtained in the presence of *trans*-ACPD (i) show almost identical properties to the simulations depicted in (b(i)). Also, the current versus frequency plot for the experimental scenario (ii) possesses a similar form to that shown in (b(ii)). (Reproduced with permission from Hughes *et al.* (2002b); copyright © 2002 Cell Press.)

oscillations. Therefore, it is likely that *in vivo* I_{CAN} and the Ca^{2+} -mediated upregulation of I_h may work in tandem to control the various types of oscillatory activities evident in TC neurons during sleep. It is important to point out, however, that due to the presence of I_{CAN} , the effects of Ca^{2+} on I_h in previous studies may have been overestimated (Luthi & McCormick 1998; but see Budde *et al.* 1997).

(e) Mechanism of the slow (less than 1 Hz) sleep rhythm within the thalamocortical loop

In summary, therefore, activation of mGluR1a *in vitro* brings about a slow oscillation in TC neurons identical to that observed *in vivo* during the slow (less than 1 Hz) sleep rhythm. The slow oscillation in TC neurons is brought about by a dynamical interaction between $I_{Twindow}$, I_{Leak} and I_h , and requires the presence of an I_{CAN} for the full expression of its various subtypes. These findings indicate that the suggested mechanism for the slow oscillation in TC neurons, i.e. a simple reflection of recurring periods of firing and quiescence in corticothalamic afferents, while contributing to overall TC neuron activity during this rhythm, is not the main process leading to a bistable condition, with two membrane potentials separated by up to 25 mV. This notion is supported by the finding that, even in the absence of mGluR1a activation, 15% of TC neurons are capable of exhibiting the slow

oscillation *in vitro* (Pirchio *et al.* 1997; Hughes *et al.* 1999), though its full expression requires activation of these receptors (Hughes *et al.* 2002b).

During the onset of sleep, the overall activity in cortical neurons is reduced (Steriade *et al.* 2001), leading to a decreased, though not abolished, modulatory influence on TC neurons and the unmasking of the slow (less than 1 Hz) oscillation. The idea that the mGluR1a present in TC neurons is substantially activated by the corticofugal afferents during sleep is well supported by numerous studies showing that in this state, cortical neurons are far from inactive and continue to generate sustained periods of action potential firing (Steriade *et al.* 1993c,d, 1996, 2001; Contreras & Steriade 1995, 1997a,b). Indeed, during sleep, although the rhythmic recurrence of the 'down' states of the slow oscillation in cortical neurons will lessen the overall output of the cortex to the thalamus in comparison to wakefulness, the 'up' state present in cortical neurons supports action potential firing rates that are actually comparable with those observed during the wakeful state (Steriade *et al.* 2001).

As I_T is present in a variety of neurons in different brain areas, including cortex and thalamic non-relay nuclei, a physiologically relevant $I_{Twindow}$ and a mechanism similar to the one described for the bistability of TC neurons might also underlie some of the dynamical behaviours exhibited by these neurons under certain conditions.

Indeed, our preliminary data indicate that NRT neurons, which also possess I_{CAN} (Bal & McCormick 1993) and mGluRs (Cox & Sherman 1999), are also able to generate a slow (less than 1 Hz) oscillation *in vitro* with a mechanism identical to those of TC neurons, and can give rise to 'grouped' sleep spindle oscillations. This finding is not surprising as $\alpha 1I$, the main subunit present in NRT neurons (Craig *et al.* 1999; Talley *et al.* 1999), exhibits a much larger $I_{Twindow}$ than the other two subunits that are expressed in TC neurons (Lee *et al.* 1999). In addition, together with the synaptic barrage of intracortical networks, an interaction between $I_{Twindow}$ and I_{Leak} might also represent a necessary component of the slow (less than 1 Hz) sleep rhythm expressed by cortical neurons, as they also express $\alpha 1G$, $\alpha 1H$ and $\alpha 1I$ subunits (Craig *et al.* 1999; Talley *et al.* 1999).

(f) $I_{Twindow}$ -based bistability as a cellular mechanism for detecting novel stimuli

The presence of a physiologically relevant $I_{Twindow}$ in TC neurons, and its stereotypical voltage expression, raise two important issues concerning the electrical behaviour of these neurons, and thus of the thalamocortical loop.

- (i) On the basis of the steady-state activation and inactivation properties of I_T , it is generally assumed that the LTCP-mediated burst firing evoked in TC neurons by EPSPs or IPSPs may only occur when the membrane potential is set negative or positive, respectively, to *ca.* -65 mV, and in particular to result from the generation of LTCPs superimposed upon EPSPs or following the termination of IPSPs (Jahnsen & Llinas 1984; Crunelli *et al.* 1987; Crunelli & Leresche 1991; Bal *et al.* 1995; Sherman 1996). This interpretation has strongly influenced ideas concerning the setting of the membrane potential of TC neurons during different behavioural stages (Steriade *et al.* 1993b; Steriade 2000) and in neurological disorders (Llinas *et al.* 1999; Crunelli & Leresche 2002). The presence of a physiologically relevant $I_{Twindow}$ drastically changes this accepted notion, as the switching off of this current can provide a hyperpolarization that meets the time and voltage requirement for removal of I_T inactivation. For example, from holding potentials positive to -65 mV, LTCP-mediated burst firing can be triggered by small (less than 5 mV), physiologically relevant, postsynaptic potentials irrespective of their sign (i.e. EPSPs or IPSPs) and duration (figure 5a; Williams *et al.* 1997). In other words, from the same holding potential the generation of burst firing in TC neurons is not ruled by the form of driving postsynaptic potentials.
- (ii) From what is mentioned above, it follows that even at relatively depolarized membrane potentials of -55 or -60 mV an EPSP may be able to lead to the formation of a burst response in TC neurons in alert animals (Guido & Weyand 1995; Sherman 1996, 2001a; Weyand *et al.* 2001), as an LTCP-mediated burst firing always follows the switching off of $I_{Twindow}$. As we have previously suggested, therefore, in this situation $I_{Twindow}$ will bring about an amplification of the input signal, increasing the

sensitivity to small-amplitude isolated synaptic inputs (i.e. novel stimuli) via the generation of action potential burst firing (Williams *et al.* 1997). In this respect, therefore, $I_{Twindow}$ might represent one of the cellular mechanisms underlying the burst firing that is observed during the alert state and has been suggested to increase the detectability of novel stimuli (i.e. the so-called 'wake-up call'; Sherman 2001a,b). Within this scenario, the delay between a sensory evoked postsynaptic potential and the occurrence of the LTCP-mediated burst is not set, but will strictly depend on the membrane potential and could be finely tuned by (i) the neuron I_{Leak} , which takes into account the overall background synaptic activity, and (ii) the strength of I_h , which is known to be modulated by some thalamic transmitters (McCormick 1992). The latter effect can be easily appreciated by observing the response of TC neurons to intracellular current steps of varying amplitude and duration, mimicking different sizes of I_h (see fig. 2b in Williams *et al.* 1997). This $I_{Twindow}$ -based process for generating burst firing during wakefulness that we are proposing here seems more plausible than a brainstem-mediated, 15–20 mV hyperpolarization, which would necessitate a sudden and marked decrease in the tone of the various depolarizing inputs originating from this brain area (Steriade *et al.* 1997). Similarly, the increase in spontaneous firing and changes in receptive field properties evoked by GABA_B receptor antagonists in TC neurons (Lee *et al.* 1994) would also suggest that GABA_B IPSPs (originating either from NRT cells or interneurons) might not be involved in the hyperpolarization that must precede the LTCP-mediated action potential burst that is associated with novel stimuli.

3. ELECTRICAL COUPLING AMONG TC NEURONS

(a) Spikelets are present in adult TC neurons

A number of *in vivo* studies have described the presence of small depolarizing potentials (so-called fast prepotentials) in TC neurons (Deschenes *et al.* 1984; Steriade *et al.* 1991; Pinault & Deschenes 1992; Timofeev & Steriade 1997). Some of these events exhibit an increase in amplitude in response to membrane hyperpolarization, but no change in frequency in response to depolarizations, and have therefore been identified as conventional EPSPs (Pinault & Deschenes 1992; Timofeev & Steriade 1997). In other cases, however, the amplitude of the fast prepotentials has been shown to be insensitive to membrane potential, and their generation to be critically dependent on neuronal depolarization (Deschenes *et al.* 1984; Steriade *et al.* 1991; Timofeev & Steriade 1997). A closer analysis of the latter type of fast prepotentials indicates that they do not represent a homogeneous population but consist of either: (i) short-duration (4–10 ms), single-component, stereotypical events (figure 6a), which can appear as isolated rhythmic potentials (Deschenes *et al.* 1984; Steriade *et al.* 1991) or in combination with EPSPs (Timofeev & Steriade 1997); or (ii) longer-duration (10–20 ms) events which exhibit multiple components (Steriade *et al.* 1991).

Figure 5. I_{Twindow} -based bistability allows for LTCP-mediated burst firing to be elicited at -60 mV by synaptic potentials, irrespective of their sign. (a)(i), Single EPSPs ($n=3$ traces), elicited by electrical stimulation of the optic tract, evoke an LTCP and burst firing in a TC neuron recorded at -60 mV in the presence of bicuculline and CGP 35348. (ii) Single GABA_B receptor-mediated IPSPs ($n=3$ traces), evoked by direct electrical stimulation of the NRT, give rise to a similar burst firing response in a TC neuron recorded at -60 mV in the presence of APV, CNQX and bicuculline. The initial period of the response in (i) and (ii) is also shown at a faster time base. (b) Voltage traces ($n=3$ in each group) evoked by positive current steps of three different amplitudes demonstrate the ability of depolarizing pulses to elicit burst responses from a resting membrane potential of -66 mV. Note how the time from the start of the pulse to the LTCP/burst firing generation is inversely related to the amplitude of the pulse. In (a) and (b), action potential amplitude has been truncated for clarity. (Reproduced with permission from Williams *et al.* (1997); copyright © 1997 The Physiological Society.)

During intracellular recordings performed *in vitro* from TC neurons in the cat dorsal lateral geniculate nucleus in the presence of mGluR agonists (either *trans*-ACPD or dihydroxyphenylglycine, a selective mGluR1a agonist) or high (5 mM) extracellular K^+ , we have recently observed depolarizing potentials (hereafter called spikelets), either in isolation or accompanying single action potential firing (figure 6b), and often with a striking rhythmicity (Hughes *et al.* 2002a) (figure 6e). These spikelets are never observed in the absence of mGluR activation or high K^+ , and occur in 20% of TC neurons, which otherwise have morphological and electrophysiological features typical of this type of thalamic neuron. In addition, they are present in slices from young (postnatal day 8) and adult (postnatal week 16) cats. The amplitude of the spikelets recorded *in vitro* may vary among different neurons (2–7 mV), but does not change in an individual neuron even after a long recording session (2–3 h) or as a result of membrane polarization (figure 6f), leading to an apparent all-or-none nature (Hughes *et al.* 2002a). These spikelets are always of a short duration (9 ms) and exhibit a stereotypical waveform, with a fast rising phase (time to peak *ca.* 1 ms) and a relatively slower decay (decay time constant *ca.* 0.8 ms) (Hughes *et al.* 2002a) (figure 6c). Indeed, comparison of a spikelet and an action potential shows the former to have slightly longer time to peak and decay than the latter (figure 6c).

In the absence of injected DC, the mean spikelet frequency is 10 Hz (range: 2.2–24.8 Hz). Interestingly, this value is not significantly different from either (i) the frequency of single action potential firing recorded in the absence of DC from TC neurons that do not exhibit spikelets in the presence of *trans*-ACPD (12 Hz; range: 4.4–20.0 Hz) or (ii) the mean frequency of spontaneous extra-

cellular spikes recorded from TC neurons under the same conditions (10 Hz; range: 2.6–19.2 Hz) (Hughes *et al.* 2002a). Increasing membrane depolarization leads to an increase in spikelet frequency (figure 6e,f), whilst hyperpolarization often abolishes the spikelets.

Given the similarity in both the waveform and properties of the short-duration fast prepotentials observed *in vivo* and the spikelets observed *in vitro* during mGluR activation, it is highly likely that they represent the same entity. This proposal is further supported by (i) the similar proportion of TC neurons displaying clearly identifiable short-duration fast prepotentials *in vivo* (22%) (Timofeev & Steriade 1997), and that exhibiting spikelets *in vitro* (19%) (Hughes *et al.* 2002a), and (ii) the finding that spikelets are unmasked following the activation of mGluRs and the observation *in vivo* that synchronized oscillatory activity involving short-duration fast prepotentials is potentiated by cholinergic input (Steriade *et al.* 1991), which depolarizes TC neurons (McCormick 1992).

(b) Spikelet pharmacology and dye-coupling among TC neurons

Spikelets *in vitro* are routinely observed in the presence of antagonists of fast chemical synaptic transmission involving glutamate and GABA synapses (Hughes *et al.* 2002a). In addition, spikelets are not a manifestation of dendritic Ca^{2+} spikes, as they are resistant to the blockade of both low- and high-voltage-activated Ca^{2+} channels with either Ni^{2+} , Cd^{2+} or selective toxins (Hughes *et al.* 2002a). However, spikelets are abolished by TTX, indicating that they are dependent on the activation of Na^+ channels (Hughes *et al.* 2002a).

In a large proportion of TC neurons (88%) exhibiting spikelets, injection of either biocytin or neurobiotin leads

Figure 6. Spikelets and dye-coupling in TC neurons. (a) Fast prepotentials recorded from a TC neuron *in vivo*. (b) Spikelets recorded in the presence of *trans*-ACPD in a TC neuron from the dorsal lateral geniculate nucleus *in vitro* show similar features to those of the fast prepotentials recorded *in vivo*. A movie showing intracellular recordings with spikelets can be seen at http://www.thalamus.org.uk/html/neuroscience110_3.html. (c) Enlarged spikelet and action potential from the cell in (b). The overlay of the spikelet and the action potential shows that the spikelet has a slightly longer time to peak and substantially slower decay than the action potential. (d) Inverted image of a 100 μ M section of a cat dorsal lateral geniculate slice showing dye-coupling between three TC neurons (soma labelled 1, 2 and 3). During image acquisition the focus was adjusted to optimize the appearance of neurons 1 and 3. The corresponding electrophysiological recording, showing rhythmic spikelets and the voltage-dependence of their frequency, is shown in (e). (f) The effect of varying membrane potential (through the injection of steady DC) on the spikelet frequency (i), and amplitude (squares) and time to peak (circles) (ii). (Reproduced with permission from Hughes *et al.* (2002a); copyright © 2002 Elsevier Science.)

to the staining of additional cells, i.e. dye-coupling (Hughes *et al.* 2002a) (figure 6d). Even when spikelets are not evident in intracellular recordings, dye-coupling is observed to occur regularly between TC neurons: 37% of neurons, in fact, show dye transfer to at least one (and up to a maximum of four) neighbouring cell(s), with their soma located within 80 μ m from each other (Hughes *et al.* 2002a). Interestingly, dye-coupling in the cat dorsal lateral geniculate nucleus does not involve TC neurons in adjacent laminae, even when the dye-coupled neurons are located very close to the lamina borders, indicating a certain degree of segregation within the neuronal population of this thalamic relay nucleus. If no correlation existed between spikelets and dye-coupling, then one would expect to see a similar proportion of neurons exhibiting dye-coupling, regardless of the number that also display spikelets. But this is clearly not the case, as the probability that the sampled neurons showing spikelets are also involved in dye-coupling solely by chance is 0.005.

All these findings demonstrate that spikelets are not generated by either conventional, i.e. chemical, synaptic transmission or intrinsic conductances (see below for explanation of TTX block). It is also unlikely that spikelets result from electric field effects (Jefferys 1995; Dudek *et al.* 1998), as in this case their waveform would have a shorter rise time than the action potentials (as it would be

similar to their first derivative; figure 6e), their frequency would be insensitive to DC injection (figure 6c), and there would not be such a strong correlation with the occurrence of dye-coupling. Another unlikely possibility is that spikelets are generated by the simultaneous impalement of a neuron soma with the axon or dendrite of an additional neuron, leading to fusion of the two cells and also explaining the presence of dye-coupling (Kaneko *et al.* 1981). Given the fine structure of dendrites and axons, in fact, it is improbable that as many as one in five of our microelectrode impalements would consist of double recordings, and, furthermore, that they would exhibit a consistent long-term stability and predictable electrical properties among different cases. In addition, if all our dye-coupling results were due to double impalement, a prevalence of 37% would seem unfeasibly large, and clearly would not account for dye-coupling results where more than two TC neurons are labelled. Finally, the distinct variations in the degree of staining between groups of dye-coupled TC neurons would not be expected to occur from fused membranes (Connors *et al.* 1983).

(c) **Spikelets represent electrical coupling among TC neurons**

Thus, the most parsimonious explanation for the spikelets that are recorded in cat TC neurons *in vitro* is that

they represent electrotonic coupling via gap junctions: indeed, their properties are similar to those known to be generated by electrotonic coupling in other neurons (MacVicar & Dudek 1981; Logan *et al.* 1996; Draguhn *et al.* 1998; Galarreta & Hestrin 1999; Gibson *et al.* 1999; Tamas *et al.* 2000; Venance *et al.* 2000), which also exhibit dye-coupling. In particular, as spikelets are (i) sensitive to TTX, and (ii) dependent on the presence of mGluR activation (or high extracellular K^+), which bring about depolarization and rhythmic action potential firing in TC neurons at similar frequencies, they may represent attenuated action potentials generated in electrotonically coupled cells (hence the block by TTX). Indeed, carbenoxolone, a putative gap junction blocker (Davidson & Baumgarten 1988), reversibly blocks the occurrence of spikelets in all TC neurons tested, without any change in passive membrane properties.

Electrical coupling is a well-established phenomenon among GABAergic (i.e. inhibitory) neurons in a number of brain areas, including, recently, the GABAergic neurons in the young rat NRT (Landisman *et al.* 2002). The presence of electrical coupling among excitatory (i.e. glutamatergic) neurons, however, appears less common (MacVicar & Dudek 1981). Notwithstanding the fact that thalamic relay nuclei have not traditionally been associated with gap junctions, ultrastructural investigations have shown that rat TC neurons make extensive filamentous contacts and exhibit occasional close membrane appositions that may be associated with gap junctions (Lieberman & Spacek 1997). In addition, mRNA coding for various connexins, the molecular substrate of gap junctions, has been detected in a number of rat thalamic relay nuclei (Micevych & Abelson 1991; Parenti *et al.* 2000), and we are currently performing similar investigations in the cat thalamus.

At the electrophysiological level, too, a large number of investigations using both sharp and patch electrode recordings, as well as single neuron injection, have been unable to detect the presence of electrical and/or dye-coupling in TC neurons. While our data indicate that the prevalence of these phenomena is low and the activation of mGluRs is essential to uncover electrical and dye-coupling in these cells, previous studies have not reported the occurrence of spikelets following application of *trans*-ACPD or other mGluR agonists (McCormick & von Krosigk 1992; von Krosigk *et al.* 1999; Turner & Salt 2000; Zhan *et al.* 2000; Gutierrez *et al.* 2001). As gap junctions are exceptionally sensitive to metabolic and mechanical stress (Martinez & Saez 2000), and extremely susceptible to even small and brief changes in oxygen and pH (Budunova *et al.* 1993), mostly in an irreversible manner, it might be that careful preparation, handling and maintenance of thalamic slices is essential for revealing spikelets and dye-coupling in TC neurons.

(d) *Functional significance of electrical coupling among TC neurons*

In accordance with data from other brain areas (Adamchik *et al.* 2000), the relative prevalence of spikelets and dye-coupling among TC neurons indicates that the incidence of such coupling would be reasonably low. In addition, the discrete, stereotypical nature of spikelets and the uncommon occurrence of dye-coupling between more

than two cells indicates that individual TC neurons would rarely be coupled to more than one other neuron. As it has also been shown for other groups of neurons (Draguhn *et al.* 1998; Adamchik *et al.* 2000), however, these factors do not preclude the possibility that electrotonic coupling would have a profound effect on thalamic synchronization both of physiological (Steriade *et al.* 1993b; Sillito *et al.* 1994; Alonso *et al.* 1996; Neuenschwander & Singer 1996; Weliky & Katz 1999) functions (i.e. sensory processing or sleep waves) and pathological (Pinault *et al.* 1998; Llinas *et al.* 1999; Crunelli & Leresche 2002) activities (i.e. absence epilepsy or Parkinson's disease). Indeed, our preliminary observations indicate that carbenoxolone disrupts the synchronization of oscillatory activities in simultaneously recorded pairs of TC neurons *in vitro*. It would be interesting to ascertain whether both high and low frequency thalamic oscillations are synchronized by gap junctional potentials, or whether this gap junction-based mechanism shows some degree of selectivity with respect to particular frequencies of thalamic activities. Interestingly, the fast prepotentials, which are commonly observed *in vivo* (Deschenes *et al.* 1984) and have similar properties to the spikelets observed in TC neurons *in vitro*, are thought to be involved in the generation of synchronized fast (20–40 Hz) oscillations within the thalamocortical loop (Steriade *et al.* 1991; Timofeev & Steriade 1997).

4. THALAMIC ASTROCYTES DRIVE TC NEURON EXCITATION

(a) *Spontaneous $[Ca^{2+}]_i$ transients in thalamic astrocytes*

Up until a decade ago, glial cells in the CNS were considered as having merely supportive roles for neurons. This view drastically shifted with the discovery that astrocytes in culture could respond with increases in $[Ca^{2+}]_i$ to synaptic stimulation and the application of neurotransmitters (Cornell-Bell *et al.* 1990; Charles *et al.* 1991; Dani *et al.* 1992). As these evoked $[Ca^{2+}]_i$ transients can propagate as Ca^{2+} waves through gap-junctionally coupled astrocytes in organotypic slice culture, it has been argued that astrocytes can participate in a form of slow, long-distance information processing (Dani *et al.* 1992). Another important discovery, both in neuron–astrocyte cocultures (Charles 1994; Nedergaard 1994; Hassinger *et al.* 1995; Araque *et al.* 1998) and *in situ* (Pasti *et al.* 1997; Kang *et al.* 1998; Newman & Zahs 1998), has been that chemically or mechanically evoked $[Ca^{2+}]_i$ transients in astrocytes are able to modulate and possibly integrate the activity of adjacent neurons, by releasing neurotransmitters such as glutamate (Parpura *et al.* 1994; Bezzi *et al.* 1998; Innocenti *et al.* 2000), through a probable exocytotic, Ca^{2+} -dependent mechanism (Araque *et al.* 2000).

Together these findings, therefore, have led to the recognition of bidirectional astrocyte–neuron communication (Pasti *et al.* 1997), and the hypothesis of a 'tripartite synapse' (Kang *et al.* 1998; Araque *et al.* 1999; Carmignoto 2000), in which the astrocyte, in addition to pre- and postsynaptic neurons, is a functional component of the synapse. In this scenario, however, astrocytes are still reactive elements, i.e. they *respond* to synaptic activity with rises in $[Ca^{2+}]_i$ and *subsequently* release neurotransmitters to have a feedback effect on synaptic transmission.

Figure 7. Spontaneous $[Ca^{2+}]_i$ transients in rat VB astrocytes. (a) Fluorescence image of a region of an acutely isolated VB slice, where (i), (ii) and (iii) denote the positions of astrocytes that displayed the spontaneous $[Ca^{2+}]_i$ transients shown in (b) (plots of $\Delta F\%$ over time). The image was taken at 220 s after beginning of recording, and displays absolute fluorescence level. A movie showing spontaneous $[Ca^{2+}]_i$ transients in VB astrocytes, including those illustrated here can be seen at http://www.thalamus.org.uk/html/natureneuroscience4_8.html.

Recently, we have observed that astrocytes in the rat VB (Parri *et al.* 2001) and other thalamic nuclei display spontaneous astrocytic $[Ca^{2+}]_i$ transients *in situ* which are independent of neuronal activity. Figure 7 shows an example of such astrocytic $[Ca^{2+}]_i$ oscillations imaged in a VB slice loaded with the membrane-permeable Ca^{2+} indicator Fluo-4AM, using an experimental condition where both action potential-dependent release and transmitters for GABA, glutamate and ATP had been blocked by TTX and specific antagonists, respectively.

Over a recording period of 10 min, two or more spontaneous $[Ca^{2+}]_i$ transients (figure 7b) occur in the majority of astrocytes (67%), whereas the remaining ones only display a single transient. Interestingly, in about 4% of astrocytes we have observed spontaneous rhythmic pacemaker $[Ca^{2+}]_i$ oscillations (Parri & Crunelli 2001). In the case of multiple transients and for the pacemaker $[Ca^{2+}]_i$ oscillations the average frequency is 0.01 Hz, while their average duration, measured at half-height, is *ca.* 15 s (at room temperature) (Parri & Crunelli 2001). Similar spontaneous $[Ca^{2+}]_i$ oscillations have recently been described in the hippocampus (Nett *et al.* 2002).

(b) Mechanism of spontaneous $[Ca^{2+}]_i$ transients

The number of spontaneous $[Ca^{2+}]_i$ transients in an astrocyte, and the percentage of astrocytes showing pacemaker transients, markedly increase following either an increase in extracellular Ca^{2+} from 2 to 5 mM, or application of the L-type Ca^{2+} channels agonist BayK8644. The number of astrocytes showing spontaneous $[Ca^{2+}]_i$ transients is not affected by Ni^{2+} , whereas the N, P, Q high-voltage-activated Ca^{2+} channel blocker ω -conotoxin-MVIIC produces a small (20%), non-significant reduction. By contrast, the L-type Ca^{2+} channel blocker nifedipine causes a marked (65%) reduction in astrocytic activity (Parri *et al.* 2001). After addition of the non-spe-

cific Ca^{2+} channel blocker Co^{2+} , or removal of extracellular Ca^{2+} , only a single $[Ca^{2+}]_i$ transient is observed in any given astrocyte (generally 3–4 min after the experimental procedure) (Parri *et al.* 2001).

The latter findings clearly indicate that extracellular Ca^{2+} is not required for the expression of $[Ca^{2+}]_i$ transients and suggest a role for intracellular Ca^{2+} stores in their generation. Indeed, the SERCA pump blockers thapsigargin or cyclopiazonic acid, which lead to a depletion of intracellular Ca^{2+} stores, abolish spontaneous astrocytic activity (Parri *et al.* 2001). In addition, spontaneous $[Ca^{2+}]_i$ oscillations are abolished by incubation with the IP_3 receptor antagonist 2-aminoethyldiphenylborate, but are not affected by ryanodine, implicating the activation of IP_3 , rather than ryanodine, receptors as the mechanism of intracellular Ca^{2+} release underlying these astrocytic oscillations.

In summary, the mechanism underlying the generation of spontaneous $[Ca^{2+}]_i$ oscillations seems to involve an IP_3 -receptor-mediated release from intracellular stores, with store refilling dependent on extracellular Ca^{2+} via a dihydropyridine sensitive pathway.

(c) Correlated spontaneous $[Ca^{2+}]_i$ transients lead to localized astrocytic $[Ca^{2+}]_i$ waves

Using probability analysis it can be shown that spontaneous $[Ca^{2+}]_i$ transients occurring in neighbouring astrocytes are correlated, with a much greater incidence than expected solely by chance, and that this correlated activity is restricted to a maximum of five astrocytes. In addition, the distance between correlated pairs of astrocytes is *ca.* 70 μm , and the proportion of astrocytic $[Ca^{2+}]_i$ transients in one cell that leads to a transient in the other cell is *ca.* 30%.

One of the most characteristic features of astrocytic Ca^{2+} signalling in culture studies is the propagation of

intercellular Ca^{2+} waves through astrocytic networks (Giaume & Venance 1998). As shown in figure 8, spontaneous $[\text{Ca}^{2+}]_i$ transients *in situ* give rise to intercellular waves that propagate between astrocytes (Parri *et al.* 2001), with a speed of *ca.* $3.5 \mu\text{m s}^{-1}$, a value comparable with that observed in culture studies (Finkbeiner 1993). In contrast to astrocytes in culture (Nedergaard 1994; Giaume & McCarthy 1996; Giaume & Venance 1998), however, spontaneous $[\text{Ca}^{2+}]_i$ waves in the thalamus *in situ* do not appear to involve gap junctions, as they are unaffected by carbenoxolone. In addition, filling single astrocytes with lucifer yellow via patch pipettes fails to show dye-coupling in adjacent astrocytes.

Thus, spontaneous $[\text{Ca}^{2+}]_i$ waves *in situ* propagate among few thalamic astrocytes within a localized area, and their underlying mechanism probably involves chemical transmission.

(d) Spontaneous astrocytic $[\text{Ca}^{2+}]_i$ waves elicit large and long-lasting NMDA currents in adjacent TC neurons

Astrocytic $[\text{Ca}^{2+}]_i$ waves imaged at 33°C have an apparent speed of propagation of $7.5 \mu\text{m s}^{-1}$, while the average duration of individual $[\text{Ca}^{2+}]_i$ transients at half-maximal amplitude, is *ca.* 10 s. In this physiological condition, but still in the presence of TTX, large inward currents are observed in the majority (70%) of TC neurons, and are correlated with spontaneous, propagating $[\text{Ca}^{2+}]_i$ waves in neighbouring astrocytes (Parri *et al.* 2001; figure 8c). The distance between the neuron and astrocytes displaying correlated activity is *ca.* $35 \mu\text{m}$, compared with maximum dendritic extensions of *ca.* $70 \mu\text{m}$ for rat VB TC neurons at this developmental time (see below). As the spontaneous $[\text{Ca}^{2+}]_i$ transients uncorrelated to a neuronal inward current are 38% smaller ($p < 0.005$) than the correlated events (Parri *et al.* 2001), the spontaneous astrocyte–neuron signalling mechanism that underlies the generation of this inward currents in TC neurons *in situ* is dependent on the magnitude of the astrocytic $[\text{Ca}^{2+}]_i$ transient, and thus consistent with a Ca^{2+} -dependent process. A causal role for astrocytic $[\text{Ca}^{2+}]_i$ transients as the signal for the generation of the inward currents in TC neurons is also confirmed by the findings that stimulation of astrocytic $[\text{Ca}^{2+}]_i$ transients by *trans*-ACPD is also accompanied by slowly decaying inward currents in adjacent neurons (Parri *et al.* 2001).

Three striking features characterize the astrocyte-driven inward currents recorded in TC neurons in the presence of TTX: (i) a large amplitude (100 pA) which would be capable of depolarizing TC neurons sufficiently to elicit action potentials (Parri & Crunelli 1998); (ii) slow kinetics, including a τ_{act} of $145 \pm 48 \text{ ms}$ and a τ_{decay} of $580 \pm 356 \text{ ms}$; and (iii) a full block by APV, indicating that they are mediated by NMDA receptors (Parri *et al.* 2001). Indeed, in the absence of Mg^{2+} and at 23°C , the amplitude of the inward currents is *ca.* 160 pA, and their kinetics shows larger τ_{act} and τ_{decay} ($332 \pm 115 \text{ s}$ and $2.7 \pm 0.7 \text{ s}$, respectively) (Parri *et al.* 2001). As would be expected from neuronal NMDA receptor activation, TC neurons patch-clamped with electrodes containing Fluo-4 show $[\text{Ca}^{2+}]_i$ increases during spontaneous $[\text{Ca}^{2+}]_i$ transients in adjacent astrocytes (Parri *et al.* 2001), indicating that as well as electrical excitability, astrocyte-driven sig-

nalling to neurons results in neuronal biochemical changes.

In summary, the presence of spontaneous $[\text{Ca}^{2+}]_i$ waves propagating among localized groups of astrocytes in the VB thalamus and subsequent signalling to TC neurons provides the first demonstration in the CNS *in situ* of an astrocytic network parallel to, and intercommunicating with, neuronal networks, that processes information on a slower time-scale, using Ca^{2+} as a medium, compared with fast neuronal electrical processing.

(e) Spontaneous astrocytic $[\text{Ca}^{2+}]_i$ waves are developmentally regulated

The spontaneous $[\text{Ca}^{2+}]_i$ transients observed in the VB thalamus *in situ* are developmentally regulated, as the number of spontaneous astrocytes remains constant from postnatal day 4 (P4) until P9, then starts to decline by P11, reaching by P14 a plateau that remains constant to *ca.* 70% reduction until P23 (Parri *et al.* 2001; figure 9a). Whereas large-scale neuronal biochemical changes (Yuste *et al.* 1992; Spitzer 2002) and spontaneous neuronal electrical activity (Wong 1993; Yuste & Sur 1999) are recognized as important signals in the developing nervous system, an involvement of spontaneous astrocytic $[\text{Ca}^{2+}]_i$ waves and associated neuronal NMDA currents has not been considered. Before suggesting potential functions for this spontaneous astrocyte–neuron communication process, therefore, it seems worthwhile to review briefly some key developmental features in the thalamus (figure 9b).

A somatotopic map of the whisker field (the ‘barreloid’ map) exists in the VB thalamus of adult rats, and like other thalamic nuclei, this thalamic complex also receives corticothalamic afferents from its cortical projection target (i.e. the barrel cortex), so forming the thalamocortical loop. At birth, before sensory input has fully shaped the sensory pathway, dye injections in the barrel cortex retrogradely fill cells in the VB barreloids, indicating that a rudimentary corticothalamic pathway exists. However, the first two postnatal weeks result in major developmental changes in the thalamus and the thalamocortical loop. While at P7 there are few synaptic complexes, these increase threefold by P12 up to P15, after which the rate of synaptogenesis is attenuated (Matthews *et al.* 1977; figure 9b). A large proliferation in corticothalamic afferents also occurs within a similar developmental time (i.e. P8 to P15; Ivy & Killackey 1981; figure 9b).

At this time, the neuronal elements of the VB thalamus are also undergoing radical changes. While at P7, TC neurons are characterized by short stubby irregular dendrites with distinct growth cones and filipodia, by P12 they show dendritic elongation with secondary and tertiary branching and the appearance of spinous protrusions (Matthews *et al.* 1977; figure 9b). The matching of dendritic elongation and generation of secondary and tertiary branches with the proliferation of thalamocortical afferents agrees with the findings in the dorsal lateral geniculate nucleus that corticothalamic afferents synapse distally while sensory afferents synapse proximally on TC neurons (Wilson *et al.* 1984). During this postnatal period, the complement of receptors mediating synaptic transmission on TC neurons also changes. Corticothalamic synaptic stimulation at early postnatal ages produces synaptic responses largely due to NMDA receptors; during maturation the duration

Figure 8. Localized, spontaneous astrocytic $[Ca^{2+}]_i$ waves elicit inward currents in adjacent TC neurons. (a) Image showing the position of active astrocytes 1, 2 and 3, and the position of the recorded TC neuron soma (n). (b) Consecutive pseudocolour images (at times indicated) superimposed on a monochrome image, showing $\Delta F\%$ during a spontaneous wave that starts in astrocyte 1 and propagates over the position of recorded neuron n to astrocyte 2 and 3. (c) Plots of $\Delta F\%$ for astrocytes 1, 2 and 3, and the current recorded from neuron n. As the spontaneous wave passes through the position of the recorded neuron a large inward current is seen (dotted line in plots denotes the time of current peak). Stimulation of a $[Ca^{2+}]_i$ wave by local application of *trans*-ACPD (at site indicated by X in (a) at time indicated by arrow) causes $[Ca^{2+}]_i$ increases in astrocytes 1, 2 and 3, and an inward current in the recorded neuron (asterisk in current trace). Movies of spontaneous astrocytic $[Ca^{2+}]_i$ waves and the correlated generation of an inward current in a VB TC neuron can be seen at http://www.thalamus.org.uk/html/natureneuroscience4_8.html. (Reproduced with permission from Parri *et al.* (2001); copyright © 2001 Nature Publishing Group.)

of postsynaptic responses in the dorsal lateral geniculate nucleus decreases and the proportion of the postsynaptic response due to AMPA/kainate receptor activation increases (Golshani *et al.* 1998).

Within this developmentally regulated expression of NMDA receptors, their NR2D subtype displays an interesting postnatal profile in the VB thalamus, with high levels of NR2D mRNA from birth to P10, while expression decreases between P10 and P21 when it is virtually absent (Monyer *et al.* 1994; Wenzel *et al.* 1996). Receptors containing the NR2D subunit have a weaker Mg^{2+} block, a much slower current decay, and a lower EC_{50} for glutamate than NMDA receptors with different subunit compositions (Wyllie *et al.* 1998). Indeed, recombinant NMDA receptors containing the NR2D subunit show currents with τ_{decay} of ca. 4.5 s, a value similar to that of

native receptors containing this subunit (ca. 3 s) (Wyllie *et al.* 1998). As NMDA receptors with the NR2D subunit are believed to be located extrasynaptically (Misra *et al.* 2000), they must need glutamate spill-over from an adjacent synapse or an extrasynaptic source of glutamate for their activation. Clearly, the glutamate released by the astrocytes via their spontaneous $[Ca^{2+}]_i$ waves would be ideally suited for the activation of these extrasynaptic receptors in the VB thalamus, and the slow decay of the astrocyte-driven neuronal currents indicates that the NMDA receptors involved in this thalamic astrocyte–neuron signalling might preferentially contain the NR2D subunit. Nonetheless, it cannot be excluded at present that the long duration of the neuronal currents elicited by the spontaneous astrocytic wave simply results from the long duration of the glutamate release process.

Figure 9. Developmental context of spontaneous astrocytic $[\text{Ca}^{2+}]_i$ oscillations in the VB. (a) Developmental profile showing the number of astrocytes in a given imaged field that display spontaneous $[\text{Ca}^{2+}]_i$ transients. All data obtained in the presence of TTX ($1 \mu\text{M}$). (b) Horizontal bars represent the time courses of the indicated events from P0 to P20. The relative magnitude of the indicated process, i.e. the amount of vimentin staining or the rate of synaptogenesis, is expressed by the darkness of the bar at a particular age. Dotted lines in upper bar indicate that the level of spontaneous activity at these ages is unknown. Bars that stop before P20 do so due to the age range used in the particular studies. ((a) reproduced with permission from Parri *et al.* (2001); copyright © 2001 Nature Publishing Group.)

(f) Physiological significance of astrocyte-driven neuronal excitation

As the astrocytes exhibiting spontaneous $[\text{Ca}^{2+}]_i$ waves are essentially providing a localized excitatory signal to thalamic neurons, one possibility to consider is perhaps that of a local developmental signal. In particular, as NMDA receptors have been implicated in the control of dendritic arborization (Rajan & Cline 1998), and a substantial dendritic development occurs in the TC VB neurons within the first two postnatal weeks (figure 9b), the spontaneous astrocytic activity may play a critical role by allowing Ca^{2+} entry into these neurons via NMDA receptors. By activating neuronal NMDA receptors, spon-

taneously active astrocytes could therefore be facilitating and/or strengthening synapse formation, providing an extremely useful way of coordinating small clusters of neurons. Indeed, immature astrocytes, immunopositive to vimentin, as are those showing spontaneous $[\text{Ca}^{2+}]_i$ oscillations in the VB thalamus (Parri *et al.* 2001), have been implicated in the developmental plasticity of the visual cortex (Muller & Best 1989).

Another potential role for the spontaneous astrocytic $[\text{Ca}^{2+}]_i$ waves is suggested by the large magnitude of the NMDA current that they elicit in TC neurons (Parri & Crunelli 1998). Such a current, in fact, would undoubtedly elicit action-potential firing in TC neurons and thus lead to the excitation of cortical neurons. This, in turn, would be expected to feed back to the thalamus, via corticothalamic afferents, and due to the duration of the thalamic NMDA stimulation, would perhaps facilitate synapse formation onto the very same neuron or group of neurons, that initiated the activity. In this respect, it is worth stressing the similar developmental profile within the VB complex of the astrocyte-driven neuronal NMDA currents, the NR2D subtype expression, and the increase in the number of corticothalamic afferents arriving into the thalamus. Such a putative role for spontaneous astrocytic $[\text{Ca}^{2+}]_i$ waves in the somatotopic wiring of the thalamocortical loop, however, must be considered in relation to the relatively large amount of sensory activity occurring at this developmental time.

Finally, the presence of spontaneous astrocytic $[\text{Ca}^{2+}]_i$ waves, and associated neuronal NMDA currents, in other thalamic nuclei or areas, indicates that this astrocyte–neuron signalling mechanism might possess more widespread brain functions. In addition, the large and long-lasting neuronal NMDA currents that these astrocytic waves are capable of eliciting, even in the absence of action-potential-driven glutamate release, should now be carefully taken into account when investigating brain glutamatergic synaptic transmission, in particular during the first three postnatal weeks. As spontaneous astrocytic $[\text{Ca}^{2+}]_i$ oscillations are still present, though to a lesser extent, after this period, it is also possible that a subset of astrocytes have a functional role in the adult thalamus.

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GLOSSARY

4-AP: 4-aminopyridine	I_{Leak} : leak current
ADP: after depolarization	IP_3 : inositol-(1,4,5)-triphosphate
APV: DL-2-amino-5-phosphonovaleric acid	IPSP: inhibitory postsynaptic potential
BAPTA: bis-(<i>o</i> -aminophenoxy)- <i>N,N,N',N'</i> -tetraacetic acid	I_{T} : T-type Ca^{2+} current
$[\text{Ca}^{2+}]_{\text{i}}$: intracellular Ca^{2+}	I_{Twindow} : T-type Ca^{2+} current window
CNS: central nervous system	LTCP: low threshold spike
DC: direct current	mGLUR: metabotropic glutamate receptor
EEG: electroencephalogram	NMDA: <i>N</i> -methyl-D-aspartate
EGTA: ethylene glycol-bis(<i>b</i> -aminoethyl ether)- <i>N,N,N',N'</i> -tetraacetic acid	NRT: nucleus reticularis thalami
EPSP: excitatory postsynaptic potential	R_{N} : apparent input resistance
GABA: γ -aminobutyrate	SERCA: sarco(endo)plasmic reticulum Ca^{2+} -ATPase
I_{CAN} : Ca^{2+} -activated non-selective cation current	TC: thalamocortical
I_{h} : inward rectifying current	<i>trans</i> -ACPD: ($\pm$)-1-aminocyclopentane- <i>trans</i> -1,3-dicarboxylic acid
	TTX: tetrodotoxin
	VB: ventrobasal