

## PROPERTIES OF A HYPERPOLARIZATION-ACTIVATED CATION CURRENT AND ITS ROLE IN RHYTHMIC OSCILLATION IN THALAMIC RELAY NEURONES

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### SUMMARY

1. The physiological and functional features of time-dependent anomalous rectification activated by hyperpolarization and the current which underlies it,  $I_h$ , were examined in guinea-pig and cat thalamocortical relay neurones using *in vitro* intracellular recording techniques in thalamic slices.

2. Hyperpolarization of the membrane from rest with a constant-current pulse resulted in time-dependent rectification, expressed as a depolarizing sag of the membrane potential back towards rest. Under voltage clamp conditions, hyperpolarizing steps to membrane potentials negative to approximately  $-60$  mV were associated with the activation of a slow inward current,  $I_h$ , which showed no inactivation with time.

3. The activation curve of the conductance underlying  $I_h$  was obtained through analysis of tail currents and ranged from  $-60$  to  $-90$  mV, with half-activation occurring at  $-75$  mV. The time course of activation of  $I_h$  was well fitted by a single-exponential function and was strongly voltage dependent, with time constants ranging from greater than 1–2 s at threshold to an average of 229 ms at  $-95$  mV. The time course of de-activation was also described by a single-exponential function, was voltage dependent, and the time constant ranged from an average of 1000 ms at  $-80$  mV to 347 ms at  $-55$  mV.

4. Raising  $[K^+]_o$  from 2.5 to 7.5 mM enhanced, while decreasing  $[Na^+]_o$  from 153 to 26 mM reduced, the amplitude of  $I_h$ . In addition, reduction of  $[Na^+]_o$  slowed the rate of  $I_h$  activation. These results indicate that  $I_h$  is carried by both  $Na^+$  and  $K^+$  ions, which is consistent with the extrapolated reversal potential of  $-43$  mV. Replacement of  $Cl^-$  in the bathing medium with isethionate shifted the chloride equilibrium potential positive by approximately 30–70 mV, evoked an inward shift of the holding current at  $-50$  mV, and resulted in a marked reduction of instantaneous currents as well as  $I_h$ , suggesting a non-specific blocking action of impermeable anions.

5. Local (2–10 mM in micropipette) or bath (1–2 mM) applications of  $Cs^+$  abolished  $I_h$  over the whole voltage range tested ( $-60$  to  $-110$  mV), with no consistent effects on instantaneous currents. Barium (1 mM, local; 0.3–0.5 mM, bath) evoked a steady

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inward current, reduced the amplitude of instantaneous currents, and had only weak suppressive effects on  $I_h$ .

6. Block of  $I_h$  with local application of  $\text{Cs}^+$  resulted in a hyperpolarization of the membrane from the resting level, a decrease in apparent membrane conductance, and a block of the slow after-hyperpolarization that appears upon termination of depolarizing membrane responses, indicating that  $I_h$  contributes substantially to the resting and active membrane properties of thalamocortical relay neurones.

7. A sub-population of cat dorsal lateral geniculate nucleus neurones generated rhythmic high-frequency bursts of action potentials with an interburst frequency of 1–2 Hz. Intracellular recordings indicate that this activity results from the interaction of the low-threshold  $\text{Ca}^{2+}$  current  $I_t$  with the hyperpolarization-activated cation current  $I_h$ . It is proposed that the presence and physiological characteristics of  $I_h$  contribute substantially to the physiological properties of thalamic neurones during periods of inattentiveness and slow-wave sleep.

## INTRODUCTION

Thalamocortical relay neurones possess two basic modes of action potential generation which vary according to the state of the animal: (1) single-spike firing occurs during attentiveness and waking and (2) rhythmic burst firing occurs during periods of inattentiveness and sleep (Steriade & Deschênes, 1984; Steriade & Llinás, 1988). Intracellular investigations both *in vivo* and *in vitro* have revealed that these two modes of action potential generation depend upon both the intrinsic electrophysiological properties of thalamic neurones and the interconnections of the different subtypes of thalamic cells, especially between the  $\gamma$ -aminobutyric acid-containing neurones of the nucleus reticularis and the neurones located in individual relay nuclei (Jahnsen & Llinás, 1984*a, b*; Steriade & Deschênes, 1984; Steriade & Llinás, 1988). One of the prominent features of thalamic relay neurones is that upon hyperpolarization of the membrane there appears strong time-dependent inward rectification which moves the membrane potential back towards the normal resting value (Crunelli, Kelly, Leresche & Pirchio, 1987*a*; McCormick & Pape, 1988; Lightowler, Hynd, Pollard & Crunelli, 1989). Since the membrane potential of thalamic relay neurones that are rhythmically oscillating *in vivo* continuously fluctuates within the voltage range in which this rectification is present (Steriade & Deschênes, 1984), the underlying current may be of importance for shaping the electrical behaviour of these neurones, although its ionic basis and voltage dependence have not yet been characterized.

In many different types of electrically responsive cells, including heart cells, and neurones in the sensory and sympathetic ganglia, spinal cord, brain stem, cerebellum, hippocampus and cerebral cortex, hyperpolarization of the membrane results in a depolarizing sag back towards rest (Hotson, Prince & Schwartzkroin, 1979; Halliwell & Adams, 1982; Mayer & Westbrook, 1983; DiFrancesco, 1985; Crepel & Penit-Soria, 1986; Spain, Schwindt & Crill, 1987; Bobker & Williams, 1989; Eng, Gordon, Kocsis & Waxman, 1990; Tokimasa & Akasu, 1990). The current underlying this phenomenon is carried by both  $\text{Na}^+$  and  $\text{K}^+$  ions, activates upon membrane hyperpolarization (typically beyond  $-60$  mV), and de-activates upon depolarization. In sino-atrial cells and Purkinje fibres of the heart, this type of current,

termed  $I_f$ , appears to be important in determining the rate of action potential generation (Noble, DiFrancesco & Denyer, 1989). In the central nervous system, in contrast, the role of hyperpolarization-activated cation currents is less clear. Potential roles in determining the resting membrane potential and modulating the response to hyperpolarizing inputs have been suggested (Halliwell & Adams, 1982; Spain *et al.* 1987). An additional possibility is that  $I_h$  contributes to rhythmic cellular oscillations (Alonso & Llinás, 1989). In particular, the recent demonstration of endogenous oscillations in thalamocortical relay neurones maintained *in vitro* at membrane potentials in which time-dependent anomalous rectification is prevalent (Haby, Leresche, Jassik-Gerschenfeld, Soltesz & Crunelli, 1988; McCormick & Prince, 1988) suggests a possible important contribution of  $I_h$  to this type of electrophysiological behaviour.

Here we describe some of the physiological characteristics of the current which underlies this form of anomalous rectification and investigate its contribution to the electrophysiological behaviour of thalamocortical relay cells.

#### METHODS

Thalamic slices were prepared from male or female guinea-pigs (200–300 g) or cats (four; approximately 2.5 kg) as described previously (Crunelli, Leresche, Hynd, Patel & Parnavelas, 1987*b*; McCormick & Pape, 1988; McCormick & Prince, 1988). The animals were deeply anaesthetized (pentobarbitone, 40 mg/kg i.p., guinea-pigs; ketamine, 25 mg/kg i.m., followed by pentobarbitone, 36 mg/kg i.v., cats) and killed by decapitation. A block of tissue containing the dorsolateral aspect of the thalamus was removed and placed in physiological saline at a temperature of 5 °C. Slices were prepared as 400  $\mu$ m thick coronal sections on a Vibratome (Ted Pella, Model 1000) and placed in an interface-type recording chamber (Fine Science Tools). Slices were maintained at  $36 \pm 1$  °C and continuously superfused with a solution containing (in mM): NaCl, 126; KCl, 2.5; MgSO<sub>4</sub>, 1.2–2; NaHCO<sub>3</sub>, 26; NaH<sub>2</sub>PO<sub>4</sub>, 1.25; CaCl<sub>2</sub>, 2; dextrose, 10; saturated with 95% O<sub>2</sub>, 5% CO<sub>2</sub> to a final pH of 7.4. Recording commenced 2 h after preparation of the slices.

Intracellular recordings were obtained with thin-wall micropipettes (World Precision Instruments, TW-100), prepared on a micropipette puller (Flaming–Brown, P-80/PC) and filled with 4 M-potassium acetate. Final resistances ranged between 35 and 55 M $\Omega$ .

An Axoclamp 2A amplifier (Axon Instruments) was used for both current and voltage clamp recordings. During current clamp recordings, the bridge balance was continuously monitored. During single-electrode voltage clamp, head stage output was continuously monitored to ensure adequate settling time. Sampling frequencies were between 3.5 and 5.5 kHz and the amplifier gain was typically around 1 nA/mV. Voltage clamp experiments were performed using the pClamp software program (Axon Instruments) operating on an IBM AT computer. The degree of 'space clamp' obtained in these neurones under our conditions is unknown. However, the finding that the rate and voltage dependence of activation of  $I_h$  were both well behaved, in that they were smooth and continuous functions of the membrane potential, suggests that sufficient clamp was obtained in order to avoid serious errors. Data were digitized (Neurocorder DR-384) and stored on magnetic tape with a video cassette recorder for later analysis or collected on-line with the computer. Only neurones having stable membrane potentials negative to  $-55$  mV, resting input resistances above 30 M $\Omega$  and action potentials which overshoot 0 mV were included for analysis. In the cat dorsal lateral geniculate nucleus, recordings were obtained from neurones located in the A-laminae.

Changing the ionic composition of the bathing medium was achieved by the following equimolar substitutions: (a) extracellular sodium concentration was decreased by replacing NaCl with choline chloride; (b) extracellular potassium concentration was increased by substitution of KCl for NaCl; and (c) extracellular chloride concentration was reduced by replacing NaCl by sodium isethionate, and KCl by potassium acetate. DC offsets due to changes in junction potential at the Ag–AgCl reference electrode in different chloride concentrations were compensated in the following way: the membrane potential at which single-spike firing was elicited was determined in normal bathing

solution. During introduction of a different chloride solution, this firing threshold was repeatedly monitored and its reading was kept constant by adding current to the input offset circuitry of the amplifier. Complete exchange, indicated by a constant reference potential, was typically achieved in 30–60 min. At the end of the experiment, the change in reference potential was controlled by measuring the DC offset of the electrode in normal bathing solution. Finally, when  $\text{Mn}^{2+}$  or  $\text{Ba}^{2+}$  were added to the superfusate,  $\text{MgCl}_2$  was substituted for  $\text{MgSO}_4$  and  $\text{NaH}_2\text{PO}_4$  was removed to avoid precipitation.

Caesium and barium were applied to thalamic neurones either in the bathing medium ( $\text{Cs}^+$ , 1–2 mM;  $\text{Ba}^{2+}$ , 500  $\mu\text{M}$ ) or locally to the exposed surface of the slice with a broken micropipette ( $\text{Cs}^+$ , 2–20 mM;  $\text{Ba}^{2+}$ , 1 mM). Drug-applying micropipettes had a tip diameter of approximately 2–5  $\mu\text{m}$  and substances were extruded in volumes of 5–15 pl by applying a brief pressure pulse of nitrogen (10–20 ms, 207–345 kPa) to the pipette. In some experiments,  $\text{Ni}^{2+}$  (1 mM), 4-aminopyridine (4-AP, 0.5–1 mM), tetraethylammonium (10 mM) or tetrodotoxin (TTX, 0.2  $\mu\text{M}$ ) were introduced in the perfusion medium. Averaged data are represented as mean  $\pm$  standard deviation.

### RESULTS

The data presented here are based largely upon recordings obtained from neurones in the guinea-pig dorsal lateral geniculate nucleus (LGND;  $n = 67$ ). Additional recordings from cells in the LGND of the cat ( $n = 14$ ), as well as from the medial geniculate nucleus ( $n = 9$ ) of both species, revealed similar results and therefore were combined for analysis. A representative sample of twenty guinea-pig LGND neurones possessed a resting membrane potential of  $-66 \pm 3$  mV, apparent input resistance of  $46 \pm 12$  M $\Omega$  and spike amplitude of  $91 \pm 6$  mV. All neurones reported here exhibited large rebound burst discharges upon repolarization of the membrane after a brief hyperpolarization, strong inward rectification when hyperpolarized, and delayed onset to firing when depolarized. These electrophysiological properties suggest that these neurones were thalamocortical relay neurones (Jahnsen & Llinás, 1984a, b; McCormick & Pape, 1988) and therefore we refer to them as such.

Hyperpolarization of thalamic relay neurones beyond approximately  $-60$  mV resulted in a slowly activating rectification, apparent as a depolarizing sag of the membrane potential back towards the normal resting value (Fig. 1A, arrows). Both the amplitude of this rectification and the apparent rate of its activation increased markedly with incremental increases in the hyperpolarizing current pulse (Fig. 1A). In neurones located in other parts of the nervous system, termination of a hyperpolarizing current pulse is often associated with the generation of a rebound depolarizing potential, resulting in part from the slow de-activation of the current underlying anomalous rectification (Mayer & Westbrook, 1983; Spain *et al.* 1987). In thalamic relay cells, the membrane potential changes upon termination of a hyperpolarizing response are complicated by the interacting effects of de-activation of anomalous rectification with activation of other currents, such as the low-threshold calcium current (Jahnsen & Llinás, 1984a, b; Coulter, Huguenard & Prince, 1989; Crunelli, Lightowler & Pollard, 1989; Hernández-Cruz & Pape, 1989) and transient potassium currents (Jahnsen & Llinás, 1984a, b; D. A. McCormick, unpublished observations).

In single-electrode voltage clamp, hyperpolarizing voltage steps from a holding potential of  $-55$  mV resulted in a slow inward relaxation, the time course and amplitude of which was dependent on the amplitude of the voltage step (Fig. 1B, arrows). The injection of short duration conductance test pulses (50 ms duration, 10 mV amplitude) during the development of this inward relaxation revealed a

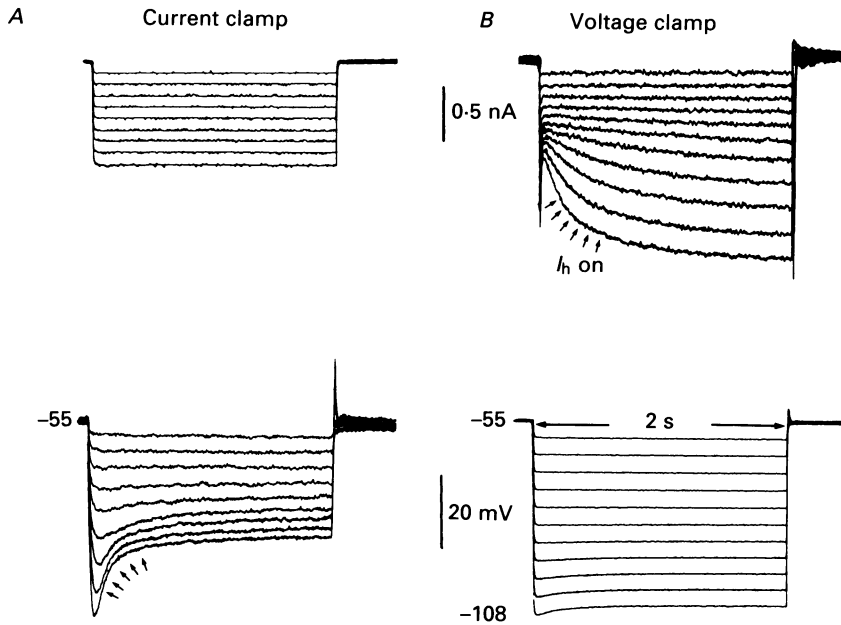


Fig. 1. Rectification of hyperpolarizing responses evoked in a guinea-pig LGND relay cell. Upper traces are membrane current; lower traces are membrane potential. Recordings are from the same cell, depolarized to  $-55$  mV. *A*, in current clamp conditions hyperpolarizing current pulses (duration 1.9 s) evoke membrane hyperpolarizations that show a voltage- and time-dependent depolarizing 'sag' (arrows). *B*, under voltage clamp conditions, hyperpolarizing voltage steps elicit a slow inward current ( $I_h$ , arrows) whose amplitude and rate of activation increase with increasing hyperpolarization.

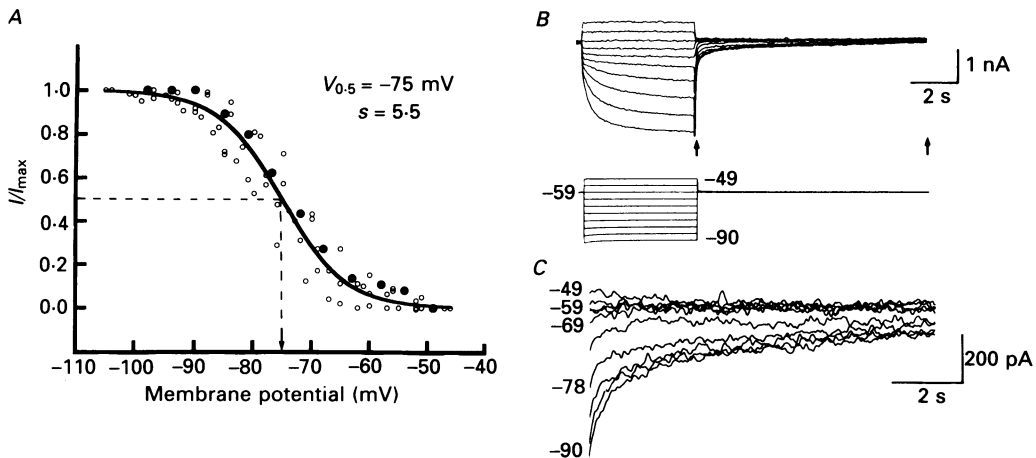


Fig. 2. Activation curve of the conductance,  $G_h$ , which underlies  $I_h$ . *A*, plot of the activation curve for  $G_h$  for seven different LGND relay neurones. Filled circles represent data obtained from cell illustrated in *B* and *C*. Line represents best fit using eqn (1) for data illustrated. *B*, incremental increases in hyperpolarizing steps from  $-40$  to  $-90$  mV progressively increase the amount of  $I_h$  which is activated. Tail currents between arrows are expanded for detail in *C*. Care was taken in measuring tail currents so as to minimize the contribution of capacitive or non- $I_h$  active currents by allowing sufficient time for these events to dissipate before measuring  $I_h$ . See text for details.

substantial increase in membrane conductance (mean =  $19.6 \pm 5.7$  nS;  $n = 5$ ;  $-55$  to  $-95$  mV), indicating that it represented the activation of an inward current, rather than de-activation of an outward current.

In the following we will refer to this slow inward current as  $I_h$  because it is activated by hyperpolarization. In agreement with the results in current clamp, the amplitude of  $I_h$  increased markedly with increases in the size of the hyperpolarizing voltage step (Fig. 1*B*). The threshold for activation of  $I_h$  appeared to be approximately  $-60$  to  $-65$  mV and at  $-95$  mV the amplitude of  $I_h$  ranged from  $0.5$  to  $2.0$  nA (e.g. Figs 1–3). The current did not decay during voltage steps longer than  $1$  min in duration, indicating very little, if any, inactivation of the conductance underlying  $I_h$ .

#### Activation curve

The activation curve of the membrane conductance underlying  $I_h$  ( $G_h$ ) was constructed by measuring tail currents elicited by repolarizing the membrane to holding level (typically  $-60$  mV) following voltage steps of  $4$ – $5$  s to between  $-55$  and  $-105$  mV (Fig. 2). This experimental protocol was chosen to reduce contamination of  $I_h$  tail currents by other currents, the possible contribution of which was further minimized in some neurones by including  $\text{Ni}^{2+}$  ( $1$  mM) to block low-threshold calcium currents (Coulter *et al.* 1989; Crunelli *et al.* 1989; Hernández-Cruz & Pape, 1989), 4-aminopyridine ( $0.5$ – $1$  mM) to block transient potassium outward currents (such as  $I_A$  and  $I_D$ , Storm, 1988), and tetrodotoxin (TTX,  $0.2$   $\mu\text{M}$ ) to block transient and persistent voltage-gated sodium currents and spontaneous synaptic potentials. The tail current amplitudes were normalized to the maximal amplitude and plotted against the membrane potential to which the neurone was stepped during activation of  $I_h$ . The resulting data were well fitted by the Boltzmann equation:

$$I/I_{\max} = (1 + \exp((V_m - V_{0.5})/s))^{-1}, \quad (1)$$

where  $V_m$  is the membrane potential,  $V_{0.5}$  is the membrane potential at which  $G_h$  is half-activated,  $I$  is the amplitude of the tail current at the beginning of  $I_h$  de-activation and  $s$  is the slope factor which determines the steepness of the fitted curve (e.g. Mayer & Westbrook, 1983; Hagiwara & Irisawa, 1989).

In this manner, the activation range of  $G_h$  was between  $-60$  and  $-95$  mV, with half-activation occurring at an average of  $-74.6 \pm 3.4$  mV ( $n = 7$ ) with a slope factor ( $s$ ) of  $5.5 \pm 0.8$  (Fig. 2*A*).

#### Time course of activation and de-activation

The rate of activation of  $I_h$  increased markedly as the membrane potential was hyperpolarized (Figs 1*B* and 3). Over the whole voltage range tested,  $I_h$  was well

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Fig. 3. Time constants of  $I_h$  activation and de-activation. *A*, plot of the voltage dependence of  $I_h$  activation and de-activation as calculated from the data presented in *B*–*E*. Progressive hyperpolarizing voltage steps activate  $I_h$  (*B*). The current traces during the  $3$  s interval indicated are expanded in *D* in which the data are fitted with a single-exponential function (circles represent actual data; line is best fit). Voltage dependence of  $I_h$  de-activation was examined by fully activating  $I_h$  and then stepping to membrane potentials between  $-83$  and  $-55$  mV (*C*). The current traces during the  $3$  s interval indicated were fitted with a single-exponential function (*E*). Data obtained after block of transient outward current with 4-aminopyridine and voltage-activated  $\text{Na}^+$  currents with tetrodotoxin.

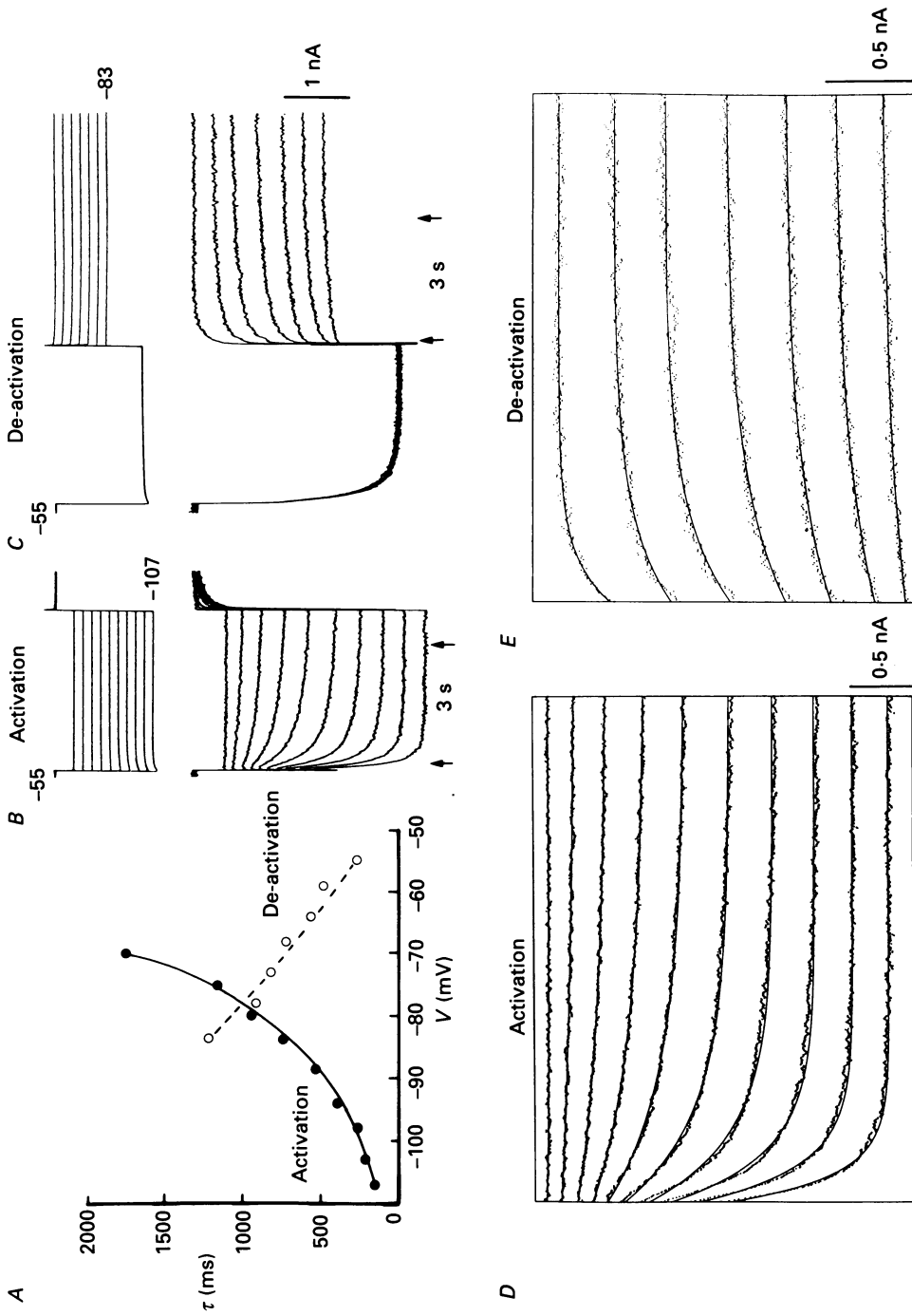


Fig. 3. For legend see facing page.

fitted ( $r > 0.98$ ) by a single-exponential function of the form:

$$I_t = A_0 + A_1 e^{-t/\tau}, \quad (2)$$

where  $I_t$  is the amplitude of the current at time  $t$ ,  $A_0$  and  $A_1$  are constants and  $\tau$  is the time constant (Fig. 3*D*).  $\tau$  ranged from greater than 1–2 s at membrane potentials just past threshold (e.g.  $-65$  to  $-70$  mV) to an average of 229 ms ( $\pm 57$  ms;  $n = 7$ ) at  $-95$  to  $-100$  mV (Fig. 3*A*).

The rate of de-activation of  $I_h$  was determined through examining tail currents associated with depolarization of the membrane from a hyperpolarized level (e.g.  $-105$  mV) or through envelope analysis. When examining tail currents, the transient outward potassium currents were typically blocked with local application of 4-aminopyridine (1–2 mM;  $n = 5$ ) after block of synaptic transmission with local application of tetrodotoxin (10  $\mu$ M). The amplitude and time course of  $I_h$  during de-activation was well fitted ( $r > 0.95$ ) by a single-exponential function (Fig. 3*E*). The rate of de-activation of  $I_h$  was strongly voltage dependent (Fig. 3*A*) ranging from  $> 1$  s at approximately  $-85$  mV to an average of 347 ms ( $\pm 97$ ;  $n = 5$ ) at  $-55$  mV. The latter value of de-activation of  $I_h$  was confirmed through the use of envelope analysis (Mayer & Westbrook, 1983; Tokimasa & Akasu, 1990). In this protocol, the membrane potential was held at  $-95$  mV for a sufficient time to fully activate  $I_h$  (e.g. 5 s) and was then transiently depolarized to between  $-60$  and  $-55$  mV for a varying length of time. The membrane was then repolarized to  $-95$  mV and the amplitude of  $I_h$  activated by this repolarization was measured. Due to the lack of inactivation of  $I_h$ , this amplitude corresponds to the amount of  $I_h$  which de-activated during the transient depolarization. Using this experimental protocol, we determined the rate of de-activation of  $I_h$  to average  $426 \pm 121$  ms ( $n = 6$ ) at  $-60$  to  $-55$  mV, a value which is in good agreement with the time constants obtained through analysis of tail currents.

#### *Ionic composition of $I_h$*

The nature of the ions carrying  $I_h$  through the membrane was investigated by changing the extracellular concentration of  $K^+$ ,  $Na^+$  and  $Cl^-$ . Raising the extracellular  $K^+$  concentration ( $[K^+]_o$ ) from 2.5 to 7.5 mM resulted in an inward current, an increase in membrane conductance, and an enhancement of  $I_h$ , as measured by the difference between the steady-state and instantaneous  $I$ - $V$  relations (Fig. 4*A* and *B*;  $n = 4$ ). Possible contamination of the steady-state currents by the  $K^+$ -carried inward rectifier, which at physiological intracellular magnesium concentrations is activated by hyperpolarization beyond the potassium equilibrium potential,  $E_K$  (Matsuda, Saigusa & Irisawa, 1987; Ishihara, Mitsuiye, Noma & Takano, 1989), can be assumed to be small, since the range of hyperpolarizing voltage pulses was largely positive to  $E_K$  (presumably  $-105$  and  $-73$  mV in 2.5 and 7.5 mM  $[K^+]_o$ , respectively; McCormick & Prince, 1987). These data indicate that a significant portion of  $I_h$  is carried by  $K^+$ .

Decreasing  $[Na^+]_o$  from 153 to 26 mM resulted in a marked reduction of the amplitude of the hyperpolarization-activated steady-state current and a slower time course of activation at a given voltage (Fig. 5*A*;  $n = 6$ ). In addition, the de-activating currents, emerging as tail currents after stepping back to the holding



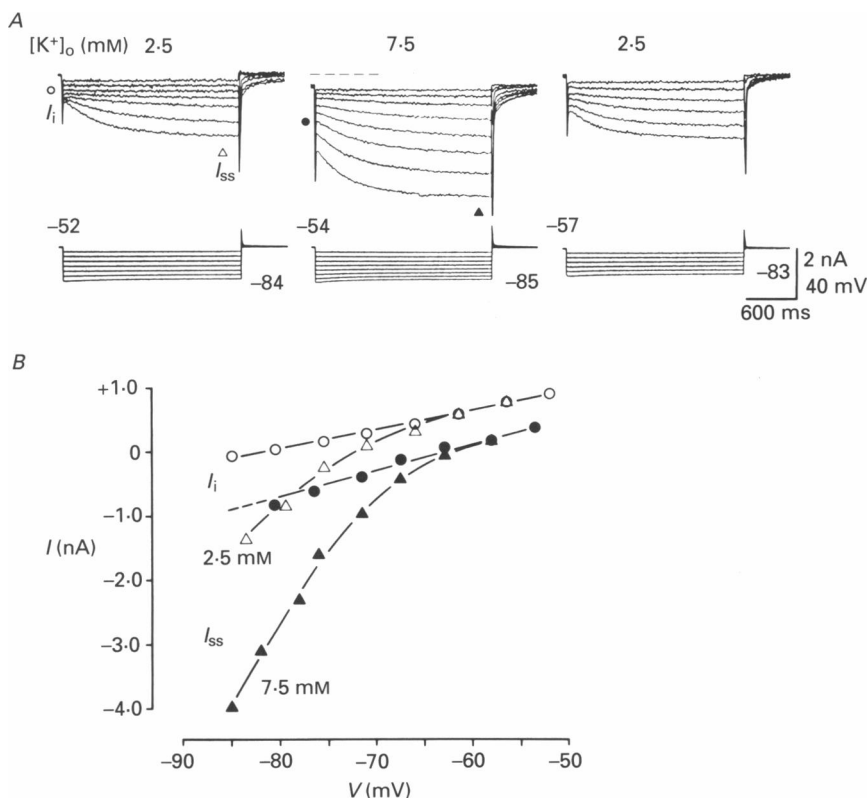


Fig. 4. Effects of changing the extracellular concentration of potassium ions,  $[K^+]_o$ , upon hyperpolarization-activated currents. *A*, family of currents (upper traces) evoked in a LGND neurone by hyperpolarizing voltage steps (lower traces) in control solution (2.5 mM  $[K^+]_o$ , left), during increased  $[K^+]_o$  (7.5 mM, middle; dashed line for comparison in baseline holding current), and during recovery to control  $[K^+]_o$  (2.5 mM, right). Holding potential and most negative steady-state step potential are indicated near traces; step amplitude is 5 mV, step duration is 2 s. *B*,  $I-V$  relationship for instantaneous currents ( $I_i$ , circles) and steady-state currents after 2 s ( $I_{ss}$ , triangles) obtained from experiment in *A*. Open symbols indicate control condition; filled symbols indicate elevated  $[K^+]_o$ .  $I_i$  at larger hyperpolarizations extrapolated (dashed line) from instantaneous  $I-V$  relationships at smaller hyperpolarizations, that were directly determined from measurement of current traces. Tail currents are contaminated by transient  $Ca^{2+}$  and  $K^+$  currents. Note increased slope of the steady-state  $I-V$  relation during increased  $[K^+]_o$  that exceeds increase in slope of the instantaneous  $I-V$  relationship.

potential of  $-50$  mV, changed from small inward to clear outward currents in the low- $Na^+$  solution, indicating a negative shift of the reversal potential. Current *versus* voltage ( $I-V$ ) relationships revealed a negative shift of the steady-state  $I-V$  relation in low  $Na^+$ , and no significant change in instantaneous current (Fig. 5C). These results indicate that  $Na^+$  ions also contribute to  $I_h$ .

A possible contribution of  $Cl^-$  to  $I_h$  was examined by decreasing  $[Cl^-]_o$  from 132.5 to 4 mM using isethionate. The low- $Cl^-$  solution shifted  $E_{Cl^-}$ , which normally is approximately  $-75$  mV, by up to 70 mV more positive, as indicated by the reversal potential of the muscimol- (0.1 mM, local application) induced chloride current. If a

large anion conductance contributed to  $I_h$ , this positive shift of  $E_{Cl}$  is expected to increase  $I_h$  in size. However, the low- $Cl^-$  solution resulted in a substantial decrease in size of both the steady-state currents and the instantaneous currents activated by hyperpolarizing voltage steps, and in addition elicited an inward shift of the holding

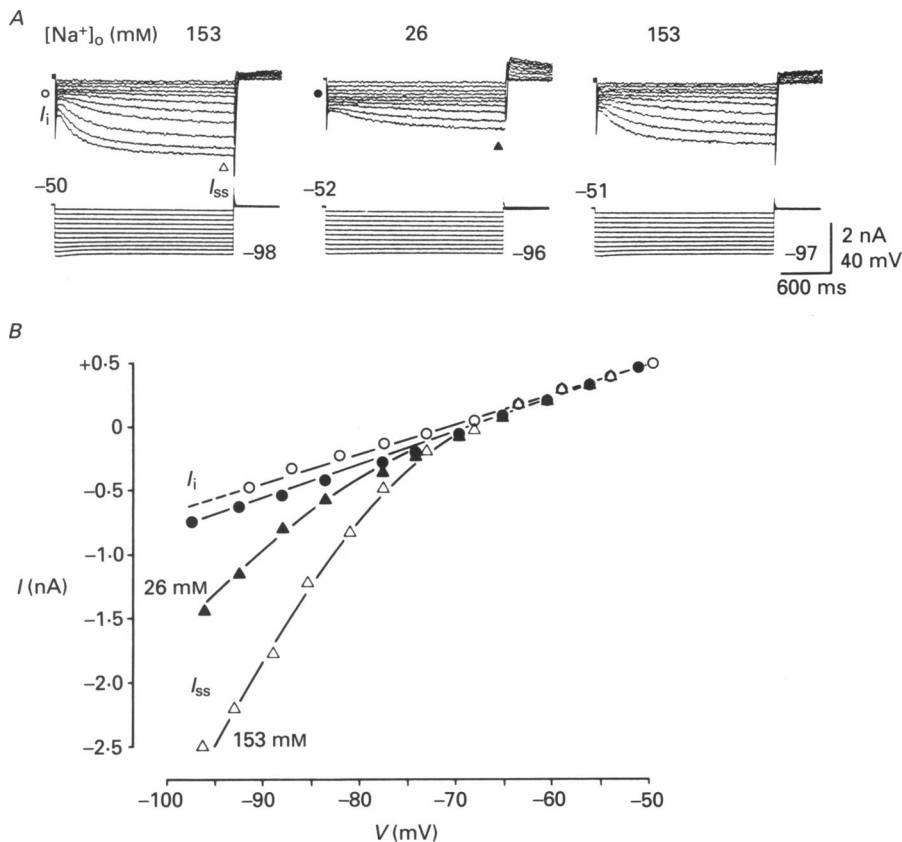


Fig. 5. Effects of changing the extracellular concentration of sodium ions,  $[Na^+]_o$ , upon  $I_h$ . *A*, family of currents (upper traces) evoked by hyperpolarizing voltage steps (lower traces) in control solution (153 mM  $[Na^+]_o$ , left), during reduced  $[Na^+]_o$  (26 mM, middle), and during recovery to control  $[Na^+]_o$  (153 mM, right). Pulse protocol as in Fig. 4. Note the reduced size and time course of activation in the steady-state currents, and the change in polarity of the de-activating tail currents in the low- $Na^+$  solution. *B*,  $I-V$  relationship obtained from data in *A*, with instantaneous currents ( $I_i$ ) plotted as circles and steady-state currents ( $I_{ss}$ ) plotted as triangles; open symbols represent control conditions and filled symbols reduced  $[Na^+]_o$ . Recordings in the same LGND neurone.

current at a potential of  $-50$  mV (Fig. 6*A* and *B*;  $n = 3$ ). Thus, the amplitude of  $I_h$  was significantly reduced in the low- $Cl^-$  solution, suggesting a blocking action of isethionate ions on  $I_h$ . Interestingly, the time course of activation of  $I_h$  appeared unchanged during substitution of  $[Cl^-]_o$ . Similar blocking effects of impermeable anion substitutes on anomalous rectifier currents have been reported in sino-atrial node cells (Yanagihara & Irisawa, 1980) and sensory ganglion neurones (Mayer & Westbrook, 1983).

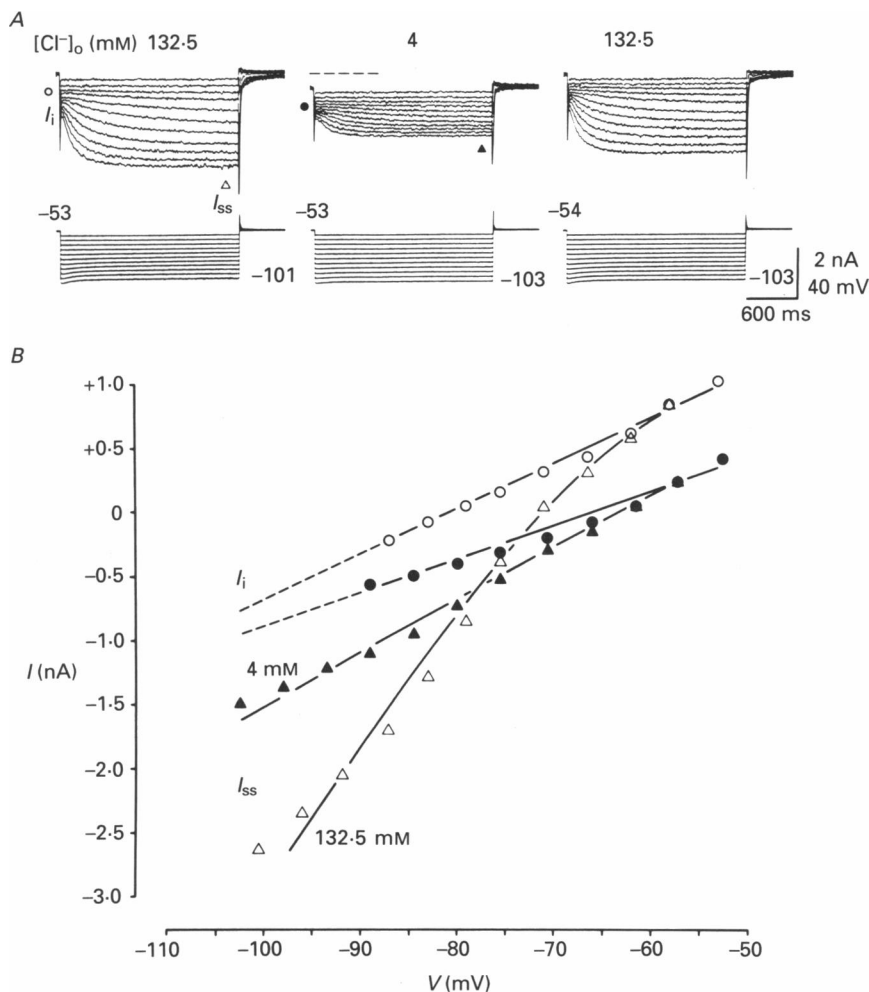


Fig. 6. Effects of changing the extracellular concentration of chloride ions,  $[Cl^-]_o$ , upon  $I_h$ . A, family of currents (upper traces) elicited by hyperpolarizing voltage commands (lower traces) in a single LGND neurone in control solution (132.5 mM  $[Cl^-]_o$ , left), during substitution of chloride ions by isethionate ions (4 mM  $[Cl^-]_o$ , middle; dashed line for comparison in baseline holding current), and during recovery in control solution (right). Pulse protocol as in Fig. 4. B,  $I-V$  relationship from experiment in A. Open symbols, control; filled symbols, reduced  $[Cl^-]_o$ . Low- $Cl^-$  solution substantially reduces  $I_h$ , i.e. the difference between instantaneous currents ( $I_i$ ) and steady-state currents ( $I_{ss}$ ).

We conclude that both  $Na^+$  and  $K^+$  ions carry the hyperpolarization-activated current,  $I_h$ , in thalamic neurones, and suggest that  $Cl^-$  ions do not contribute substantially to this inwardly rectifying process.

#### Sensitivity of $I_h$ to extracellular $Cs^+$ and $Ba^{2+}$

In many different cell types low concentrations of extracellular caesium ions ( $Cs^+$ ), but not barium ions ( $Ba^{2+}$ ), selectively block hyperpolarization-activated cation currents (Halliwell & Adams, 1982; Mayer & Westbrook, 1983; DiFrancesco, 1985;

Spain *et al.* 1987). In thalamocortical neurones, local (2–10 mM) or bath (1–2 mM) application of  $\text{Cs}^+$  resulted in a selective reduction of  $I_h$  (Fig. 7*A* and *B*;  $n = 12$ ). In contrast,  $\text{Cs}^+$  appeared to have no consistent effect on the instantaneous components of the current responses (Fig. 7*A* and *B*).

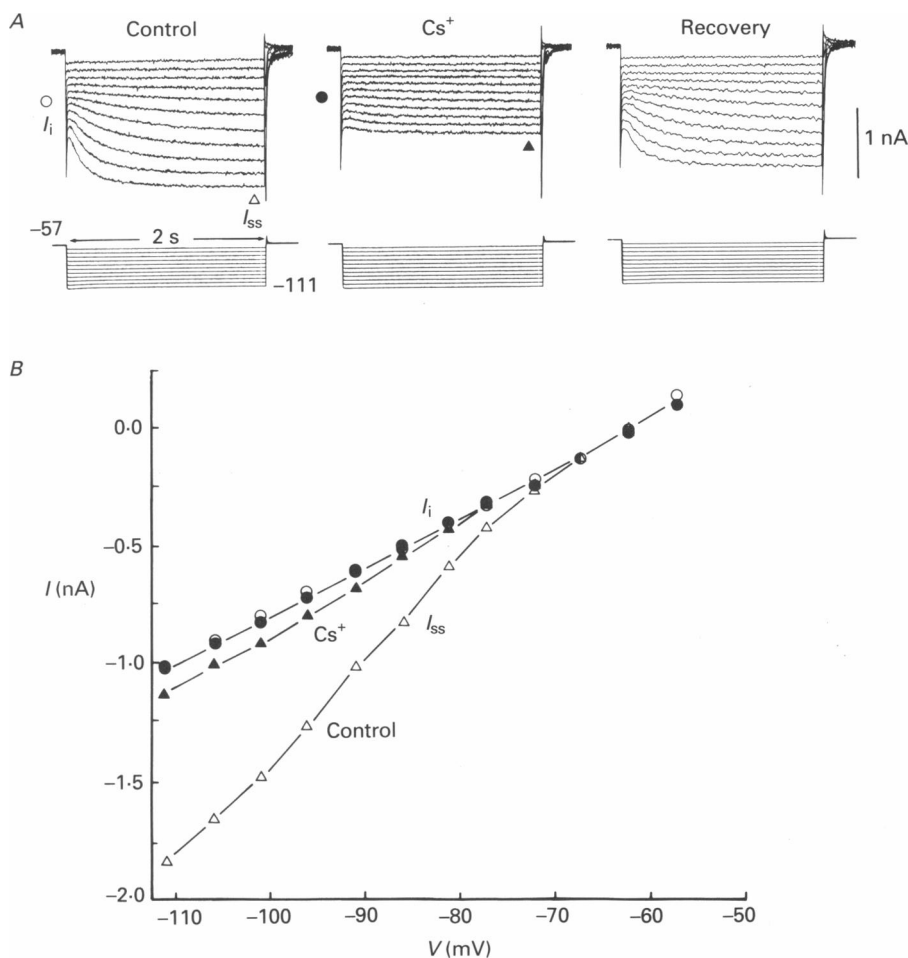


Fig. 7. Extracellular caesium blocks  $I_h$ . *A*, family of currents (upper traces) evoked by hyperpolarizing voltage steps (lower traces) under control conditions (left), after local application of caesium ( $\text{Cs}^+$ , 10 mM in micropipette; middle), and after recovery from  $\text{Cs}^+$  (right). Holding potential and most negative step potential are indicated near traces; step amplitude is 5 mV; step duration is 2 s. *B*,  $I$ - $V$  relationship obtained from experiment in *A*. Instantaneous current ( $I_i$ ) is plotted as circles; steady-state current ( $I_{ss}$ ) after 2 s is plotted as triangles. Open symbols represent currents under control condition; filled symbols are currents after application of  $\text{Cs}^+$ .  $\text{Cs}^+$  blocks the time- and voltage-dependent inward current with no effect on instantaneous current.

In current clamp recordings, local or bath applications of  $\text{Cs}^+$  resulted in a complete or near-complete block of the depolarizing 'sag' activated by hyperpolarization (e.g. Fig. 13*A*), confirming that this feature of electroresponsiveness is due to activation of  $I_h$ .

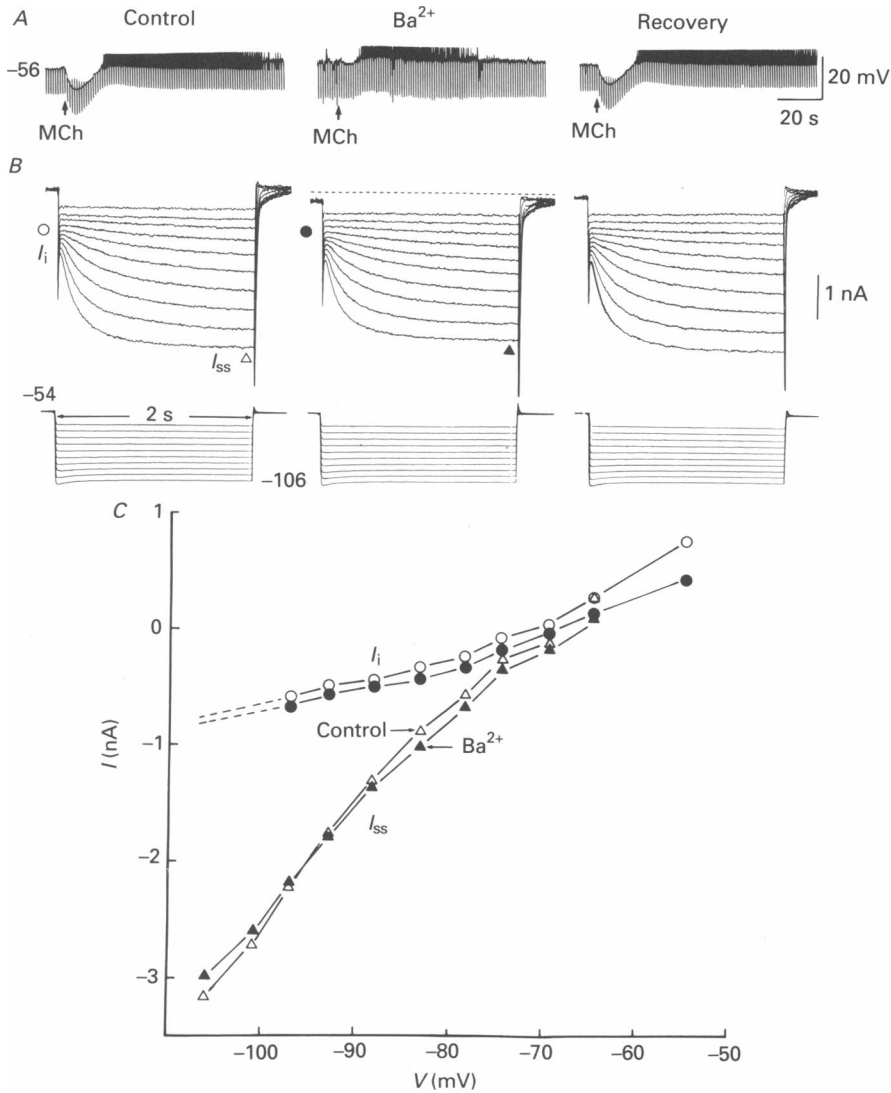


Fig. 8. Extracellular barium does not block  $I_h$ . *A*, hyperpolarizing–depolarizing response elicited by local application of the muscarinic-specific agonist acetyl- $\beta$ -methylcholine (MCh, 5 mM), due to increase and decrease of membrane potassium conductances (McCormick & Prince, 1987). The hyperpolarizing response is reversibly antagonized by local application of  $Ba^{2+}$  (1 mM). *B*, same cell as in *A*. Inward currents (upper traces) elicited by stepping from a holding potential of  $-54$  mV to  $-106$  mV in increments of 5 mV (lower traces).  $Ba^{2+}$  evokes a small steady inward current (indicated by dashed line) and reduction in instantaneous current ( $I_i$ ), but has very little, if any, effect on  $I_h$  (difference between  $I_{ss}$  and  $I_i$ ). *C*,  $I$ – $V$  relationship obtained from experiment in *B*. Open symbols represent control currents; filled symbols are currents during  $Ba^{2+}$ . Instantaneous current ( $I_i$ , circles) is slightly reduced by  $Ba^{2+}$ , whereas the steady-state current ( $I_{ss}$ , triangles) is relatively unaffected.

Extracellular application of  $\text{Ba}^{2+}$  either locally (1 mM) or in the bath (300–500  $\mu\text{M}$ ) to relay neurones held near firing threshold resulted in a small inward current, a reduction of instantaneous currents evoked by hyperpolarizing voltage pulses from about  $-50$  mV, and only mild suppressive effects on  $I_h$  (Fig. 8B and C;  $n = 4$ ).

In current clamp recordings,  $\text{Ba}^{2+}$  resulted in depolarization of the membrane, a decrease in apparent input conductance, and a potent and reversible block of the hyperpolarizing response elicited by the muscarinic agonist acetyl- $\beta$ -methylcholine (5 mM, local application; Fig. 8A), known to be due to an increase in membrane potassium conductance (McCormick & Prince, 1987). In contrast,  $\text{Ba}^{2+}$  had no consistent effect on the depolarizing 'sag' activated by hyperpolarization (not shown).

#### *Reversal potential*

Determination of the reversal potential of  $I_h$  was achieved through two different methods. First, under voltage clamp conditions, the membrane potential was stepped from approximately  $-95$  mV to potentials varying from  $-75$  to  $-35$  mV and the magnitude and polarity of the emerging tail currents during de-activation of  $I_h$  were measured. To minimize contamination of  $I_h$  tails by other currents, the cells were exposed to a number of channel blockers including  $\text{Ni}^{2+}$  (0.5 mM), 4-aminopyridine (0.5–1 mM), tetraethylammonium (10 mM),  $\text{Ba}^{2+}$  (1 mM) and tetrodotoxin (0.2  $\mu\text{M}$ ). In this manner,  $I_h$  tail currents reversed from inward to outward at approximately  $-43 \pm 6$  mV ( $n = 3$ ).

To corroborate this reversal potential, a second method was used which takes advantage of the finding that  $I_h$  is selectively and potently blocked by local application of  $\text{Cs}^+$  (see above). The neurone was voltage clamped well into the activation range of  $I_h$  (e.g.  $-90$  mV) and voltage steps to varying potentials were applied (Fig. 9A). The instantaneous current at the beginning of each voltage step was determined, and a  $I$ - $V$  relationship was constructed (Fig. 9B). Next,  $I_h$  was blocked through local application of  $\text{Cs}^+$  (5–10 mM) and instantaneous  $I$ - $V$  relationships were again determined. The extrapolated meeting point of the instantaneous  $I$ - $V$  curves with and without contribution of  $I_h$  corresponds to the reversal potential of  $I_h$ . In this manner, the reversal potential was determined to be  $-43 \pm 9$  mV ( $n = 8$ ), in agreement with the tail current analysis method above.

#### *Functional properties of $I_h$*

The ability of extracellular  $\text{Cs}^+$  to selectively block  $I_h$  was used to investigate the contribution of  $I_h$  to the electrophysiological behaviour of thalamic neurones. Using this protocol, we have found  $I_h$  to contribute to three different electrophysiological characteristics of thalamic neurones: determination of the resting membrane potential, the occurrence of rhythmic burst firing, and the appearance of an after-hyperpolarization after removal of a depolarization.

#### *Contribution of $I_h$ to resting membrane potential*

The resting membrane potential of thalamocortical relay neurones ranges from  $-62$  to  $-75$  mV which is within the activation range of  $I_h$  (see Fig. 2). Therefore,  $I_h$  may contribute significantly to the resting membrane properties of these neurones.

Indeed in current clamp recordings we found that local application of  $\text{Cs}^+$  (5–20 mM;  $n = 14$ ) while the cell was at rest resulted in a hyperpolarization of 5–10 mV (Figs 11–13) and a substantial increase in the amplitude of electrotonic responses induced by a hyperpolarizing current pulse (see Fig. 10A in McCormick & Pape, 1990).

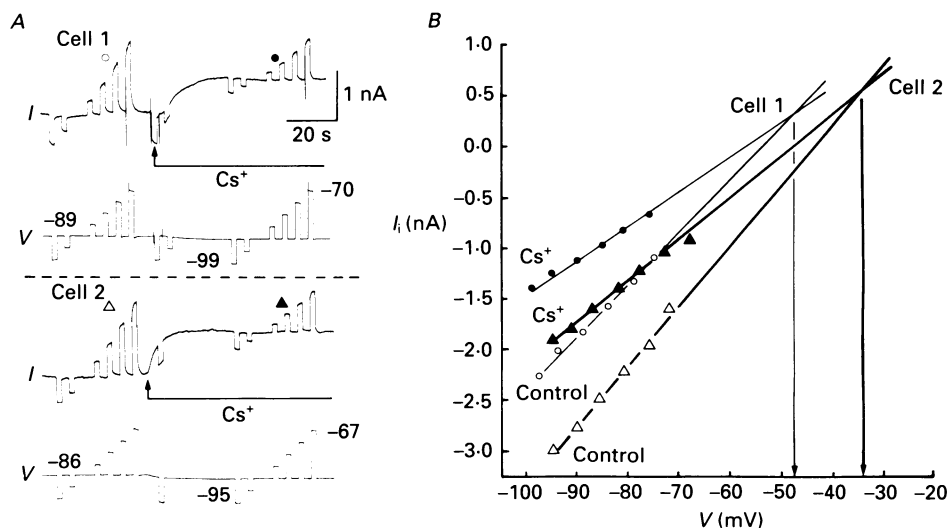


Fig. 9. Extrapolated reversal potential of  $I_h$ . *A*, inward and outward current relaxations (upper traces) upon hyperpolarizing and depolarizing voltage steps (lower traces) from two LGND cells, held at  $-89$  mV (cell 1) and  $-86$  mV (cell 2). Indicated near the voltage traces are the holding potential, the most positive and most negative step potentials. Step amplitude is 5 mV; step duration is 2 s. The slow inward and outward relaxations following the instantaneous currents represent activation and de-activation of  $I_h$ , respectively. Local application of caesium ( $\text{Cs}^+$ , 10 mM) results in a steady outward current and strongly reduced current relaxations, due to block of  $I_h$ . *B*, instantaneous  $I-V$  relationships obtained from the experiments shown in *A*. Cell 1 is plotted as circles, cell 2 as triangles. Open symbols represent instantaneous currents obtained under control conditions; filled symbols are instantaneous currents during  $\text{Cs}^+$ . Extrapolated intersection of instantaneous  $I-V$  curves with (control) and without ( $\text{Cs}^+$ ) contribution of  $I_h$  occurs at the reversal potential of  $I_h$ . Curves fitted by eye. See text for further details.

In voltage clamp, these local applications of  $\text{Cs}^+$  were associated with a selective and readily reversible reduction of  $I_h$  (see Fig. 7). These results indicate that  $I_h$  contributes substantially to determining the resting membrane potential of thalamocortical relay neurones.

#### Contribution of $I_h$ to rhythmic burst firing

Extracellular recordings in cat LGND (but not guinea-pig LGND) revealed that a sub-population of thalamic relay neurones spontaneously and rhythmically generates high-frequency (300–500 Hz) bursts of two to six action potentials with an interburst frequency of 1–2 Hz (Haby *et al.* 1988; McCormick & Prince, 1988). Intracellular recordings from oscillating cat LGND neurones revealed that the slow 1–2 Hz oscillation is characterized by the regular occurrence of low-threshold  $\text{Ca}^{2+}$  spikes

(Fig. 10) which appear to be separated by an after-hyperpolarization (AHP). The oscillation was not dependent upon the occurrence of fast  $\text{Na}^+$ - $\text{K}^+$  action potentials, since at certain membrane potentials, the rhythmic low-threshold  $\text{Ca}^{2+}$  spikes were subthreshold for the generation of these fast spikes (not shown). Depolarization or

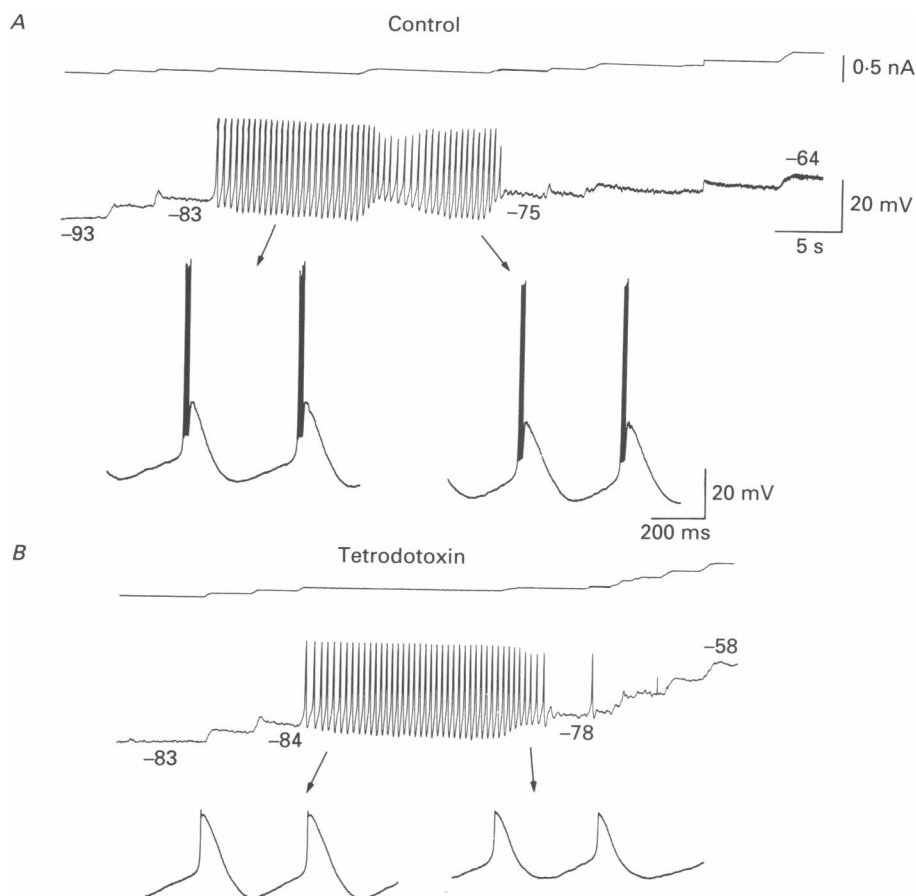


Fig. 10. Voltage dependence of 1–2 Hz oscillation in a cat LGND relay neurone. *A*, at membrane potentials between  $-93$  and  $-83$  mV, no oscillation or synaptic activity is seen. Slight depolarization of the neurone above  $-83$  mV elicits a burst which is followed by rhythmic burst generation (see expanded traces). Further depolarization above approximately  $-75$  mV abolishes rhythmic activity. *B*, local application of tetrodotoxin ( $10\text{ }\mu\text{M}$ ) completely blocks fast action potentials and synaptic activity, but does not abolish rhythmic occurrence of low-threshold  $\text{Ca}^{2+}$  spikes. Both recordings from a cat LGND neurone.

hyperpolarization of the membrane through current injection revealed a small range of membrane potentials between approximately  $-85$  and  $-70$  mV at which this type of rhythmic activity can occur, and a relative lack of membrane or synaptic events which might be triggering the 1–2 Hz bursting. These data suggest that the rhythmic burst activity is an intrinsic property of the thalamocortical relay cell that



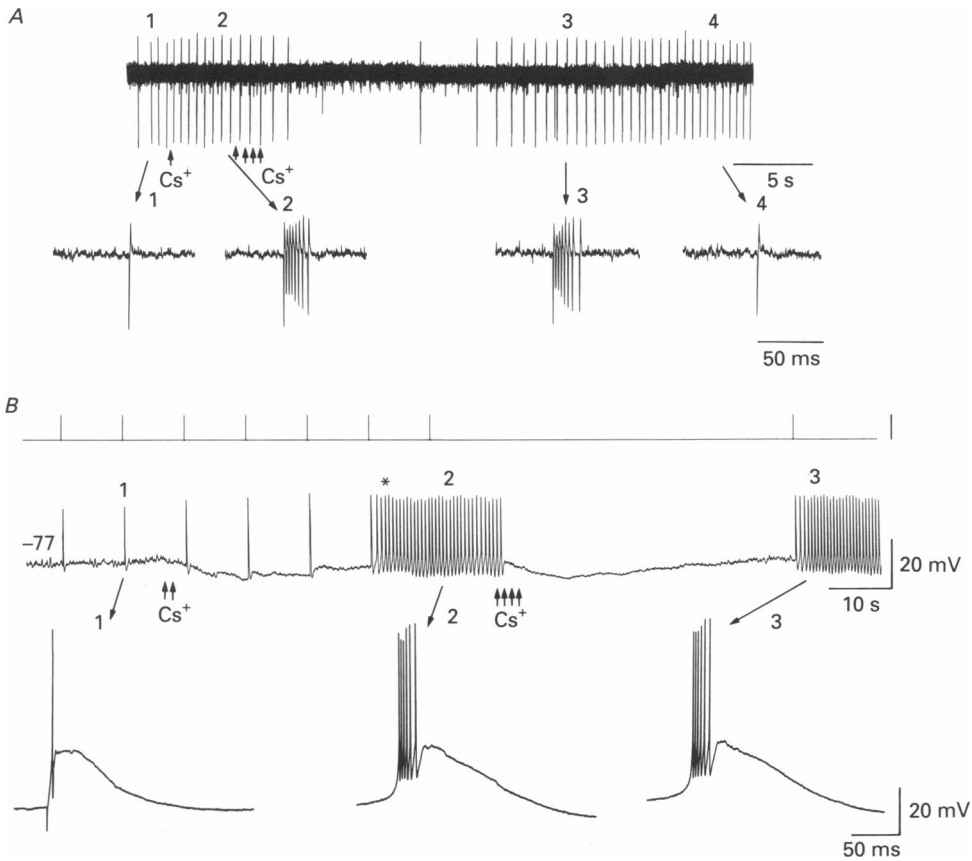


Fig. 11. Caesium enhances and blocks rhythmic burst firing in cat LGND neurones. *A*, extracellular recording of a spontaneously active LGND neurone. A single application of  $\text{Cs}^+$  enhances rhythmic activity from the rhythmic generation of a single spike (*A1*) to rhythmic occurrence of bursts of eight action potentials (*A2*). Further applications of  $\text{Cs}^+$  initially slows the rate at which bursts occur and finally abolishes all activity. Spontaneous activity slowly reappears with strong rhythmic bursts (*A3*) which gradually lessen back to single action potentials (*A4*). *B*, intracellular recording from a cat LGND neurone at  $-77$  mV. Intracellular injection of a short duration (3 ms) depolarizing current pulse triggers a low-threshold  $\text{Ca}^{2+}$  spike and a single fast action potential, followed by a small AHP (*B1*). Application of  $\text{Cs}^+$  results in a hyperpolarization and an enhancement of the burst, which now contains six fast action potentials. As the hyperpolarization lessens, rhythmic 1–2 Hz bursting activity can be triggered by the current pulse (asterisk). Further applications of  $\text{Cs}^+$  can reversibly block this spontaneous activity.

can only occur at membrane potentials where both the low-threshold  $\text{Ca}^{2+}$  current is available for activation (Jahnsen & Llinás, 1984*a, b*) and  $I_h$  is prevalent. The endogenous nature of this activity was confirmed by the finding that local application of tetrodotoxin ( $10 \mu\text{M}$ ) completely blocked fast action potentials and synaptic activity, but did not abolish the rhythmic oscillation (Fig. 10*B*). In addition, simultaneous extracellular recordings from two oscillating neurones with the same electrode revealed that each neurone exhibited its own particular frequency

of oscillation which was slightly different from that of its neighbour ( $n = 6$ ). Thus, the oscillatory activity in the two cells slowly drifted into and out of phase, as would be expected by two independent oscillators (not shown).

The presence of rhythmic oscillation only at membrane potentials within the range of  $I_h$  activation suggests an important contribution of this current. This hypothesis

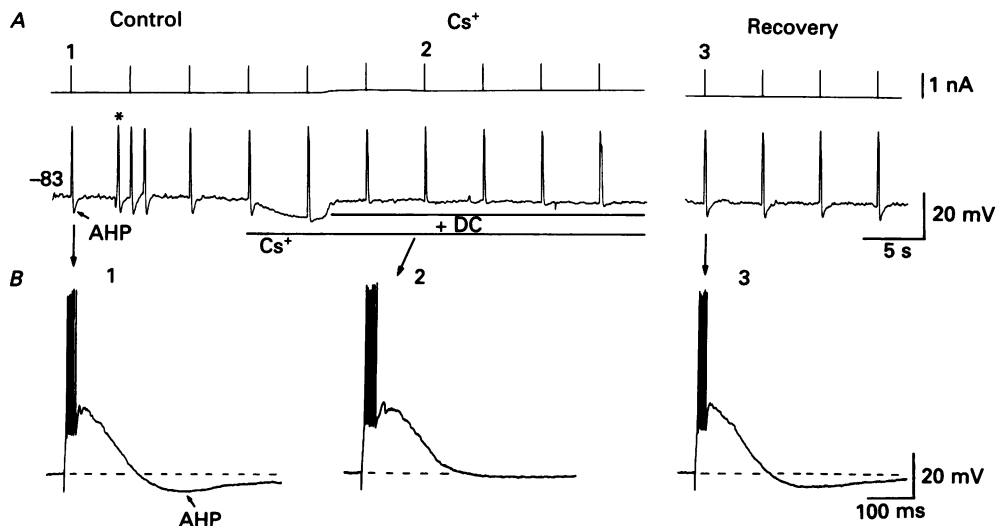


Fig. 12. Local application of  $\text{Cs}^+$  blocks the burst after-hyperpolarization (AHP). *A*, intracellular injection of a short duration (3 ms) depolarizing current pulse into a cat LGND neurone at  $-83$  mV results in a burst of six action potentials, followed by an AHP (*A1* and *B1*), which can occasionally trigger rhythmic activity (asterisk). Local application of  $\text{Cs}^+$  (10 mM) results in a hyperpolarization of the membrane potential and a reduction in the amplitude of the AHP. Compensation for the hyperpolarization with intracellular injection of current (+ DC) reveals that the AHP is greatly reduced or abolished (*A2* and *B2*). This effect is reversible (*A3* and *B3*).

was tested through the selective block of  $I_h$  with local application of  $\text{Cs}^+$  (5–10 mM). Interestingly, local application of  $\text{Cs}^+$  to spontaneously oscillating neurones resulted in two effects, depending upon the dose administered. With small applications (e.g. one 10–15  $\mu\text{l}$  microdrop placed on the surface of the slice within 50  $\mu\text{m}$  of the entry point of the recording electrode), the rhythmic burst firing was markedly enhanced in that the number of spikes generated per burst was increased (Fig. 11, compare *A1* and *A2*;  $n = 11$ ). Further applications of  $\text{Cs}^+$  resulted in further increases in the number of spikes per burst as well as a lengthening of the interburst interval which was generally followed by a complete and reversible cessation of activity (Fig. 11*A*). These results suggest that this pattern of rhythmic burst activity is strongly influenced by the presence and amplitude of  $I_h$ .

Intracellular recordings revealed that local application of small amounts of  $\text{Cs}^+$  enhanced the probability of obtaining rhythmic burst firing ( $n = 6$ ) while larger doses blocked this activity. For example, in the cell illustrated in Fig. 11*B*, injection of a short duration (3 ms) depolarizing current pulse activated a low-threshold  $\text{Ca}^{2+}$  spike and one fast action potential, but no oscillatory activity (Fig. 11*B1*). Local

application of  $\text{Cs}^+$  resulted in hyperpolarization of the membrane. As this lessened 1–2 Hz rhythmic burst firing appeared, each phase of which was associated with a low-threshold  $\text{Ca}^{2+}$  spike which generated a burst of fast action potentials and a pronounced AHP (Fig. 11*B2*). Further applications of  $\text{Cs}^+$  could disrupt this oscillation and replace it with a hyperpolarization. During repolarization, the rhythmic activity either spontaneously reappeared, or could be triggered by a single short depolarizing current pulse (Fig. 11*B3*).

These data suggest that the presence and characteristics of 1–2 Hz oscillation are strongly influenced by the presence and amplitude of  $I_h$ . One possible mechanism by which  $I_h$  may contribute to rhythmic burst firing is through the generation of a hyperpolarization following each burst. Indeed, generation of a single low-threshold  $\text{Ca}^{2+}$  spike was followed by the occurrence of a pronounced after-hyperpolarization (AHP; Fig. 12*A* and *B*), which could result in the occurrence of a brief period of rhythmic oscillation (Fig. 12*A*, asterisk). Application of small amounts of  $\text{Cs}^+$  resulted in hyperpolarization of the membrane and a reduction in the AHP even though the burst response was enhanced. Compensation for the membrane hyperpolarization with intracellular injection of current revealed the burst AHP to be strongly reduced or abolished (Fig. 12*A2* and *B2*). These results suggest that the burst AHP is generated by the de-activation of  $I_h$  during the burst and the subsequent activation of  $I_h$  after the burst. This hypothesis gives rise to a number of predictions. First, the AHP should not depend upon the occurrence of a low-threshold  $\text{Ca}^{2+}$  spike or fast action potentials, but rather should be present even after a passive depolarization. Second, the amplitude of the AHP should increase with increases in the duration of the depolarization. Third, the membrane conductance of the cell should increase as the AHP lessens, and fourth, the AHP should not occur at membrane potentials positive to the activation range of  $I_h$ .

All of these predictions were confirmed with intracellular recordings. Removal of a tonic depolarization which does not generate either a low-threshold  $\text{Ca}^{2+}$  spike or fast-action  $\text{Na}^+$ – $\text{K}^+$  action potentials is associated with the generation of an apparent slow AHP (Fig. 13*A* and *A1*). This apparent AHP increases in amplitude with increases in duration of the depolarization (Fig. 13*B*), and the rising phase of the AHP could activate a low-threshold  $\text{Ca}^{2+}$  spike (Fig. 13*B*). This ability of the slow AHP to activate a low-threshold  $\text{Ca}^{2+}$  spike only occurs at membrane potentials around  $-75$  mV (Fig. 13*C*) and the slow AHP all but disappears if the neurone is tonically depolarized above  $-60$  mV, even if a large number of action potentials are generated (Fig. 13*C*). In addition, injection of conductance test pulses during the generation of the slow AHP reveals that the membrane conductance is lowest at the peak of the hyperpolarization and slowly increases until the membrane potential once again stabilizes at the resting membrane potential (not shown). These properties suggest that this hyperpolarizing ‘overshoot’ is due to repolarization of the membrane through leakage channels which then shifts the membrane potential into a range in which  $I_h$  is active. Activation of  $I_h$  subsequently results in a steady depolarization which is both smooth and slow in time course. In this manner, the initial hyperpolarizing and subsequent depolarizing phase of the apparent after-hyperpolarization are due to a decreased and increased level of  $I_h$  activation, respectively.

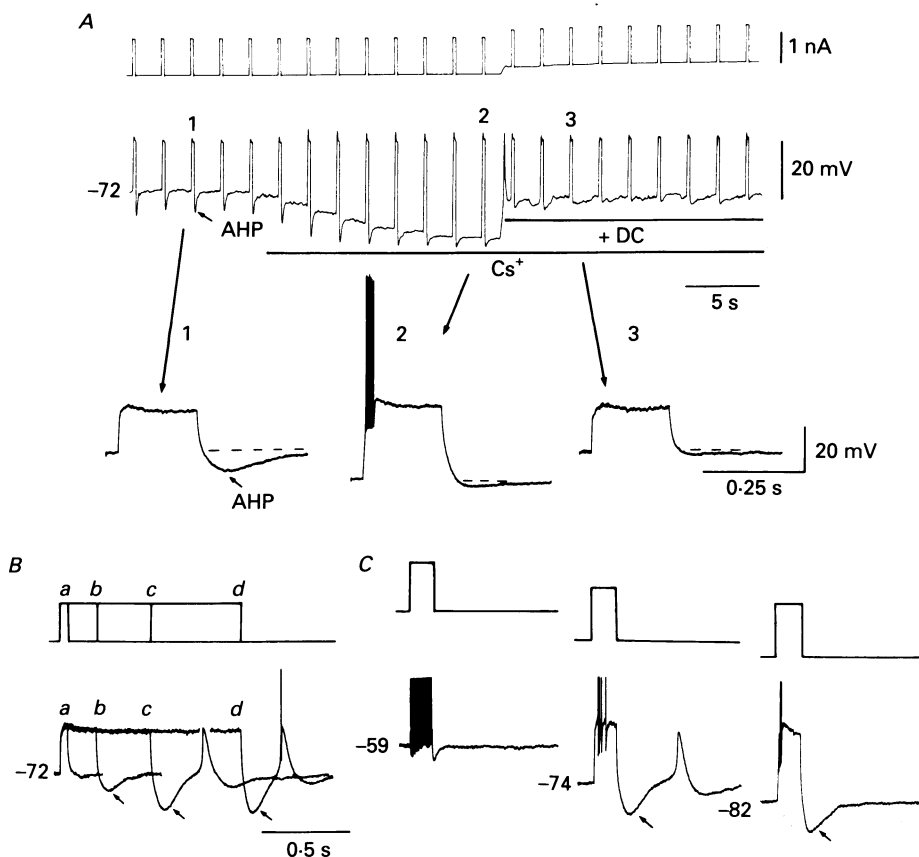


Fig. 13. Contribution of  $I_h$  to the resting potential and to the after-hyperpolarization (AHP). *A*, depolarization from normal resting potential ( $-72$  mV) by intracellular injection of a current pulse, which does not result in a low-threshold  $\text{Ca}^{2+}$  spike or the generation of action potentials, is followed by a substantial after-hyperpolarization (trace expanded in *A1*). Local application of  $\text{Cs}^+$  (20 mM in micropipette) results in a substantial hyperpolarization of the membrane, that de-inactivates the low-threshold  $\text{Ca}^{2+}$  spike, thereby activating a burst of fast action potentials (*A2*). Compensation for the hyperpolarization with the intracellular injection of current (+ DC) reveals that the AHP is nearly abolished (compare *A1* and *A3*). *B*, increasing the duration of the depolarizing current pulse results in a progressively larger AHP (arrows), that could generate a rebound low-threshold  $\text{Ca}^{2+}$  spike (*c* and *d*). *C*, voltage dependence of the AHP. A prolonged train of action potentials, elicited by a current pulse at  $-59$  mV, is followed by only a short duration AHP. Hyperpolarization of the neurone to  $-74$  mV results in the appearance of large AHP (arrow) even though the current pulse now causes only four action potentials. At this membrane potential, the AHP is associated with the generation of a rebound  $\text{Ca}^{2+}$  spike. Further hyperpolarization of the membrane to  $-82$  mV abolishes the ability of the AHP to trigger a  $\text{Ca}^{2+}$  spike. All data collected from the same cat LGND layer A relay neurone. Spike amplitudes are truncated. Current calibration in *A* for *A*–*C*; voltage calibration in *A3* to *A1*–*A3*, *B* and *C*. Time calibration in *B* for *B* and *C*.

These results indicate that  $I_h$  contributes substantially to the determination of the resting membrane potential, the generation of a slow after-hyperpolarization following a depolarization, and the occurrence of slow 1–2 Hz rhythmic burst firing (Fig. 14*B*). In this manner, thalamocortical relay cells display two firing modes. In

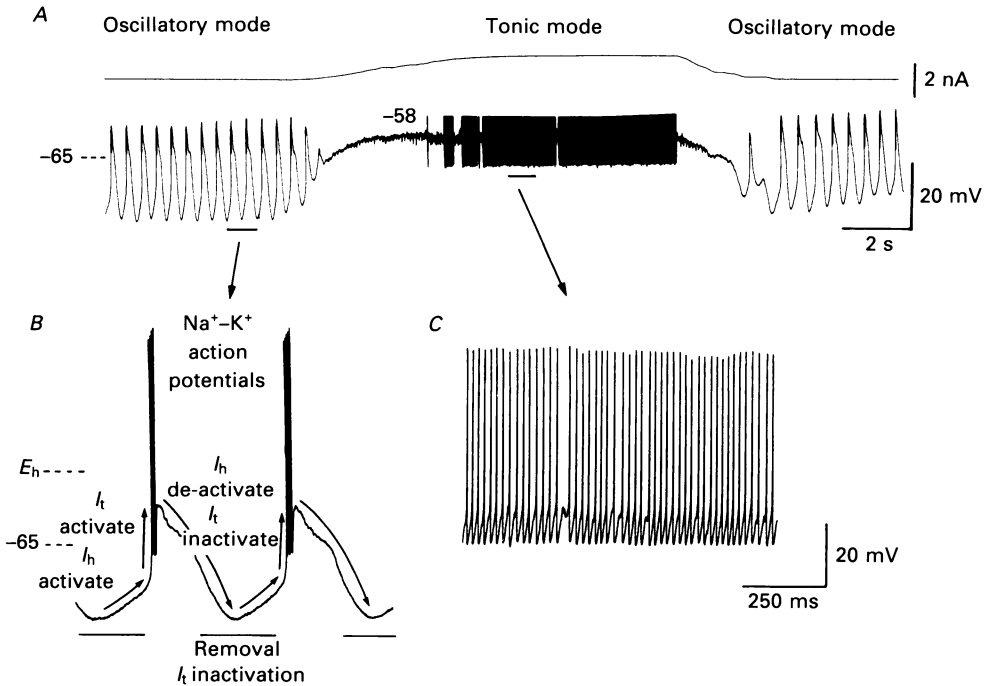


Fig. 14. Two different firing modes of thalamic relay neurones and the proposed ionic substrate of rhythmic burst firing. *A*, this cat LGND neurone generated rhythmic burst firing at a rate of about 2 Hz. Depolarization of the cell to  $-58$  mV with the intracellular injection of current (top trace) halted the rhythmic activity and switched the neurone to the tonic, or single-spike, mode of action potential generation. Removal of the depolarization reinstated the oscillatory activity. *B*, expanded trace of oscillatory activity and the proposed currents which largely mediate it. Activation of the low-threshold calcium current,  $I_t$ , depolarizes the membrane towards threshold for a burst of  $\text{Na}^+$ - and  $\text{K}^+$ -dependent fast action potentials. The depolarization de-activates the portion of  $I_h$  that was active immediately before the  $\text{Ca}^{2+}$  spike. Repolarization of the membrane due to  $I_t$  inactivation is followed by a hyperpolarizing overshoot, due to the reduced depolarizing effect of  $I_h$ . The hyperpolarization in turn de-inactivates  $I_t$  and activates  $I_h$ , which depolarizes the membrane towards threshold for another  $\text{Ca}^{2+}$  spike. *C*, expanded trace of single-spike activity.

the lack of synaptic inputs, these neurones may exhibit endogenous rhythmic burst firing (Fig. 14*A*). We suggest that this rhythmic burst firing arises largely from the interaction of two dominant currents,  $I_t$  and  $I_h$  (see Discussion). Upon tonic depolarization of the membrane, as occurs for example during awakening from sleep (Hirsch, Fourment & Marc, 1983), this rhythmic activity is abolished by moving the membrane potential out of the range in which  $I_t$  and  $I_h$  are active (Fig. 14*A*), and switches the neurone to the tonic, or single-spike, mode of action potential generation (Fig. 14*C*), that allows faithful transfer of synaptic information (Steriade & Llinás, 1988).

## DISCUSSION

*Inward rectification in thalamic neurones*

The present results demonstrate that thalamocortical relay neurones respond to hyperpolarization of the membrane with a time- and voltage-dependent inward rectification. The characteristics of the current,  $I_h$ , which underlies this rectification include permeability to both  $\text{Na}^+$  and  $\text{K}^+$  ions, a reversal potential ( $-43$  mV) substantially positive to normal resting potential, and a sensitivity to extracellular  $\text{Cs}^+$ . The exact contribution of  $\text{Cl}^-$  to  $I_h$  in thalamic neurones remains to be delineated.

Hyperpolarization-activated ionic conductances are a prevalent feature of electrically excitable cells, including frog skeletal muscle fibres, heart, photoreceptors, neurones in the peripheral and central nervous system, and some types of axons (Halliwell & Adams, 1982; Mayer & Westbrook, 1983; DiFrancesco, 1985; Crepel & Penit-Soria, 1986; Baker, Bostock, Grafe & Martius, 1987; Spain *et al.* 1987; Bobker & Williams, 1989; Eng *et al.* 1990). In general, two different types of hyperpolarization-activated inward currents have been distinguished. One is carried by  $\text{K}^+$  ions, is blocked by  $\text{Ba}^{2+}$  and  $\text{Cs}^+$  ions, has fast activation kinetics, and has an activation range which is dependent on the difference between membrane potential and potassium equilibrium potential (reviewed in Rudy, 1988). This hyperpolarization-activated  $\text{K}^+$  current has traditionally been referred to as the inward rectifier. The other type is permeable to both  $\text{Na}^+$  and  $\text{K}^+$  ions, is selectively blocked by extracellular  $\text{Cs}^+$  but not  $\text{Ba}^{2+}$ , has comparatively slow activation kinetics, and the activation range shows no dependence on the extracellular  $\text{K}^+$  concentration (Halliwell & Adams, 1982; Mayer & Westbrook, 1983). The characteristics of the inward rectifier of thalamocortical relay cells resemble those of the  $\text{Ba}^{2+}$ -insensitive conductance in terms of ion selectivity, block by extracellular  $\text{Cs}^+$  and activation kinetics. However, compared with the time- and voltage-dependent anomalous rectifier in hippocampal pyramidal cells (Halliwell & Adams, 1982), cerebellar Purkinje cells (Crepel & Penit-Soria, 1986), and cerebral cortical pyramidal cells (Spain *et al.* 1987) the corresponding current in thalamic neurones ( $I_h$ ) appears to possess the distinctive feature of a much slower time course of activation which depends strongly on voltage. These characteristics make the inward rectifier in thalamic neurones similar to inwardly rectifying conductances found in sensory and sympathetic ganglion cells (Mayer & Westbrook, 1983; Tokimasa & Akasu, 1990) and heart cells (DiFrancesco, 1985). The differences in time course of activation suggest that there may exist more than one type of hyperpolarization-activated, time-dependent cation current in neurones. These differences may reflect either the existence of molecularly distinct subclasses of ionic channels or they may result from post-translational modifications of one common type of ionic channel, although available data cannot distinguish between these and other possibilities.

*Functional role of  $I_h$* 

The exact role of  $I_h$  in the complicated electrophysiological behaviour of thalamic neurones *in vivo* is not yet known. However, comparing the results obtained from recent intracellular recordings of the activity of thalamic neurones during different

neuronal states *in vivo* (reviewed by Steriade & Deschênes, 1984; Steriade & Llinás, 1988) with the physiological characteristics of  $I_h$  reported here suggests some strong possibilities.

*Contribution of  $I_h$  to the resting membrane potential*

An important function of  $I_h$  in thalamic neurones is the contribution to the normal resting potential. The value of the membrane potential critically determines the excitability and pattern of action potentials which are generated by thalamocortical relay neurones (Fig. 14; Jahnsen & Llinás, 1984*a, b*). Patterns of activity range from spontaneous single-spike firing at membrane potentials positive to  $-55$  mV (Fig. 14*C*) to the rhythmic generation of burst discharges at more hyperpolarized levels (Fig. 14*B*). The normal resting potential of non-oscillating thalamic neurones of  $-60$  to  $-70$  mV is substantially positive to the presumed potassium equilibrium potential ( $-105$  mV, McCormick & Prince, 1987), indicating that depolarizing currents contribute substantially to determining the membrane potential, even in the absence of synaptic inputs. A significant contribution of the inward current  $I_h$  to the resting potential is indicated by the hyperpolarization of the membrane from rest during extracellular application of  $\text{Cs}^+$ . This hyperpolarization was in general large enough to move the cell into the burst mode of action potential generation (Fig. 13*A*). Thus, recruitment of  $I_h$  in these cells can be assumed to move the membrane potential from a region where the low-threshold  $\text{Ca}^{2+}$  current is de-inactivated (i.e. able to be activated) into a region closer, but still subthreshold, to single-spike firing and in which the low-threshold  $\text{Ca}^{2+}$  current is inactivated. Thus  $I_h$  may contribute to establishing a true 'resting' condition between burst activity and generation of single spikes. In addition, the voltage sensitivity of  $I_h$  and the transient potassium currents which are activated by depolarization (D. A. McCormick, unpublished observations) serve to stabilize the membrane potential by compensating for either depolarizing or hyperpolarizing influences. Depending on the functional state of the brain, stronger inputs can move the membrane potential out of the indifferent region into a region of repetitive single-spike firing that would allow faithful transmission of incoming synaptic information or into a region of rhythmic burst discharges (reviewed in Steriade & Llinás, 1988).

*Contribution of  $I_h$  to intrinsic thalamic oscillation*

During certain periods of sleep, or under the influence of barbiturate anaesthesia, thalamic neurones generate high-frequency (300–500 Hz) bursts of two to six action potentials (McCarley, Benoit & Barrionuevo, 1983; Steriade & Deschênes, 1984). These high-frequency bursts may occur in a rhythmic or non-rhythmic manner. The two main types of rhythmic activity which have been observed *in vivo* are spindle waves and 1–2 Hz rhythmic bursting (Lamarre, Filion & Cordeau, 1971; Steriade & Deschênes, 1984; Steriade, Deschênes, Domich & Mulle, 1985). Spindle waves appear as an 8–12 Hz oscillation in the electroencephalogram and local thalamic field potential which steadily increases and then decreases in amplitude over approximately a 2 s period (Steriade & Deschênes, 1984). Spindle waves appear to be generated entirely within the thalamus and arise from the interaction of the GABAergic neurones of the thalamic reticular nucleus amongst themselves and

with thalamocortical relay neurones. Intracellular recordings from thalamocortical relay neurones *in vivo* during spindle waves indicate that this activity results from the periodic occurrence of low-threshold  $\text{Ca}^{2+}$  spikes activated by the offset of rhythmically occurring IPSPs which presumably are induced by burst discharges occurring in the inhibitory neurones of the thalamic reticular nucleus (Steriade & Deschênes, 1984).

In contrast to spindle wave generation, slow rhythmic (1–2 Hz) burst firing appears to be an endogenous property of cat thalamocortical relay neurones (Haby *et al.* 1988; McCormick & Prince, 1988). This type of rhythmic activity has been reported to occur in some stages of sleep (Lamarre *et al.* 1971) and is markedly enhanced by isolation of thalamocortical relay nuclei either through surgical knife cuts *in vivo* (Steriade *et al.* 1985) or through the maintenance of thalamic slices *in vitro* (McCormick & Prince, 1988).

In both types of rhythmic activity, the membrane potential of thalamic relay neurones oscillates within the activation range of  $I_h$  (e.g. between  $-60$  and  $-85$  mV), although the contribution of  $I_h$  to these two types of activity is likely to be somewhat different. The slow 1–2 Hz oscillation appears to depend critically upon the presence and strength of  $I_h$  (Figs 11–13).  $I_h$  may contribute to slow rhythmic burst firing in three distinct ways: (1) by causing an after-hyperpolarization following the generation of a burst; (2) by determining the overall range of membrane potential within which the neurone oscillates; and (3) by affecting the input conductance of the cell and therefore determining the responsiveness of the membrane potential to fluctuations in current flow.

The properties of the after-hyperpolarization which occurs after a depolarization or after the generation of a burst indicate that  $I_h$  contributes substantially to this physiological feature of thalamic neurones (Figs 12 and 13). The AHP occurs in a voltage range which is widely overlapping with the activation range of  $I_h$ , it can be elicited by largely passive depolarizations, its amplitude increases with increases in duration of the depolarization, and it is readily and reversibly blocked by  $\text{Cs}^+$ . In other neuronal systems, an AHP which is similar in time course is associated with the activation of a  $\text{Ca}^{2+}$ -activated potassium current,  $I_{\text{AHP}}$  (Pennefather, Lancaster, Adams & Nicoll, 1985; Lancaster & Adams, 1986). However, this current appears to be small or absent in LGND relay neurones (D. A. McCormick, unpublished observations).

The interaction of  $I_h$  and the low-voltage-activated  $\text{Ca}^{2+}$  current,  $I_t$ , appears to determine the 1–2 Hz rhythmic activity that is intrinsic to thalamocortical relay neurones (Fig. 14). The occurrence of a low-threshold  $\text{Ca}^{2+}$  spike is associated with de-activation of  $I_h$ . As the  $\text{Ca}^{2+}$  spike declines, due to inactivation of  $I_t$ , the lessened depolarizing influence of  $I_h$  creates a hyperpolarizing overshoot (the initial phase of the slow AHP). This hyperpolarization subsequently results in two important effects: removal of inactivation of  $I_t$  and activation of  $I_h$  (Fig. 14B). Activation of  $I_h$  depolarizes the membrane potential towards the threshold for activation of  $I_t$  and subsequently promotes the generation of a low-threshold  $\text{Ca}^{2+}$  spike. The generation of an additional  $\text{Ca}^{2+}$  spike results in the activation of a burst of fast  $\text{Na}^+$ - $\text{K}^+$ -mediated action potentials, and so the cycle begins again.

Although the voltage-dependent properties of  $I_h$  and  $I_t$ , the behaviour of the AHP,



and the potent blocking ability of  $\text{Cs}^+$ , are all consistent with the proposed ionic substrate of slow rhythmic bursting, some additional points require further investigation. First, the rate of  $I_h$  de-activation (Fig. 3) appears slow compared with the duration of the  $\text{Ca}^{2+}$  spike (typically between 150 and 200 ms). However, one must take into account the strong voltage dependence of the rate of  $I_h$  de-activation (Fig. 3). For example, the time constant of  $I_h$  de-activation averages 347 ms at  $-55$  mV, the most positive voltage at which it could be determined. During a typical  $\text{Ca}^{2+}$  spike, that is elicited from about  $-80$  mV, the membrane depolarizes to between  $-50$  and  $-40$  mV. The resulting reduction of the time constant of  $I_h$  de-activation might allow a large portion of  $I_h$  to de-activate during the  $\text{Ca}^{2+}$  spike. In addition, the hyperpolarization-activated cation current,  $I_t$ , in sino-atrial cells of the heart has recently been shown to be strongly sensitive to the concentration of intracellular  $\text{Ca}^{2+}$  (Hagiwara & Irisawa, 1989). Assuming a similar sensitivity of  $I_h$  to  $[\text{Ca}^{2+}]_i$ , the occurrence of low-threshold  $\text{Ca}^{2+}$  spikes in thalamic neurones may enhance de-activation of  $I_h$  during depolarization and activation upon hyperpolarization, thereby increasing the amplitude of the after-hyperpolarization.

An additional point of consideration is that other voltage-dependent conductances might contribute to the 1–2 Hz rhythmic firing in thalamic neurones. For example, cardiac pacemaking, which is based to a large degree on the properties of a similar, hyperpolarization-activated cation current,  $I_t$  (reviewed by DiFrancesco, 1985, and Noble, 1985), is also strongly influenced by other ionic currents, such as a slow inward current ( $I_{\text{SI}}$ ) and the delayed rectifier current ( $I_{\text{K}}$ ) (see review by DiFrancesco & Noble, 1989). In thalamic neurones, the ‘classical’ delayed rectifier does not seem to contribute to the generation of rhythmic activity, since its activation threshold is not reached by the  $\text{Ca}^{2+}$  spike. However, the transient voltage-dependent potassium currents,  $I_{\text{A}}$  or  $I_{\text{D}}$  (Jahnsen & Llinás, 1984*a, b*; Storm, 1988), have voltage ranges of activation and inactivation which are largely overlapping with  $I_t$  (D. A. McCormick & H.-C. Pape, unpublished observations) and therefore may slow the approach to threshold for the  $\text{Ca}^{2+}$  spike. In addition, the persistent  $\text{Na}^+$  inward current (Jahnsen & Llinás, 1984*a, b*) could play a similar role to that demonstrated for the slow inward current in cardiac Purkinje cells. In conclusion, the present data reveal a critical role of  $I_h$  in slow thalamic oscillations. The possible influences of other ionic conductances, or ionic pumps, however, remain to be investigated in detail (e.g. see review by Noble *et al.* 1989).

The influence of  $I_h$  on spindle oscillations will differ to some extent from that on slow rhythmic burst firing due to the fact that spindle wave generation in relay cells relies upon the occurrence of rhythmic barrages of IPSPs (Steriade & Deschênes, 1984). The time- and voltage-dependent activation of  $I_h$  may contribute to spindle oscillations by influencing the amplitude and time course of these IPSPs. The amplitude of these IPSPs will be determined in part by the resting membrane conductance, which can be strongly influenced by  $I_h$ . Thus, increases in activation of  $I_h$  may be capable of decreasing the responsiveness of thalamocortical relay neurones to IPSPs (see McCormick & Pape, 1990). In addition, the activation of  $I_h$  by the hyperpolarization associated with IPSPs, particularly those mediated by increases in potassium conductance (Hirsch & Burnod, 1987; Crunelli, Haby, Jassik-Gerschenfeld, Leresche & Pirchio, 1988), will contribute to a faster repolarization

of the membrane, particularly during the repolarizing phase of the IPSP. This, in turn, may strongly influence the pattern of activity generated in response to these synaptic inputs.

In conclusion, activation and de-activation of  $I_h$  has important functional consequences in controlling the responsiveness of thalamic neurones to transient or sustained hyperpolarizations, in providing the pacemaking for slow oscillatory burst activity, and in contributing to the determination of the membrane potential during different states of neuronal function.

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