All thalamocortical neurones possess a T-type Ca^{2+} 'window' current that enables the expression of bistability-mediated activities

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1. The existence of a non-negligible steady-state ('window') component of the low threshold, T-type Ca^{2+}current (I_T) and an appropriately large ratio of I_T to I_{Leak} conductance (i.e. g_T/g_{Leak}) have been shown to underlie a novel form of intrinsic bistability that is present in about 15% of thalamocortical (TC) neurones.
2. In the present experiments, the dynamic clamp technique was used to introduce into mammalian TC neurones *in vitro* either an artificial, i.e. computer-generated, I_T in order to enhance endogenous I_T, or an artificial inward I_{Leak} to decrease endogenous I_{Leak}. Using this method, we were able to investigate directly whether the majority of TC neurones appear non-bistable because their intrinsic ionic membrane properties are essentially different (i.e. presence of a negligible I_T 'window' component), or simply because they possess a g_T or g_{Leak} conductance that is insufficiently large or small, respectively.
3. The validity of the dynamic clamp arrangement and the accuracy of artificial I_T were confirmed by (i) recreating the low threshold calcium potential (LTCP) with artificial I_T following its block by Ni^{2+} (0·5–1 mM), and (ii) blocking endogenous LTCPs with an artificial outward I_T.
4. Augmentation of endogenous I_T by an artificial analog or introduction of an artificial inward I_{Leak} transformed all non-bistable TC neurones to bistable cells that expressed the full array of bistability-mediated behaviours, i.e. input signal amplification, slow oscillatory activity and membrane potential bistability.
5. These results demonstrate the existence of a non-negligible I_T 'window' component in all TC neurones and suggest that rather than being a novel group of neurones, bistable cells are merely representative of an interesting region of dynamical modes in the (g_T, g_{Leak}) parameter space that may be expressed under certain physiological or pathological conditions by all TC neurones and other types of excitable cells that possess an I_T 'window' component with similar biophysical properties.

The primary contribution of the low threshold, transient Ca^{2+} current, I_T (Huguenard, 1996; Perez-Reyes *et al.* 1998; Bean & McDonough, 1998), to the subthreshold electrical activity of central neurones has long been considered to be the low threshold Ca^{2+} potential (LTCP) (Llinás & Yarom, 1981; Jahnsen & Llinás, 1984; Deschênes *et al.* 1984; Friedman & Gutnick, 1987; McCormick & Pape, 1990*a*; Crunelli & Leresche, 1991; Fraser & MacVicar, 1991; Jeanmonod *et al.* 1996). In a small group (15%) of thalamocortical (TC) neurones, however, I_T is also responsible for an intrinsic bistability (Williams *et al.* 1997*a*; Turner *et al.* 1997) that is manifest as: (i) input signal amplification, where responses to small current steps or synaptic potentials can be amplified in both the voltage and time domain when neurones are held in a membrane potential region centred around −60 mV, (ii) slow (0·1–1 Hz) oscillations with unusual plateau-like waveforms that differ substantially from conventional δ oscillations, and (iii) in the absence of the hyperpolarization-activated inward current, I_h, membrane potential bistability, where two resting membrane potentials separated by up to 30 mV can exist for the same values of DC current and can be 'switched' between by appropriate voltage perturbations (Williams *et al.* 1997*a*).

The origin of this intrinsic bistability has been shown to involve an interaction between the steady-state ('window') component of I_T, $I_{T\infty}$, and the leak current, I_{Leak} (Tóth *et al.* 1996; Williams *et al.* 1997*a*). In particular, bistability can exist for some value of injected DC current if, and only if, the maximal gradient, with respect to membrane potential (V), of the absolute magnitude of $I_{T\infty}$ exceeds the leak conductance, g_{Leak}, i.e. $(d|I_{T\infty}|/dV)_{max} > g_{Leak}$ (Fig. 1) (Williams *et al.* 1997*a*). In terms of maximal conductances, the above inequality can be written as $g_T/g_{Leak} > 1/[d(\theta|V - E_T|)/dV]_{max}$, where g_T is the maximal conductance of I_T, θ the product of its steady-state inactivation and the nth power of activation, and E_T the reversal potential of I_T. Therefore, any neurone that expresses a T-type Ca^{2+} current that meets this simple condition should exhibit some form of bistability-mediated phenomena as described above. In particular, the reason why the majority of TC neurones do not display such behaviours is clearly due to either the presence of a fundamentally different I_T, which exhibits negligible overlap between its steady-state activation and inactivation curves, or simply an insufficiently large g_T or excessively large g_{Leak}.

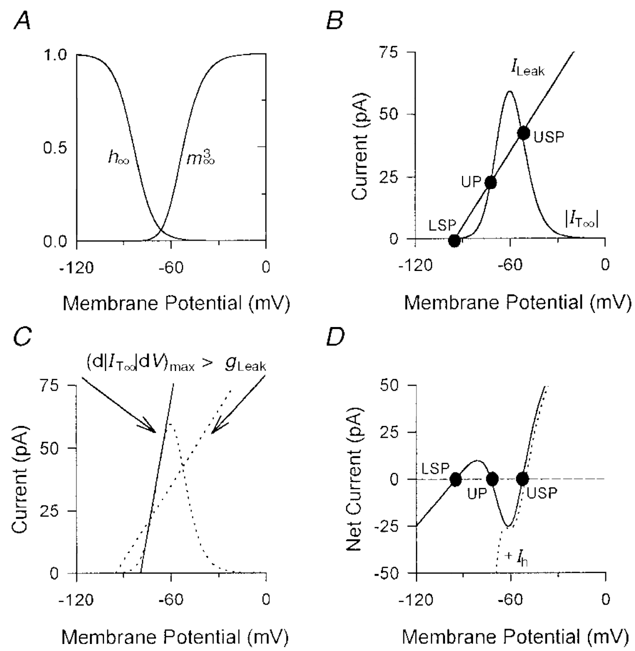

Figure 1. Biophysical mechanism underlying intrinsic bistability

A, steady-state activation (m_∞^3) and inactivation (h_∞) curves of I_T (Tóth *et al.* 1996; Williams *et al.* 1997*a*). $I_{T\infty}$ exists as the result of an area of overlap between the two curves. *B*, plot of $|I_{T\infty}|$ and I_{Leak} *vs.* membrane potential showing the three points where the net current is zero (USP, upper stable point; LSP, lower stable point; UP, unstable point) (see *D* for further details). *C*, same plot as in *B* showing the tangent $(d|I_{T\infty}|/dV)_{max}$ to $I_{T\infty}$ as a continuous line. *D*, plot of net current ($I_{T\infty} + I_{Leak}$, continuous line; $I_{T\infty} + I_{Leak} + I_h$, dotted line) *vs.* membrane potential. If g_{Leak} is smaller than $(d|I_{T\infty}|/dV)_{max}$ then in the absence of I_h and for a range of DC currents there will exist three points at which the net current is zero. The upper and lower equilibrium points are stable attractors (USP and LSP) whilst the remaining equilibrium point is unstable (UP). Thus, transient, hyperpolarizing voltage deviations elicited from USP that do not reach UP will return normally to USP, whereas voltage excursions that cross UP will cause a further hyperpolarization towards LSP due to a net outward current. The membrane potential is prevented from remaining at LSP by the activation of I_h (dashed line in *D*). In *D*, the dotted line indicating the net current in the presence of I_h has been slightly offset to the right for clarity.

In this study, in order to assess whether normally non-bistable TC neurones are endowed with the basic properties to become bistable, a dynamic clamp system (Sharp *et al.* 1993) was used to introduce either an artificial I_T in order to enhance endogenous I_T, or an artificial inward I_{Leak} thus presenting a means to decrease the endogenous I_{Leak}. Using this technique, we have been able to establish that bistability-mediated activities can be unmasked in any TC neurone by solely making adjustments to the ratio g_T/g_{Leak}. In conjunction with previous findings (Williams *et al.* 1997*a*), this demonstrates in all TC neurones the presence of a non-negligible $I_{T\infty}$ whose functional expression may be brought about by enhancements of I_T (cf. Chung *et al.* 1993; Tsakiridou *et al.* 1995) or reductions of I_{Leak} (cf. McCormick & Prince, 1987; McCormick & VonKrosigk, 1992; Steriade *et al.* 1994) that underlie certain physiological and pathological conditions (McCormick, 1992; Jeanmonod *et al.* 1996). Some of these results have been published in preliminary form (Hughes *et al.* 1998; Cope *et al.* 1998).

METHODS

Slice preparation and recording solutions

Male Wistar rats (150–200 g) were deeply anaesthetized (1·5% halothane) and killed by decapitation. Adult cats of either sex (1–1·5 kg) were deeply anaesthetized with a mixture of O_2 and NO_2 (2:1) and 1% halothane. A wide craniotomy was performed and the meninges were removed. The animals were killed by a coronal cut at the level of the inferior colliculus, and, following transection of the optic tracts, the brain was removed. The preparation and maintenance of rat and cat dorsal lateral geniculate nucleus (LGN) slices were as described previously (Williams *et al.* 1996, 1997*a*). Slices (400–450 μm) were perfused with a warmed (35 ± 1 °C) continuously oxygenated (95% O_2, 5% CO_2) medium containing (mM): NaCl, 134; KCl, 2; KH_2PO_4, 1·25; $MgSO_4$, 1; $CaCl_2$, 2; $NaHCO_3$, 16; glucose, 10; 6-cyano-7-nitroquinoxaline-2,3-dione, 0·02; DL-2-amino-5-phosphonovaleric acid, 0·1; bicuculline methiodide, 0·03; and *P*-(3-aminopropyl)-*P*-diethoxymethyl-phosphinic acid, 0·5. When $NiCl_2$ (0·5–1 mM) was added to the recording medium, PO_4^{3-} and SO_4^{2-} were replaced with Cl^-, and in some slices 4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methyl-amino)-pyrimidinium chloride (ZD 7288) (300 μM) was used to block I_h (Williams *et al.* 1997*b*).

Sharp microelectrode recording and data analysis

Impaled neurones were identified as TC neurones by the presence of a robust LTCP on release from hyperpolarization, and strong inward and outward rectification (Williams *et al.* 1996; Turner *et al.* 1997). Intracellular recordings, using the current clamp technique, were performed with standard or thin-walled glass microelectrodes filled with 1 M potassium acetate (resistance: 80–120 MΩ and 30–50 MΩ, respectively) and connected to an Axoclamp-2A amplifier (Axon Instruments). Voltage and current records were stored either on a Biologic DAT recorder (IntraCel, Royston, UK) or directly on the hard disk of an IBM compatible personal computer and later analysed using pCLAMP (Axon Instruments). The apparent input resistance (R_N) was calculated from small voltage responses evoked at −60 mV. The apparent leak conductance (g_{Leak}) was taken as the reciprocal of R_N. Numerical results are expressed in the text as means ± S.E.M. and statistical significance was tested ($P = 5\%$) using Student's *t* test.

The dynamic clamp

The dynamic clamp system (Sharp *et al.* 1993) was implemented using a personal computer connected to a DigiData 1200A interface (Axon Instruments). Membrane potential (V) was sampled, and artificial current was updated, at 10–50 kHz. The equations used to describe artificial I_T are:

$$I_{T(artificial)} = g_{T(artificial)} m^3 h (V - E_{Ca}),$$

$$m_\infty = 1/[1 + \exp(-(V + 63)/7{\cdot}8)],$$

$$h_\infty = 1/[1 + \exp((V + 83{\cdot}5)/6{\cdot}3)],$$

$$\tau_m = 2{\cdot}44 + 0{\cdot}02506 \exp(-0{\cdot}984\,V),$$

$$\tau_h = 7{\cdot}66 + 0{\cdot}2868 \exp(-0{\cdot}1054\,V),$$

where $E_{Ca} = 180$ mV is the Ca^{2+} reversal potential and $g_{T(artificial)}$ is the maximal conductance (Tóth *et al.* 1996; Williams *et al.* 1997*a*). The activation and inactivation variables, m and h, obey the differential equation:

$$\mathrm{d}y/\mathrm{d}t = (y_\infty - y)/\tau_y,$$

where $y = m$ or h. Accordingly, y_∞ represents the steady-state values of the activation and inactivation variables (m_∞ and h_∞), and τ_y the corresponding time constants (τ_m and τ_h). The equation used to describe artificial I_{Leak} is:

$$I_{Leak(artificial)} = g_{Leak(artificial)}(V - E_K),$$

where E_K (= −95 mV) is the K^+ reversal potential and $g_{Leak(artificial)}$ is the maximal conductance.

For experiments with I_{Leak}, $g_{Leak(artificial)}$ was changed until a satisfactory reproduction (see below) of the bistability-related phenomena observed in the naturally bistable TC neurones was achieved. A similar approach was used for $g_{T(artificial)}$. Neither E_K and E_{Ca} were allowed to change. The ratio of $g_{T(artificial)}$ or $g_{Leak(artificial)}$ to g_{Leak}, in addition to their absolute values, is quoted to aid comparison of the artificial current used in different neurones and experimental conditions. Except for the experiments involving the reproduction and elimination of LTCPs, the values of $g_{T(artificial)}/g_{Leak}$ and $g_{Leak(artificial)}/g_{Leak}$ given in the results indicate the amount of current needed for bistability-mediated behaviours similar to those observed in normally bistable TC neurones to become clearly apparent (i.e. an inflection point in the charging pattern, appearance of two stable membrane potentials, etc.). These values may not represent, therefore, the minimum amount of current needed to bring about bistability.

RESULTS

The experiments presented here are based on a total of 61 neurones recorded in the rat ($n = 7$) and cat ($n = 54$) LGN, whose electrophysiological properties ($R_N = 143 \pm 16$ MΩ, resting $V = -64 \pm 1$ mV, $n = 36$) under control conditions were similar to those of morphologically identified TC neurones (Williams *et al.* 1996, 1997*a*). The dynamic clamp arrangement was initially tested by either (i) using artificial I_T to recreate LTCPs following their block by Ni^{2+} (0·5–1 mM) ($g_{T(artificial)} = 67{\cdot}14 \pm 11{\cdot}05$ nS; $g_{T(artificial)}/g_{Leak} = 7{\cdot}66 \pm 1{\cdot}16$, $n = 15$) (Fig. 2*A*), or (ii) introducing an artificial outward I_T to block LTCPs ($g_{T(artificial)} = -90{\cdot}87 \pm 9{\cdot}20$ nS; $g_{T(artificial)}/g_{Leak} = -11{\cdot}08 \pm 1{\cdot}76$, $n = 23$) (Fig. 2*B*). In both cases, the results confirmed the accuracy of the system.

Input signal amplification

Voltage domain

In all TC neurones that under control conditions showed no evidence of any bistability-mediated behaviours, but exhibited a passive response to small negative current steps (< 100 pA) elicited from −60 mV (Fig. 3*B*1 and *C*1, insets), either addition of artificial I_T ($g_{T(artificial)} = 53{\cdot}5 \pm 10{\cdot}48$ nS; $g_{T(artificial)}/g_{Leak} = 8{\cdot}87 \pm 1{\cdot}10$, $n = 10$); or artificial inward I_{Leak} ($g_{Leak(artificial)} = -5{\cdot}58 \pm 1{\cdot}08$ nS; $g_{Leak(artificial)}/g_{Leak} = -0{\cdot}59 \pm 0{\cdot}06$, $n = 6$) led to behaviour characterized by (i) large amplitude voltage deviations in response to small negative current steps, such that their R_N was in the gigaohm range, and (ii) an accompanying time-dependent

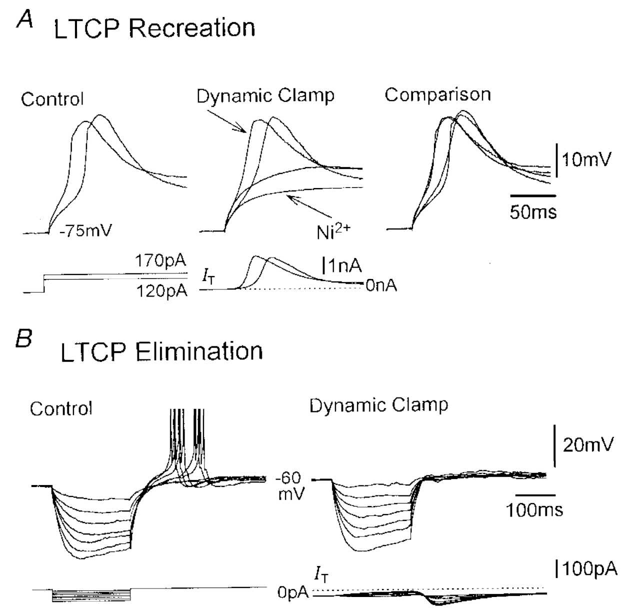

Figure 2. Recreation and elimination of LTCPs using artificial I_T

A, dynamic clamp ($g_{T(artificial)} = 45$ nS) recreation of LTCPs following their block by Ni^{2+} (0·5 mM) in a cat TC neurone. Tetrodotoxin (1 μM) and ZD7288 (300 μM) were present in the recording medium. *B*, elimination of LTCPs using an artificial outward I_T ($g_{T(artificial)} = -25$ nS) in a rat TC neurone. In *A* and *B*, control and dynamic clamp traces were obtained using identical current steps, respectively. In this and all subsequent figures (i) action potential amplitude has been truncated for clarity; (ii) current traces labelled I_T or I_{Leak} show the artificial currents injected by the dynamic clamp system; and (iii) unlabelled current traces indicate the current steps. In *B*, the current calibration applies to both the current steps and artificial I_T.

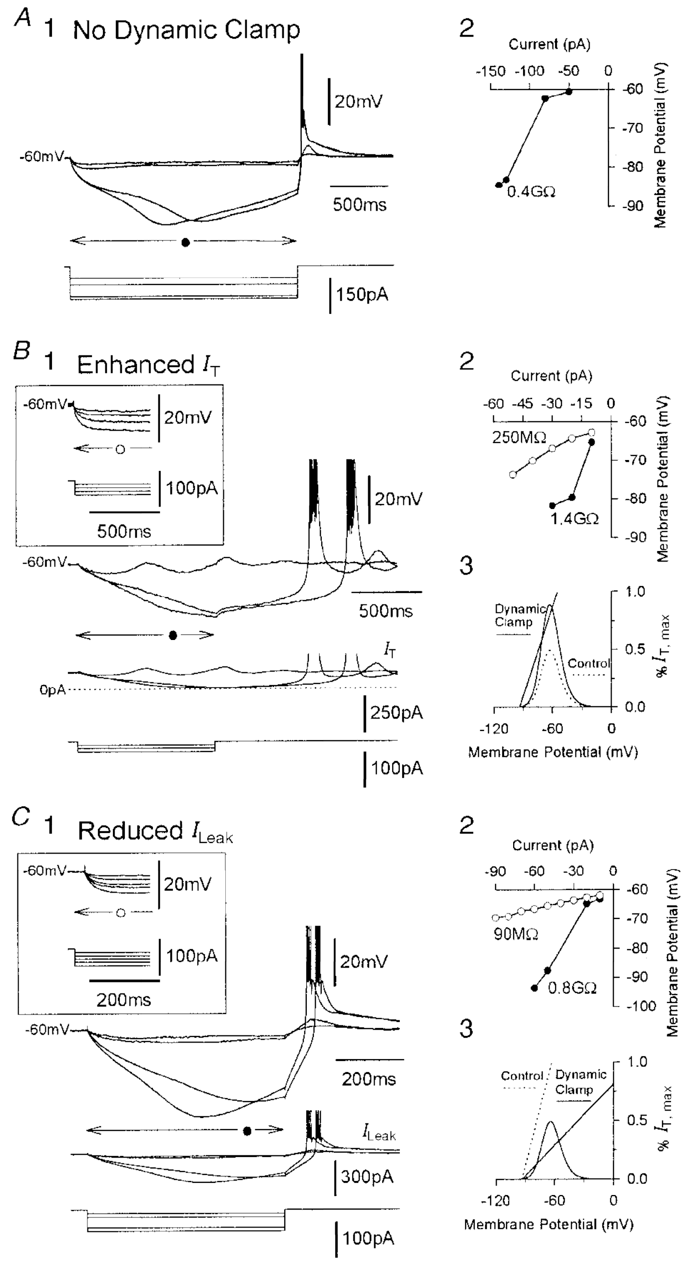

Figure 3. Input amplification in the voltage domain

*A*1, bistability-mediated input amplification in a cat TC neurone in control conditions. The two smaller current steps are insufficient to drive the membrane potential beyond UP (see Fig. 1), wheras the two larger ones enable the neurone to reach this threshold causing the membrane potential to move toward LSP before being repolarized by I_h. *A*2, the voltage–current (*V–I*) relationship for the neurone in *A*1 reveals a large R_N. In this and all other *V–I* relationships in this figure, the voltage responses were measured at their peak deflection (arrow under the voltage records in *A*1, *B*1 and *C*1) during the current steps. *B*1, voltage amplification in a different cat TC neurone following the addition of artificial I_T ($g_{T(artificial)} = 55$ nS). The inset shows the response of the neurone without dynamic clamp. *B*2, *V–I* relationships for the neurone in *B*1 illustrates the increase in R_N following the addition of artificial I_T (○ control; ● enhanced I_T). *B*3, schematic representation of the transformation to a bistable system that underlies the response shown in *B*3. *C*1, voltage amplification in a rat TC neurone following a reduction in I_{Leak} using artificial inward I_{Leak} ($g_{Leak(artificial)} = -7$ nS). The inset shows the response of the neurone without dynamic clamp. *C*2, *V–I* relationships for the neurone in *C*1 (○ control; ● reduced I_{Leak}). *C*3, schematic representation of the biophysical changes underlying the response shown in *C*2.

increase in R_N and non-exponential charging pattern (Fig. 3*B* and *C*). These input signal amplification properties were typical of those observed in a small proportion of previously recorded bistable TC neurones without dynamic clamp (Fig. 3*A*) (Williams *et al.* 1997*a*; Tuner *et al.* 1997). Note, in particular, how the similarities in membrane charging patterns and LTCP waveforms were closest between neurones with decreased I_{Leak} (Fig. 3*C*) and control neurones (Fig. 3*A*), than between this latter group (Fig. 3*A*) and neurones with enhanced I_T (Fig. 3*B*).

Time domain

In neurones displaying voltage amplification properties following either the addition of artificial I_T ($g_{T(artificial)} = 106{\cdot}67 \pm 8{\cdot}16$ nS; $g_{T(artificial)}/g_{Leak} = 10{\cdot}00 \pm 1{\cdot}15$, $n = 5$), or reduction in I_{Leak} ($g_{Leak(artificial)} = -5{\cdot}58 \pm 1{\cdot}08$ nS; $g_{Leak(artificial)}/g_{Leak} = -0{\cdot}59 \pm 0{\cdot}06$, $n = 6$), it was also observed that the response to relatively short (50–500 ms), negative current steps could often outlast the duration of the input current step by up to 2 or 3 times (Fig. 4*B* and *C*). Again, this modified behaviour was characteristic of bistable TC neurones recorded in control conditions (Fig. 4*A*) (Williams *et al.* 1997*a*), with the closest similarities being observed between neurones recorded without dynamic clamp and those with artificial inward I_{Leak} (Fig. 4*A* and *C*). It is worth noting that amplification in the voltage and time domain (or membrane potential bistability; see below) could be obtained in the same five neurones by adding artificial I_T or artificial inward I_{Leak} in two subsequent dynamic clamp tests (see Fig. 5).

Modulation of intrinsic oscillatory activity

In neurones unable to exhibit any intrinsic oscillatory activity in control conditions, either the addition of artificial I_T ($g_{T(artificial)} = 90{\cdot}0 \pm 33{\cdot}33$ nS; $g_{T(artificial)}/g_{Leak} = 8{\cdot}25 \pm 3{\cdot}26$, $n = 4$), or reduction in I_{Leak} ($g_{Leak(artificial)} = -7{\cdot}17 \pm 0{\cdot}2$ nS; $g_{Leak(artificial)}/g_{Leak} = -0{\cdot}69 \pm 0{\cdot}04$, $n = 3$) resulted in the generation of slow (0·1–1 Hz) oscillatory activity. These oscillations differed considerably from conventional δ oscillations (Leresche *et al.* 1991; Steriade *et al.* 1991; Pirchio *et al.* 1997) in that typical increases in frequency in response to increasing DC current were replaced for higher values of DC current by an uncharacteristic decline (Fig. 5*B* and *C*). The slow oscillatory activity obtained following either an artificial enhancement of I_T, or a reduction in I_{Leak} was once again equivalent to that observed in control conditions in a small group of bistable TC neurones (Fig. 5*A*) (Williams *et al.* 1997*a*; Turner *et al.* 1997). Notably, in neurones recorded following a reduction in I_{Leak}, or in control conditions, the slow oscillations generated in response to higher values of injected current were characterized by a pronounced plateau or 'shoulder' and a subsequent large hyperpolarization (Fig. 5*A* and *C*). However, in neurones possessing an artificially enhanced I_T, the behaviour was, unsurprisingly, less damped and the plateau replaced by further LTCPs (Fig. 5*B*).

In both cases, the slow oscillation is the result of what would normally be small, short duration, hyperpolarizing phases in the oscillation being amplified in both the time and voltage domain, leading to an activity largely controlled by the equilibrium points formed between $I_{T\infty}$ and I_{Leak} (see Figs 1 and 5). To further illustrate this point, in two out of the four neurones that were exhibiting slow oscillations following an enhancement of I_T, we investigated the effect of a subsequent reduction in $I_{T\infty}$ (and thus a removal of the underlying bistability) by shifting the inactivation curve of artificial I_T by −5 mV. In both neurones, this action brought about conventional δ oscillations which displayed a characteristic current–frequency relationship (Fig. 5*B*) (cf. Fig. 7 in Williams *et al.* 1997*a*; Pirchio *et al.* 1997). Conventional δ

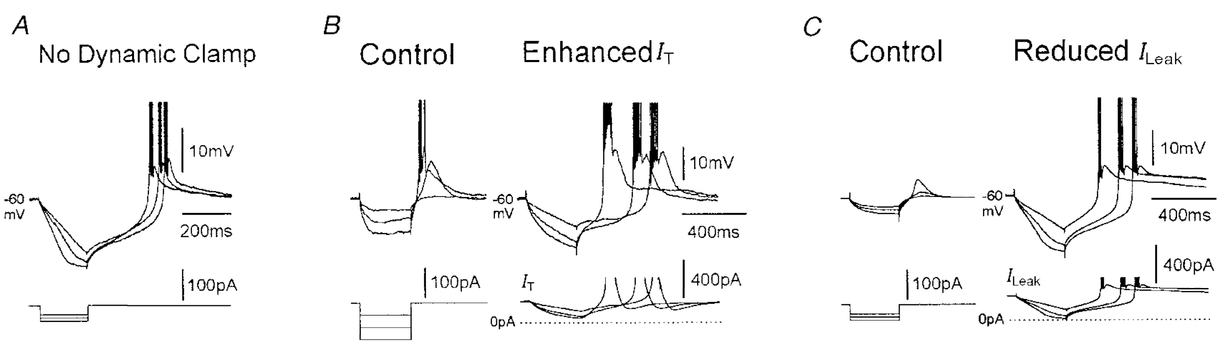

Figure 4. Input amplification in the time domain

A, example of bistability-mediated input signal amplification in both voltage and time domain recorded in a cat TC neurone in control conditions. This behaviour is due to the fact that subsequent to voltage responses reaching LSP (see Fig. 1), the net current becomes dominated by I_h, and therefore if the time taken for I_h to depolarize the membrane potential beyond UP is longer than the time remaining of the current step, the response will outlast the input. *B*, the voltage response of another cat TC neurone following an artificial enhancement of I_T ($g_{T(artificial)} = 120$ nS) exhibits amplification properties similar to those shown in *A*. *C*, the input amplification in the time domain obtained in a rat TC neurone by addition of artificial inward I_{Leak} ($g_{Leak(artificial)} = -7$ nS) is remarkably similar to that shown in *A*. In *B* and *C*, identical current steps were used in control and during dynamic clamp, respectively.

oscillations could also be obtained by reducing the amount of artificial inward $I_{\rm Leak}$ ($g_{\rm Leak(artificial)} = -5{\cdot}67 \pm 0{\cdot}41$ nS; $g_{\rm Leak(artificial)}/g_{\rm Leak} = -0{\cdot}55 \pm 0{\cdot}04$, $n = 3$) (Fig. 5*C*).

Membrane potential bistability

Following pharmacological blockade of $I_{\rm h}$ with ZD7288 (300 μM) (Williams *et al.* 1997*b*) and either the addition of artificial $I_{\rm T}$ ($g_{\rm T(artificial)} = 80{\cdot}0 \pm 24{\cdot}94$ nS; $g_{\rm T(artificial)}/g_{\rm Leak} = 7{\cdot}55 \pm 2{\cdot}54$, $n = 4$) or artificial inward $I_{\rm Leak}$ ($g_{\rm T(artificial)} = -4{\cdot}75 \pm 1{\cdot}52$ nS; $g_{\rm Leak(artificial)}/g_{\rm Leak} = -0{\cdot}54 \pm 0{\cdot}07$, $n = 4$), membrane potential bistability became apparent in previously non-bistable neurones whereby two stable voltage levels separated by 14–25 mV co-existed for the same value of injected DC current, and could be 'switched' between by appropriate membrane potential perturbations (Fig. 6*B* and *C*). This phenomenon was identical to that observed in bistable TC neurones recorded in the presence of ZD7288 (300 μM) without dynamic clamp (Fig. 6*A*) (Williams *et al.* 1997*a*), and re-enforces the point that whilst in the absence of $I_{\rm h}$ intrinsic bistability is clearly apparent as two separate, steady-state membrane potentials, in the presence of $I_{\rm h}$ this behaviour is transformed into input signal amplification and slow oscillations.

Since the two stable voltage levels exhibited during membrane potential bistability correspond directly to the upper and lower stable equilibrium points (Fig. 1*C*), it should be possible to change and predict these values by varying the amounts of either artificial $I_{\rm T}$ (or $I_{\rm Leak}$) and/or

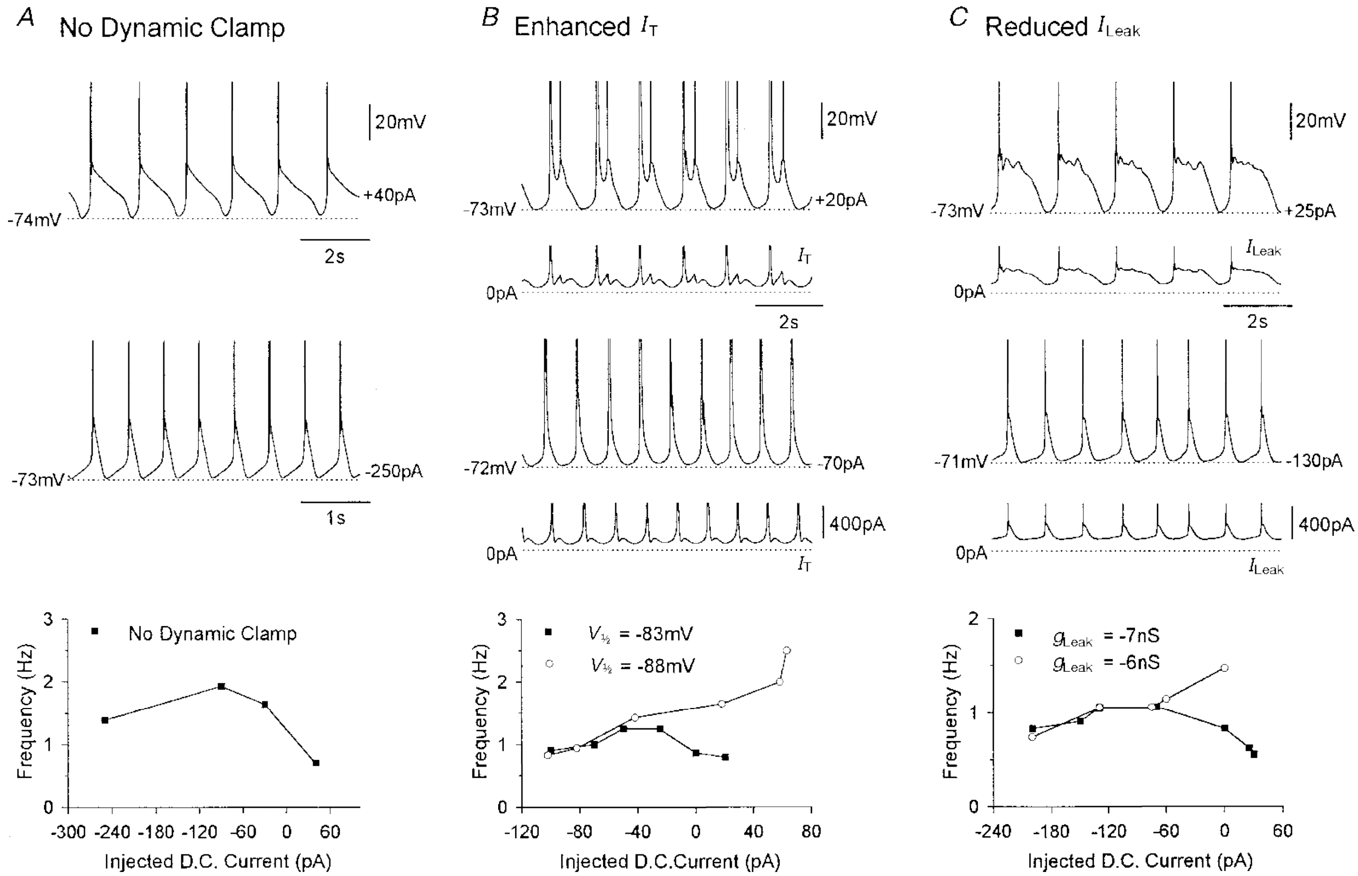

Figure 5. Slow oscillatory activity

A, bistability-mediated oscillatory activity recorded in a cat TC neurone under control conditions. Note the shoulder following the burst of action potentials in the upper trace and the uncharacteristic form of the frequency–current relationship. At lower values of injected DC current, the existence of the oscillation is dominated by the kinetic and steady-state properties of $I_{\rm T}$ and $I_{\rm h}$. At higher values of injected DC current however, the slow activity is largely controlled by equilibrium points formed between $I_{\rm T\infty}$ and $I_{\rm Leak}$. *B*, slow oscillatory behaviour induced following an artificial enhancement of $I_{\rm T}$ ($g_{\rm T(artificial)} = 100$ nS) in another cat TC neurone. This atypical form of the frequency–current relationship can be transformed to the conventional pattern for δ oscillations (10) by reducing $I_{\rm T\infty}$ via a small negative shift (−5 mV) in the half-inactivation voltage ($V_{½}$) of artificial $I_{\rm T}$. *C*, similar activity unveiled in the same TC neurone shown in *B* following addition of artificial inward $I_{\rm Leak}$ ($g_{\rm Leak(artificial)} = -7$ nS). Following injection of a smaller amount of artificial inward $I_{\rm Leak}$ ($g_{\rm Leak(artificial)} = -6$ nS) the neurone displayed δ oscillations and their characteristic frequency–current relationship. In *A*, *B* and *C*, values of injected DC current are indicated on the right of each voltage trace.

the injected DC current. Indeed, when artificial I_T was used to replace entirely the endogenous I_T that had been blocked by Ni^{2+} (0·5–1 mM), it was found that the voltage difference between the two resting membrane potentials exactly matched the one predicted by the amount of artificial I_T and DC current (Fig. 6*D*). The accuracy of these predictions further endorses the suggestion that the behaviours observed in this study following either an enhancement of I_T or reduction of I_{Leak} are indeed the result of an intrinsic bistability as described previously (Tóth *et al.* 1996; Williams *et al.* 1997*a*).

Validation of results obtained with artificial I_T

To exclude the possibility that attainment of bistability-mediated behaviours through the enhancement of I_T was not merely due to the interaction of artificial I_T and endogenous I_{Leak}, we compared the values of g_{Leak} with that of $(d|I_{T\infty}|/dV)_{max}$ for artificial I_T. It was found that $(d|I_{T\infty(artificial)}|/dV)_{max}$ (3·81 ± 1·14 nS) was significantly smaller than g_{Leak} (6·44 ± 1·31 nS) ($P < 0·05$; $n = 8$), and thus the properties induced by the dynamic clamp were indeed a product of the interaction of I_{Leak} and an effective I_T, formed by the superposition of endogenous and artificial I_T. It is also important to note: (i) the similarity in the amount of $g_{T(artificial)}$ (or $g_{Leak(artificial)}$) relative to g_{Leak} (i.e. $g_{T(artificial)}/g_{Leak}$ or $g_{Leak(artificial)}/g_{Leak}$) required to induce input amplification, slow oscillatory activity and membrane potential bistability, and their small variability (i.e. standard errors) amongst neurones; and (ii) that all these phenomena could be reproduced entirely by

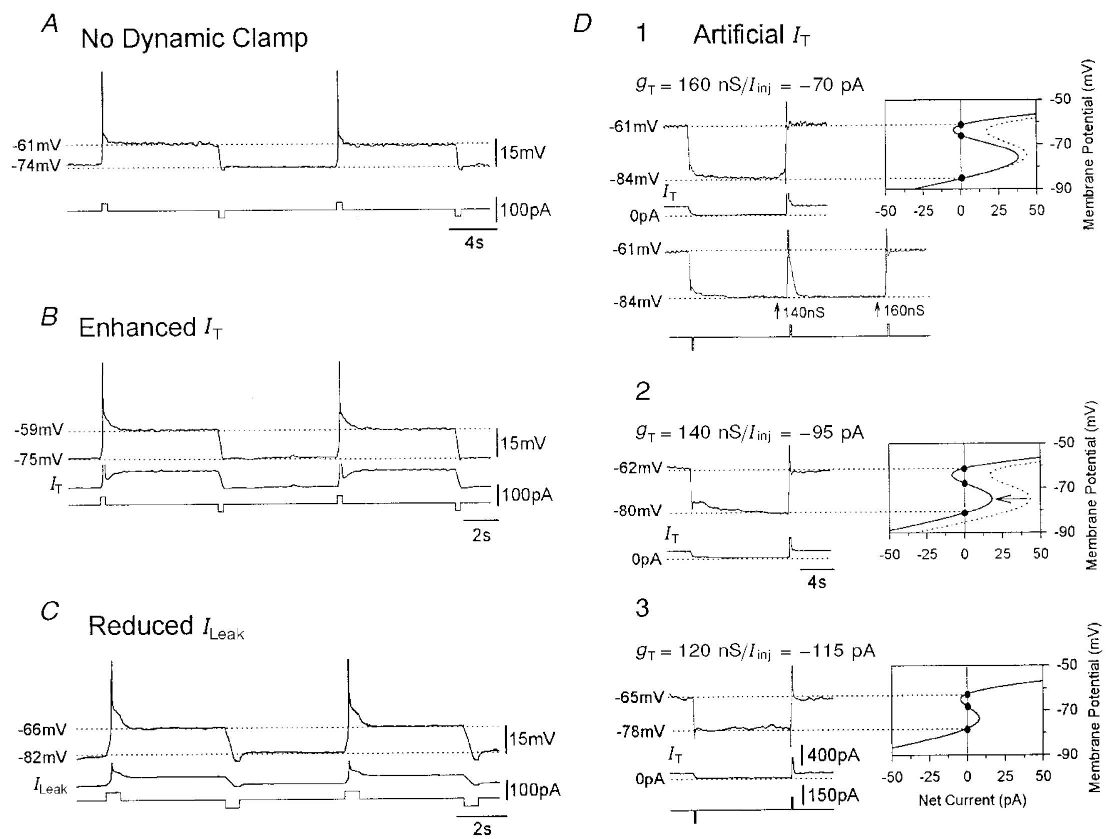

Figure 6. Membrane potential bistability

A, membrane potential bistability observed in a cat TC neurone in the presence of ZD7288 (300 μM). The two stable membrane potentials, corresponding to USP and LSP, can be 'switched' between if the membrane potential is perturbed beyond UP (see Fig. 1). *B* and *C*, membrane potential bistability induced in two different cat TC neurones in the presence of ZD7288 (300 μM) following the addition of artificial I_T ($g_{T(artificial)} = 100$ nS), and artificial inward I_{Leak} ($g_{Leak(artificial)} = -7$ nS), respectively. *D*, in another cat TC neurone (*D*1), traces recorded in the presence of Ni^{2+} (0·5 mM) and ZD7288 (300 μM) show the two resting membrane potentials to match accurately the points of current balance in the voltage *vs.* net current plot, constructed using this neurone's g_{Leak} and the indicated artificial I_T and injected DC current (I_{inj}) used in this experiment (continuous line in plot). Note how the decrease in artificial I_T from 160 to 140 nS without an accompanying change in I_{inj} failed to elicit bistability (left arrow in bottom voltage record) since only one equilibrium point was present for this combination of g_{Leak}, artificial I_T and I_{inj} (dotted line in plots in *D*1 and 2). Membrane potential bistability could be re-instated for an artificial I_T of 140 nS when I_{inj} was changed from −70 to −95 pA (continuous line in *D*2), or for an even smaller I_T and a larger I_{Leak} (*D*3). Current calibrations in *D*3 also apply to *D*1 and 2 and time calibration in *D*2 applies to *D*1 and 3.

the addition of artificial I_T following an almost complete removal of endogenous I_T by Ni^{2+} (0·5–1 mM) ($g_{T(artificial)}$ = 147·5 ± 28·82 nS; $g_{T(artificial)}/g_{Leak}$ = 17·65 ± 1·19, $n = 4$) (cf. Fig. 6*D*).

DISCUSSION

The main finding of this study is that any TC neurone in two species can be transformed to a bistable neurone exhibiting input signal amplification, slow oscillations and membrane potential bistability following either an enhancement of endogenous I_T or a reduction in endogenous I_{Leak}. These results, therefore, demonstrate the existence of a non-neglible, physiologically relevant $I_{T\infty}$ in all rat and cat TC neurones which may enable them to be bistable under certain physiological and pathological conditions.

Dynamic clamp

The use of the dynamic clamp allowed us to finely control the properties of both I_T and I_{Leak} in a manner more flexible than pharmacological manipulations, and more realistic than computer simulations. It is unlikely that the limitations of this technique (e.g. single point current source, lack of emulation of $[Ca^{2+}]_i$ dynamics, etc.) will have strongly affected these results since (i) when endogenous I_T channels were blocked, the introduction of artificial I_T yielded an accurate reproduction of LTCPs, (ii) in experiments involving the enhancement of endogenous I_T, this current continued to function normally alongside the artificial current, (iii) there was a good agreement between artificially induced phenomena and those recorded previously without dynamic clamp in a small number of TC neurones (Williams *et al.* 1997*a*; Turner *et al.* 1997), and (iv) the magnitude of the artificial I_T required to induce all bistability-related phenomena was comparable amongst different experiments/neurones.

Existence of $I_{T\infty}$

All the results obtained in this study are consistent with our biophysical description of intrinsic bistability. In particular, the agreement between the values of the stable membrane potentials following the complete replacement of endogenous I_T with artificial I_T and those predicted theoretically confirms that $I_{T\infty}$ and I_{Leak} combine in the manner set out in our biophysical hypothesis to produce certain non-linear phenomena in TC neurones. Previous experimental evidence has shown that intrinsic bistability does not depend on Na^+, K^+ and high threshold Ca^{2+} currents (Williams *et al.* 1997*a*). Therefore, since (i) in this study all neurones that were tested only with a reduction of I_{Leak} displayed bistability-mediated phenomena, (ii) in experiments involving an enhancement of I_T, artificial $I_{T\infty}$ alone was too small to account for bistable behaviour, and (iii) apart from the unique interaction between $I_{T\infty}$ and I_{Leak}, we are unaware of any other possible ionic mechanism for generating subthreshold bistable behaviour with similar properties to those exhibited here, we can conclude that, contrary to findings in previous studies (see review by Huguenard, 1996), a non-negligible, physiologically relevant $I_{T\infty}$ exists in all TC neurones. This also firmly suggests that the reason why not all TC neurones are intrinsically bistable under control conditions is due to differing ratios of g_T to g_{Leak} rather than to fundamentally different biophysical properties of I_T within the TC neuronal population (Tarasenko *et al.* 1997; see also Avery & Johnston, 1996; Bean & McDonough, 1998; Meir & Dolphin, 1998).

Source of intrinsic bistability in control conditions

Although it is impossible from the present experiments to determine the precise magnitude of g_T/g_{Leak} required to bring about bistability-mediated behaviours, it is worth stressing that the absolute value of $g_{T(artificial)}/g_{Leak}$ necessary for the expression of these phenomena in the presence of endogenous I_T was almost half of that required in the absence of endogenous I_T, and comparable to that needed to either reproduce or eliminate LTCPs. Together with the finding that a value of around −0·5 for $g_{Leak(artificial)}/g_{Leak}$ is necessary to obtain intrinsic bistability, this result suggests that TC neurones exhibiting bistability-related phenomena in control conditions (i.e. in the absence of dynamic clamp) possess either a g_{Leak} 50% smaller, or a g_T 100% larger than normal. In other words, the value of g_T/g_{Leak} required to bring about intrinsic bistability is probably about twice that typically observed *in vitro*.

The putative 50% decrease in g_{Leak} observed in this study is in close agreement with investigations on somal shunt (Rall *et al.* 1992) and distributed injury conductances (Spruston & Johnston, 1992; Staley *et al.* 1992). Furthermore, bistability-related phenomena can be more easily observed in TC neurones with a relatively larger g_{Leak} or in the presence of Ba^{2+} (Williams *et al.* 1997*a*; Turner *et al.* 1997), and in this study there was a clear and consistently greater degree of similarity between behaviours observed in neurones with an artificially reduced I_{Leak} and those without dynamic clamp, compared with that between this latter group of neurones and those with an enhanced I_T. Therefore, although we cannot exclude the possibility that a small group of TC neurones possessing a larger than usual g_T has remained undetected in the large number of studies on I_T (Huguenard, 1996), the available experimental and theoretical evidence favours a reduced g_{Leak} as responsible for only a minority of these neurones being able to show bistability-related phenomena under control conditions. In particular, *in vitro*, this reduced g_{Leak} may be due to either normal variations, minimized somal shunt or, appealingly, the presence of differing amounts of neurotransmitters that are known to affect I_{Leak} in TC neurones (McCormick & Pape 1990*b*; McCormick & VonKrosigk, 1992; Steriade *et al.* 1994).

Bistability in normal and pathological thalamic processes

Although it may be argued that the bistability-mediated phenomena described here are unlikely to occur *in vivo* due to the intense and constant synaptic actvity causing a substantial increase in apparent input conductance (Bernander *et al.* 1991; Rall *et al.* 1992), it should be noted that a number of pathways exist which may potentially lead to the unmasking of bistability in TC neurones.

Firstly, during periods of wakefulness, acetylcholine-containing neurones of the mesopontine nuclei and noradrenaline-containing neurones of the locus coeruleus, both of which project onto TC neurones, exhibit a pronounced increase in firing rate (Aston-Jones & Bloomfield, 1981; Steriade *et al.* 1990). Moreover, noradrenaline and acetylcholine have both been shown to cause reductions of I_{Leak} in TC neurones (McCormick & Prince, 1987; McCormick & Pape, 1990*b*). Thus, it appears feasible that in addition to causing membrane potential depolarization and a shift from burst to tonic firing (McCormick & Prince, 1987; McCormick & Pape, 1990*b*), activity in certain brainstem nuclei may cause the dynamics of some TC neurones to undergo a state-dependent transformation from a monostable to a bistable nature. Such a change in the properties of TC neurones could have important consequences for the processing of sensory information in the thalamus. For example, both excitatory and inhibitory postsynaptic potentials evoked in bistable TC neurones via stimulation of sensory afferents can cause large, long-lasting amplified hyperpolarizations (Williams *et al.* 1997*a*). During these hyperpolarizations, TC neurones will be less likely to fire single action potentials in response to further sensory input and so their ability to be involved in the relay of peripheral information to the cerebral cortex may be transiently reduced via a self-limiting mechanism. However, the instigation of a large hyperpolarization by afferent stimuli may be indicative of a scenario whereby TC neurones can be primed to generate LTCP-mediated burst firing as a more specific signal in response to subsequent sensory input (Guido & Weyland, 1995). Secondly, activation of metabotropic glutamate receptors, either via stimulation of corticothalamic fibres (McCormick & VonKrosigk, 1992) or not (Turner & Salt, 1998), has also been shown to cause a reduction of I_{Leak}. Therefore, it is not unreasonable to suggest that activity in layer VI cortical neurones, or other fibres presynaptic to metabotropic glutamate receptors, may also lead to the formation of bistable behaviour in TC neurones causing their information transfer properties to be radically altered via a novel feedback mechanism. Additionally, the region of negative slope conductance known to accompany the slow response of central neurones to NMDA (Nowak *et al.* 1984; Crunelli & Mayer, 1984) may act to facilitate bistability in both of the above scenarios.

Alternatively, intrinsic bistability might be envisaged to accompany the transition from a high to a low conductance state that is associated with a reduction in synaptic input as would be expected during the onset of periods of decreased arousal. In this condition, synaptic activity, via periodic activation of NMDA (Leresche *et al.* 1991), non-NMDA (Soltesz & Crunelli, 1992*a*), $GABA_A$ (von Krosigk *et al.* 1993; Bal *et al.* 1995) and $GABA_B$ (Crunelli & Leresche, 1991; Soltesz & Crunelli, 1992*b*; von Krosigk *et al.* 1993; Bal *et al.* 1995) receptors, takes on a largely discrete, synchronized form. As such, synaptic signals appear as well defined, isolated stimuli (Bernander *et al.* 1991) and, therefore, rather than preventing bistability would become subject to bistability-mediated amplification (Williams *et al.* 1997*a*). This in turn would increase the propensity of TC neurones to produce both intrinsic (Leresche *et al.* 1991; Curró-Dossi *et al.* 1992; Williams *et al.* 1997*a*), and network (Steriade *et al.* 1993; von Krosigk *et al.* 1993; Bal *et al.* 1995) oscillations.

Apart from a reduction of I_{Leak}, bistable behaviour could emerge as a result of a large g_T such as that suggested (Jeanmonod *et al.* 1996), or shown (Chung *et al.* 1993; Tsakiridou *et al.* 1995) to correspond to certain pathological conditions. Thus, the appearance of bistable TC neurones may play an incisive role in initiating and controlling both physiological (Steriade *et al.* 1993; von Krosigk *et al.* 1993; Bal *et al.* 1995) and pathological (von Krosigk *et al.* 1993; Steriade *et al.* 1994; Bal *et al.* 1995; Pinault *et al.* 1998) rhythms in the thalamus. In conclusion, $I_{T\infty}$-dependent bistability is an integral part of the electroresponsiveness of TC neurones that may also underlie crucial components in a variety of neuronal mechanisms in other excitable cells that possess an I_T with similar biophysical properties (Huguenard, 1996; Perez-Reyes *et al.* 1998).

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