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ELECTROPHYSIOLOGY OF A SLOW (0·5–4 Hz) INTRINSIC OSCILLATION OF CAT THALAMOCORTICAL NEURONES *IN VIVO*

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SUMMARY

1. Electrophysiologically identified thalamocortical neurones have been intra- and extracellularly recorded in acutely prepared cats, under different anaesthetic conditions.

2. A slow (0·5–4 Hz) membrane potential oscillation was observed in thalamocortical cells recorded in motor, sensory, associational and intralaminar thalamic nuclei. The oscillation consisted of rhythmic low-threshold spikes alternating with after-hyperpolarizations.

3. About 80% of the neurones with intact cortical connections were set into the slow oscillatory mode by bringing their membrane potential to between −68 and −90 mV. The oscillation did not depend upon the occurrence of fast action potentials and did not outlast the imposed hyperpolarization.

4. Anatomical or functional disconnection from related cortical areas resulted in a membrane potential hyperpolarization of about 9 mV and in the occurrence of spontaneous slow oscillations in virtually all recorded neurones. The intrinsic nature of the phenomenon was supported by the lack of rhythmic postsynaptic potentials as the cells were prevented from oscillating by outward current injection.

5. In contrast with other thalamic nuclei, the slow oscillation has not been observed in anterior thalamic neurones despite their having similar basic electrophysiological properties.

6. Barbiturate administration suppressed the slow oscillatory mode, an effect accompanied by a decrease in the membrane input resistance.

7. Multiunit recordings of spontaneously oscillating cells showed epochs characterized by phase-related firing. This synchronous discharge was paralleled by a clear-cut build-up of field potentials in the frequency range of electroencephalogram slow or delta waves.

8. These results demonstrate that the majority of thalamocortical neurones are endowed with electrophysiological properties allowing them to oscillate at 0·5–4 Hz, if they have a membrane potential more negative than −65 mV and a high input resistance. Such a condition is physiologically achieved in the deepest stages of electroencephalogram-synchronized sleep, as a result of brain stem–thalamic as well

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as cortico-thalamic deafferentation. We postulate a thalamic contribution in the genesis of electroencephalogram delta waves during slow wave sleep, once independently oscillating thalamocortical cells become in phase.

INTRODUCTION

The epitome of electroencephalogram (EEG) synchronization, with widespread appearance of rhythmic and high-amplitude field potential waves, is seen during the behavioural states of drowsiness and quiet sleep. In these conditions the electrocortical scenario is dominated by spindle oscillations and slow waves.

Spindle waves are defined as high-amplitude, waxing and waning waves at 7–14 Hz, clustered in sequences that last for 1·5–2 s and which recur periodically every 5–10 s (Steriade & Deschênes, 1984). Spindles originate in the thalamus (Morison & Bassett, 1945) and their electrogenesis has been intensively investigated (Andersen & Andersson, 1968; Steriade, Jones & Llinás, 1990*b*). They characterize the early stages of sleep.

As sleep deepens, slow or delta EEG waves (0·5–4 Hz) progressively prevail. The persistence of cortical slow waves in athalamic animals (Villablanca, 1974) suggested that their site of origin is quite different from that of spindles waves. Furthermore, laminar analyses and unit recordings have demonstrated an intracortical generator of slow waves between layers II–III and layer V (Calvet, Calvet & Scherrer, 1964; Ball, Gloor & Schaul, 1977; Petsche, Pockberger & Rappelsberger, 1984). In addition to synaptic mechanisms, intrinsic currents of pyramidal cells (Connors, Gutnick & Prince, 1982; Schwindt, Spain, Foehring, Chubb & Crill, 1988*a*; Schwindt, Spain, Foehring, Stafstrom, Chubb & Crill, 1988*b*) have also been implicated in the genesis of cortical delta waves (Buzsáki, Bickford, Ponomareff, Thal, Mandel & Gage, 1988; Steriade & Buzsáki, 1990). These and other results led to the common assumption that delta waves exclusively originate in the cerebral cortex. However, focal waves within the frequency range of delta waves have also been recorded in the ventrolateral (Steriade, Apostol & Oakson, 1971) and in the reticular (Steriade, Domich & Oakson, 1986) thalamic nuclei during EEG-synchronized epochs. Although this suggests the possibility of a thalamic contribution to the generation of EEG delta waves, the lack of a known intrathalamic slow oscillatory mechanism left this possibility merely speculative.

The ionic nature of an intrinsic 0·5–3 Hz membrane potential oscillation in some dorsal thalamic neurones of cat, rat and guinea-pig has been recently investigated *in vitro* (McCormick & Pape, 1990*a*; Leresche, Lightowler, Soltesz, Jassik-Gerschenfeld & Crunelli, 1991; Soltesz, Lightowler, Leresche, Jassik-Gerschenfeld, Pollard & Crunelli, 1991). This activity resulted from an interplay between the low-threshold transient Ca^{2+} current (I_t) (Jahnsen & Llinás, 1984*a*, *b*; Coulter, Huguenard & Prince, 1989; Crunelli, Lightowler & Pollard, 1989; Suzuki & Rogawski, 1989) and the hyperpolarization-activated cation current (I_h) (Pape & McCormick, 1989; McCormick & Pape, 1990*a*, *b*; Soltesz *et al.* 1991).

Here we report that the vast majority of physiologically identified thalamocortical cells *in vivo* are endowed with a slow (0·5–4 Hz) intrinsic oscillatory capability, which becomes actual at membrane potentials physiologically reached during slow-wave sleep (SWS). In addition to the membrane conductances underlying the slow

oscillation at the unitary level, thalamocortical systems appear also to be equipped with network properties leading to synchronicity (Steriade, Curró Dossi & Nuñez, 1991). As a direct consequence, thalamic delta waves are generated and transmitted towards the cortical mantle. A new view about the oscillatory state of the sleepy thalamus is then proposed, not just limited to spindling, but involving delta waves as well.

METHODS

Experiments were conducted on adult cats of either sex (2·5–3·5 kg). Animals were deeply anaesthetized with urethane (1·8 g/kg), paralysed with gallamine triethiodide and artificially ventilated with control of the end-tidal CO_2 concentration at $3{\cdot}7 \pm 0{\cdot}2$ %. Internal temperature (37–39 °C) and heart beat were continuously monitored. All the wounds and pressure points were generously infiltrated with lidocaine and the depth of anaesthesia maintained to obtain a constant pattern of EEG synchronization as during SWS. A mixture of halothane and nitrous oxide was used after an initial ketamine administration (40 mg/kg I.M.) in six animals, while four other cats were anaesthetized with sodium pentobarbitone (35 mg/kg). The short-acting barbiturate sodium thiamylal (2 mg/kg I.V.) was injected in four cases, in already anaesthetized animals.

Stability of the recordings was insured by cisternal drainage and bilateral pneumothorax. Cortex and fornix overlying the thalamus were routinely removed by suction to facilitate the passage of the micropipettes. To ascertain whether the inevitable interruption of corticothalamic fibres caused by this procedure was responsible for the peculiar spontaneous firing pattern observed in the lateroposterior (LP) thalamic nucleus (see Results), we sometimes extended the decortication to involve other thalamic territories. This was achieved either surgically (lobectomy or hemispherectomy) or chemically by means of spreading depression. The latter was induced by applying a 2 M-KCl-soaked filter paper on cortical areas related to the recording site.

Coaxial stimulating electrodes were inserted in various prethalamic structures such as brachium conjunctivum, dorsal column nuclei, optic chiasm and mammillary body, as well as in the internal capsule, for the electrophysiological identification of thalamocortical neurones. Criteria for antidromic invasion were fixed latency, ability to follow high-frequency stimuli ($\gtrsim 250$ Hz) and collision with spontaneously occurring or orthodromically-evoked action potentials. The location of recording electrodes was established by combining connectivity tests with micrometer readings during the experiments according to conventional stereotaxic co-ordinates (Berman & Jones, 1985).

Tungsten or low-impedance glass microelectrodes were used for extracellular recordings. Signals entered an AC amplifier (bandpass 0·3–10000 Hz) for the analysis of both unitary and field potential activity.

Intracellular recordings were performed by means of 3 M-potassium acetate-filled micropipettes (DC resistance 25–40 MΩ). A high-impedance amplifier with active bridge circuitry was used to record and inject current into the cells. Only stable intracellular recordings from neurones having a membrane potential (V_m) negative to -55 mV and an overshooting action potential were considered. Signals were stored on a magnetoscope after digital conversion.

At the end of the experiments animals were deeply anaesthetized with sodium pentobarbitone (50 mg/kg) and perfused intracardially with 0·5 l of 0·9% saline solution, followed by 1 l of 4% paraformaldehyde. The location of stimulating electrodes was verified on 80 μm thionin-stained frontal sections.

RESULTS

Data base and neuronal identification

A total of 305 thalamocortical cells were recorded in the course of this study. Since similar results were obtained under various anaesthetics apart from barbiturates, these data will be considered together.

Intracellular recordings

Seventy thalamocortical cells with intact cortical connections were studied intracellularly. In this sample we found a V_m ranging from -55 to -68 mV ($-60{\cdot}3 \pm 0{\cdot}4$ mV, mean $\pm$ S.E.M.). Action potential amplitudes averaged

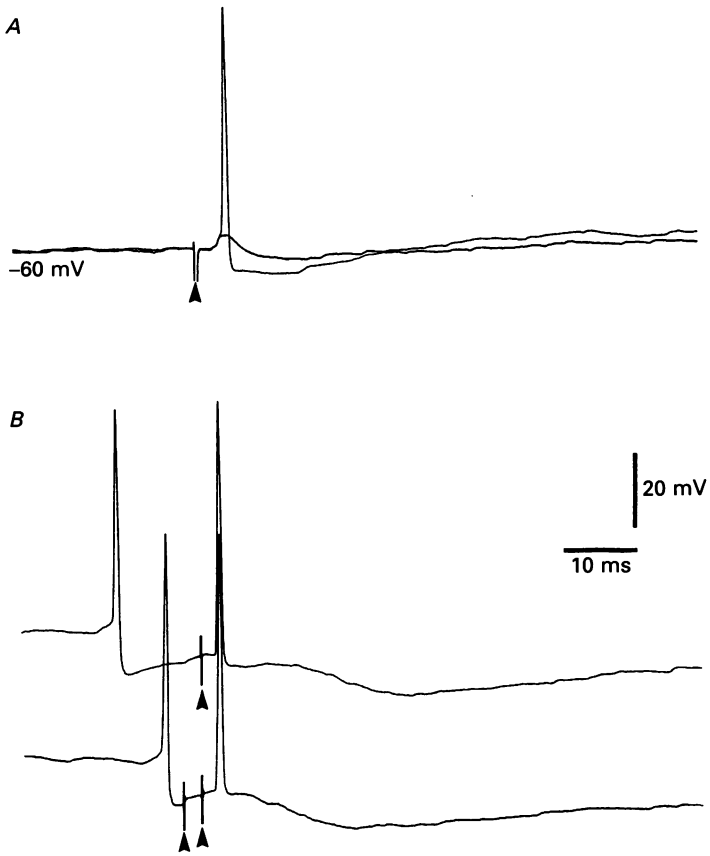

Fig. 1. Electrophysiological identification of a VL thalamocortical neurone. *A*, orthodromic response to brachium conjunctivum (BC) stimulation: two superimposed traces taken at slightly different stimulation intensities. *B*, antidromic invasion from the internal capsule (IC). In the upper trace the antidromic spike did not collide with an orthodromic action potential apparently triggered by an excitatory postsynaptic potential. In the lower trace only the second IC stimulus of the doublet succeeded in antidromically firing the soma. Arrow-heads mark stimulus artifacts. In this and following figures V_m is indicated.

$62{\cdot}2 \pm 0{\cdot}5$ mV. Suprathreshold depolarizing current pulses delivered at rest usually induced tonic firing, while low-threshold spikes (LTSs) crowned by high-frequency (> 250 Hz) bursts of action potentials were triggered at V_m more negative than -65 mV. The apparent membrane input resistance, measured at the resting level by injecting small constant-current hyperpolarizing pulses ($\lesssim 0{\cdot}5$ nA, > 50 ms) and recording the induced voltage deflections, averaged $17{\cdot}3 \pm 0{\cdot}8$ MΩ, ranging from 10–

26 MΩ. These neurones were recorded in ventroanterior–ventrolateral (VA–VL), centrolateral–paracentral (CL-PC) and anterior thalamic (AT) nuclei. The electrophysiological identification of a VL thalamocortical neurone is depicted in Fig. 1.

Another fifty-eight cells were recorded after surgical ablation of cortical-related areas. In the cases without spontaneous oscillations, or whenever real periods of rest could be observed, V_m was $-69{\cdot}4 \pm 0{\cdot}7$ mV, ranging from -65 to -76 mV. Action potential amplitudes averaged $70{\cdot}8 \pm 0{\cdot}8$ mV. Suprathreshold depolarizing current pulses delivered at rest were able to trigger LTSs, and tonic firing could be obtained only under steady depolarizing current. The apparent membrane input resistance was $22{\cdot}1 \pm 1{\cdot}5$ MΩ, ranging from 15 to 37 MΩ. These neurones were mostly recorded in the LP nucleus, but also in VA–VL, CL–PC and dorsal lateral geniculate (dLG) thalamic nuclei.

Extracellular recordings

Extracellular recordings of 177 thalamocortical neurones were performed in the LP, VA–VL, CL–PC, dLG, ventroposterior, mediodorsal and AT thalamic nuclei. In all those cases, related cortical territories were removed or were chemically inactivated by means of potassium-induced spreading depression. With the exception of AT cells (see below), no differences in firing pattern were observed among the other groups which will therefore be considered together.

Induced slow (delta) oscillations in neurones with intact cortical afferents

Most cells (82%) with intact cortical connections were set into a slow oscillatory mode as their V_m was brought more negative than -68 mV ($-71{\cdot}5 \pm 0{\cdot}9$ mV) by means of hyperpolarizing current pulses. The frequency ranged from 0·5 to 4 Hz. The oscillation consisted of rhythmic LTSs alternating with after-hyperpolarizations (AHPs) (Figs 2 and 3). Each LTS was triggered by a depolarizing sag which, for reasons which will become clearer in what follows, will be referred to as a pacemaker depolarization,borrowing the term from heart physiology. While the first pacemaker depolarization appeared to follow the initial passive voltage response, each of the ensuing ones represented the decaying phase of corresponding AHPs.

When long hyperpolarizing current pulses were applied, the oscillation was riding on a slow depolarizing sag, able to shift the V_m generally from 4 to 20 mV with respect to the peak voltage response (Figs 2 and 3). This phenomenon was voltage dependent, its amount and rate being higher as the imposed voltage step increased. The time required to reach a stable V_m ranged from 0·35 to 2·5 s. From now on it will be referred to as slow V_m relaxation. As a result of this depolarizing shift the oscillation could fade. This aspect was characterized by a progressive dampening in both LTS and AHP, which could eventually be replaced by subthreshold oscillations at the same frequency (see Fig. 2*A*).

Upon termination of the current pulse, a depolarizing tail characterized the return to the resting level. Depending on the V_m prior to, and the voltage step upon the pulse break, a rebound LTS could be triggered. The occurrence of an LTS appeared not to affect the duration of the depolarizing tail, which ranged from 0·25 to 2 s (Figs 2 and 3).

The intrinsic nature of the delta oscillation was strongly suggested by its

occurrence regardless of the presence of action potentials which could have engaged synaptically other network elements (Figs 2 and 3*A*). Figure 3 demonstrates the voltage dependence of the oscillation. At more negative V_m the pacemaker depolarization clearly became steeper, resulting in an earlier and more powerful

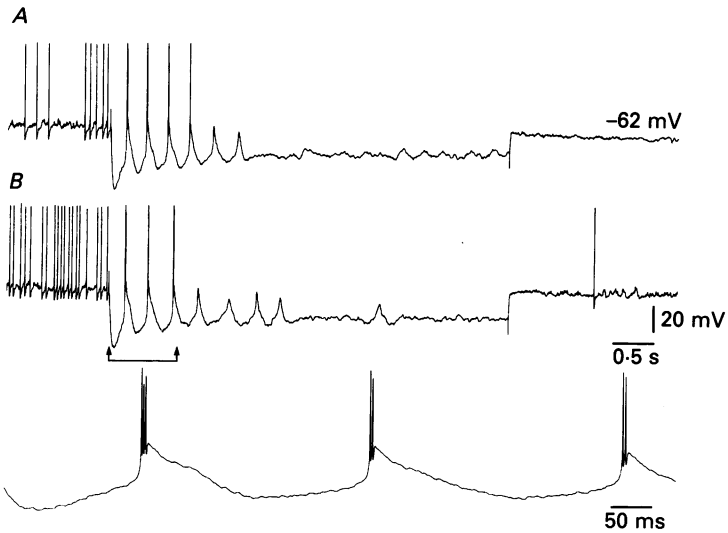

Fig. 2. Induced delta oscillation in a neurone with intact cortical afferents. *A* and *B* represent the same VL thalamocortical cells, switched from tonic to slow oscillatory mode by injecting 2 nA hyperpolarizing current pulses. Note the dampening of the oscillation with appearance of subthreshold events as the slow V_m relaxation progressively depolarized the membrane. Upon removal of the pulse, V_m recovered after a tail depolarization. The portion between arrows in *B* is depicted in the bottom part at higher speed, to show high-frequency bursts of spikes crowning the LTSs, each of them triggered by a pacemaker depolarization.

activation of the LTS; this led to a higher frequency of the oscillation and a reduced temporal jitter. A dampening in rhythmicity with appearance of subthreshold oscillations accompanied the slow V_m relaxation observed with long current pulses. We found a direct relation between the number of cycles and the induced voltage steps. This is shown in Fig. 3*B*, with data collected from four different VL neurones. In all likelihood, this complex phenomenon resulted from the voltage dependence of pacemaker depolarization, V_m relaxation rate, AHP depth and LTS activation properties.

The slow oscillation has not been observed at V_m beyond -90 mV, although V_m relaxation and tail depolarization were still present at these levels (see Fig. 5*D*).

Spontaneous intrinsic delta oscillations after disconnection from related cortical areas

A spontaneous 0·5–4 Hz rhythmicity was firstly and repeatedly observed in the LP nucleus. Virtually all extracellularly recorded neurones ($n = 105$) displayed clocklike bursts or single spikes which, at the intracellular level ($n = 53$), appeared to result from rhythmic LTSs (Figs 4, 8 and 11*B*).

Similarly to what was found in other thalamic nuclei, each LTS was triggered by a pacemaker depolarization representing the decaying phase of an AHP. The

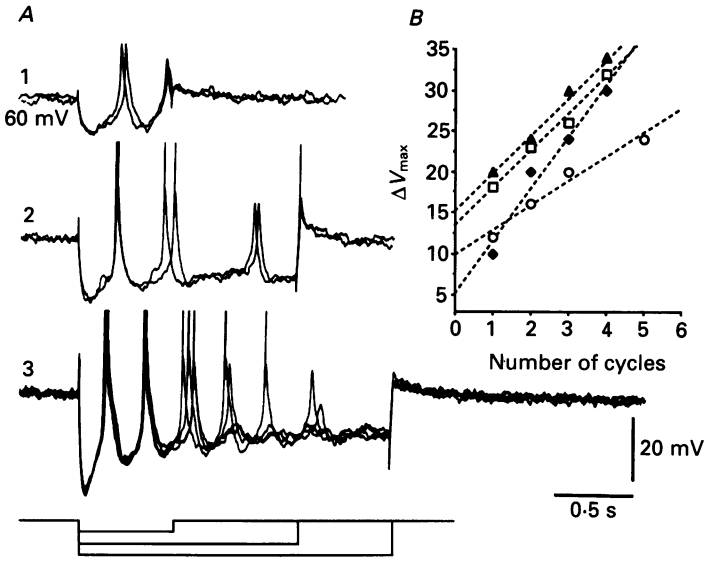

Fig. 3. Voltage dependence of the delta oscillation. VL neurone with intact cortical afferents. *A*, higher number of elicited cycles obtained as the pulse amplitude increased (0·5, 1, 1·5 nA from 1 to 3). Traces have been superimposed to show stereotypy of the oscillation for a given test condition. Action potentials truncated. Current monitor at bottom. *B*, relation between number of elicited cycles and induced peak voltage deflection for four different VL cells, tested with hyperpolarizing current pulses at a holding V_m of −60 mV.

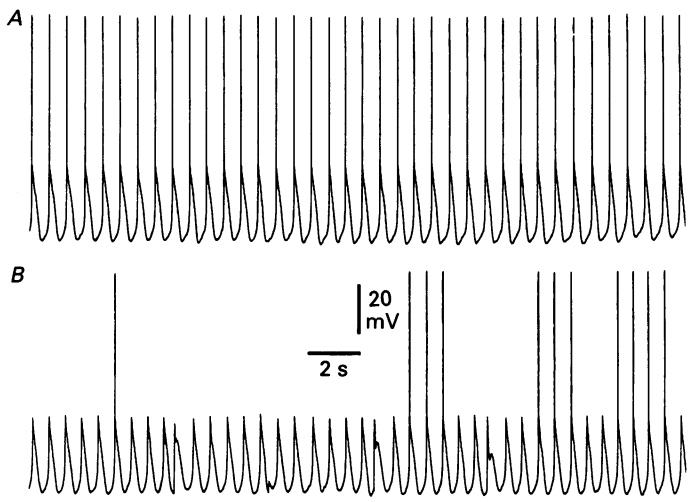

Fig. 4. Spontaneous delta oscillations in a nucleus deprived of cortical afferents. LP neurone displaying an uninterrupted oscillation at about 1·2 Hz. *A*, at this level of polarization (AHP peak at −75 mV), each LTS was crowned by action potentials. *B*, as the V_m spontaneously depolarized by 3 mV, the oscillation mostly consisted of LTSs in isolation, some of them triggered by arhythmically occurring fast pre-potentials. Note in both traces complete repolarizing power of AHP, and virtual lack of slow V_m relaxation.

oscillation could be stopped by DC depolarization, with the appearance of a depolarizing tail (not depicted). Interestingly, no rhythmic postsynaptic potentials that could possibly act as extrinsic drives were uncovered by this manoeuvre. The delta oscillation appeared within a V_m window between -65 mV (-70.8 ± 0.9 mV)

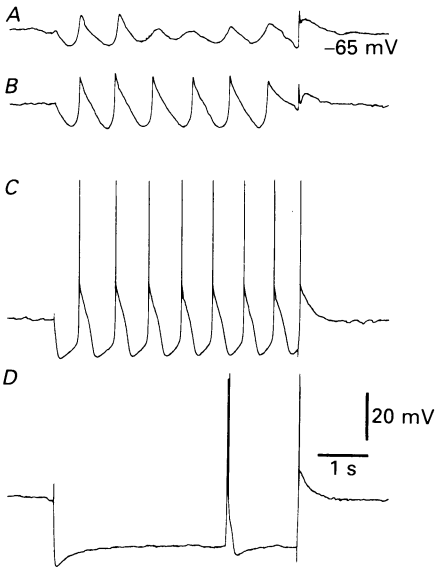

Fig. 5. Hyperpolarization-induced slow oscillations in an LP thalamocortical neurone. Hyperpolarizing current pulses of increasing amplitude were applied at a holding V_m of -65 mV to prevent spontaneous oscillations occurring at rest (-68 mV). Progressive development of a delta oscillation (*A–C*) and its disappearance at the most hyperpolarized level (*D*). Note, in *D*, survival of V_m relaxation and tail depolarization. From top to bottom current pulse intensities were 0·05, 0·1, 0·4 and 0·7 nA.

and -90 mV (-85.7 ± 1.8 mV). When hyperpolarizing pulses of increasing amplitudes were applied to a neurone prevented from oscillating by steady depolarizing current (Fig. 5), it was possible to appreciate the transition from V_m just enough for subthreshold oscillations, to fully developed oscillations and, then, to levels out of the oscillatory range. Only a modest V_m relaxation could be detected.

The electrophysiological peculiarity of LP neurones was their dramatic oscillatory trend. Both the hyperpolarized V_m and the higher membrane input resistance found in these cells (see Data base and neuronal identification) could account for it. We hypothesized that this was due to the routine removal of cortical areas related to LP (see Methods). That this was indeed the case was demonstrated by extending the decortication and recording the same type of activity in other thalamic territories. This has been observed both extra- and intracellularly in virtually all thalamocortical neurones recorded under these experimental conditions. Furthermore, extracellular recordings with metal microelectrodes in LP ($n = 8$) and VL ($n = 5$) before and after cortical spreading depression by means of potassium, showed that tonic discharges changed into rhythmic firing in the delta frequency range as the electrocortical activity in the related cortical areas disappeared (Fig. 6).

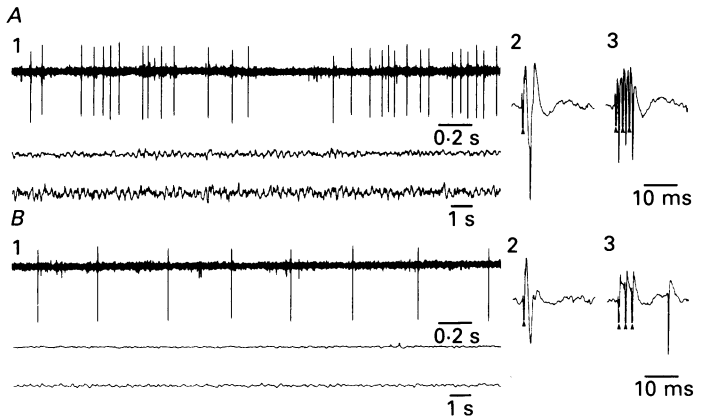

Fig. 6. Appearance of spontaneous delta activity after chemical inactivation of related cortical areas. Thalamocortical neurone extracellularly recorded in CL before (*A*) and after (*B*) potassium-induced spreading depression (SD) of the ipsilateral pericruciate area. The change from tonic (*A*1) to rhythmic (*B*1) firing was time related with the flattening of the EEG. At right, a reduction in the excitability of the cell was demonstrated during SD by ortho- and antidromic stimulation, to BC (*A*2 and *B*2) and pericruciate area (*A*3, *B*3), respectively. Arrow-heads mark stimulus artifacts.

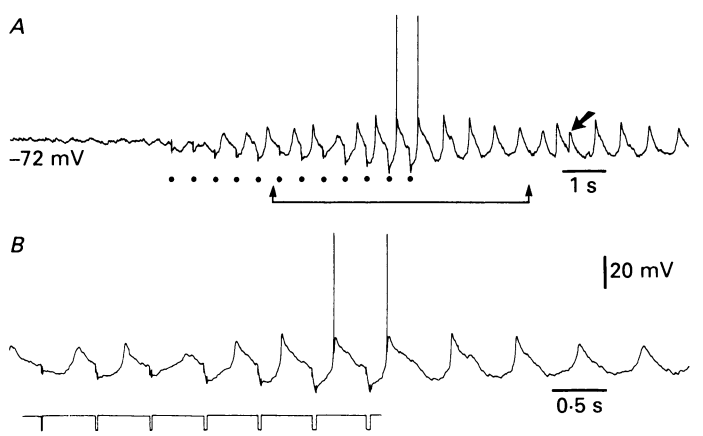

Fig. 7. Induction of self-sustaining delta oscillations by injecting short rhythmic hyperpolarizing current pulses. *A*, LP neurone recorded at 'rest' during an oscillation-free period. The frequency of the pulse was chosen according to previously recorded oscillatory epochs. Pulse duration was progressively increased until rebound LTSs with sodium spikes could be triggered. Pulse amplitude was 0·4 nA. The portion between arrows is depicted at higher speed in *B* with the current monitor trace. Note progressive development from subthreshold to suprathreshold self-sustaining delta oscillations. Oblique arrow in *A* marks the spontaneous occurrence of a fast pre-potential.

The high propensity to oscillate of neurones deprived of cortical afferents was demonstrated intracellularly during oscillation-free periods by injecting short hyperpolarizing current pulses at a frequency resembling the natural one (Fig. 7). If the stimulation was interrupted after the induction of overt LTSs, the cell could oscillate by itself, regardless of the occurrence of action potentials.

Another electrophysiological characteristic encountered under these experimental conditions was the virtual absence of slow V_m relaxation during periods of uninterrupted delta oscillations (Figs 4, 8 and 11). This phenomenon, probably reflecting the high membrane input resistance of these neurones (see Discussion),

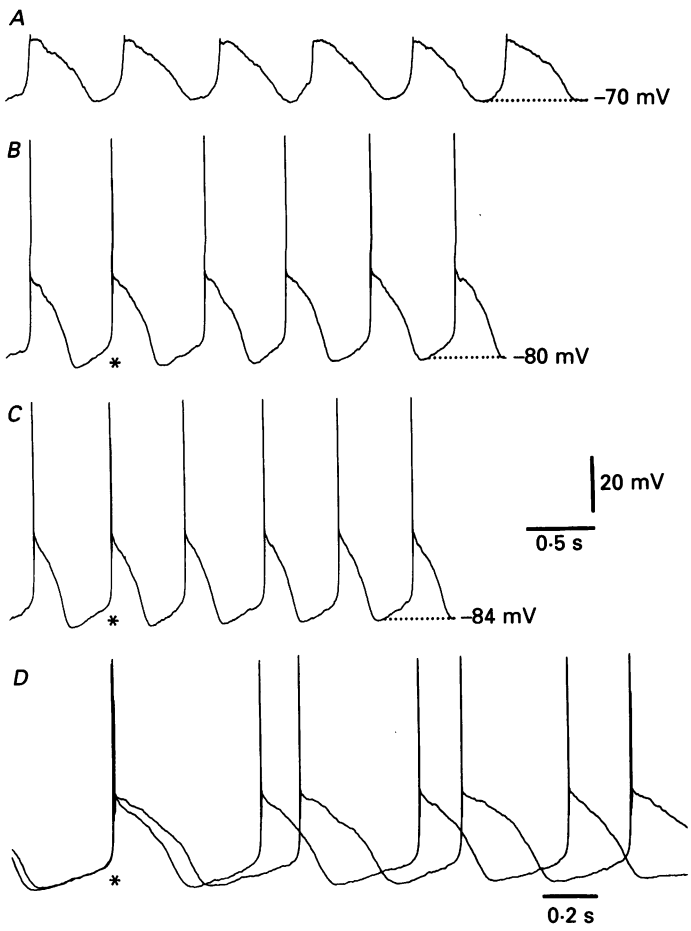

Fig. 8. Voltage-dependent changes in delta rhythmicity. LP neurone displaying uninterrupted delta oscillations recorded at increasing levels of membrane polarization. Dotted lines indicate V_m at the AHP peak. Six cycles are depicted in *A*, *B*, and *C*. Note increased steepness of the pacemaker depolarization from *A* to *C*. The increase in frequency at more negative V_m was also dependent upon a sharpening of the LTS. This aspect is demonstrated in more detail in *D*, where *B* and *C* traces are superimposed, beginning with the LTSs marked by asterisks.

enabled us to study the effect of the V_m on the frequency of the oscillation in quasi-steady-state conditions. Usually the frequency was higher at increased levels of membrane polarization (Figs 5 and 8). As depicted in Fig. 8, the frequency change could be accounted for by at least two distinct mechanisms. In addition to an increased pacemaker depolarization steepness, a sharpening in the LTS silhouette

was also evident at more hyperpolarized levels. Neither of the aforementioned aspects was a peculiarity of LP cells, in that similar observations have been made in other thalamic nuclei as well.

Absence of delta intrinsic oscillations in the anterior thalamic nuclei

Among the dorsal thalamic neurones investigated in this study, only AT cells ($n = 12$) did not display the 0·5–4 Hz intrinsic oscillation.

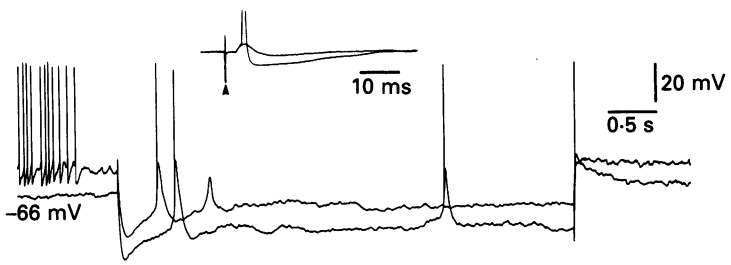

Fig. 9. Lack of delta oscillations in an AT neurone. Two superimposed hyperpolarizing pulses (1·5 nA) delivered at rest (−66 mV) and at −55 mV. In the latter case, two LTSs 0·5 s apart were obtained, seemingly triggered by pacemaker-like depolarizations. V_m relaxation was prominent in both sweeps, while a tail depolarization characterized the break of the pulse injected at −66 mV. Note delayed LTS take-off, increasing at more hyperpolarized levels, indicative of an A-current. Inset, two superimposed sweeps showing the identification from the mammillary body at slightly different stimulation intensities. Arrow-head marks stimulus artifact. Action potential truncated.

The only response to long hyperpolarizing current pulses consisted of V_m relaxation and tail depolarization at the onset and at the break of the pulse, respectively (Figs 9 and 10). Sometimes, the rate of relaxation was fast enough to trigger an LTS and a second cycle could exceptionally follow (Fig. 9, upper trace, recorded at −55 mV). Nevertheless, a true regenerative oscillatory process has not been observed.

Sometimes pseudorhythmic bursts appeared under hyperpolarizing current. Although the bursts could be in the frequency range of delta oscillations, all the underlying LTSs appeared to be triggered by fast pre-potentials (FPPs) (Fig. 10). This failure to induce the oscillation by intracellular current injections was paralleled by the lack of 0·5–4 Hz rhythmic firing at the extracellular level ($n = 9$), after large excisions of the related cortical areas (data not shown).

Sensitivity to barbiturate administration

Hyperpolarizing current pulses in intact brain neurones under deep barbiturate anaesthesia did not result in a clear-cut delta oscillation ($n = 14$). As depicted in Fig. 11*A*, the response only consisted of V_m relaxation and tail depolarization. Often, the depolarizing slope of the V_m relaxation triggered single LTSs. During epochs characterized by spindle oscillations, care was taken to apply the pulses during interspindle lulls. Furthermore, administration of a short-acting barbiturate

reversibly blocked the spontaneously on-going oscillation in LP cells ($n = 4$), the effect not being dependent upon the occurrence of intracellular spindle oscillations (Fig. 11*B*).

Neurones with intact cortical connections recorded under barbiturate anaesthesia displayed a V_m of $-68{\cdot}4 \pm 0{\cdot}7$ mV, ranging from -65 to -70 mV. No further

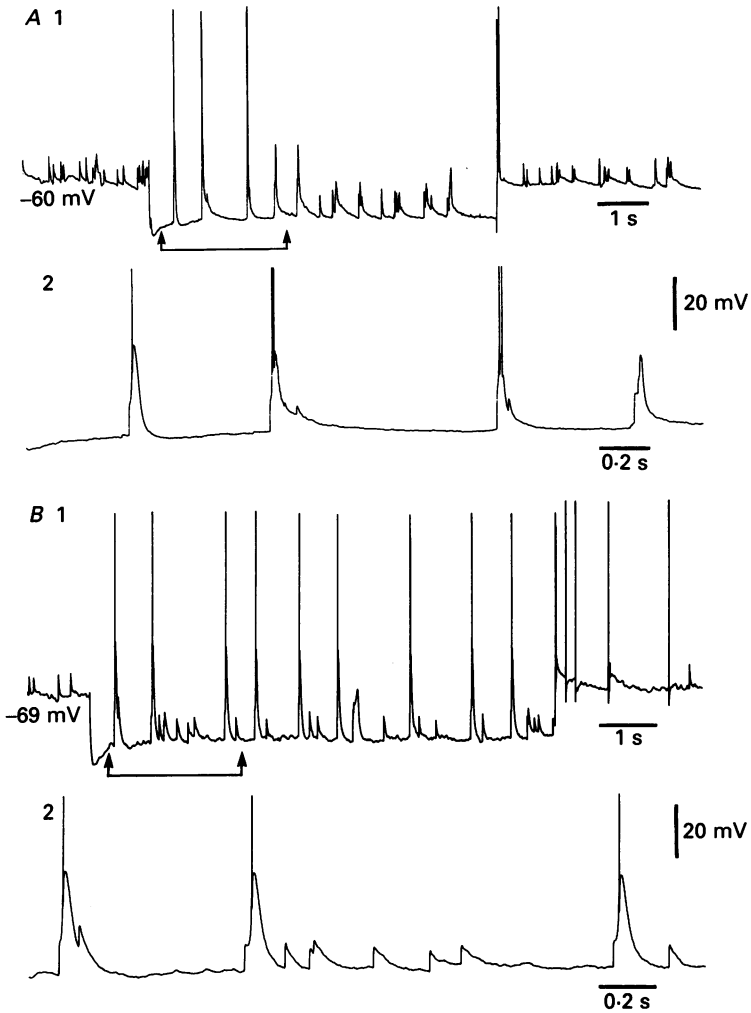

Fig. 10. Pseudorhythmic activity in AT neurones. Two different thalamocortical cells. *A*1 and *B*1, V_m relaxation with quasi-rhythmic LTSs obtained by injecting hyperpolarizing current pulses (1·5 and 1 nA, respectively). The portions between arrows are depicted at higher speed in *A*2 and *B*2 with truncated action potentials. Each LTS was clearly triggered by an FPP, appearing as an inflexion in the rising phase.

hyperpolarization was observed after barbiturate administration in cells whose related cortical areas had been ablated. Under both experimental conditions we found a membrane input resistance of $10{\cdot}5 \pm 0{\cdot}3$ MΩ i.e. in the lowest range observed

in this study. We believe that this change in the passive properties of the membrane could partially explain the suppressing effect of barbiturates upon the phenomenon under study (see Discussion).

Thalamic delta-wave generation

The final issue we addressed was whether the activity of simultaneously oscillating neurones was somehow correlated and, if so, whether it could result in field potentials

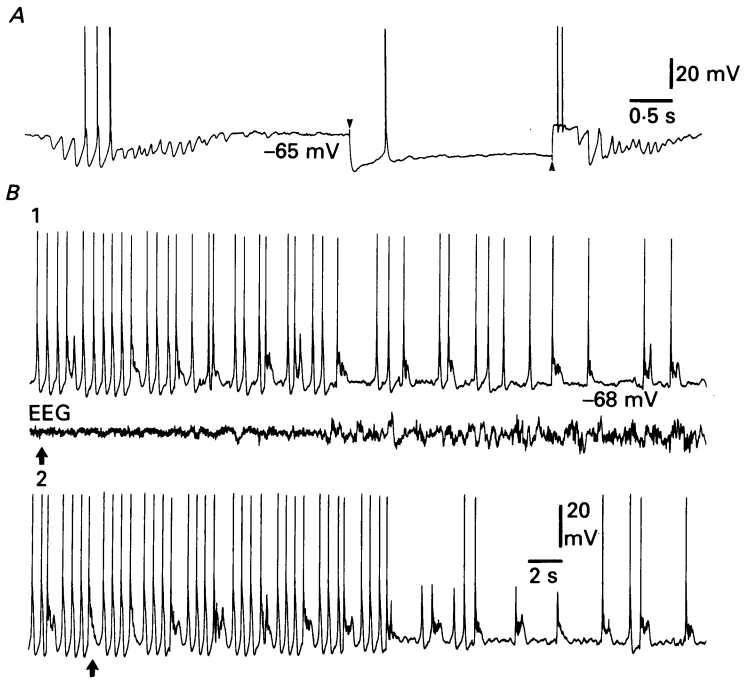

Fig. 11. Sensitivity of delta oscillations to barbiturate administration. *A*, CL neurone recorded under sodium pentobarbitone. A 2·5 nA hyperpolarizing current pulse (between arrow-heads) was applied during an interspindle lull. Note V_m relaxation and a single LTS seemingly triggered by its depolarizing slope. *B*1, LP neurone displaying spontaneous delta oscillatory activity. Administration of the short-acting barbiturate sodium thiamylal (arrow) reversibly blocked the oscillation and simultaneously increased the EEG synchronization. *B*2, same effect, obtained after the cell recovered its delta activity.

in the frequency range of delta waves. The experimental design consisted of multiunit recordings in a nucleus deprived of cortical afferents.

Often the activity of pairs of oscillating cells resembled that of independent oscillators, with slight frequency differences.

Nevertheless we observed limited epochs with spontaneous phase-locked firing between different units ($n = 12$) (Fig. 12). At a macrophysiological level, focal slow waves became evident or were greatly enhanced during these epochs. Since synchronous cellular firing did not appear a *sine qua non* condition for the build-up of focal delta waves (see left part of the top traces on Fig. 12), slow extracellular currents produced by each LTS could play a critical role.

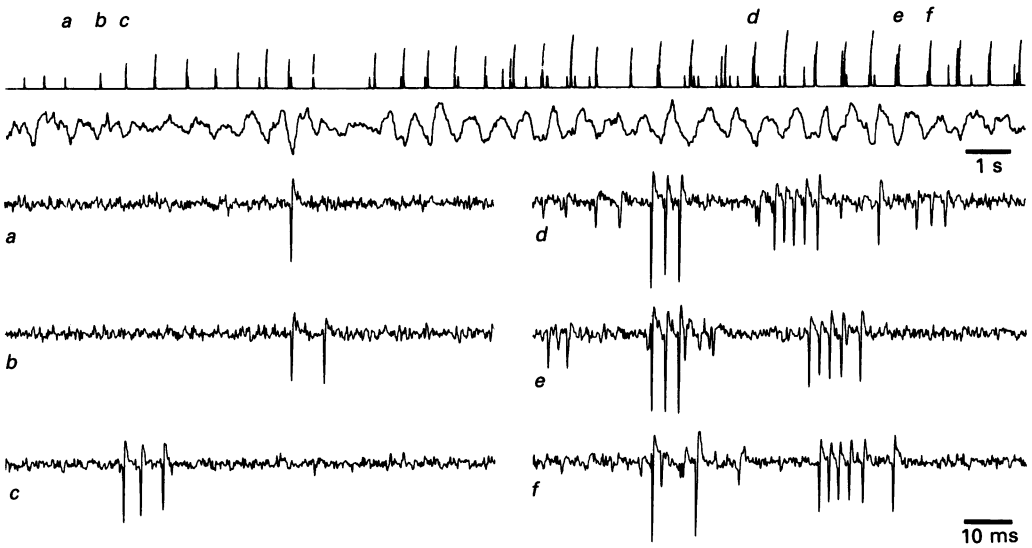

Fig. 12. Relation between multiunitary activity and focal delta waves. Extracellular recordings in the LP nucleus with a low-impedance microelectrode. At top, traces of multiunitary activity and focal slow waves. Oscilloscopic traces of the cellular activity in *a–f* are depicted at bottom. Note parallel build-up of focal delta waves and synchronous firing.

DISCUSSION

Mechanisms underlying slow (0·5–4 Hz) intrinsic oscillations in thalamic neurones

The present study provides the first evidence that thalamocortical neurones *in vivo* possess an intrinsic capability to oscillate at 0·5–4 Hz. Similar V_m oscillations have been described recently in thalamic neurones *in vitro*. An oscillation consisting of 0·5–2 Hz LTS–AHP sequences has been recorded in slices of cat medial geniculate, parataenial (McCormick & Prince, 1988) and dLG nuclei (McCormick & Pape, 1990*a*, *b*). A 0·5–3 Hz oscillation was obtained in thalamic slices of cat and rat by using a low-Mg^{2+}/high-Ca^{2+} bathing medium (Leresche, Jassik-Gerschenfeld, Haby, Soltesz & Crunelli, 1990; Leresche *et al.* 1991; Soltesz *et al.* 1991). In all these studies the oscillatory activity was present at a hyperpolarized V_m (-70 to -90 mV) and appeared to be generated by the interplay of at least two membrane intrinsic currents, I_t and I_h. The oscillation was never observed in cells lacking the low-threshold transient Ca^{2+} conductance and it was abolished by Ni^{2+} and Cs^+, known to block the I_t and the I_h, respectively (Leresche *et al.* 1990; McCormick & Pape, 1990*a*; Soltesz *et al.* 1991).

I_h is a non-inactivating inward (anomalous) rectifier, carried by Na^+ and K^+. In dorsal thalamic neurones, I_h is operative at V_m more negative than -55 mV (McCormick & Pape, 1990*a*; Soltesz *et al.* 1991). In current clamp recordings, its activation upon hyperpolarization appears as a depolarizing sag back towards rest and its deactivation upon depolarization results in a depolarizing tail component. Both processes are quite slow, their time constants ranging from hundreds to

thousands of milliseconds, as measured under voltage clamp (McCormick & Pape, 1990*a*; Soltesz *et al.* 1991). In sino-atrial cells and in cardiac Purkinje fibres, this mixed cationic current appears critically involved in generating the diastolic pacemaker depolarization (for a review see DiFrancesco & Noble, 1989). In the thalamus, as in the heart, the suitability of I_h for producing oscillatory activities derives from its particular kinetic properties. If I_h displaces the V_m in a direction where its conductance closes and the I_h deactivation results in V_m repolarization, the cycle could ideally repeat forever, provided that these two opposite processes alternate with one another. Physiologically, this does not seem to be the case, the regenerative phenomenon being strongly dependent upon the occurrence of LTSs. The LTS could be essential in quickly shifting the V_m towards a region where I_h deactivation prevails. The termination of both I_h and I_t would deprive the membrane of their depolarizing pressure, resulting in a V_m displacement towards values where prevailing I_h activation could restart the cycle by triggering another LTS. According to this interpretation, the AHP appears as a passive response and, indeed, the membrane conductance has been reported to be lowest at the most hyperpolarized level and to increase as V_m regains its resting value (McCormick & Pape, 1990*a*). Furthermore, in our *in vivo* model, an AHP could follow a depolarizing pulse delivered at a V_m negative to -65 mV regardless of the occurrence of LTS (not shown). Nevertheless, repolarization of the membrane could be also actively achieved, for instance, through the Ca^{2+}-activated potassium current I_{AHP} (Jahnsen & Llinás, 1984*b*; Roy, Clercq, Steriade & Deschênes, 1984).

The depolarizing sag we observed in response to long-lasting (> 100 ms) hyperpolarizing current pulses is indicative of anomalous rectification. More specifically, the time course and voltage dependence of the phenomenon are consistent with the involvement of the I_h. This sag may be steep enough to trigger an LTS, and subsequent LTSs can be generated by similar depolarizing ramps, referred to as pacemaker depolarizations; the latter appear as the decaying phases of corresponding AHPs. In neurones deprived of cortical connections, the AHP is deep enough to repolarize the membrane completely after each LTS, in which case the oscillation can go on indefinitely (Figs 4, 8 and 11*B*). By contrast, partial repolarization appears to follow the oscillatory LTS of neurones with intact cortical afferents, the phenomenon resulting in a progressive depolarizing shift, referred to as slow V_m relaxation (Figs 2 and 3). This aspect might depend on a higher membrane conductance of synaptic origin, globally reducing the voltage responsivity of the cell. Because of a partial shunt of the depolarizing surge provided by the LTS, the I_h deactivation could be impaired, and each consecutive AHP become less pronounced. This change in AHP might also reflect a similar shunt of actively involved outward currents. With prominent V_m relaxation, the pacemaker depolarization becomes less steep, seemingly because of a lessening in the I_h activation rate (see Fig. 3 in McCormick & Pape, 1990*a*), and finally fails to trigger the LTS (Figs 2 and 3). Once the oscillation has completely dampened, a sustained V_m plateau is usually observed, probably reflecting a dynamic equilibrium reached by the I_h conductance. The fading of the oscillation appears to be a continuum, which apparently contrasts with the all-or-none nature of the LTS. This phenomenon in fact results in V_m waves that have been called subthreshold oscillations (Figs 2, 3 and 5). They may arise from the

activity of T-channel patches. Clearly, further investigations are required to clarify this matter. The I_h–I_t interplay, necessary in providing the I_h on–off alternation, is also disrupted by an excessively polarized membrane (Fig. 5*D*). This phenomenon has been ascribed to imbalance between the two currents (McCormick & Pape, 1990*a*; Leresche *et al.* 1991).

The sensitivity of the oscillation to barbiturates (Fig. 11) explains why delta oscillations have not been previously reported in studies performed under this anaesthetic condition (Deschênes, Paradis, Roy & Steriade, 1984). We have observed a higher membrane conductance under barbiturate anaesthesia and an increase in GABA-induced conductance by barbiturates has been reported in thalamic relay neurones *in vitro* (Sykes & Thomson, 1989). On the other hand, the failure to trigger delta oscillations during epochs with barbiturate-induced spindles (Fig. 11*B*) might be accounted for by both the effect of the drug and the one of spindles themselves. In fact, a parallel study conducted in this laboratory (Nuñez, Curró Dossi & Steriade, 1991) has demonstrated that spindles *per se*, as occurring spontaneously in *cerveau isolé* preparations, could greatly depress the 0·5–4 Hz oscillation.

The case of the anterior thalamic nuclei

Another unsolved question refers to the lack of delta oscillations in AT neurones. It is known that these cells appear endowed with the same basic electrophysiological characteristics (Paré, Steriade, Deschênes & Oakson, 1987) that are common to other dorsal thalamic neurones (Deschênes *et al.* 1984; Jahnsen & Llinás, 1984*a*). In spite of this, injection of hyperpolarizing current pulses only resulted in a depolarizing sag upon application and in a tail depolarization upon removal. In all likelihood this phenomenon reflects the turning on and off of the I_h (Figs 9 and 10). Isolated LTSs were sometimes triggered by the depolarizing sag, but a real oscillation has not been observed. The unusual finding of more than one LTS might suggest that cell damage related to the impalement could have disrupted a weak oscillatory capability. However, the lack of rhythmic 0·5–4 Hz firing at the extracellular level following the removal of the related cortical areas, appears to support the assumption that AT thalamocortical neurones do not exhibit this oscillation. *In vitro* experiments with voltage-clamp analysis should shed some light on this phenomenon.

Delta waves in thalamocortical systems

During behavioural states globally characterized by a decreased alertness, such as drowsiness and SWS, the thalamus operates in an oscillatory mode. So far, spindle waves have been the only known thalamic oscillation during sleep. Spindles are generated in the thalamic reticular RE nucleus (RE) as a result of intrinsic and synaptic mechanisms. The oscillation is transmitted from this pacemaker structure to the dorsal thalamus as barrages of rhythmic inhibitory postsynaptic potentials and then to the whole neocortical convexity by means of spike bursts riding on rebound LTSs (Steriade, Domich, Oakson & Deschênes, 1987; Steriade & Llinás, 1988).

As sleep deepens, EEG delta (0·5–4 Hz) waves become more and more represented at the expense of spindle waves, so that one should hypothesize either a reduced cortical transfer of on-going thalamic spindles or a change in the thalamic functional

state. In the latter case the thalamus could either be just a resonant structure, tuned on cortical delta waves generators (see Introduction,) or an active oscillatory device. Until recently, it has been generally assumed, without conclusive evidence, that EEG delta waves originate exclusively in the cortex (Steriade, Gloor, Llinás, Lopes da Silva & Mesulam, 1990), the thalamic contribution to sleep-related EEG oscillations being limited to spindling. The present *in vivo* demonstration of a slow delta oscillatory propensity intrinsic to thalamocortical cells and operational at V_m typical of SWS (Hirsch, Fourment & Marc, 1983) strongly suggests that the oscillatory state of the thalamus can transcend spindling. This new electrophysiological property which, by definition, is generated as a unitary event can in fact affect the whole thalamocortical system through the recruitment of one or more synchronizing devices.

Although 'spontaneous' synchronicity in the firing of simultaneously recorded cells in a nucleus deprived of cortical afferents is sometimes possible (see Fig. 12), a parallel study conducted in this laboratory (Steriade *et al.* 1991) has demonstrated that the stimulation of corticothalamic pathways is able to reinforce and synchronize the delta oscillatory activity of multiple units. At the intracellular level this effect was accompanied by slight V_m hyperpolarization. Because these experiments have been carried out in thalamic nuclei deprived of their cortical projection areas and cortical stimulation potentiated slow oscillations in not directly related nuclei, the most probable synchronizer is the reticular thalamic nucleus (RE), because of its widespread projections to the dorsal thalamus. Nevertheless its role would substantially differ from the pacemaking activity exerted during spindling. In fact, as far as the delta rhythmicity is concerned, the source is intrinsic to each thalamocortical cell. RE neurones would instead provide a synchronous hyperpolarizing drive, resulting in a rhythmic thalamo-RE and thalamo-cortical output. The recruitment of GABAergic RE and thalamic local circuit cells would reinforce the delta activity. Indeed, we have shown that when a steadily hyperpolarized thalamocortical neurone is subject to phasic hyperpolarizations, it can be set in a self-sustaining oscillatory state (Fig. 7). When the thalamic circuitry is artificially deprived of its cortical link, the activity of neighbouring cells often resembles that of independent oscillators. Under these circumstances, it appears as if none of the different oscillatory foci has succeeded in imposing its rhythmicity upon the others. It is reasonable to assume that the participation of the cerebral cortex would help the dominant frequency to emerge, by means of corticothalamic resonant loops, where synaptic and intrinsic intracortical mechanisms (see Introduction) would play an important role as well.

The same study (Steriade *et al.* 1991) also demonstrated how the intracellular activity of a thalamocortical neurone can be dominated by spindling or slow delta oscillations in a voltage-dependent manner. The synaptically driven spindle rhythmicity, prevalent at more depolarized V_m levels, was overwhelmed by the slow intrinsic oscillation as the cells were hyperpolarized. This sort of incompatibility between delta and spindle oscillations appears corroborated by another study conducted in this laboratory (Nuñez *et al.* 1991), showing an impairment of intracellular delta oscillations by spontaneously occurring spindle sequences. During SWS the V_m of thalamocortical cells progressively hyperpolarizes as a result of

decreased activity in brain stem modulatory systems and a reduced corticothalamic depolarizing pressure (Steriade & McCarley, 1990). This phenomenon could be the determining factor accounting for the switch from spindling to delta waves in thalamocortical systems. On the other hand, the V_m depolarization upon natural arousal or stimulation of brain stem cholinergic aggregates (Curró Dossi, Paré & Steriade, 1991), should effectively set thalamocortical cells out of the oscillatory range. Indeed, stimulation of brain stem cholinergic nuclei is able to block on-going delta oscillations in thalamocortical cells, the effect being prevented by anti-cholinergic drug administration (Steriade *et al.* 1991).

In addition to its involvement in physiological sleep EEG rhythms, the delta oscillation could be implicated in pathophysiological conditions globally characterized by rhythmic activities in thalamocortical systems, like some forms of epilepsy. In particular, the ability of corticothalamic volleys to trigger a synchronous self-sustaining thalamic oscillation of 0·5–4 Hz (Steriade *et al.* 1991) might play an important role in the secondary bilateral synchrony, where one or more cortical epileptic spikes can induce generalized spike-and-wave complexes at 2–3 Hz.

The functional state of a thalamocortical neurone is the result of an interplay between intrinsic and network properties and the oscillation described in this study provides a framework for a better understanding of this crucial physiological aspect.

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