Journal of Physiology (1988), **399**, *pp.* 153–176
With 10 *text-figures*
Printed in Great Britain

Cl^-- AND K^+-DEPENDENT INHIBITORY POSTSYNAPTIC POTENTIALS EVOKED BY INTERNEURONES OF THE RAT LATERAL GENICULATE NUCLEUS

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(*Received 4 August 1987*)

SUMMARY

1. Hyperpolarizing potentials evoked by electrical stimulation of the optic tract were studied in projection cells of the rat dorsal lateral geniculate nucleus (LGN) *in vitro*. In the same cells the effects of γ-amino butyric acid (GABA), baclofen and acetylcholine (ACh) were also investigated.

2. In the majority of cells a short- (SHP) (34 ms) and a long-lasting (LHP) (240 ms) hyperpolarizing potential could be recorded in the presence and in the absence of a preceding EPSP. They were blocked by tetrodotoxin (1 μM) and were more sensitive than the monosynaptic EPSP to a low-Ca^{2+}–high-Mg^{2+} solution.

3. The SHP was associated with a marked decrease (75%) in input resistance, was blocked by bicuculline (1–100 μM) and its reversal potential (−67 mV) was dependent on the extracellular Cl^- concentration.

4. The LHP was associated with a smaller decrease (45%) in input resistance and its reversal potential (−76 mV) was dependent on the extracellular K^+ concentration. It was increased by bicuculline (100% at 50 μM) and nipecotic acid (30% at 10 μM), blocked by Ba^{2+} (1 mM), and unaffected by eserine (1–10 μM), neostigmine (1–10 μM) or by recording with EGTA-filled electrodes. In the presence of bicuculline, a *single* LHP was able to evoke, as a rebound response, a low-threshold Ca^{2+} spike that was, however, not followed by another LHP (or any other long-lasting hyperpolarization).

5. Ionophoretic applications of GABA evoked in the same cell a Cl^--dependent hyperpolarization (reversal potential: −65 mV) and/or depolarization, both of which were associated with a marked decrease (91%) in input resistance and abolished by bicuculline. GABA was also able to evoke a bicuculline-insensitive, K^+-dependent hyperpolarization that had a reversal potential of −75 mV and was associated with a smaller decrease (43%) in input resistance.

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6. Baclofen, applied by ionophoresis, pressure ejection or in the perfusion medium (1–100 μM), produced a hyperpolarization that had a reversal potential of −79 mV and was associated with a decrease (45%) in input resistance.

7. In the majority of cells (thirty-seven out of forty) ACh evoked a slow depolarization and only in three cells a hyperpolarization which had a reversal potential of −80 mV.

8. These results suggest that the SHP and LHP recorded in rat LGN projection cells following stimulation of the optic tract are both inhibitory postsynaptic potentials (IPSPs) originating from LGN interneurones: the SHP is a $GABA_A$ IPSP and the LHP is a late, K^+-dependent IPSP probably mediated by $GABA_B$ receptors and represents the main synaptic conductance underlying the long-lasting hyperpolarizations observed during the 5–6 Hz rhythmic oscillations of LGN and other thalamic projection cells.

INTRODUCTION

Inhibition is known to play a major role in the modulation of visual inputs performed by projection cells in the dorsal lateral geniculate nucleus (LGN) (Singer & Creutzfeldt, 1970; Sherman & Spear, 1982; Sillito & Kemp, 1983; Ahlsen, Lindstrom & Lo, 1985; Eysel, Pape & Van Schayck, 1987). The physiological basis of this inhibition is presumed to be γ-aminobutyric acid (GABA)-mediated inhibitory postsynaptic potentials (IPSPs) originating from LGN interneurones and from cells of the adjacent nucleus reticularis thalami (Guillery, 1966; Lieberman, 1973), both of which show a positive reaction product when treated with antibodies raised against glutamic acid decarboxylase and GABA (Montero & Scott, 1981; Ohara, Lieberman, Hunt & Wu, 1983; Ottersen & Störm-Mathisen, 1984; Montero & Singer, 1984, 1985; Gabbott, Somogyi, Stewart & Hamori, 1986).

IPSPs have also been implicated in the mechanism controlling rhythmic oscillations recorded in the LGN and other thalamic nuclei under particular physiological conditions (Purpura & Schofer, 1963; Andersen & Sears, 1964). Recently, an interplay mainly between Cl^--dependent IPSPs, Ca^{2+}-activated K^+ conductances and low-threshold Ca^{2+} spikes has been suggested as the mechanism underlying the rhythmic oscillations (5–6 Hz) observed in thalamic projection cells (Jahnsen & Llinás, 1984; Roy, Clerq, Steriade & Deschenes, 1984).

However, no direct evidence is available on the presence of such GABA-mediated IPSPs in the LGN, and in the rat the very existence of an inhibition mediated by LGN interneurones has been strongly questioned (Sumitomo, Nakamura & Iwana, 1976; but cf. Kelly, Godfraind & Maruyama, 1979). Moreover, the synaptic or intrinsic nature of the various conductances suggested to underlie the rhythmic oscillations of thalamic projection cells, as well as the presence of a strong Ca^{2+}-activated K^+ conductance, are still controversial.

Using an *in vitro* preparation of the rat LGN (Crunelli, Kelly, Leresche & Pirchio, 1987*a*, *b*) we report here that two hyperpolarizing potentials can be recorded in LGN projection cells following electrical stimulation of the optic tract. The present results strongly suggest that both hyperpolarizing potentials are synaptic in origin and mediated in a feed-forward manner by LGN interneurones: the first is a $GABA_A$ IPSP while the second, long-lasting IPSP is K^+ dependent, likely to be mediated by

$GABA_B$ receptors and to underlie the long-lasting hyperpolarizations present during the 5–6 Hz rhythmic oscillations of LGN projection cells. A preliminary report of some of these results has been published (Crunelli, Haby, Jassik-Gerschenfeld & Leresche, 1987*d*).

METHODS

The preparation and maintenance of LGN slices were similar to those described in previous papers (Crunelli *et al.* 1987*a*, *b*). Briefly, male Wistar rats (200 g) were decapitated and a block of tissue containing the LGN was separated from the rest of the brain by two cuts made parallel to the plane of the optic tract. Using a Vibroslice (Campden Instruments), slices of the LGN (300–400 μm thick) were then cut at room temperature, transferred to a recording chamber and perfused with a warmed (35 °C) continuously oxygenated (95% O_2, 5% CO_2) medium of composition (mM): NaCl, 134; KCl, 5; KH_2PO_4, 1·25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 16; and glucose, 10. In all these procedures maximal care was taken to avoid excessive mechanical damage to the final few millimetres of the optic tract. Intracellular glass microelectrodes (GC 120F, Clark Electronic Instruments) were filled with 1 M-potassium acetate, 3 M-potassium chloride or 0·2 M-potassium EGTA in 0·2 M-potassium acetate. A tungsten bipolar electrode was used to stimulate the optic tract (20–100 μs, 1–40 V). Potentials were recorded with an electrometer (WP Instrument, model 707) which was also used to inject current through the recording microelectrode.

An independently mounted multibarrel micropipette (4–6 μm tip diameter) was used to eject ionophoretically the following substances: GABA (0·5 M, pH 4·5), acetylcholine (ACh) (1 M, pH 4·5), baclofen (20 mM, pH 3) and bicuculline methiodide (10 mM in 200 mM-NaCl, pH 4·5). A barrel containing NaCl (1 M) was used for automatic current balancing, and retaining currents were applied to the individual barrels when necessary. Before measuring the response to one of these substances, the ionophoretic pipette was positioned 20–100 μm away from the recording electrode, lowered into the slice at roughly the same depth as the impaled neurone and then slowly advanced (5–10 μM steps) until the shortest possible delay in the onset of the response was achieved.

To halve the Cl^- concentration, 50% of the sodium chloride was replaced with equimolar sodium isethionate, and when the concentration of K^+ was changed, appropriate changes in the NaCl concentration were made. In the barium-containing solution KH_2PO_4 was omitted and $MgCl_2$ was substituted for $MgSO_4$. Drugs were obtained from the following sources: tetrodotoxin (TTX), atropine sulphate, eserine hemisulphate, neostigmine methylsulphate, nipecotic acid, Sigma; bicuculline methiodide, Pierce. Pirenzepine chloride was generously supplied by Boehringer Ingelheim, U.K. and The Boots Company, U.K. and (±) baclofen by Ciba Geigy, France.

Results were stored on a Racal FM 4D tape-recorder and later analysed with a PDP11/23 computer. Numerical results are all expressed in the text as mean ± S.E. of the mean.

RESULTS

The experiments described in this paper are based on 251 neurones of the dorsal LGN from which stable, long-lasting intracellular recordings were made. Their active and passive membrane properties were indistinguishable from those previously described for projection cells of the rat dorsal LGN *in vitro* (Crunelli *et al.* 1987*a*) (resting membrane potential: -62 ± 2 mV; input resistance: 69 ± 5 MΩ; membrane time constant: 21 ± 3 ms). To exclude completely the possibility that even a small portion of the nucleus reticularis could be present in our *in vitro* preparation, in a few experiments slices were prepared so that they contained only the most lateral $\frac{2}{3}$ of the LGN. The results obtained from thirty-four cells recorded in such slices were identical to those obtained in the normal slices.

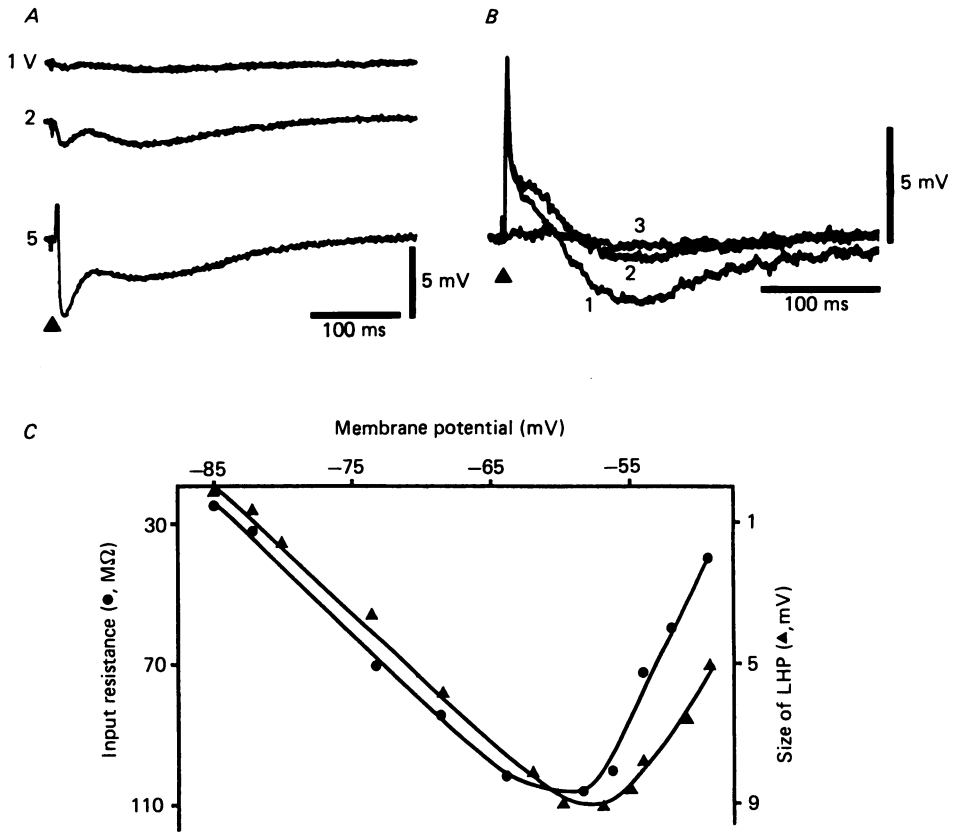

Fig. 1. *A*, intracellular voltage records show the short- (SHP) and long-lasting (LHP) hyperpolarizing potentials evoked in a rat LGN projection cell by electrical stimulation of the optic tract (▲). Using low intensities of stimulation (1 and 2 V) both the SHP and the LHP could be evoked in the absence of a preceding EPSP, while at higher intensities (5 V) the complete sequence of potentials (EPSP, SHP and LHP) was generated. The small depolarizing hump present between the SHP and the LHP is similar to the one observed *in vivo* (cf. Fig. 1 of Roy *et al.* 1984). In *B*, three superimposed traces show the effect of a solution containing low Ca^{2+} (0·2 mM) and high Mg^{2+} (7·8 mM) on the LHP (control solution is trace 1). Eight minutes after starting the perfusion with this solution the LHP was almost completely blocked, while the EPSP was only slightly reduced (trace 2). Following an additional 3 min no synaptic response to stimulation of the optic tract remained (trace 3). This cell was recorded in the presence of bicuculline (1 μM) to block the SHP and it is the same cell as in Fig. 8*D*. In this and in the following Figures arrow-heads indicate the stimulus artifact, unless mentioned otherwise. All traces in *A* and *B* were recorded at −60 mV. In *C*, the amplitude of the LHP (▲) evoked by stimulation of the optic tract and the input resistance of the same cell (●) (measured from hyperpolarizing electrotonic potentials while holding the cell at various membrane potentials) are plotted against the membrane potential. At potentials more positive and more negative than the resting membrane potential (−60 mV) the amplitude of the LHP decreased, closely following changes in the input resistance of the cell.

Physiological properties of the hyperpolarizing potentials

Electrical stimulation of the optic tract evoked a complex sequence of potentials in LGN projection cells (Fig. 1*A*, bottom trace). The first component of this response was the fast monosynaptic excitatory postsynaptic potential (EPSP) whose properties have been described previously (Crunelli *et al.* 1987*b*). In 183 cells, this EPSP was followed by two hyperpolarizing potentials which could be easily characterized, in the first instance, by their different latency to peak, duration and amplitude (Fig. 1*A*, bottom trace). Thus the first hyperpolarizing potential had a short latency to onset and peak and a short duration (short hyperpolarizing potential,

TABLE 1. Properties of the short- (SHP) and long-lasting (LHP) hyperpolarizing potentials

	SHP †	LHP ‡	LHP in bicuculline ‡
Latency to onset (ms)	3·6±0·3	—	38±1
Latency to peak (ms)	8·8±0·7	—	112±3
Duration (ms)	34±2	242±21	301±8*
50% decay time (ms)	4·5±0·5	67±8	72±3
Amplitude (mV) (range)	6·8±0·4 (0·7–10)	1·8±0·4 (0·5–3·5)	5·8±0·5** (3–18)

* $P < 0·05$, ** $P < 0·001$ compared to LHP in the absence of bicuculline (50 μM).
† Measured at −55 mV ($n = 22$).
‡ Measured at −60 mV ($n = 26$).

SHP), while the second one had a longer latency to onset and peak and a much longer duration (up to 240 ms) (long hyperpolarizing potential, LHP) (Fig. 1*A* and Table 1). The amplitude of both the SHP and LHP increased with higher stimulus intensities and the SHP could reach up to 10 mV (measured at −55 mV). In control solution the amplitude of the LHP ranged from 1 to 3 mV even at the maximal stimulation used, but it markedly increased in the presence of bicuculline (see below) and could be as high as 18 mV (Table 1). In an additional twenty-seven cells the SHP could be recorded in the absence of the LHP and in another forty-one cells the LHP could be observed in the absence of the SHP. Moreover by carefully decreasing either the intensity and/or the duration of stimulation, it was possible in almost every cell to evoke the SHP and/or the LHP in the absence of a preceding EPSP (Figs 1*A*, 2*B*, 3*A*, 5*A*, 6*E* and *F*). Both the SHP and LHP were blocked by tetrodotoxin (TTX) (1 μM) ($n = 15$) and were more sensitive than the optic tract EPSP to a solution containing low Ca^{2+} (0·2 mM) and high Mg^{2+} (7·8 mM) ($n = 10$). Thus at a time when the EPSP was only slightly decreased in the presence of this solution, the SHP and LHP could be observed to be almost abolished (Fig. 1*B*). It is worth mentioning at this point the remarkable similarity between the small depolarizing hump observed during the long-lasting hyperpolarization of the first cycle of oscillation recorded *in vivo* (cf. Fig. 1 of Roy *et al.* 1984) and the one present in our recordings between the SHP and the LHP (Figs 1*A* and 5). Since this depolarizing hump was observed only when the SHP was present, it is possible that it does not represent, as it has been suggested, a dendritic Ca^{2+} conductance (Roy *et al.* 1984), but that it is simply due to the summation of the decay phase of the SHP with the rising phase of the LHP.

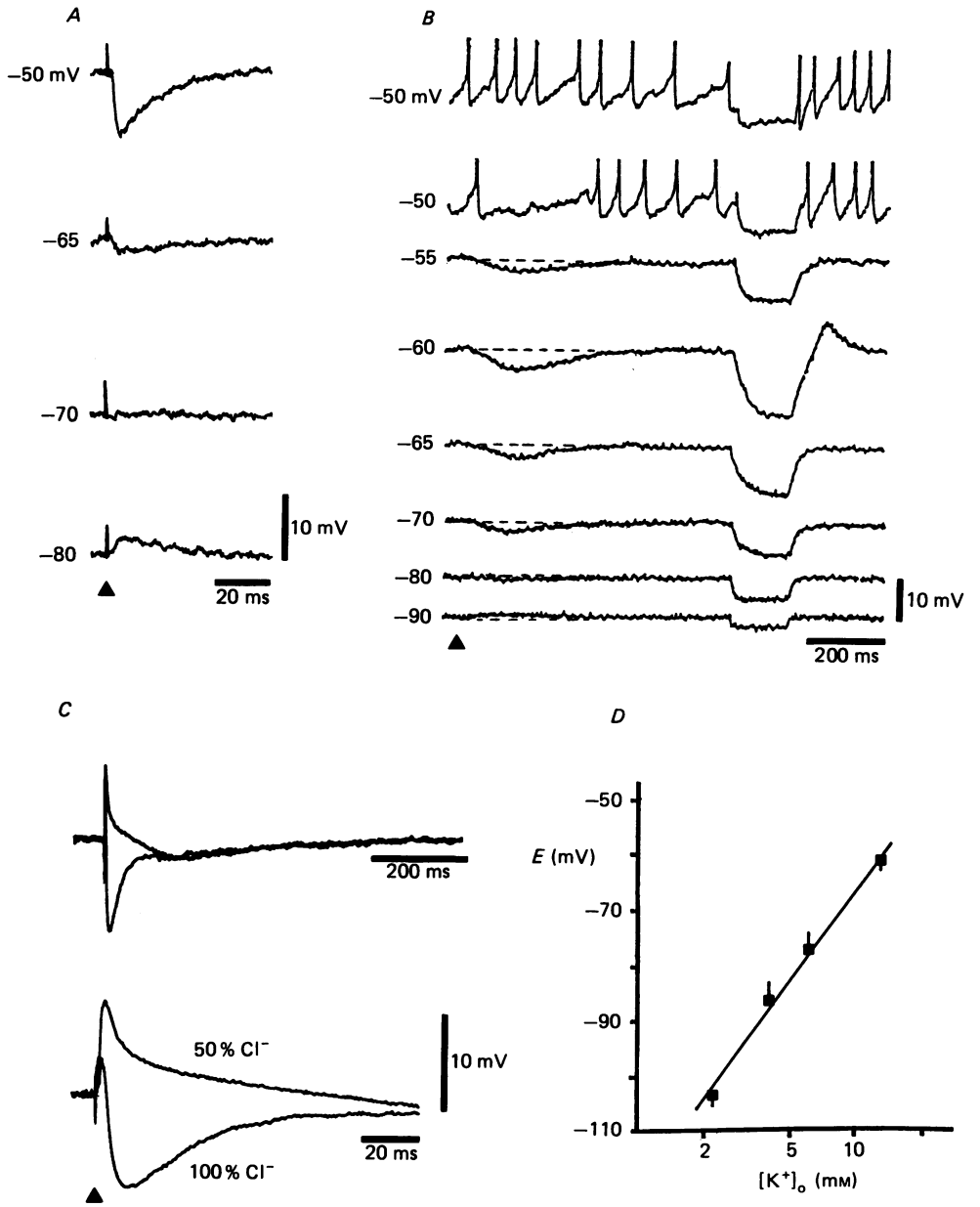

Fig. 2. The reversal potential and the ionic dependency of the SHP and LHP. In *A*, intracellular voltage records show the SHP evoked by stimulation of the optic tract at the four membrane potentials indicated on the left-hand side of each trace. The SHP clearly reversed in polarity at about −70 mV. In *B*, records from another cell (recorded in 4·25 mM-K^+) where the SHP was blocked by bicuculline (10 μM), show the reversal potential of the LHP. Hyperpolarizations from resting membrane potential (−60 mV) decreased the amplitude of the LHP which reversed in polarity at −85 mV. At membrane potentials positive to −60 mV, the amplitude of the LHP also decreased, partly as a consequence of the membrane rectification. (This could be seen by the decrease in the size of the hyperpolarizing electronic potentials obtained in response to a constant-current

Voltage–current plots performed on and off the SHP showed that it was associated with a marked decrease ($75 \pm 12\%$, $n = 18$) in the input resistance of the cell and that its amplitude was linearly related to the membrane potential, at least in the voltage range -45 to -75 mV. On the contrary, the LHP was associated with a smaller decrease ($45 \pm 7\%$, $n = 15$) in input resistance and its peak amplitude was non-linearly related to membrane potential (Fig. 1*C*). In fact it had a maximum in the voltage region close to resting membrane potential (-55 to -65 mV), while decreasing in size at more hyperpolarized and depolarized levels of membrane potential (Figs 1*C* and 2*B*). Although the LHP became smaller when the cell was depolarized, it was still able to decrease the probability of firing, as can be clearly seen by comparing the top two traces of Fig. 2*B* which were both recorded at -50 mV, one in the presence and one in the absence (top trace) of synaptic stimulation.

The reversal potential of the SHP (-67 ± 2 mV, $n = 18$) (Fig. 2*A*) was shifted towards more depolarized levels of membrane potential by recording with KCl-filled electrodes ($n = 5$) or when the extracellular Cl^- concentration was decreased by substituting 50% of NaCl with sodium isethionate (Fig. 2*C*) ($n = 7$). Probably due to the strong inward rectification present in the hyperpolarized region of LGN cells (Fig. 1*C*; cf. Crunelli *et al.* 1987*a*; Crunelli, Leresche & Parnavelas, 1987*c*), only in about 50% of the cells the LHP could be clearly reversed in polarity (Fig. 2*B*), while in the remaining cells (mostly those recorded in a low extracellular K^+ concentration) the reversal potential of the LHP had to be calculated by extrapolation from plots similar to the one in Fig. 1*C*. The reversal potential of the LHP (-76 ± 4 mV, $n = 24$) (Fig. 2*B*) was more negative than the one of the SHP and it closely followed changes in the extracellular K^+ concentration. As shown in Fig. 2*D*, a tenfold change in the K^+ concentration produced a 54 mV shift in the reversal potential of the LHP, a value which is similar to the one predicted by the Nernst equation for a simple K^+ conductance (60 mV). As mentioned before, the LHP became smaller at potentials more positive than the resting potential, but no reversal of the LHP could be observed in this region, at least up to -35 mV. Changes in the extracellular K^+ concentration had no effect on the reversal potential of the SHP, and similarly, a 50% decrease in the extracellular Cl^- concentration had no effect on the reversal potential of the LHP (-78 ± 5 mV, $n = 7$) (Fig. 2*C*).

Addition of 1 mM-Ba^{2+} to the perfusion medium abolished the LHP ($n = 5$) (Fig. 3*C*) and recordings performed with EGTA-filled microelectrodes ($n = 8$) had no effect on the peak amplitude and duration of the LHP at a time when the slow

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pulse of 0·2 nA). However, even at -50 mV the LHP, though small, was still able to reduce the firing probability of the cell, as can be seen by comparing this trace with the upper one that was still recorded at -50 mV but in the absence of optic tract stimulation. Both in *A* and *B* the intensity of stimulation was adjusted so that no EPSP was evoked and in *B* spikes have been truncated. In *C*, each pair of traces (recorded at -60 mV) show, at two different time bases, the effect of halving the Cl^- concentration on the SHP and LHP. The SHP changed from an hyperpolarizing to a depolarizing potential while the LHP was unaffected. In *D*, the reversal potential of the LHP (*E*) is plotted against the extracellular K^+ concentration $[K^+]_o$. The slope of the regression line ($r = 0{\cdot}96$) is similar to the one predicted by the Nernst equation for a simple K^+ conductance.

component of the spike after-hyperpolarization, suggested to be a Ca^{2+}-activated K^+ conductance (Jahnsen & Llinás, 1984), was abolished. In addition, the Ca^{2+} entry due to a low-threshold Ca^{2+} spike that followed an optic tract EPSP did not increase the amplitude of the LHP (Fig. 3*A* and *B*).

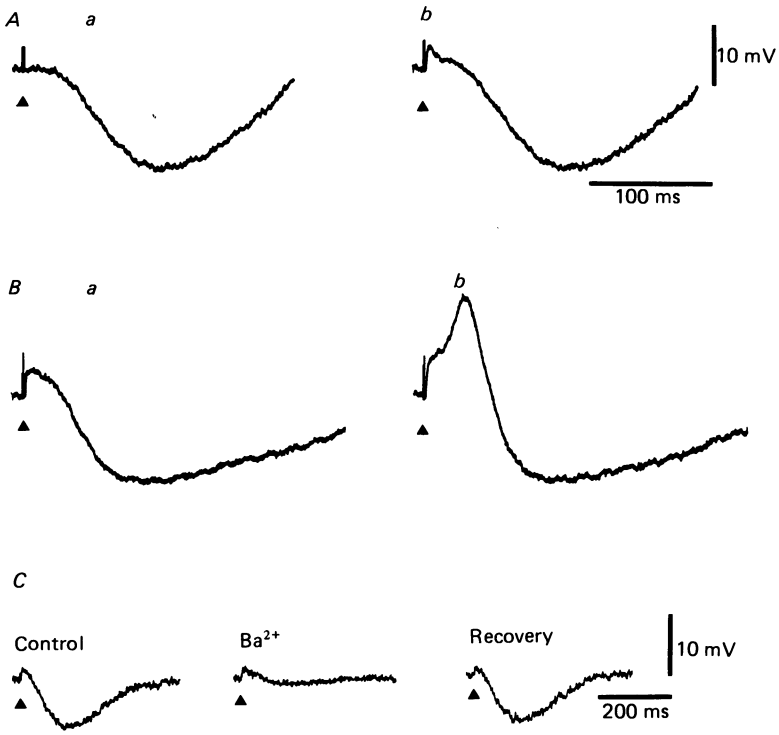

Fig. 3. The LHP is unaffected by a preceding EPSP and/or a low-threshold Ca^{2+} spike but is blocked by Ba^{2+}. *Aa* shows the LHP evoked by stimulation of the optic tract in the absence of an EPSP. By slightly increasing the intensity of stimulation a small EPSP could be generated (*Ab*), but almost no change was observed in the amplitude of the LHP. *Ba* shows the EPSP–LHP sequence recorded in another cell. A slight increase in the intensity of stimulation evoked a low-threshold Ca^{2+} spike at the peak of the EPSP, but the Ca^{2+} entry due to this potential only slightly increased the amplitude of the LHP (*Bb*). *C* shows the blockade of the LHP produced by 1 mM-Ba^{2+}. Perfusion of the slice with the Ba^{2+}-containing solution depolarized the cell by 8 mV. The membrane potential was then hyperpolarized to the control level (by steady DC current injection) and the LHP observed to be blocked. The recovery was obtained 25 min after switching back to the control solution. All experiments in this Figure were carried out in the presence of bicuculline (1 μM) to block the SHP, and calibrations in *A* also apply to the records in *B*. Traces in *A* and *C* were recorded at -60 mV, those in *B* at -65 mV.

Both SHP and LHP showed a constant decline in amplitude with increasing frequencies of stimulation. While the SHP decreased in amplitude only at frequencies higher than 0·3–0·5 Hz (Fig. 4*A*), the LHP was already smaller at frequencies higher than 0·1 Hz, reaching a maximum decrease of 25% at 0·25–0·5 Hz (Fig. 4*Ca*). In some cells the amplitude of the LHP could become smaller at frequencies of stimulation as low as 0·05 Hz.

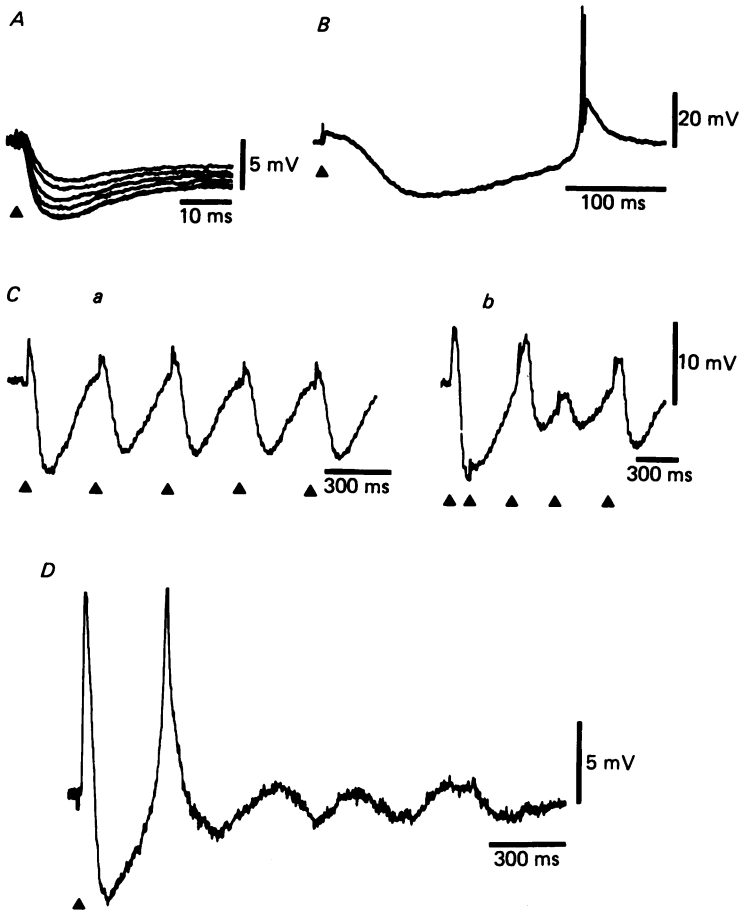

Fig. 4. In *A*, six superimposed traces show the decrease in the amplitude of the SHP at increasing frequencies of stimulation (from top to bottom: 3, 2, 1, 0·5, 0·35 and 0·25 Hz). Note how no decrease could be observed at the two lowest frequencies (traces obtained at 0·35 and 0·25 Hz are identical). In *B*, stimulation of the optic tract evoked a small EPSP followed by an 18 mV LHP. On return of the membrane potential to its resting level a low-threshold Ca^{2+} spike with a burst of Na^{+} action potential was generated. However, no LHP (or any other long-lasting hyperpolarization) followed the low-threshold Ca^{2+} spike, a result similar to that obtained when a low-threshold Ca^{2+} spike is evoked by a hyperpolarizing electrotonic potential (cf. Jahnsen & Llinás, 1984; Crunelli *et al.* 1987*a*, *c*). In *C*, rhythmic responses could be obtained by stimulating the optic tract at 3·4 Hz so that after each LHP the stimulus was in time with the return of the voltage to its resting value. Note how the first LHP is bigger than the following ones, all of which have a similar amplitude. When a stimulus was delivered at the peak of the LHP (*Cb*, second stimulus) only a small EPSP and LHP were evoked, while the return of the voltage to its resting value was delayed by about 200 ms (cf. Fig. 5 of Andersen & Sears, 1964). In *D*, a chart record from another cell shows small rhythmic oscillations following a low-threshold Ca^{2+} spike evoked by the LHP. All experiments in this Figure (except those in *A*) were carried out in the presence of bicuculline (50 μM). Traces in *A* were recorded at -55 mV, those in *B*, *C* and *D* at -60 mV.

In the presence of bicuculline a *single* shock delivered to the optic tract was able to evoke a LHP of large enough amplitude to evoke a low-threshold Ca^{2+} spike as a rebound response. In some cells this could reach threshold for the fast Na^{+}-dependent action potentials (Fig. 4*B*), thus generating a single, full cycle of oscillation similar to the one described in the LGN and other thalamic nuclei *in vivo* (cf. Fig. 5 of Andersen & Sears, 1964; Fig. 1 of Roy *et al.* 1984; Fourment, Hirsch & Marc, 1985).

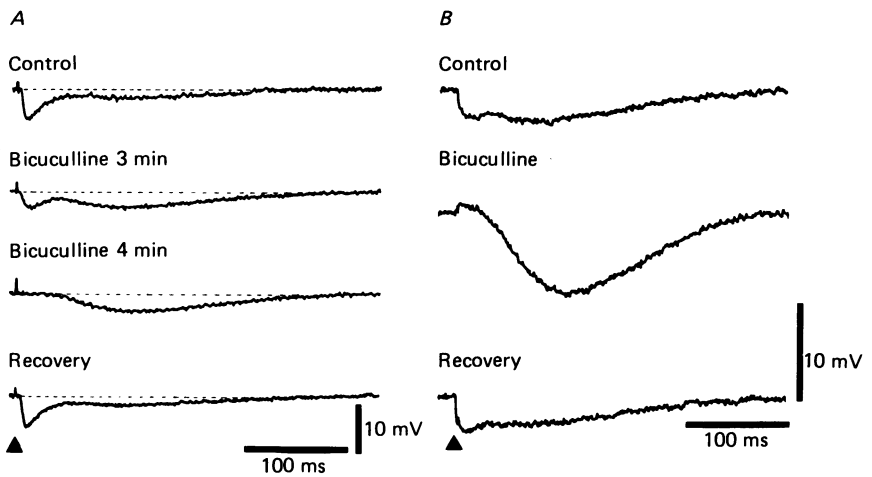

Fig. 5. The effect of bicuculline on the SHP and LHP. Bicuculline applied in the perfusion medium (10 μM) (*A*) abolished the SHP and increased the amplitude and the duration of the LHP. Ionophoretic application of bicuculline (80 nA, 2 s) (*B*) markedly increased the amplitude of the LHP, but it did not, in this cell, prolong its duration. In both *A* and *B* the intensity of stimulation was adjusted so that in the control experiments no EPSP could be evoked although in *B* the block of the SHP revealed a clear, though small, EPSP preceding the LHP. Note the small depolarizing hump present in the control records of *A* and *B*, and when the SHP was not completely blocked (Bicuculline 3 min) (cf. Fig. 1*A*). Records in *A* were obtained at -55 mV, those in *B* at -60 mV.

In contrast to thalamic projection cells recorded *in vivo*, the low-threshold Ca^{2+} spike was not followed by another LHP or any other long-lasting hyperpolarization (Fig. 4*B*). However, if after each LHP another stimulus was delivered close to the point where the membrane potential returned to its resting value, a series of rhythmic responses similar to those obtained *in vivo* after a single shock (Andersen & Sears, 1964; Roy *et al.* 1984) could be observed (Fig. 4*Ca*). If the stimulus occurred at the peak or along the decay phase of the LHP, the whole cycle of oscillation was prolonged (Fig. 4*Cb*), an observation similar to that made *in vivo* (cf. Fig. 5 of Andersen & Sears, 1964). Finally it should be mentioned that in four cells a series of very small membrane oscillations (2–3 mV) followed the low-threshold Ca^{2+} spike evoked by a single LHP (Fig. 4*D*).

Pharmacological properties of the hyperpolarizing potentials

Bicuculline (1–100 μM) as well as picrotoxin (50 μM) (not shown) reversibly blocked the SHP without any change in the passive membrane properties of the cells ($n = 35$) (Fig. 5*A*). The amplitude and the duration of the LHP were strongly

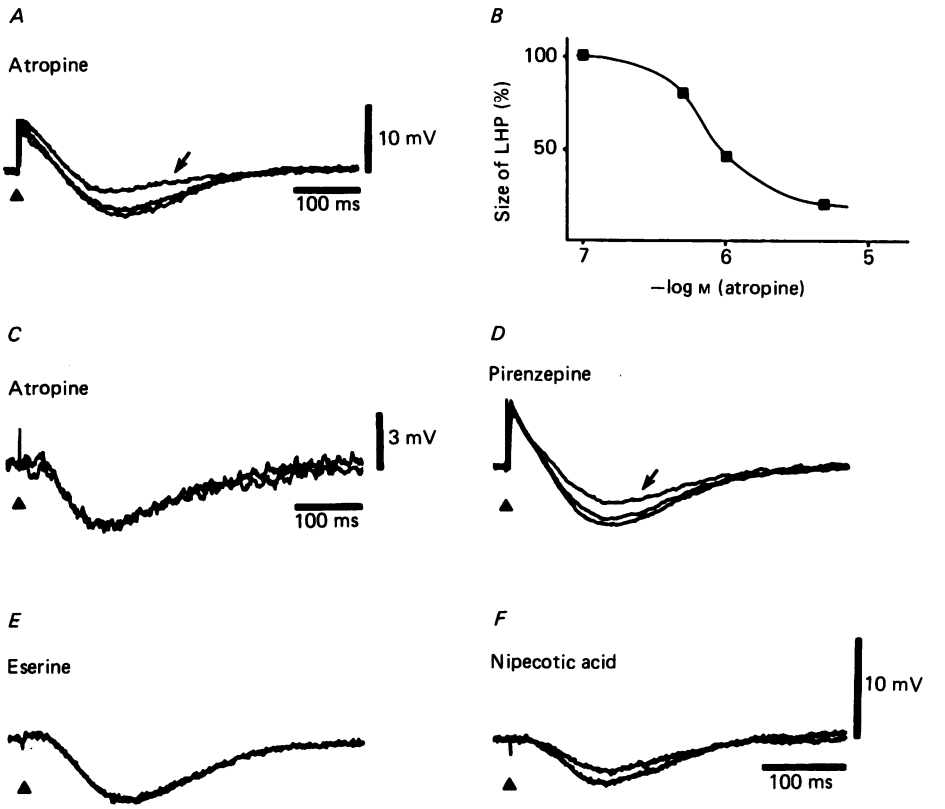

Fig. 6. The effect of cholinergic drugs and nipecotic acid on the LHP (all records, except those in *C*, obtained in the presence of bicuculline, 50 μM). In *A*, three superimposed traces, obtained in control solution, during perfusion with atropine (↙) (5 μM) and during the recovery period, show the reversible decrease (70%) in the amplitude of the LHP produced by this muscarinic antagonist. Data from this cell and seven other cells (where two or more concentrations of atropine could be tested in the same cell) were averaged to construct the dose–response curve shown in *B*. From this plot the concentration of atropine which inhibited the LHP by 50% was calculated as 0·8 μM. In *C*, two superimposed traces, one obtained in control solution, the other in the presence of atropine (10 μM), show the lack of effect of the muscarinic antagonist on the LHP in the absence of bicuculline. In *D*, three superimposed traces, obtained in control solution, during perfusion with pirenzepine (↙) (0·3 μM) and during the recovery period, show the reversible decrease (50%) in the amplitude of the LHP produced by this muscarinic antagonist. In *E*, two superimposed traces, one obtained in control solution, the other in the presence of 1 μM-eserine, show the lack of effect of this cholinesterase inhibitor on the LHP. In *F*, two superimposed traces, one obtained in control solution the other in the presence of nipecotic acid (10 μM), show the increase (40%) in the amplitude of the LHP produced by this GABA uptake blocker. Calibrations in *F* also apply to the records in *D* and *E*, and all traces were recorded at −65 mV.

increased in the presence of these substances and no change was observed in the latency to peak (Fig. 5*A* and Table 1). Although the time course of this effect was remarkably similar to that of the block of the SHP, in some cells the amplitude of the LHP still continued to increase for a few minutes after a complete block of the

SHP had occurred. Bicuculline applied by ionophoresis (20–180 nA, 5–60 s, $n = 8$) produced effects on the SHP and LHP that were identical to those described above. However, in three cells an increase only in the amplitude of the LHP, but not in its duration, was observed (Fig. 5*B*). Nipecotic acid (1–10 μM), a GABA uptake blocker, was able to increase (30 ± 7 %, $n = 7$) the amplitude of the LHP only in the presence of bicuculline (Fig. 6*F*).

Since ACh has recently been shown to produce a muscarinic hyperpolarization associated with an increase in K^+ conductance in cells of the nucleus reticularis and other thalamic nuclei (McCormick & Prince, 1986, 1987), we also examined the effect of atropine, pirenzepine and cholinesterase inhibitors on the LHP. In fifteen cells, in the presence of bicuculline, atropine reversibly inhibited the LHP with an IC_{50} of 0·8 μM and had no effect on the passive membrane properties of the cells (Fig. 6*A* and *B*). However, in six neurones where the action of atropine was tested in the absence of bicuculline, this muscarinic antagonist was unable to inhibit the LHP (Fig. 6*C*). Pirenzepine, when applied at a concentration of 0·3 μM (presumed to be selective for M_1 receptors, Hammer & Giachetti, 1984), inhibited the LHP by 80 ± 13 % ($n = 4$) (Fig. 6*D*), while eserine (1–10 μM, $n = 8$) (Fig. 6*E*) and neostigmine (1–10 μM, $n = 3$) (not shown) had no effect both in the presence and in the absence of bicuculline.

The action of GABA, baclofen and ACh

The reversal potential, the Cl^- dependency and the blockade by bicuculline strongly suggested that the SHP was a $GABA_A$-mediated IPSP. To confirm this possibility and in an attempt to indentify the transmitter responsible for the LHP, the effect of GABA, baclofen and ACh on LGN projection cells was examined.

GABA applied by ionophoresis was effective on every cell ($n = 43$). The type of response observed was very much dependent on the intensity of the ejection current and on the relative position of the ionophoretic and the recording electrode (Fig. 8*A*). Using small ejection currents (5–50 nA) and/or with careful positioning of the ionophoretic pipette some 20–50 μm away from the impaled cell, GABA produced a hyperpolarization which was rather fast in onset and decay and was associated with a marked decrease in input resistance (91 ± 5 %) (Figs 7 and 8*A*). The reversal potential of this GABA effect (-65 ± 2 mV, $n = 16$) (Fig. 7) was shifted to more positive potentials by decreasing the extracellular Cl^- concentration. With higher ejection currents (and whatever distance between the ionophoretic and the recording electrode) GABA invariably produced a depolarization that was also associated with a massive decrease in input resistance (89 ± 7 %) (Fig. 8*A*). We did not measure the reversal potential of this depolarization directly, but by extrapolation it was calculated to be around -45 mV. With high ejection currents both responses could often be observed, with the hyperpolarization generally preceding the depolarization (Fig. 8*A*). Both the hyperpolarization and the depolarization evoked by GABA could be reversibly blocked by bicuculline (and picrotoxin) applied by ionophoresis (Fig. 9*Ca*) or in the perfusion medium.

In the presence of bicuculline (10–100 μM), GABA could, with some difficulty, also be shown to produce a hyperpolarization that had different properties from the one described above ($n = 12$). In fact, it markedly outlasted the duration of the ionophoretic application, was associated with a smaller decrease in input resistance

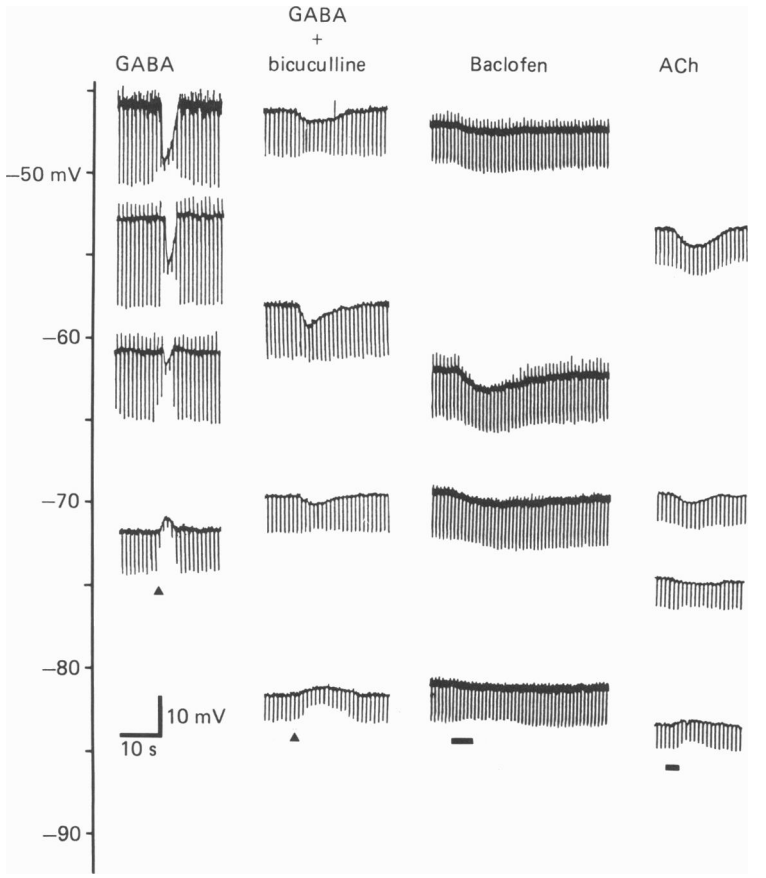

Fig. 7. The reversal potential of the hyperpolarizing responses of LGN projection cells to GABA, baclofen and ACh. The fast, Cl^--dependent hyperpolarization evoked by GABA reversed in polarity at −68 mV. In the presence of bicuculline (100 μM) GABA produced a hyperpolarization that was slower in onset and much longer in duration, and that reversed at −77 mV. The duration of the ionophoretic ejection of GABA (arrow-heads) was 500 ms in both cases. Baclofen evoked a hyperpolarization that was slow in onset, rather long in duration, and that was associated with a 25% decrease in the input resistance of the cell. In this cell, the baclofen-induced hyperpolarization could not be reversed but it only flattened out at about −80 mV. Note how the responses to baclofen and GABA (in the presence of bicuculline) were smaller at −50 mV than when recorded at −60 mV. The hyperpolarization evoked by ACh was associated with a small decrease in input resistance and reversed in polarity at −81 mV. Note that the hyperpolarizing response to ACh was only obtained in three out of forty cells. Bars below the ACh and baclofen responses indicate the duration of the ionophoretic ejection and downward deflections in all traces represent hyperpolarizing electrotonic potentials obtained in response to a constant pulse of current.

(43±9%) and its reversal potential (−75±4 mV, $n = 14$) was K^+ (but not Cl^-) dependent (Figs 7 and 8*B*). This bicuculline-insensitive, K^+-dependent hyperpolarization evoked by GABA showed a maximum amplitude in the voltage region close to resting membrane potential, while decreasing at more hyperpolarized and depolarized levels of membrane potential (Fig. 7).

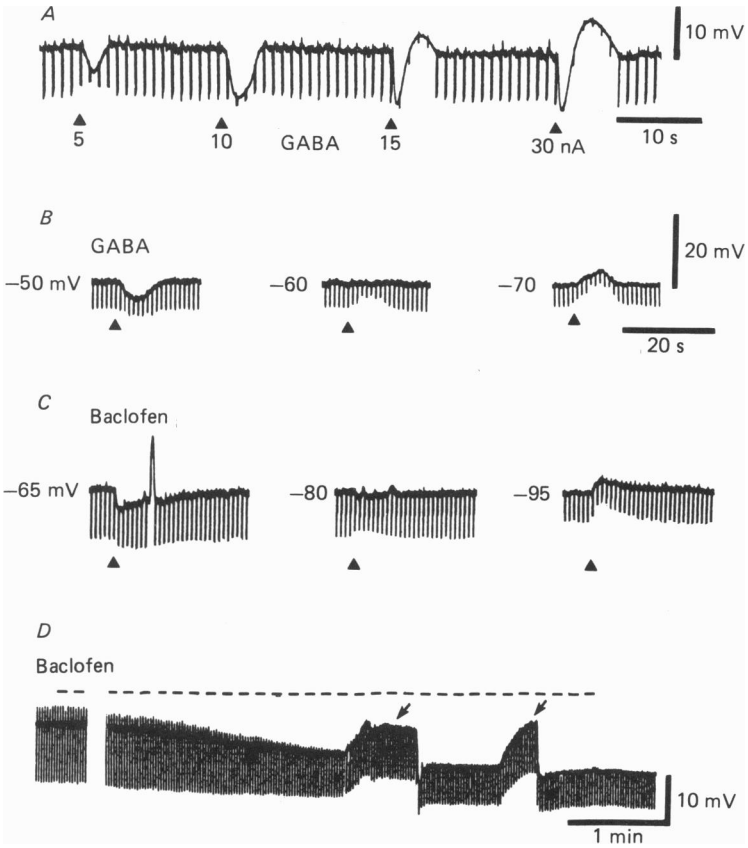

Fig. 8. The effect of GABA and baclofen on LGN projection cells. *A*, in the absence of bicuculline, using small ionophoretic currents (500 ms, 5–10 nA) GABA evoked pure hyperpolarizing responses. Increasing the intensity of the ejection current (15–30 nA) resulted in a bigger hyperpolarization which was then followed by a depolarization. Both effects were associated with a marked decrease in the input resistance of the cell as indicated by the decrease in the amplitude of the hyperpolarizing electrotonic potentials (downward deflections in the trace). In *B*, the bicuculline (50 μM)-insensitive GABA hyperpolarizations (500 ms, 30 nA) are shown to reverse in polarity at about −60 mV when recorded in a 11·5 mM-extracellular K^+ concentration. *C*, in contrast to the effect shown in Fig. 7, the response of this cell to baclofen (500 ms, 50 nA) was much faster (time to peak of 1–2 s) and clearly reversed in polarity at about −80 mV. Note the spontaneous depolarizations (50–500 ms in duration) present during the slow return to resting potential. Arrow-heads in all records indicate the time of the ionophoretic ejection. The chart record in *D* shows the hyperpolarizing effect of baclofen (1 μM) recorded in a low-Ca^{2+} (0·2 mM) and high-Mg^{2+} (7·8 mM) solution at a time when synaptic transmission was blocked (same cell as in Fig. 1 *B*) (the break in the trace corresponds to 3 min). During the response to baclofen the cell was depolarized back to resting membrane potential by steady DC current injection (↙), and the input resistance of the cell observed to be decreased by about 25%. Downward deflections in all traces represent hyperpolarizing electrotonic potentials obtained in response to a constant pulse of current, and calibrations in *B* also apply to the records in *C*.

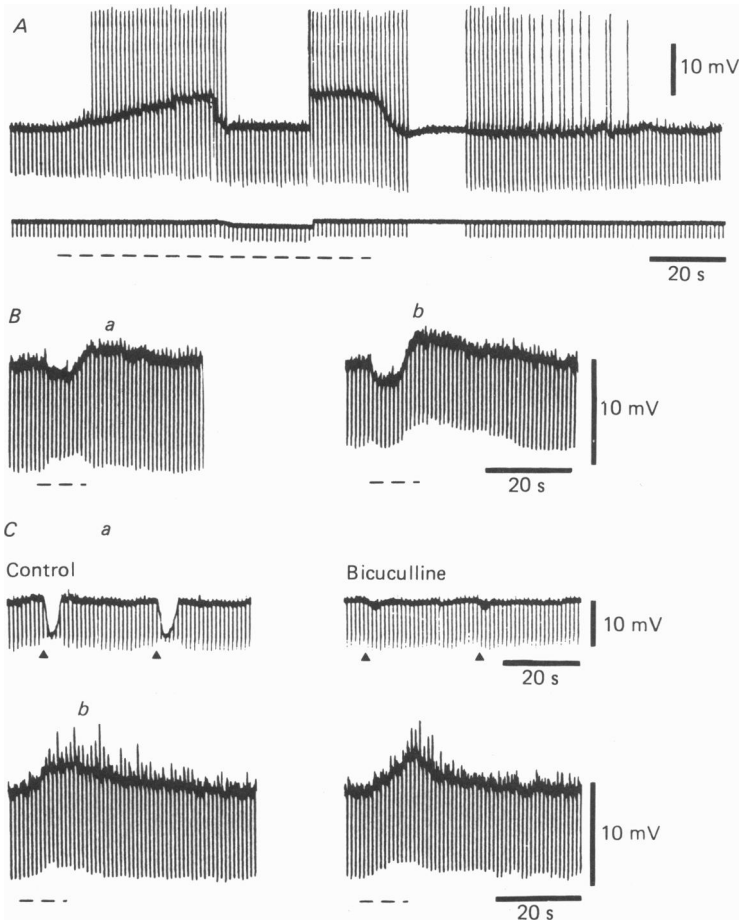

Fig. 9. The effect of ACh (dashed lines) on rat LGN projection cells. A slow depolarization was the common response to ionophoretic application of ACh (80 nA) (*A*). Manual voltage clamp showed a small increase in input resistance during the plateau depolarization. In another cell (*B*), ACh (50 nA) evoked a hyperpolarization followed by a depolarization. Note how at −55 mV (*Bb*) the amplitude of both the hyperpolarizing and the depolarizing response to ACh is bigger than at −65 mV (*Ba*). In *C*, the response of the same cell to GABA and ACh in the presence and in the absence of bicuculline is shown. At a time when bicuculline applied by ionophoresis (20 nA, 20 s) blocked the Cl^--dependent GABA response (*Ca*), the ACh depolarization (50 nA) was unaffected nor any hyperpolarization unmasked (*Cb*). Arrow-heads in *Ca* indicate the time of the ionophoretic application of GABA (500 ms, 10 nA) and downward deflections in all traces represent hyperpolarizing electrotonic potentials obtained in response to a constant pulse of current.

Baclofen applied either by ionophoresis ($n = 7$), pressure ejection ($n = 4$) or in the perfusion medium (1–100 μM) ($n = 10$) hyperpolarized LGN neurones reversibly and in a dose-dependent manner (Figs 7 and 8). This effect of baclofen was associated with a 20–50 % decrease in input resistance, although in three out of the ten cells where it was bath applied no change in resistance could be observed. The time to

peak of the response to ionophoretically applied baclofen could range from less than 1 to 5 s, while the decay of its response was always rather long in duration, outlasting the ejection period by several seconds. During this slow return to the resting membrane potential some cells showed spontaneous depolarizations (5–20 mV in amplitude, 50–500 ms in duration) (Fig. 8*C*). The baclofen hyperpolarization showed a non-linear relationship with the membrane potential (maximal amplitude in the range of -55 to -60 mV), and had a reversal potential of -79 ± 4 mV ($n = 9$) (Figs 7 and 8*C*). All these effects of GABA and baclofen persisted in the presence of TTX (1 μM) and when using a low-Ca^{2+} (0·2 mM) and high-Mg^{2+} (7·8 mM) solution (Fig. 8*D*). However, in eight cells recorded in 0 mM-Ca^{2+} (and 8 mM-Mg^{2+}), baclofen applied in the perfusion medium had no effect.

The most common response of LGN cells (thirty-seven out of forty) to ionophoretic application of ACh was a slow depolarization that was never bigger than 8–10 mV (Fig. 9*A*). By manually voltage clamping the membrane potential this action of ACh was observed to be associated with a small increase (15–30%) or sometimes no change in input resistance. Upon termination of the ionophoretic ejection of ACh, the membrane potential returned to its resting level in a couple of seconds. However, the input resistance of the cell was often still increased and, as a result, hyperpolarizing electrotonic potentials could evoke rebound low-threshold Ca^{2+} spikes that could reach threshold for firing. The overall effect of the ACh depolarization on the pattern of excitability of LGN cells was, however, dependent on the resting potential of the cell. In fact if the cell was at a membrane potential more negative than -65 mV, ACh transformed a burst type of activity into a continuous firing pattern. Only in three cells did ACh produce a hyperpolarization that was associated with a clear, though small, decrease in input resistance (15–35%) and that had a reversal potential of -80 ± 2 mV (Fig. 7). In another cell the ACh-evoked hyperpolarization was followed by a depolarization (Fig. 9*B*). TTX (1 μM) had no effect on the ACh depolarization, but it decreased the amplitude of the ACh hyperpolarization by 30% in the only cell tested. We also examined the effect of ACh in the presence of bicuculline ($n = 4$). As shown in Fig. 9*C*, bicuculline, applied with an ejection current sufficient to block the Cl^--dependent GABA hyperpolarization, had no effect on the ACh depolarization nor did it unmask any ACh hyperpolarization. Using higher ejection currents of bicuculline no ACh hyperpolarization could be unmasked and now bicuculline decreased the ACh depolarization by 20–30%.

DISCUSSION

The main conclusion of this investigation is that the SHP and LHP recorded in rat LGN projection cells following electrical stimulation of the optic tract represent two distinct IPSPs both originating from LGN interneurones. The SHP is a $GABA_A$ IPSP while the LHP is a late, long-lasting, K^+-dependent IPSP that is mediated by GABA acting on $GABA_B$ receptors. The LHP appears to be the main synaptic conductance underlying the long-lasting hyperpolarizations observed during rhythmic oscillations of LGN and other thalamic projection cells.

The SHP and LHP as IPSPs from LGN interneurones

The SHP and LHP were abolished by treatment known to block synaptic transmission but they could still be evoked in the absence of a preceding EPSP, and the LHP in the absence of the SHP. Moreover, the amplitude of the LHP was not decreased by recording with EGTA-filled microelectrodes nor increased in the presence of low-threshold Ca^{2+} spikes evoked by the EPSP. Taken together these results indicate that the SHP and LHP are both synaptic in origin and that they are not mediated by a membrane conductance (i.e. a Ca^{2+}-activated Cl^- and K^+ conductance respectively) intrinsic to LGN projection cells and which is activated by the preceding potential. This alternative possibility might have been true if we were rcording at the reversal potential of the optic tract EPSP, but this is close to -10 mV (Crunelli *et al.* 1987*b*) and we are unaware of any other synaptic event in these cells with a reversal potential close to resting membrane potential. Thus the SHP and LHP are two IPSPs and could be mediated by the release of a transmitter either directly from optic tract fibres or indirectly from other cells present in the slice. No putative transmitter known to possess a hyperpolarizing action similar to the SHP and LHP appears to be released from optic tract fibres (Sandberg & Lindström, 1983). Moreover the fact that the two IPSPs were more sensitive than the monosynaptic EPSP to solutions containing low Ca^{2+}–high Mg^{2+} would support the hypothesis that they are mediated by other cells present in the slice through a polysynaptic pathway.

On the basis of anatomical evidence only three groups of cells could be responsible for these IPSPs: the cells of the nucleus reticularis thalami, the LGN projection cells and the LGN interneurones (Jones, 1985). The cells of the first group are known to be activated through axon collaterals of LGN projection cells and to send their axons back to the LGN where they form GABAergic synapses on projection cells (Montero & Scott, 1981; Hale, Sefton, Baur & Cottee, 1982; Ottersen & Störm-Mathisen, 1984). However, the nucleus reticularis is not present in our slices and the SHP and LHP could be recorded in slices containing only the most lateral $\frac{2}{3}$ of the LGN and in the absence of a preceding EPSP. These findings clearly exclude the possibility that the recurrent pathway through the nucleus reticularis is responsible for the two IPSPs. It is also highly unlikely that the SHP and LHP are generated by neighbouring LGN projection cells. In fact, axon collaterals of rat LGN relay cells only project outside the nucleus and do not appear to impinge on other LGN projection cells (Jones, 1985). Even if this was the case, the axons of LGN projection cells are known to mediate excitatory responses in the cortex (references in Sherman & Spear, 1982), and therefore it seems unlikely that they could mediate hyperpolarizing potentials in the LGN. Thus the LGN interneurones, the majority if not all of which are known to form GABAergic synapses on projection cells (Ohara *et al.* 1983; Montero & Singer, 1985; Gabbott *et al.* 1986), appear as the only possible source of origin of the SHP and LHP. The possibility of recording both IPSPs in the absence of an optic tract-evoked EPSP and the lack of evidence in favour of intrageniculate axon collaterals of rat LGN projection cells would also support a feed-foward nature for the SHP and LHP.

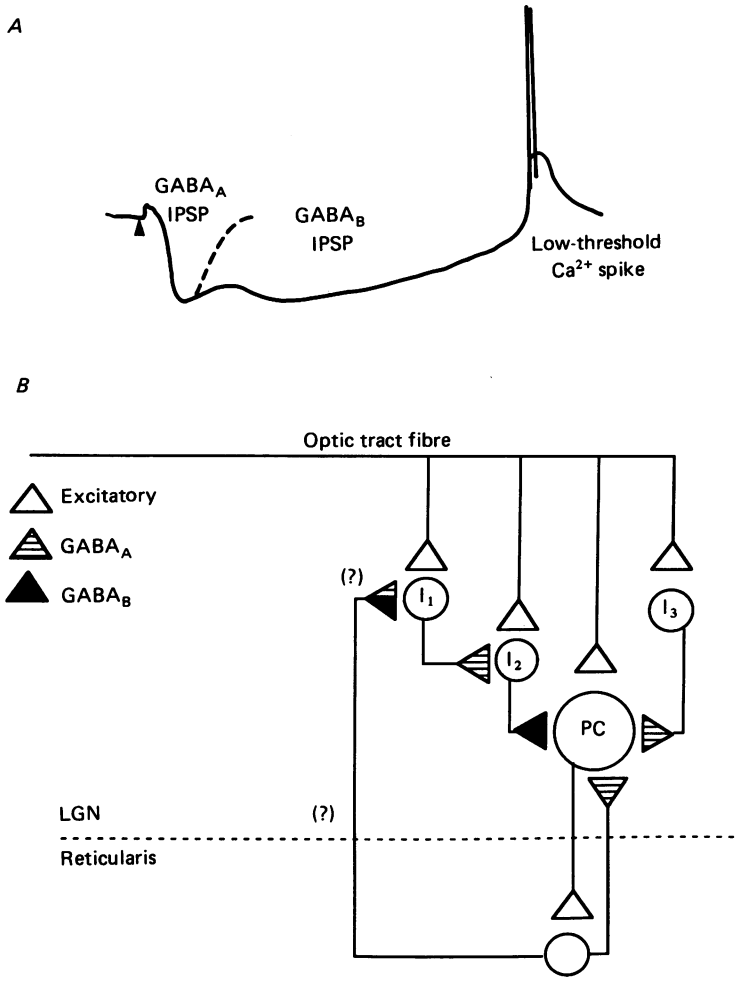

Fig. 10. *A*, schematic drawing of the response of a LGN projection cell to stimulation of the optic tract showing the different synaptic conductances responsible for a full cycle of membrane oscillation. The monosynaptic EPSP is followed by a $GABA_A$ IPSP and by a $GABA_B$ IPSP. The hyperpolarization produced by the two IPSPs is large and long enough to deinactivate the Ca^{2+} conductance responsible for a low-threshold Ca^{2+} spike. The contribution of the transient K^+ current, I_A, and a small Ca^{2+}-activated K^+ conductance are not shown. *B*, schematic drawing showing the pathways responsible for the $GABA_A$ and $GABA_B$ IPSPs recorded in LGN projection cells (PC) following stimulation of the optic tract. Location of terminals should not be taken to indicate the exact morphological location of a synapse but only the existence of a synaptic connection between two cells. The $GABA_A$ and $GABA_B$ IPSPs are generated in a feed-forward manner by interneurones I_3 and I_2 respectively. The interneurone responsible for the $GABA_B$ IPSP (I_2) is under the control of another LGN interneurone (I_1) through $GABA_A$ synapses. The latter is also under the inhibitory control ($GABA_A$ or $GABA_B$ IPSPs (?)) of cells in the nucleus reticularis. As mentioned in the text, our results are also compatible with the possibility that the same interneurone (I_2 or I_3) could be responsible for both the $GABA_A$ and the $GABA_B$ IPSPs. The synapses responsible for the recurrent IPSP generated in projection cells by neurones of the nucleus reticularis are also shown. The anatomical evidence of the innervation of rat LGN interneurones by cells of the nucleus reticularis is lacking (?).

The transmitter of the SHP and of LHP

The SHP possessed electrophysiological and pharmacological properties identical to a $GABA_A$ IPSP. These included the Cl^- dependency, the bicuculline and picrotoxin sensitivity and the similarity of its reversal potential to that of the Cl^--dependent, bicuculline-sensitive hyperpolarization evoked by GABA. Thus the present results constitute strong direct evidence for the existence in rat LGN projection cells of a $GABA_A$ IPSP generated by LGN interneurones (Fig. 10) (cf. Kelly *et al.* 1979).

The lack of inhibition by bicuculline and the K^+ dependency of the LHP were similar to those reported for long-lasting IPSPs in other areas of the CNS (Thalmann & Ayala, 1982; Satou, Mori, Tazawa & Takagi, 1982; Lancaster & Wheal, 1984; Alger, 1984) and recently in the LGN (Hirsch & Burnod, 1985). Some of these late IPSPs have been suggested to be mediated by $GABA_B$ receptors (Newberry & Nicoll, 1985). Indeed baclofen, a $GABA_B$ receptor agonist, and GABA (in the presence of bicuculline) evoked hyperpolarizations which had properties similar to the LHP, suggesting that this potential is mediated by GABA acting on $GABA_B$ receptors.

However, the LHP might have been mediated by cholinergic fibres activated by some spread of current from the stimulating electrode positioned in the optic tract. In fact, although the majority of cholinergic fibres from the brain stem reach the LGN through its centro-lateral border (Jones, 1985), other ACh-containing fibres have been described to run in close proximity to and intermix with the optic tract (Wilson, 1985). This cholinergic involvement in the LHP might have been in the form of a direct, K^+-dependent hyperpolarizing action of ACh through M_2 receptors located on projection cells (cf. McCormick & Prince, 1986, 1987), or indirectly, via the activation of M_1 receptors located on LGN interneurones (Dolabela de Lima, Montero & Singer, 1985). However, only a very small percentage of projection cells responded to ionophoretic application of ACh with a hyperpolarization, while the LHP was present in the majority of LGN neurones. Moreover, no ACh-evoked hyperpolarization could be unmasked in the presence of bicuculline. Thus, in contrast to the cat and guinea-pig LGN, presumed M_2-mediated hyperpolarizations appear to be rare in the rat LGN (cf. McCormick & Prince, 1987). The inhibition of the LHP by a concentration of pirenzepine supposed to be selective for M_1 receptors would also argue against the possibility that the LHP is mediated by M_2 receptors. As for the involvement of M_1 receptors, a complete inhibition of the LHP by atropine occurred at concentrations higher than 10 μM and only in the presence of bicuculline. Thus, rather than by a muscarinic receptor antagonism, an unselective effect (such as K^+ channel blockade) might better explain the action of atropine on the LHP. Finally, the insensitivity of the LHP to eserine and neostigmine, in the presence and in the absence of bicuculline, as well as the inhibition of the LHP by γ-D-glutamylglycine, a broad-spectrum excitatory amino acid antagonist (our unpublished observations), exclude the presence of a cholinergic synapse in the generation of the LHP. Therefore we suggest that the LHP is also mediated by GABA from LGN interneurones but via the activation of $GABA_B$ receptors (Fig. 10). Recent autoradiographic studies showing a high density of $GABA_B$ receptors in the

rat LGN (Bowery, Hudson & Price, 1987) strongly support this suggestion, although conclusive evidence awaits the development of a selective $GABA_B$ antagonist.

The two IPSPs could originate from two separate groups of LGN interneurones, one group responsible for the SHP, the other for the LHP (I_3 and I_2 in Fig. 10), or, alternatively, the same interneurone could mediate both the SHP and LHP. In the latter case it would be interesting to investigate whether the SHP and LHP are evoked at the same synaptic site (cf. ACh action in *Aplysia*; Kehoe, 1972) or, because of the peculiar features of LGN interneurones (Guillery, 1966; Lieberman, 1973), one IPSP is generated in the glomerulus through a dendritic release in the triad and the other IPSP via a more classical axonal release.

The enhancement of the LHP by bicuculline indicates that the LGN interneurones responsible for this IPSP (I_2 in Fig. 10) are under the inhibitory control of other LGN interneurones (I_1 in Fig. 10) through $GABA_A$ synapses. It is interesting to note that similar results were obtained for the late, long-lasting, K^+-dependent IPSP recorded in hippocampal CA_1 neurones (Newberry & Nicoll, 1985). In the LGN the anatomical evidence of such interneurone–interneurone (i.e. GABA–GABA) synapses are well documented (Ohara *et al.* 1983; Montero & Singer, 1985), adding support to our suggestion that the LHP is GABAergic in nature.

The relative difficulty in observing the effect of ionophoretically applied baclofen and the K^+-dependent response to GABA might be explained by the geometry of projection cell dendrites (Jones, 1985), only a few of which would be expected to be aligned with the plane of cutting of our LGN slices. Thus, unlike previous experiments in hippocampal cells (Crunelli, Forda & Kelly, 1984), we were probably unable to position the ionophoretic pipette in close proximity to the dendrites of the impaled neurone. For the same reasons no attempt was made in this study to characterize the somatic and/or dendritic nature of the Cl^--dependent hyperpolarization and depolarization evoked by GABA.

The non-linearity between membrane potential and the LHP, the baclofen response and the K^+-dependent GABA effect were probably due to strong rectifying properties present in LGN projection cells in the range -40 to -60 mV. However, a voltage sensitivity of the conductance underlying the K^+-dependent action of baclofen and GABA has been reported (Gähwiler & Brown, 1985), and thus voltage clamp experiments are needed to clarify the relative contribution of the membrane properties and the transmitter-activated conductance to the non-linearity of the LHP. The difficulties we encountered in reversing the LHP at low extracellular K^+ concentrations might be explained by the inward rectification present at hyperpolarized levels of membrane potential (for a full discussion on this and other problems associated with measurements of reversal potential cf. Crunelli *et al.* 1984, 1987*b*).

Physiological role of the SHP and the LHP

The marked drop in input resistance associated with the SHP explains the decrease in the amplitude and duration of the optic tract EPSP and of the low-threshold Ca^{2+} spike evoked by the EPSP. These effects might account for the reduction in the gain of the retinocortical transmission at the level of the LGN (Singer & Creutzfeldt, 1970; cf. Sherman & Koch, 1986) and indeed form the

physiological basis of those properties of LGN cells which are affected by bicuculline (i.e. centre–surround antagonism) (Sillito & Kemp, 1983; cf. Sherman & Spear, 1982; Sherman & Koch, 1986). However, the presence of $GABA_B$ IPSPs indicates that the strong, short-acting inhibition associated with $GABA_A$ receptor activation cannot be considered any longer the only type of GABA-mediated response originating from LGN interneurones. At present it is difficult to suggest a specific role for the LHP in the LGN. Clearly it will be interesting to investigate how the small decrease in resistance associated with the LHP, its long duration, its non-linear relationship with membrane potential and its ability to evoke low-threshold Ca^{2+} spikes relate to the gating activity of visual information performed by the LGN (cf. Sherman & Koch, 1986). In this respect the result of a recent study on shift responses (Eysel *et al.* 1987) showing X cells to be preferentially affected by a strong, short-acting type of inhibition and Y cells by a weak long-lasting inhibition might suggest a differential distribution of the SHP and LHP on these two classes of LGN cells.

One point which deserves further consideration, however, is the ability of the LHP to evoke low-threshold Ca^{2+} spikes. Recent *in vitro* (Jahnsen & Llinas, 1984) and *in vivo* (Roy *et al.* 1984) studies seem to have favoured the hypothesis that the 5–6 Hz rhythmic oscillations (θ rhythm) observed in thalamic projection cells could be mainly explained by their biophysical membrane properties. According to this hypothesis a low-threshold Ca^{2+} spike would underlie the depolarizing (i.e. excitatory) part of this type of oscillation, while a $GABA_A$ IPSP, the transient K^+ current, I_A, and mainly a Ca^{2+}-activated K^+ conductance would be responsible for the long-lasting hyperpolarization present during the oscillation. However, only a rather small (if any) Ca^{2+}-activated K^+ conductance followed low-threshold Ca^{2+} spikes evoked by the EPSP or by direct injection of current into the soma (Crunelli *et al.* 1987*a*, *c*). Thus, in contrast to what was recently suggested (Jahnsen & Llinás, 1984; Roy *et al.* 1984), the biophysical membrane properties of thalamic projection cells *alone* cannot account for the long-lasting hyperpolarization. Instead, a *single* LHP was able to deinactivate the Ca^{2+} current responsible for the low-threshold Ca^{2+} spike, indicating that the LHP is the main synaptic conductance underlying the long-lasting hyperpolarization. However, differently from *in vivo* experiments (Purpura & Schofer, 1963; Andersen & Sears, 1964; Roy *et al.* 1984) it was only in the presence of bicuculline that we could evoke a large LHP able to deinactivate a low-threshold Ca^{2+} spike. We have suggested (see above) that the interneurones responsible for the LHP (I_2 in Fig. 10) are under the inhibitory control of other LGN interneurones (I_1 in Fig. 10) through $GABA_A$ synapses. Thus a possible explanation is that *in vivo* these latter interneurones (I_1) are inhibited by inputs originating from outside the LGN which are not present in our LGN slices. A source of this extrageniculate inhibition is very likely to be the cells of the nucleus reticularis, the majority of which are GABAergic in nature (Montero & Scott, 1981; Ohara *et al.* 1983; Montero & Singer, 1985). This hypothesis is supported by the results of *in vivo* experiments showing lesions of the nucleus reticularis to abolish long-lasting hyperpolarizations in thalamic projection cells (Steriade, Deschenes, Domich & Mulle, 1985). Thus it does not appear that the nucleus reticularis *per se* mediates long-lasting hyperpolarizations but that it controls their occurrence through an intermediate GABAergic interneurone. However, it should be pointed out that in the

rat the anatomical evidence of synaptic connections between cells of the nucleus reticularis and thalamic interneurones is still lacking (but cf. Hendrickson, Ogren, Vaughn, Barber & Wu, 1983; Montero & Singer, 1985).

In conclusion we suggest that (Fig. 10): (a) $GABA_A$ and $GABA_B$ IPSPs are generated by LGN interneurones, (b) the interneurones responsible for the $GABA_B$ IPSP are under the inhibitory control of other LGN interneurones through $GABA_A$ synapses, (c) these latter interneurones are themselves controlled by inhibitory inputs originating from outside the LGN, probably in the nucleus reticularis and (d) a single $GABA_B$ IPSP underlies the long-lasting hyperpolarization capable of deinactivating the Ca^{2+} current responsible for a low-threshold Ca^{2+} spike. Clearly this hypothesis does not exclude the possibility that synaptic conductances originating in other nuclei (i.e. nucleus reticularis, brain-stem nuclei) are able to modulate long-lasting hyperpolarizations and/or to evoke a low-threshold Ca^{2+} spike in the LGN and other thalamic nuclei (cf. multiple $GABA_A$ IPSPS *in vitro* (Thomson, 1987) and *in vivo* (Steriade *et al.* 1985)).

The work was supported by the Wellcome Trust (grant 15032/1.5) and by INSERM (CRE 846013). N. L. was partly supported by a CNRS–Royal Society exchange grant, M. P. by the European Science Foundation.

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