Journal of Physiology (1991), **441**, *pp.* 175–197
With 11 *figures*
Printed in Great Britain

TWO INWARD CURRENTS AND THE TRANSFORMATION OF LOW-FREQUENCY OSCILLATIONS OF RAT AND CAT THALAMOCORTICAL CELLS

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(*Received 19 October 1990*)

SUMMARY

1. The contribution of a slow, mixed Na^+–K^+, inward rectifying current (I_h) and the T-type Ca^{2+} current (I_T) (that underlies low-threshold Ca^{2+} potentials) to the low-frequency oscillations observed in rat and cat thalamocortical (TC) cells *in vitro* was studied using current clamp and single-electrode voltage clamp recordings.

2. From a holding potential of −50 mV, voltage steps negative to −60 mV showed the presence of a slow, non-inactivating inward current, I_h. This current was unaffected by Ba^{2+} (1–4 mM), tetrodotoxin (0·5–1 μM) and TEA (20 mM, $n = 6$), reversibly blocked by Cs^+ (1–3 mM), and its reversal potential (−33·0 ± 1·2 mV) followed changes in the extracellular Na^+ and K^+, but not Cl^-, concentration.

3. Application of Cs^+ (1–3 mM) abolished the pacemaker oscillations ($n = 9$), while in six cells that did not show any oscillatory activity Cs^+ first evoked the spindle-like oscillations that, in the continuous presence of these ions, were then transformed into the pacemaker oscillations before all activities were finally blocked: all these effects were accompanied by a hyperpolarization and a progressive decrease and final blockade of I_h. Cs^+ had no effect on the '*N*-methyl-D-aspartate' (NMDA) oscillations ($n = 5$) and Ba^{2+} (2 mM, $n = 8$) did not block the pacemaker, the spindle-like and the 'NMDA' oscillations.

4. In ten cells that showed the pacemaker oscillations selective activation of β-adrenoceptors by 10–50 μM-noradrenaline (in the presence of α-noradrenergic antagonists) or by 20 μM-isoprenaline first transformed the pacemaker oscillations into the spindle-like oscillations that, in the continuous activation of β-receptors,

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were finally abolished: all these effects were accompanied by a depolarization and a progressive increase of I_h.

5. In TC cells that showed the pacemaker oscillations application of 1-octanol (50–100 μM), an antagonist of T-type Ca^{2+} currents, reversibly blocked this activity but concomitantly decreased (50%) the cell input resistance ($n = 5$). Application of Ni^{2+} (0·2–0·5 mM, $n = 13$), another antagonist of I_T reversibly blocked the pacemaker, the spindle-like and the 'NMDA' oscillations.

7. In cells showing the pacemaker oscillations it was found that the current developing from the most hyperpolarized potential of an oscillation cycle was an inward relaxation whose time course differed from that of I_h evoked at the same potential.

8. These results indicate that I_T and I_h contribute and are essential to the pacemaker, the spindle-like and the 'NMDA' oscillations, and that I_T, but not I_h, is required for the 'NMDA' oscillations to occur. In addition, they indicate that I_h is a factor involved in the transformation of the pacemaker into the spindle-like oscillations. The latter two types of oscillation, however, cannot be accounted for only by the two inward currents (I_T and I_h) acting independently.

9. The contribution of the low-frequency oscillatory activities observed in TC cells *in vitro* to those recorded in the living animal and the possibility that the pacemaker oscillations represent the basic pattern of spontaneous activity of TC cells in the absence of synaptic inputs are discussed.

INTRODUCTION

The electrophysiological features of the different types of low-frequency (< 4 Hz) oscillatory activities observed in rat and cat thalamocortical (TC) cells *in vitro* were reported in the previous paper (Leresche, Lightowler, Soltesz, Jassik-Gerschenfeld & Crunelli, 1991). Here we describe the role played in some of these oscillations by the low-threshold Ca^{2+} potentials (and their underlying T-type Ca^{2+} current, I_T) (Coulter, Huguenard & Prince, 1989*a*; Crunelli, Lightowler & Pollard, 1989; Hernandez-Cruz & Pape, 1989; Suzuki & Rogawski, 1989) and a slow, mixed Na^+–K^+, inward rectifier current (I_h) (Pollard & Crunelli, 1988; Pape & McCormick, 1989). These two currents were chosen because of the similarities (i) between the large-amplitude depolarizations present in the pacemaker, the spindle-like and the '*N*-methyl-D-aspartate' (NMDA) oscillations (Leresche *et al.* 1991) and the low-threshold Ca^{2+} potentials of TC cells (Steriade & Llinás, 1988), and (ii) between the pacemaker current of other excitable cells showing pacemaker oscillations (Hauswirth, Noble & Tsien, 1968; Brown, DiFrancesco & Noble, 1979; Brown & DiFrancesco, 1980) and the slow, mixed Na^+–K^+, inward rectifier current present in TC cells (Pollard & Crunelli, 1988; Pape & McCormick, 1989). It will be shown that both inward currents contribute to the pacemaker and the spindle-like oscillations and that modulation of I_h brings about the reversible transformation of the pacemaker into the spindle-like oscillations. The 'NMDA' oscillations, instead, require low-threshold Ca^{2+} potentials but can be observed in the absence of I_h. The role of neurotransmitters in the modulation of the spontaneous low-frequency oscillations observed *in vitro* and their possible functional significance will be

discussed. Preliminary reports of some of the results described in this paper have been published (Pollard & Crunelli, 1988; Lightowler, Pollard & Crunelli, 1990).

METHODS

The methods used in this study are similar to those described in the accompanying paper (Leresche *et al.* 1991). When single-electrode voltage clamp recordings were made, the preamplifier (Axoclamp 2A) was switched from current clamp to discontinuous single-electrode voltage clamp mode using the procedure described previously (Crunelli *et al.* 1989). This involved the optimal adjustment of the capacitance neutralization, phase, gain and sampling rate controls commensurate with complete settling of the continuously monitored input voltage and voltage-step settling times of < 3 ms. Sampling rates were in the range of 5–7 kHz. The Na^+–K^+ inward current, I_h, was activated by voltage steps negative to -60 mV from holding potentials in the range of -50 to -55 mV. This resulted in a slowly developing, non-inactivating, inwardly rectifying current superimposed on an instantaneous, inward 'leak' current. The size of I_h was calculated by subtracting the 'leak' current from the total inward current caused by the hyperpolarizing step. The instantaneous current was calculated by extrapolating the developing current back to the beginning of the voltage step. To study some of the properties of I_h a K^+ concentration of 6·3 mM was used, and to block the Ca^{2+} current I_T 1 mM-Ni^{2+} was added to the perfusion medium while the Mg^+ and Ca^{2+} concentrations were adjusted to 3 and 0·5 mM, respectively. It was not necessary to block outward voltage-dependent K^+ currents (i.e. I_A, delayed rectifier) since both had activation thresholds positive to -45 mV (Llinás, 1988; Crunelli *et al.* 1989) which is outside the range of membrane potentials used to study I_h.

The time course of I_h activation was fitted by the mathematical function:

$$I = a\exp(bt) + c, \tag{1}$$

where I = membrane current, b = rate constant, t = time (ms), a and c = constants. The time constant of I_h activation (in ms) was therefore given by the reciprocal of b (cf. Crunelli *et al.* 1989). Data points from I_h activation studies were fitted by the equation:

$$I/I_{max} = 1/\{1+\exp[(V-V_0)/k]\}, \tag{2}$$

where I = current amplitude, I_{max} = maximum current amplitude, V = membrane voltage, V_0 = membrane voltage at half-maximum activation and k = steepness coefficient (cf. Crunelli *et al.* 1989).

When using $BaCl_2$, SO_4^{2-} was substituted with Cl^- and $H_2PO_4^-$ was omitted from the perfusion medium, and when changes in extracellular K^+ were required equimolar changes in Na^+ were made. The following drugs (obtained from Sigma) were applied in the perfusion medium: noradrenaline, yohimbine, prazosin, isoprenaline, tetrodotoxin (TTX), 1-octanol, amiloride and tetraethylammonium (TEA). All quantitative data in the text are expressed as means ± S.E.M. Statistical comparisons were made by Student's t test.

RESULTS

The mixed Na^+–K^+ inwardly rectifying current, I_h

Some of the low-frequency oscillations observed in TC cells, in particular the pacemaker type, appeared similar to the cardiac pacemaker oscillations observed in sino-atrial and Purkinje fibres where a mixed Na^+–K^+ inward rectifying current, named I_f, has been shown to contribute to the mechanism responsible for their generation (Hauswirth *et al.* 1968; Brown *et al.* 1979). Evidence in support of the presence of a similar current in TC cells is visible in earlier studies (Jahnsen & Llinás, 1984*a*, *b*; Crunelli, Kelly, Leresche & Pirchio, 1987; McCormick & Pape, 1988), where, during current clamp recordings, relatively large, square hyperpolarizing current pulses, from an holding potential of -50 to -60 mV, produced electrotonic

potentials with a slowly developing depolarizing 'sag' (Fig. 1). Under voltage clamp, from a holding potential of −50 mV voltage steps negative to −60 mV evoked a slow, non-inactivating, inward relaxation (I_h), superimposed on an instantaneous inward 'leak' current, over the same membrane potential range as that producing

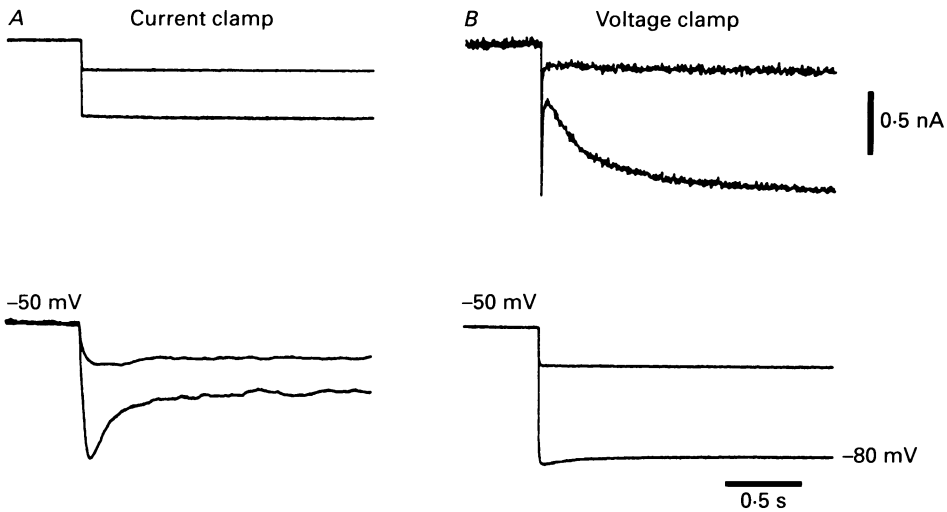

Fig. 1. The slowly developing, non-inactivating, inward current, I_h. *A*, the current clamp recordings show the presence of a slowly developing, depolarizing 'sag' in the electrotonic potentials (bottom traces) of a TC cell when it was hyperpolarized to −60 and −80 mV. *B*, the voltage clamp recordings are from the same cell and were taken immediately after those in current clamp. From a holding potential of −50 mV a voltage step to −60 mV evoked a small, slowly developing, non-inactivating inward relaxation (I_h) that developed faster and became larger at −80 mV (top traces).

'sags' in hyperpolarizing electrotonic potentials (Fig. 1). Upon repolarization to the holding potential an outward relaxation was evoked (Fig. 2*A* and *D*). The instantaneous conductance measured from the current recorded upon stepping from −50 to −100 mV was 36±2 nS ($n = 15$), while the instantaneous conductance measured upon stepping from −100 to −50 mV was 46±2 nS. The difference between these two values represents an increased conductance state that is due to the activation of I_h. The conductance (g_h) associated with maximum I_h activation at −95 mV was 5·4±1 nS ($n = 15$). The development of I_h could be fitted by a single-exponential curve (eqn (1), Methods). From this, its time constant of activation was calculated and found to be voltage dependent, ranging from 0·4±0·1 s (at −100 mV), 2·1±0·5 s (at −75 mV) to 8·3±1·9 s (at −60 mV; $n = 3$) (Methods).

The reversal potential of I_h, determined by extrapolation (details in Fig. 2; cf. Mayer & Westbrook, 1983), was −33·0±1·2 mV ($n = 10$), suggesting that Na^+, K^+ and/or Cl^- may contribute to the charge flow responsible for g_h. When the external Na^+ concentration was reduced from 150 to 50 mM by replacement with choline ions the reversal potential of I_h was shifted to a more hyperpolarized potential (−24±3·7 mV, $n = 4$, $P < 0·05$). Similarly, when the external K^+ concentration was increased from 6·3 to 18·9 mM the reversal potential of I_h was moved to a more

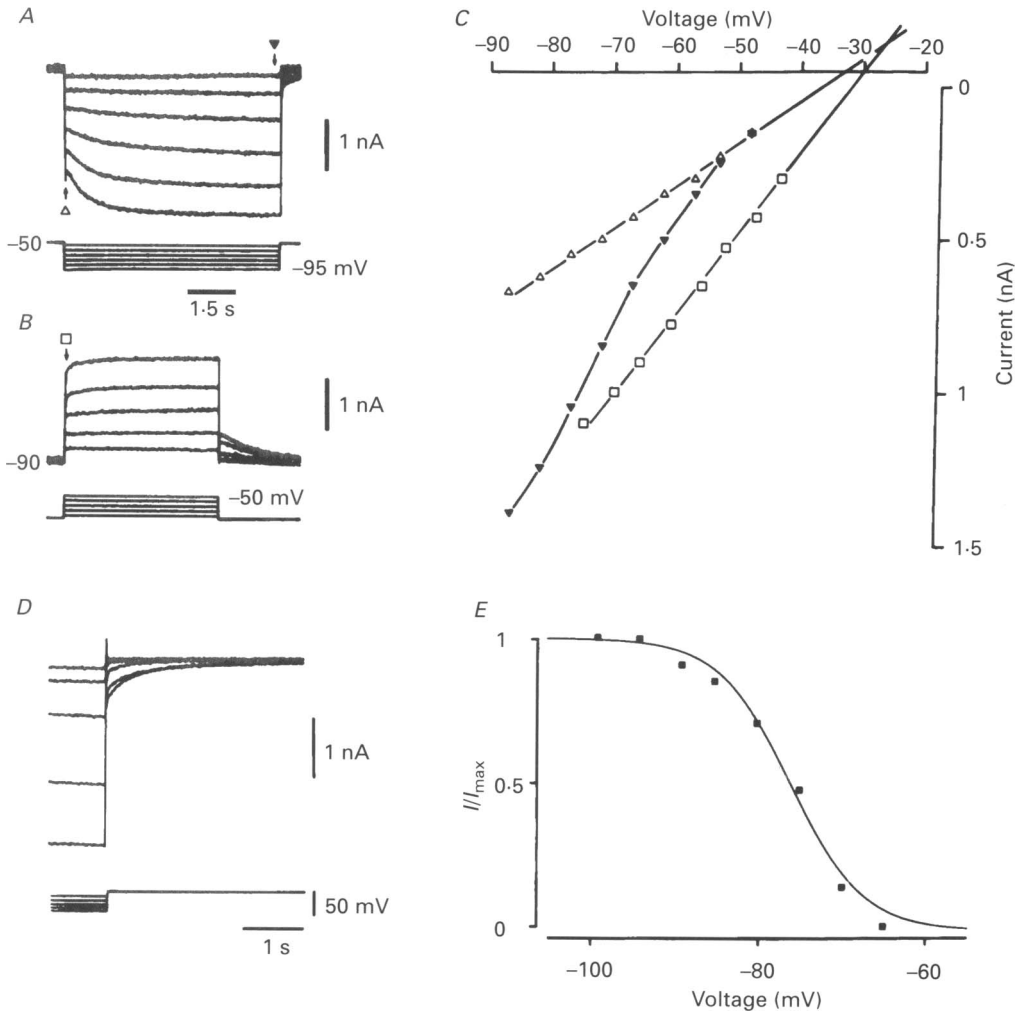

Fig. 2. The reversal potential and activation curve of I_h. *A* shows the development of I_h at different membrane potentials following hyperpolarizing steps from a holding potential of −50 mV. *B* shows tail currents due to the inactivation of I_h following depolarizing steps from a holding potential of −90 mV. In *C*, the instantaneous currents following the hyperpolarizing steps from −50 mV (△) and the depolarizing steps from −90 mV (□) have been measured in another cell and plotted against voltage. These two instantaneous current–voltage plots were linear and, when extrapolated, their intersection gave a reversal potential for I_h of −27·4 mV. The steady-state current, measured 7 s after stepping to each level in *A* (▼), forms an inwardly rectifying relationship with voltage as clearly shown in *C* (▼). *D* shows superimposed tail currents following depolarizing voltage steps to −55 mV from different holding potentials. *E* shows the steady-state activation curve of I_h from another cell. The size of the tail currents (relative to the maximum tail current obtained, I_{max}) is plotted against voltage. The curve, that was fitted to the experimental points by using eqn (2) (see Methods), shows that I_h started to activate at around −65 mV and is fully activated at −95 mV. In this cell V_0 was −76 mV and k was 4·2.

depolarized potential ($-42 \pm 1{\cdot}0$ mV, $n = 3$, $P < 0{\cdot}005$). The levels to which the reversal potential of I_h was shifted in each of these cases indicate a 32 and 29 mV/decade shift for Na^+ and K^+, respectively. The Cl^- equilibrium potential (that is close to -70 mV in TC cells; cf. Crunelli, Haby, Jassik-Gerschenfeld, Leresche & Pirchio, 1988) was shifted to more depolarized potentials either by decreasing the external Cl^- concentration from 143 to 28 mM, by replacement with isothionate ions ($n = 3$), or by recording with 1 M-KCl-filled microelectrodes ($n = 3$) (cf. Crunelli *et al.* 1988). In both conditions the reversal potential of I_h ($-33{\cdot}8 \pm 6{\cdot}0$ mV) was not significantly different from the value measured in control, though a blocking action of isothionate ions on I_h was observed (cf. Mayer & Westbrook, 1983).

The activation curve of I_h was determined by measuring the amplitude of the instantaneous tail current following depolarizing steps to -50 mV from different holding potentials, and expressing it as a percentage of the maximum tail current obtained (Fig. 2). The data were fitted with eqn (2) (see Methods) and the resulting curve showed that the activation of I_h begins at potentials negative to -55 mV and is fully activated at around -95 mV, with a V_0 of $-73{\cdot}5 \pm 4{\cdot}2$ mV and a k of $5{\cdot}8 \pm 0{\cdot}8$ ($n = 3$; Fig. 2*E*).

To investigate the time dependence of I_h de-activation, positive voltage steps (to -50 mV) of different duration from a holding potential of -90 mV were used (Fig. 3*A*). It was found that a maximum de-activation of I_h could be achieved after voltage steps longer than 1 s (Fig. 3*B*). Thus, following a depolarization of 80–350 ms (i.e. the duration of the low-threshold Ca^{2+} potentials present in the pacemaker oscillations of TC cells; Leresche *et al.* 1991), I_h was partially de-activated (20–60 %). Thus, upon re-hyperpolarization to the holding potential, there was less instantaneous inward current (open arrow in Fig. 3*A*) than at the steady-state immediately preceding the depolarizing voltage step (filled arrow in Fig. 3*A*), i.e. the current at the offset of the depolarizing step was *outward* with respect to that at the onset.

Ba^{2+}, an effective blocker of pure K^+ currents in various cell types, applied at concentrations up to 4 mM, produced only minor effects on the amplitude of I_h (9 ± 7 %, $n = 3$), as did 20 mM-TEA ($n = 3$) and 0·5–1 μM-TTX ($n = 9$). Cs^+ (1–3 mM, $n = 23$) instead, which has been found to block mixed Na^+–K^+ inwardly rectifying currents in various cell types, reversibly blocked I_h (Fig. 4*B*). In agreement with the results from previous studies (Halliwell & Adams, 1982; Constanti & Galvan, 1983; Crunelli *et al.* 1989), Cs^+ also reduced the instantaneous current particularly at potentials negative to -70 mV, a finding that probably represents either the block of a steady, partially activated I_h and/or a voltage-dependent block of a leak conductance. As previously mentioned (see Methods), when studying I_h tail currents, the perfusion medium contained 1 mM-Ni^{2+}. In experiments that were carried out in the absence of Ni^{2+} ions there seemed to be no obvious alteration in the development and size of I_h ($n = 24$). It has recently been reported that in rabbit sino-atrial node cells (Brown, Denyer & Kimura, 1989) 2 mM-Ni^{2+} alters the voltage dependence of activation of a current similar to I_h. However, because no tail current analysis could be carried out in the absence of Ni^{2+} (due to contamination by I_T) any effect that these or any other divalent cations may have on the activation and reversal potential of I_h could not be assessed.

Effect of Cs^+ and noradrenaline on the low-frequency oscillations

To investigate a possible contribution of I_h to the low-frequency oscillations observed *in vitro*, 1–3 mM-Cs^+ was added to the perfusion medium whilst recording from TC cells that showed the pacemaker oscillations. Cs^+ produced a 3–8 mV

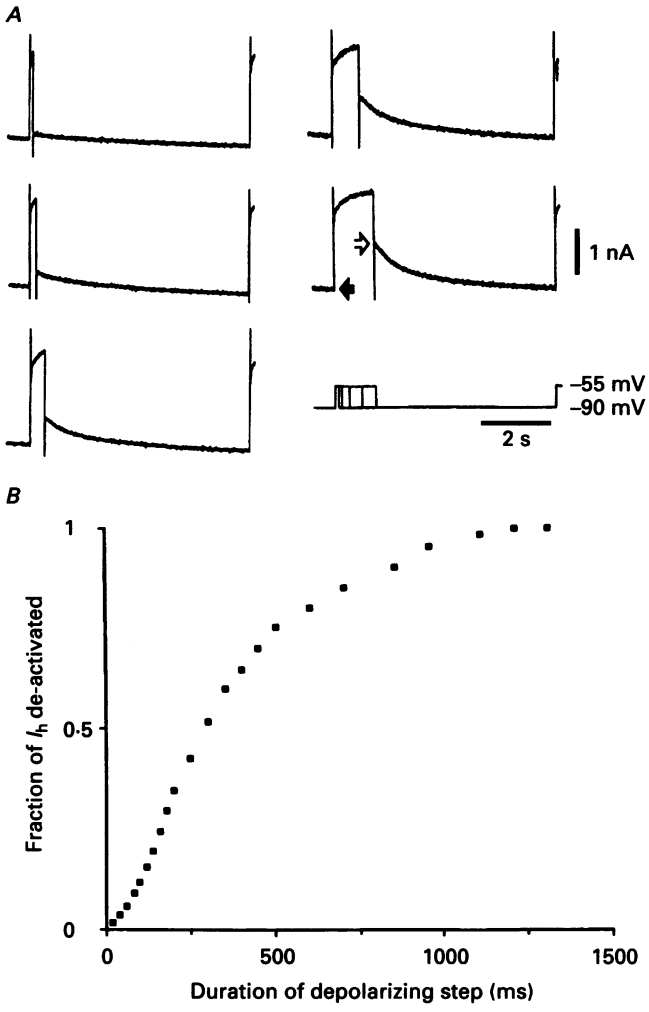

Fig. 3. The de-activation kinetics of I_h. *A*, from a holding potential of −90 mV, depolarizing voltage steps of different duration were used to calculate the time dependence of I_h de-activation. Note that, upon re-hyperpolarization to the holding potential, there was less instantaneous inward current (open arrow) than at the steady state immediately preceding the depolarizing voltage step (filled arrow). In *B*, the size of I_h (relative to the maximum that could be activated after 2 s) is plotted against the duration of the depolarization step, for the cell shown in *A*. It illustrates that a depolarization lasting more than 1 s was needed to de-activate I_h totally.

hyperpolarization accompanied by a reversible blockade of the pacemaker oscillations and of I_h ($n = 9$; Fig. 4). The blockade of the pacemaker oscillations by Cs^+ was not due to the change in membrane potential since repolarization of the cell to the control potential by steady DC current injection did not restart the oscillatory

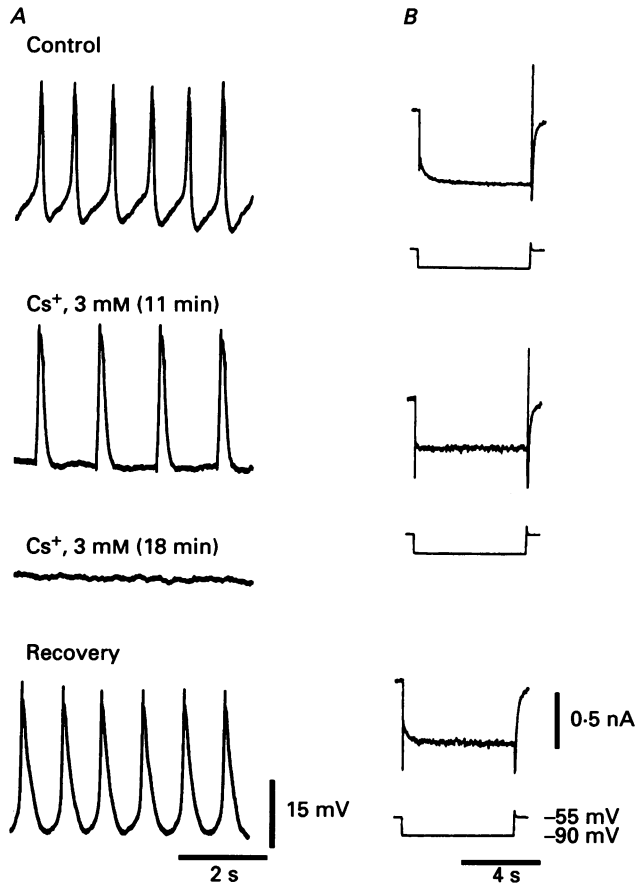

Fig. 4. The effects of Cs^+ on the pacemaker oscillations and on I_h. *A*, intracellular voltage traces show the reversible blockade by 3 mM-Cs^+ of the pacemaker oscillations in a TC cell of the cat dorsal lateral geniculate nucleus (dLGN; membrane potential at the peak of the hyperpolarization, −75 mV). Action potential height has been reduced by the frequency response of the chart recorder. The voltage clamp records in *B* show the concomitant reversible blockade of I_h by Cs^+ in the same cell and were taken immediately after the traces shown in *A*. The transient inward current evoked at the end of the hyperpolarizing voltage step is I_T which was not ideally clamped because of the unfavourable Ca^{2+}/Mg^{2+} concentration ratio (cf. Crunelli *et al.* 1989).

activity. In addition, Cs^+ did not change the pattern of frequency of the 'NMDA' oscillations recorded in five cells perfused with a 'Mg^{2+}-free' medium, indicating that I_h is not essential for this type of oscillation.

Ba^{2+} (2 mM, $n = 8$), which had no effect on I_h, did not block the pacemaker and the spindle-like oscillations, though in few cells a slight change in the frequency of

the former type of oscillation was observed after a prolonged perfusion with these ions. This finding indicates that outward K^+ conductances do not play a major role in the pacemaker and the spindle-like oscillations and they are not involved in the action of Cs^+ and noradrenaline (NA) on these spontaneous activities (see below).

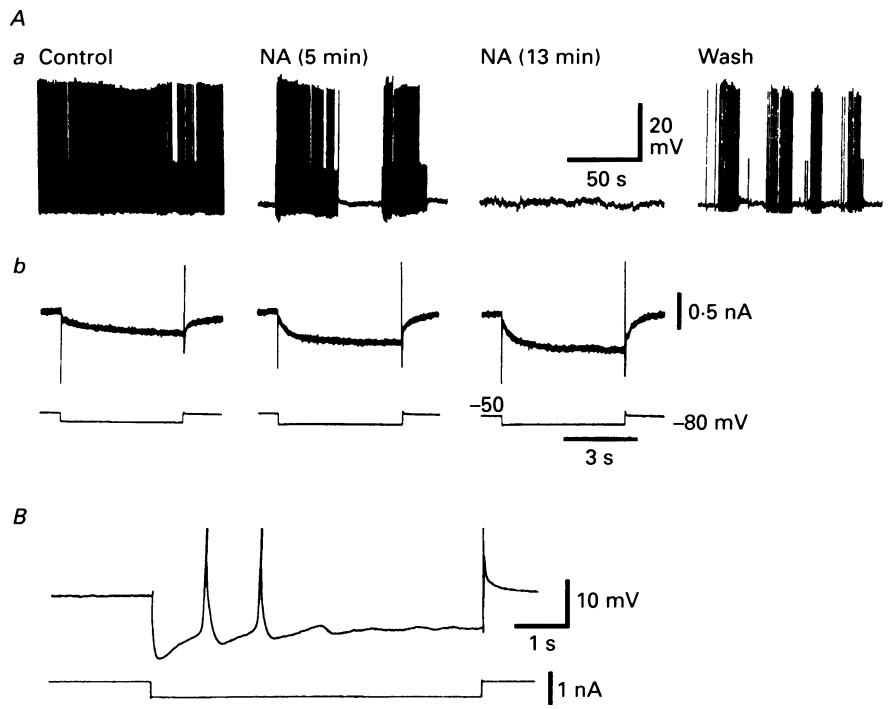

Fig. 5. Activation of β-adrenoceptors by noradrenaline (NA) transforms the pacemaker into the spindle-like oscillations. *A a*, intracellular voltage traces show the effect of 50 μM-NA in the presence of the α-antagonists yohimbine (5 μM) and prazosin (10 μM) on the pacemaker oscillations recorded in a TC cell of the cat dLGN. Five minutes after the introduction of the NA-containing medium the pacemaker oscillations were transformed into spindle-like oscillations that persisted for about 6 min with regular inter- (15–35 s) and intra-spindles (20–30 s) (membrane potential during the inter-spindle, −70 mV). After 13 min, all spontaneous activity ceased. The trace labelled 'Wash' was obtained 20 min after removing NA from the perfusion medium. *A b*, voltage clamp records show the concomitant, progressive increase in the amplitude of I_h following selective activation of β-receptors by NA in the same cell and were recorded 10–30 s after the corresponding current clamp traces in *A a*. The transient inward current present at the end of the hyperpolarizing steps represents I_T. *B*, intracellular voltage records from another TC cell of the cat dLGN where the pacemaker oscillations had been blocked, and the amplitude of I_h increased, by 8 min perfusion with a solution containing 20 μM isoprenaline (not shown). However, the cell still retained the ability to evoke a few repetitive oscillations when hyperpolarized from −55 to −70 mV. In *A a* and *B*, action potential height has been reduced by the frequency response of the chart recorder.

It has recently been shown that NA increases the amplitude of I_h in TC cells by activation of β-adrenoceptors (Pape & McCormick, 1989). Thus, it has been suggested, the NA-mediated increase in I_h should reduce the ability of TC cells to generate rhythmic burst firing that involves low-threshold Ca^{2+} potentials. We tested

this hypothesis directly by looking at the action of 10–50 μM-NA (in the presence of 5 μM-yohimbine and 10 μM-prazosin, two α-antagonists) ($n = 7$; Fig. 5*A*) or 20 μM-isoprenaline (a β-agonist) ($n = 5$) on the pacemaker oscillations. As shown in Fig. 5*A a*, the first effect of NA was to transform the pacemaker into the spindle-like

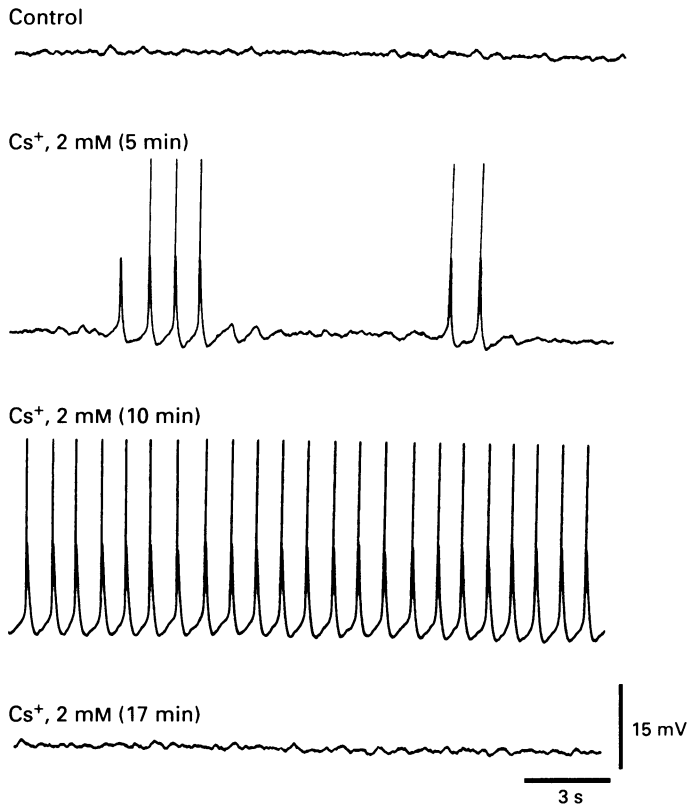

Fig. 6. The effects of Cs^+ on a cell that did not show spontaneous pacemaker oscillations. Intracellular voltage records show the effect of 2 mM-Cs^+ on a non-oscillating TC cell in the cat dLGN. Four minutes after the introduction of the Cs^+-containing medium the cell started to show the spindle-like oscillations with regular inter- (4–9 s) and intra-spindles (2–6 s) (membrane potential during the inter-spindle, −65 mV). After an additional 4 min the spindle-like oscillations were transformed into the pacemaker oscillations (1·2 Hz) that persisted for about 6 min before all activity ceased. Action potential height has been reduced by the frequency response of the chart recorder.

oscillations that persisted for a variable period of time (5–15 min, depending on the NA concentration and the flow rate of the perfusion medium). Later, in the continuous presence of NA, the spindle-like oscillations were blocked and no spontaneous oscillatory activity could be evoked at any membrane potential. However, as shown in Fig. 5*B*, even in the presence of continuous β-receptor stimulation a few repetitive oscillations could still be evoked during the initial portion of hyperpolarizing electrotonic potentials. All the effects of selective β-receptor stimulation described above were accompanied by a 2–6 mV depolarization and a progressive increase in the amplitude of I_h (Fig. 5*A b*) and were reversible. In

addition, NA did not change the time constant of decay of I_h ($0{\cdot}64 \pm 0{\cdot}2$ s before NA, $0{\cdot}61 \pm 0{\cdot}2$ s after NA, at -50 mV following activation at -85 mV, $n = 4$). However, in the same four cells, the time constant of I_h activation was unaffected in two cells (0·56 and 0·72 s before, 0·53 and 0·71 s after NA, respectively) and decreased in the other two cells (0·80 and 0·86 s before, 0·54 and 0·60 s after NA, respectively, at -85 mV).

Since an increase in I_h was capable of blocking the low-frequency oscillations (see above), it might have been possible that some of the TC cells that did not show any spontaneous oscillation (cf. Leresche *et al.* 1991) had a relatively large I_h. We tested this possibility by applying 1–3 mM-Cs^+ on six TC cells that did not display any oscillatory activity. As shown in Fig. 6, Cs^+ initially caused the cells to oscillate, not continuously, however, but in discrete periods, giving rise to the spindle-like oscillations. These lasted for about 3–11 min (depending on the Cs^+ concentration and the flow rate of the perfusion medium) before being transformed into the pacemaker oscillations. Finally, in the continuous presence of Cs^+ and after a variable period of time (4–8 min), the pacemaker oscillations were abolished and no spontaneous oscillatory activity could be evoked at any membrane potential. These effects of Cs^+ were accompanied by a progressive decrease and block of I_h and were reversible (not shown).

To confirm that the effects of Cs^+ and β-adrenoceptor stimulation were both mediated by I_h, we tested the action of these ions and isoprenaline on the same cell. As shown in Fig. 7, a TC cell showing the pacemaker oscillations was perfused with isoprenaline (20 μM). This produced the typical pattern of response: transformation of the pacemaker oscillations into the spindle-like oscillations and, then, block of all spontaneous, low-frequency oscillations. Still in the continuous presence of isoprenaline, Cs^+ (3 mM) was then added to the perfusion medium and it too produced the typical pattern of response, i.e. it evoked the spindle-like oscillations that were transformed into the pacemaker oscillations before a block of all oscillatory activity occurred.

Finally, to investigate the effects of concomitant activation of α- and β-receptor stimulation, NA was also applied to three cells in the absence of α-antagonists. In this case, TC cells were depolarized by 7–13 mV (as evident from the development of a steady inward current even at potentials positive to -50 mV where I_h is not activated), and an increase in the amplitude of I_h was observed. Concomitantly, the pacemaker oscillations were blocked both at this new and at the control membrane potential (Fig. 8). Invariably, during the wash-out of the neurotransmitter, TC cells showed a period of spindle-like oscillations (10–30 min) before the pacemaker oscillations restarted (Fig. 8).

Contribution of low-threshold Ca^{2+} potentials to low-frequency oscillations

The large depolarizations present in the pacemaker, the spindle-like and the 'NMDA' oscillations very closely resembled the low-threshold Ca^{2+} potentials described in TC cells and that have been shown to be generated by a transient inward current, I_T, involving the selective activation of T-type Ca^{2+} channels (Coulter *et al.* 1989*a*; Crunelli *et al.* 1989; Hernandez-Cruz & Pape, 1989; Suzuki & Rogawski, 1989). Indeed, the rate of rise of the large depolarizations of the pacemaker and the

spindle-like oscillations was similar to the one reported for low-threshold Ca^{2+} potentials of TC cells (Jahnsen & Llinás, 1984*a*; Deschenes, Paradis, Roy & Steriade, 1984), and, in addition, the voltage region where these two types of oscillations were observed fitted well with the voltage ranges of activation and inactivation of I_T. In

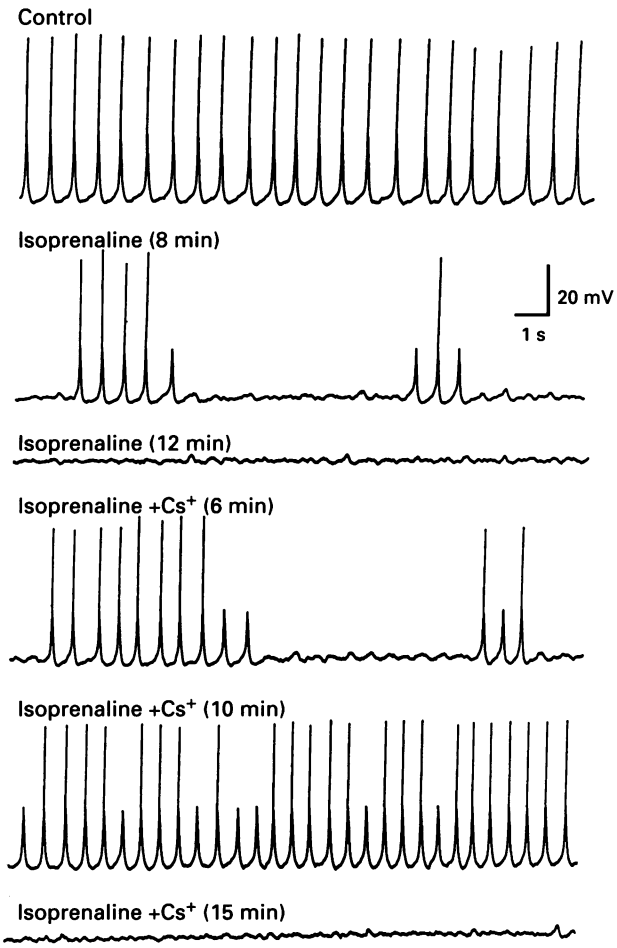

Fig. 7. Reversal by Cs^+ of the isoprenaline-induced block of low-frequency oscillations. Intracellular voltage traces from a TC cell of the cat dLGN show that 20 μM-isoprenaline was capable of transforming the pacemaker into the spindle-like oscillations (Isoprenaline, 8 min) before a full block of oscillatory activity occurred (Isoprenaline, 12 min). Still in the continuous presence of isoprenaline, addition of 3 mM-Cs^+ to the perfusion medium evoked the spindle-like oscillations (Isoprenaline + Cs^+, 6 min) that were then transformed into the pacemaker oscillations (Isoprenaline + Cs^+, 10 min) before a full block occurred (Isoprenaline + Cs^+, 15 min). Membrane potential changes during the action of isoprenaline and Cs^+ were always compensated by steady DC current injection when necessary. The block of oscillatory activity in isoprenaline and in isoprenaline + Cs^+ occurred at all membrane potentials tested (−50 to −80 mV). Action potential height has been reduced by the frequency response of the chart recorder.

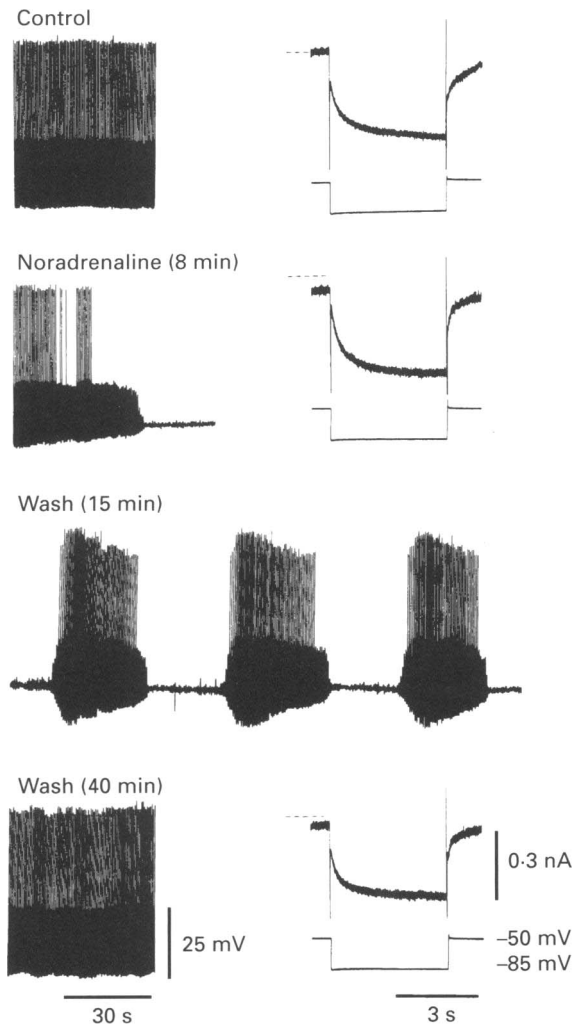

Fig. 8. The effect of α- and β-adrenoceptor stimulation by NA on the pacemaker oscillations. In a TC cell in the cat dLGN, 50 μM-NA produced a 15 mV depolarization and stopped the pacemaker oscillations, even after the cell had been repolarized to the control membrane potential by steady DC current injection. Following wash-out of NA, the membrane potential went back to its control level and the spindle-like oscillations were observed (15 min from the start of the NA wash-out), before a full recovery of the pacemaker oscillations could be seen (membrane potential during the inter-spindle, -65 mV). The three voltage clamp records show the effect of NA on the resting current (dashed line) and on I_h in the same cell. Note the increase in I_h and the generation of an inward current by NA. Action potential height has been reduced by the frequency response of the chart recorder.

order to confirm this possibility, the effects of amiloride and 1-octanol, two compounds which have been suggested to inhibit T-type calcium currents selectively (Llinás 1988; Tang, Presser & Morad, 1988), were tested. Amiloride (250–500 μM, $n = 3$) had no effect on the pacemaker nor on the 'NMDA' oscillations while 1-octanol

(50–100 μM, $n = 5$) produced a reversible block (Fig. 9*A a*). However, amiloride has only a very small effect on I_T in TC cells (Suzuki & Rogawski, 1989; S. Lightowler, C. E. Pollard & V. Crunelli, unpublished observations), while the 10–20% decrease of I_T by 50–100 μM-1-octanol (S. Lightowler, C. E. Pollard & V. Crunelli, unpublished

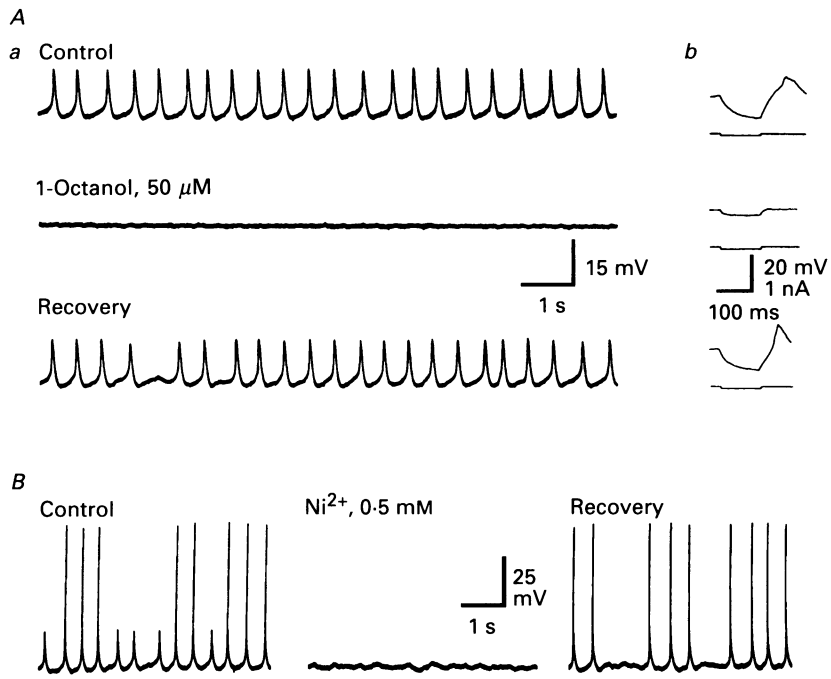

Fig. 9. The effect of 1-octanol and Ni^{2+} on the pacemaker oscillations. *A a*, intracellular voltage recordings from a TC cell in the cat dLGN show the reversible block by 1-octanol (50 μM) of the pacemaker oscillations (membrane potential at the peak of the hyperpolarization, −80 mV). *A b*, averaged ($n = 7$) hyperpolarizing electrotonic potentials (top trace in each pair) evoked by a constant pulse of current (bottom trace in each pair) show the reversible decrease in input resistance produced by 50 μM-1-octanol in the same cell as in *A a* (membrane potential, −70 mV). *B*, intracellular voltage records from a TC cell in the rat dLGN show the block of the pacemaker oscillations by 0·5 mM-Ni^{2+}, 11 min after its addition to the perfusion medium. The recovery was obtained 45 min after removing Ni^{2+} from the medium (membrane potential at the peak of the hyperpolarization, −75 mV).

observations) is accompanied by a 50% drop in the cell input resistance (Fig. 9*A b*). Thus the lack of effect by amiloride and the block of the pacemaker oscillations by 1-octanol could not be explained in terms of a selective action on I_T. Application of 0·2–0·5 mM-Ni^{2+}, however, a relatively selective blocker of I_T in TC cells (Crunelli *et al.* 1989; Hernandez-Cruz & Pape, 1989; Suzuki & Rogawski, 1989), reversibly abolished the pacemaker ($n = 5$; Fig. 9*B*), the spindle-like ($n = 3$) and the 'NMDA' oscillations ($n = 5$). As far as the 'NMDA' oscillations are concerned, it is important to note that concentrations of Ni^{2+} similar to those required to abolish I_T have been shown to block 'NMDA' receptor-mediated responses (Ault, Evans, Francis, Oakes & Watkins, 1980). However, were the latter effect the only one exerted by these ions,

the 'NMDA' oscillations would have been transformed into the pacemaker oscillations, as was the case for APV (DL-2-amino-5-phosphono-valeric acid; Leresche *et al.* 1991) which has no direct effect on I_T ($n = 8$).

In order to investigate whether, together with I_T and I_h, other currents were involved in the pacemaker oscillations we studied the time course of the current

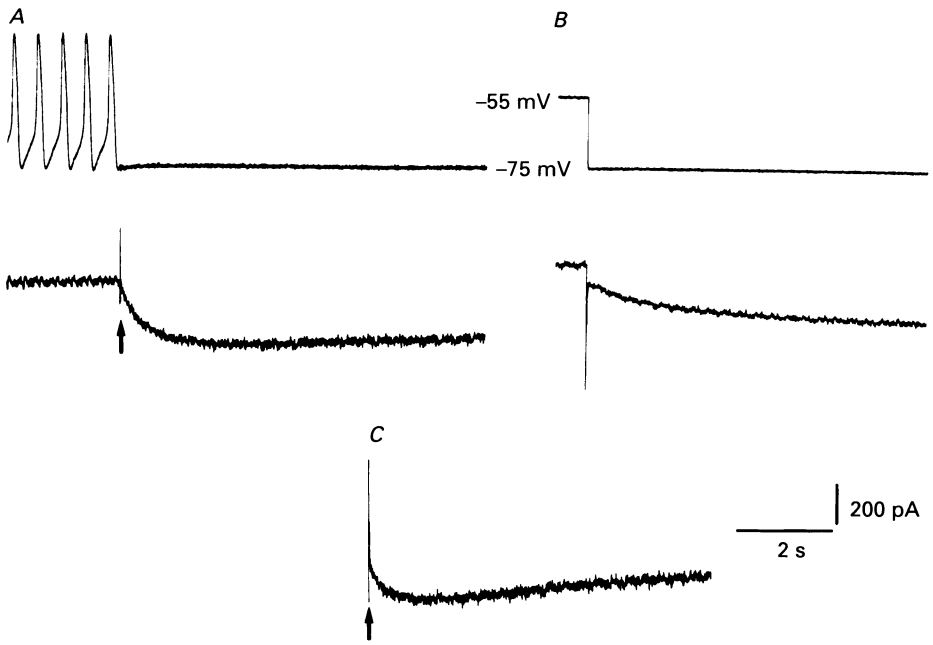

Fig. 10. Time course of current development at the peak of the hyperpolarization of the pacemaker oscillations in a TC cell of the cat dLGN. *A*, at the most hyperpolarized potential (−75 mV) of the fifth cycle of the pacemaker oscillations shown in the top trace the preamplifier was switched from current into voltage clamp to measure the total current developing (bottom trace). *B*, the bottom trace shows the size of I_h generated in the same cell as in *A* after holding the cell for 10 s (only the last 650 ms shown) at −55 mV (where I_h is inactivated) and stepping to −75 mV, the most hyperpolarized potential reached during the pacemaker oscillations. Note the different time course of the current in *A* in comparison to I_h. *C* shows the result of the digital subtraction of I_h from the current shown in *A*. The resulting current peaks at about 1 s after the peak of the hyperpolarization and then slowly decays during the subsequent 6 s.

developing from the most hyperpolarized level of a pacemaker oscillation cycle, a point in time at which I_T should be almost fully decayed (time constant of I_T decay: 25–100 ms in the range −55 to −70 mV at 25 °C, cf. Crunelli *et al.* 1989). As shown in Fig. 10, a TC cell was allowed to oscillate in current clamp and, when its membrane potential reached the most negative level of an oscillation cycle (−75 mV), the preamplifier was then switched into voltage clamp (arrow in Fig. 10*A*). For comparison, the amplitude and time course of I_h development was also measured by holding the same cell at −55 mV (where all I_h channels are de-activated) and stepping to the same, most hyperpolarized potential reached during the pacemaker oscillations (i.e. −75 mV, Fig. 10*B*). As shown in the current trace of Fig. 10*A*, from

the oscillating cell we observed an inward relaxation whose time course differed from that of I_h (bottom trace in Fig. 10*B*) since it was faster and partially decayed. In addition, the subtraction of I_h from this inward relaxation showed a current that peaked at about 1 s and then slowly, and partially, decayed reaching a steady level after 7 s ($n = 4$; Fig. 10*C*).

DISCUSSION

The main conclusions of this investigation are: (i) I_T, by generating low-threshold Ca^{2+} potentials, and I_h, by mediating the hyperpolarization that follows and the slow pacemaker depolarization that precedes a low-threshold Ca^{2+} potential, contribute and are essential to the pacemaker and the spindle-like oscillations; (ii) I_T, but not I_h, is required for the 'NMDA' oscillations to occur; (iii) I_h is a factor controlling the presence and the type of low-frequency oscillations observed in TC cells *in vitro*. Thus, transformation of the pacemaker oscillations into other types of activity can be brought about either by the additional activation of neurotransmitter-mediated conductances (i.e the 'NMDA' oscillations; Leresche *et al.* 1991) or by the neurotransmitter-mediated modulation of the voltage-activated conductances intrinsic to TC cells (i.e. the spindle-like oscillations).

Contribution of I_T to the low-frequency oscillations

The involvement of I_T in the pacemaker, the spindle-like and the 'NMDA' oscillations is supported (i) by the block of these oscillations by Ni^{2+}, (ii) by the similarities between the voltage region where these oscillations were observed (Leresche *et al.* 1991) and the voltage ranges of activation and inactivation of I_T (Crunelli *et al.* 1989) and (iii) by the absence of these oscillations in dorsal lateral geniculate nucleus (dLGN) interneurones and in cells of the ventral lateral geniculate nucleus (vLGN) (Leresche *et al.* 1991), two thalamic cell types that do not possess low-threshold Ca^{2+} potentials (Jahnsen & Llinás, 1984*a*; Crunelli *et al.* 1987; McCormick & Pape, 1988). Thus, the large-amplitude depolarizations present in the pacemaker, the spindle-like and the 'NMDA' oscillations are low-threshold Ca^{2+} potentials.

Properties of I_h in TC cells

The I_h current described in this study is a slowly developing, non-inactivating, mixed Na^+–K^+, inward current activated when TC cells are hyperpolarized to potentials negative to −55 mV. Its properties closely resemble those of mixed Na^+–K^+, inwardly rectifying currents found in other excitable cells, and variously named I_h (Mayer & Westbrook, 1983; Crepel & Penit-Soria, 1986; Lacey & North, 1988), I_f (Brown *et al.* 1979; Brown & DiFrancesco, 1980), I_Q (Halliwell & Adams, 1982), I_{AR} (Spain, Schwindt & Crill, 1987) and i (Benham, Bolton, Denbigh & Lang, 1987).

A property of this current that seems to vary between cell types is its reversal potential. This probably represents a difference in the ratio of contribution to the conductance by Na^+ and K^+ between the cell types. The similar millivolt per decade values for Na^+ and K^+ obtained in this study indicate that in TC cells these two ions contribute approximately equally to g_h. In TC cells I_h begins to activate negative to

−55 mV and is fully activated at a level of around −95 mV, a range similar to the one found in other neurones (Spain *et al.* 1987; Lacey & North, 1988). Thus, I_h is partially activated at the resting membrane potentials of TC cells *in vitro* (−65 mV; Steriade & Llinás, 1988; Llinás, 1988) and presumably plays a role in maintaining the resting membrane potential, a function that has already been suggested in other cell types (Edman, Gestrelius & Grampp, 1987; Spain *et al.* 1987). Indeed, such a role of I_h in TC cells is supported by the hyperpolarization and the depolarization produced by extracellular Cs^+ and by NA (through β-adrenoceptor stimulation), respectively.

The pharmacology of I_h was also similar to that of other mixed Na^+–K^+ currents, including its insensitivity to TTX, TEA and Ba^{2+} and its block by Cs^+. We also confirmed that selective activation of β-receptors (by NA in the presence of α-antagonists, or by isoprenaline) increases the amplitude of I_h in TC cells (Pape & McCormick, 1989; McCormick & Pape, 1990*b*).

Contribution of I_h to the pacemaker and the spindle-like oscillations

The involvement of I_h in the pacemaker and the spindle-like oscillations is supported (i) by the similarity between the membrane potentials reached during these oscillations and the voltage ranges of activation and de-activation of I_h and (ii) by the block of these oscillations by Cs^+. In addition, because of the lack of action of Ba^{2+}, it is unlikely that outward K^+ current(s), apart from those activated at potentials positive to −45 mV, play an essential role in the pacemaker and the spindle-like oscillations. Thus, the mechanism responsible for the pacemaker oscillations appears to involve mainly an interplay between the two inward currents, I_T and I_h. Starting from the most hyperpolarized level of an oscillation cycle, activation of I_h will produce a slow pacemaker depolarization until the threshold voltage of activation of I_T is reached. Since the hyperpolarization had provided the requirements in terms of time and voltage to de-inactivate I_T, a low-threshold Ca^{2+} potential is generated, which might, depending on its amplitude, evoke a single or a high-frequency burst of action potentials. The transient nature of I_T alone or with the contribution of other currents, will then terminate the depolarization and hyperpolarize the cell. Since during the depolarization associated with a low-threshold Ca^{2+} potential I_h will partly de-activate, at the end of a low-threshold Ca^{2+} potential there will be less I_h activated (i.e. less inward current) than at its beginning (cf. Fig. 3*A*) and, as a consequence, the membrane potential will hyperpolarize to a potential more negative than the threshold voltage at which I_T had been evoked. The cell will thus reach the peak of the hyperpolarization, and, since the preceding depolarization has provided the time and voltage requirements necessary for partial de-activation of the I_h channels, I_h could be re-activated and the cycle restarts again. Thus, while I_T is responsible for the large-amplitude depolarizations, I_h mediates both the slow pacemaker depolarization necessary to activate I_T and the hyperpolarization that follows low-threshold Ca^{2+} potentials. Similar conclusions on the involvement of I_T and I_h as the main voltage-activated conductances responsible for the pacemaker oscillations of guinea-pig and cat TC cells have been recently reported by McCormick & Pape (1990*a*).

Our interpretation of the finding that Cs^+ can also evoke the pacemaker oscillations is that in these, otherwise silent, cells I_h was of a relatively large-

amplitude (i.e. larger with respect to I_T) thereby preventing these cells from oscillating. Support for this interpretation of the ability of Cs^+ to evoke pacemaker oscillations comes from the finding that (i) an increase in I_h by β-adrenoceptor activation blocked the pacemaker oscillations, and that (ii) in the very same cells where I_h had previously been selectively increased by activation of β-adrenoceptors, the pacemaker oscillations were again restarted by addition of Cs^+. Thus, the pacemaker oscillations are blocked by an I_h that is either too large or too small (Fig. 11), or, in other words, I_T and I_h have to be finely tuned to allow the expression of the pacemaker oscillations.

The mechanism suggested for the spindle-like oscillations is similar to the one described above for the pacemaker oscillations, basically an interplay of I_T with, in this case, the contribution of an I_h that is relatively larger than the one required for the pacemaker oscillations. During each oscillation cycle in the intra-spindle, the larger I_h will not have sufficient time to de-activate as much as during the pacemaker oscillations (i.e. more I_h will be activated at each oscillation cycle than it can de-activate). Thus, following a certain number of oscillation cycles in the intra-spindle, the amount of I_h evoked by the repetitive oscillations will progressively and steadily increase, with the consequence of slowly depolarizing the cell and not providing a hyperpolarization sufficient for the subsequent de-inactivation of I_T. This, and the concomitant decrease in input resistance associated with the increased I_h, will reduce the ability of the cell to oscillate. Then, during the inter-spindle, a portion of I_h slowly de-activates, the membrane potential slowly hyperpolarizes and the input resistance of the cell increases, so that a new intra-spindle begins. This interpretation of the spindle-like oscillations is supported by the following evidence. Firstly, the spindle oscillations were only observed *before* the pacemaker oscillations when progressively decreasing I_h with Cs^+. Secondly, the spindle-like oscillations were only observed *after* the pacemaker oscillations when progressively increasing I_h with selective β-receptor stimulation. Thirdly, the spindle-like oscillations could be transformed into the pacemaker oscillations by depolarization of the membrane potential from -75 to -60 mV (Leresche *et al.* 1991), a voltage region where less I_h can be activated. Fourthly, the inter-spindle period was always characterized by a slow hyperpolarization comparable, both in terms of voltage range and duration, with the de-activation properties of I_h. Thus, the spindle-like oscillations simply represent the expression of a new equilibrium between I_T and an I_h relatively larger than the one required for the pacemaker oscillations (Fig. 11).

The different time courses of the inward relaxation developing from the most hyperpolarized potential of an oscillation cycle and of the development of I_h indicate that the pacemaker (and probably the spindle-like) oscillations cannot be accounted for only by the two inward currents, I_h and I_T, acting independently. In fact, in the absence of I_h, I_T decay is complete after 200 ms (Crunelli *et al.* 1989), and, in the absence of I_T, I_h does not inactivate. Thus, either these two currents are affecting each other in such a way as to modify markedly their time course and voltage ranges of activation/inactivation or there are other current(s) involved in the pacemaker oscillations. In this respect, possible membrane currents include those with voltage ranges of activation/inactivation (Llinás, 1988) that are reached during the low-frequency oscillations (Leresche *et al.* 1991), and might include, for instance, high-

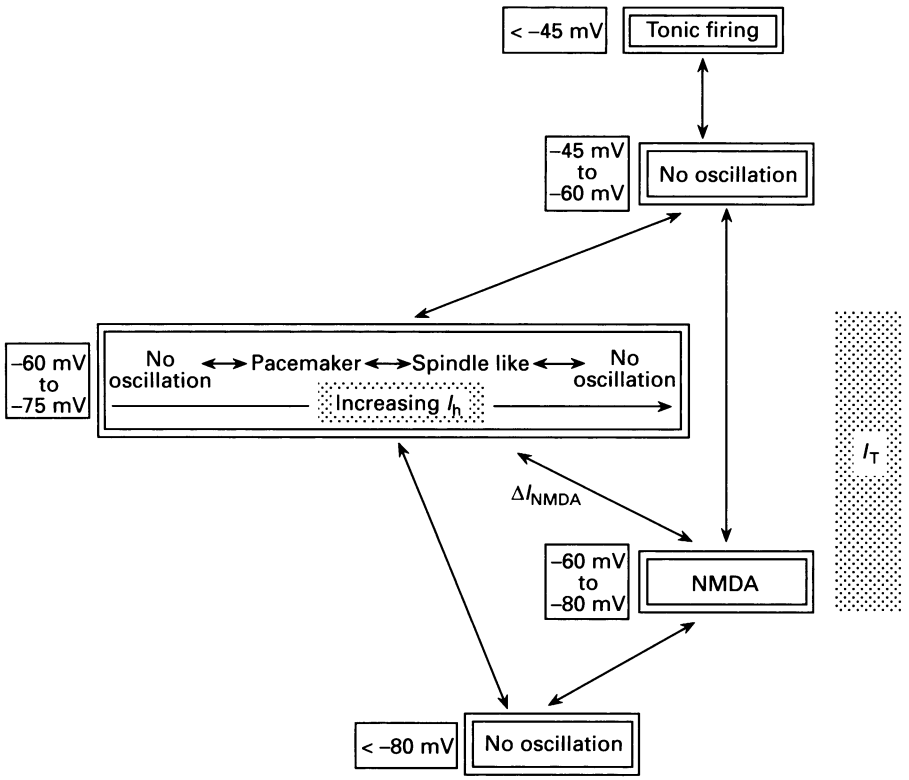

Fig. 11. Schematic representation of the relationship between the different types of spontaneous, low-frequency oscillatory activities observed in TC cells *in vitro*. The membrane potential range where the different activities (or lack of activity) are observed is indicated on the left-hand side of each box. The shaded area on the right-hand side of the figure indicates the voltage region where the low-frequency oscillations involving I_T, the current responsible for the generation of low-threshold Ca^{2+} potentials, occur. At membrane potentials positive to −45 mV, tonic firing is the predominant feature of TC cell activity. Between −45 and −60 mV, the absence of I_h and the lack of de-inactivation of I_T will not allow the expression of spontaneous, low-frequency oscillations, though synaptic potentials will be able to bring the cell to the threshold voltage for eliciting action potentials. Between −60 and −75 mV, the low-frequency oscillations involving I_T and I_h can occur: their presence and type will depend on the relative size of I_T and I_h. At membrane potentials negative to −85 mV, continuous, spontaneous, low-frequency oscillations are blocked because the threshold voltage for I_T activation cannot be reached and because a large I_h will dampen any isolated oscillation that might occur. An increase in the activation of NMDA receptors (ΔI_{NMDA}) is capable of transforming the pacemaker and the spindle-like oscillations into the 'NMDA' oscillations or, indeed, of evoking the 'NMDA' oscillations in otherwise silent cells provided their membrane potential is in the appropriate voltage range for I_T activation. The 'NMDA' oscillations may occur at more hyperpolarized potentials (−60 to −80 mV) than the pacemaker and the spindle-like oscillations because the NMDA-mediated EPSPs will provide the depolarization necessary to reach the threshold voltage for I_T activation. Note that all the events described above are reversible.

threshold Ca^{2+} current(s), Ca^{2+}-activated K^+ current(s), I_A, I_D, delayed rectifier and non-inactivating Na^+ current.

The 'NMDA' oscillations

We have shown that low-threshold Ca^{2+} potentials but not I_h are essential for the 'NMDA' oscillations to occur and that blockade of NMDA receptors transforms them into the pacemaker or the spindle-like oscillations (Leresche *et al.* 1991). Thus, a tentative mechanism for the 'NMDA' oscillations is that a spontaneously released excitatory amino acid, possibly glutamate, activates NMDA receptors present on TC cells in the rat ventrobasal nucleus and rat and cat dLGN (references in Leresche *et al.* 1991). This activation generates small-amplitude EPSPs that, in turn, will activate low-threshold Ca^{2+} potentials, i.e. the large depolarizations of the 'NMDA' oscillations. Indeed, the small-amplitude depolarizations recorded in a 'Mg^+-free' medium from cells of the vLGN and from dLGN interneurones, two types of thalamic cells that do not display low-threshold Ca^{2+} potentials (Crunelli *et al.* 1987; McCormick & Pape, 1988) were blocked by APV (Leresche *et al.* 1991) suggesting that they were NMDA receptor-mediated EPSPs. The 'NMDA' oscillations can occur at slightly more negative membrane potentials than those of the other types of oscillations since the spontaneous NMDA receptor-mediated synaptic potentials will provide the depolarization necessary to activate I_T (Fig. 11). Finally, it is important to note at this point that the spontaneous activity we have described in TC cells as the 'NMDA' oscillations is different from the NMDA-evoked oscillations described in other neurones since the latter activity is highly regular and blocked by removal of Mg^+ from the perfusion medium (Grillner & Wallen, 1985).

Functional significance

The ability of β-receptor stimulation by NA to transform the pacemaker into the spindle-like oscillations by increasing the amplitude of I_h indicates that neurotransmitters can modulate the frequency and rhythmicity of the low-frequency oscillations observed in TC cells (Fig. 11). Synaptic inputs can also affect these oscillatory activities directly via their own transmitter-gated conductances as we have shown for the NMDA receptor-mediated transformation of the pacemaker and the spindle-like oscillations into the 'NMDA' oscillations (Fig. 11; Leresche *et al.* 1991). The finding that in a few cells some of the small depolarizations present in the 'NMDA' oscillations were reversed $GABA_A$ IPSPs (Leresche *et al.* 1991) indicates that synaptic inputs mediated by this type of GABA receptor, as well as EPSPs, are capable of changing the basic frequency and disrupting the rhythmicity of the pacemaker oscillations. It therefore seems that each type of low-frequency oscillatory activities observed *in vitro* is not peculiar to a particular group of TC cells but represents a *continuum* of electrical activity present in each TC cell and that can be sculptured by the differential activation of their various voltage-dependent and transmitter-gated conductances (e.g. I_T, I_h, I_{NMDA}; Fig. 11).

Have the low-frequency oscillatory activities observed in TC cells *in vitro* any relation to the oscillatory activities described in the same cells *in vivo*? An obvious comparison is clearly with the deep stages of slow-wave sleep when the EEG (electroencephalogram) activity is characterized by slow waves occurring in the

frequency range of 0·5–4 Hz (δ-waves) (Steriade & Llinás, 1988). Because of the decreased brain stem and sensory activation during this part of the EEG-synchronized sleep (Steriade & Llinás, 1988; McCormick, 1989), TC cells hyperpolarize and those intrinsic conductances that are activated at relatively negative membrane potentials will start to prevail over the synaptic currents so that an activity similar to the pacemaker oscillations occurs. Thus, the pacemaker oscillations might represent the basic pattern of electrical activity of TC cells when the membrane potential is negative to −60 mV and synaptic inputs are diminished, though *in vivo* they will probably never be fully blocked. Indeed, rhythmic (1–4 Hz) bursts of action potentials have been recorded extracellularly from cat TC cells during slow-wave sleep (Lamarre, Filion & Cordeau, 1971). Neurotransmitters, via transmitter-gated conductances and/or transmitter-modulated voltage-dependent conductances, could then modify the rhythm and/or the frequency of the pacemaker oscillations to generate the full repertoire of low-frequency oscillations involving I_T and I_h and observed during the other stages of EEG-synchronized sleep *in vivo* (Hirsch, Fourment & Marc, 1983; Llinás, 1988; Steriade & Llinás, 1988; McCormick, 1989; Crunelli & Leresche, 1991). During REM (rapid eye movement) sleep and during wakefulness, instead, extra-thalamic inputs will depolarize the cells towards membrane potentials where the voltage-activated conductances responsible for the pacemaker oscillations (and other types of low-frequency oscillatory activities involving I_T and I_h) can no longer be operational (Fig. 11; Hirsch *et al.* 1983; Steriade & Llinás, 1988; McCormick, 1989; Crunelli & Leresche, 1991).

The spindle-like oscillations deserve a particular comment. Although this type of oscillatory activity observed *in vitro* showed some similarities with the sleep spindles recorded intracellularly *in vivo*, it is unlikely that they represent those observed *in vivo* since the latter are identified by an intra-spindle frequency of 7–14 Hz (Steriade & Llinás, 1988) and depend on the synaptic drive of the GABAergic cells of the nucleus reticularis thalami (Steriade, Deschenes, Domich & Mulle, 1985), while the spindle-like oscillations occurred in slices that did not contain this nucleus, had an intra-spindle frequency of 0·5–4 Hz and were unaffected by TTX, $GABA_A$ and $GABA_B$ receptor antagonists (Leresche *et al.* 1991). Nevertheless, our results clearly indicate that I_h plays a crucial role in the generation, modulation, and more importantly, in the termination of regular, low-frequency oscillatory activities. Thus, together with I_T and various synaptic inputs (Coulter, Huguenard & Prince, 1989*b*; Crunelli & Leresche, 1991), the role played by I_h in sleep spindles and other periodic, low-frequency oscillatory activities observed in TC cells *in vivo* during normal (Steriade & Llinás, 1988) and pathological conditions (Gloor & Fariello, 1988; Buzsaki, Smith, Berger, Fisher & Gage, 1990) should also be considered.

We wish to thank Miss Z. Emri for her invaluable help in some of the experiments. The work was supported by the Wellcome Trust (grant 17953/1.5 and 19135/1.5). I.S. was partly supported by the Herbert von Karajan Neuroscience Research Trust and S.L. by an MRC scholarship.

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