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The Slow (<1 Hz) Oscillation in Reticular Thalamic and Thalamocortical Neurons: Scenario of Sleep Rhythm Generation in Interacting Thalamic and Neocortical Networks

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As most afferent axons to the thalamus originate in the cerebral cortex, we assumed that the slow (<1 Hz) cortical oscillation described in the two companion articles is reflected in reticular (RE) thalamic and thalamocortical cells. We hypothesized that the cortically generated slow rhythm would appear in the thalamus in conjunction with delta and spindle oscillations arising from intrinsic and network properties of thalamic neurons.

Intracellular recordings have been obtained in anesthetized cats from RE (n = 51) and cortically projecting (n = 240) thalamic neurons. RE cells were physiologically identified by cortically evoked high-frequency spike bursts and depolarizing spindle oscillations. Thalamocortical cells were recognized by backfiring from appropriate neocortical areas, spindle-related cyclic IPSPs, and hyperpolarization-activated delta oscillation consisting of rhythmic low-threshold spikes (LTSs) alternating with afterhyperpolarizing potentials (AHPs).

The slow rhythm (0.3–0.5 Hz) was recorded in 65% of RE neurons. In ≈90% of oscillating cells, the rhythm consisted of prolonged depolarizations giving rise to trains of single action potentials. DC hyperpolarization increased the synaptic noise and, in a few cells, suppressed the long-lasting depolarizing phase of the slow rhythm, without blocking the fast EPSPs. In ≈10% of oscillating neurons, the hyperpolarizing phase of the oscillation was much more pronounced, thus suggesting that the slow rhythm was produced by inhibitory sculpturing of the background firing. The slow oscillation was associated with faster rhythms (4–8 Hz) in the same RE neuron. The slow rhythm of RE neurons was closely related to EEG wave complexes recurring with the same frequency, and its strong dependency upon a synchronized state of cortical EEG was observed during shifts in EEG patterns at different levels of anesthesia.

In 44% of thalamocortical cells the slow rhythm of depolarizing sequences was apparent and it could coexist with delta or spindle oscillations in the same neuron. The occurrence of the slowly recurring depolarizing envelopes was delayed by the hyperpolarizing spindle sequences or by the LTS-AHP sequences of delta oscillation. The hyperpolarization-activated delta potentials that tended to dampen after a few cycles were grouped in sequences recurring with the slow rhythm.

We finally propose a unified scenario of the genesis of the three major sleep rhythms: slow, delta, and spindle oscillations.

[Key words: reticular thalamus, dorsal thalamus, slow rhythm, delta rhythm, spindle rhythm, intracellular recordings, EEG, sleep]

Most afferent axons to the thalamus originate in the cerebral cortex. In addition to reciprocal corticothalamic projections ending in dorsal thalamic nuclei from which a given cortical field receives inputs, distinct neocortical areas have access to multiple thalamic nuclei through indirect projections relayed in GABAergic reticular (RE) thalamic neurons. The rostral pole and rostrolateral districts of the RE nuclear complex receive afferents from many cortical areas and, in turn, project to a variety of dorsal thalamic territories; among them are those with widespread cortical projections, such as the centrolateral (CL)-paracentral intralaminar and ventromedial nuclei (Steriade et al., 1984; Jones, 1985). This type of RE-mediated corticothalamocortical diffuse connection is supplemented by linkages between corticothalamic axons and GABAergic local-circuit thalamic neurons within each thalamic nucleus of felines and primates (Guillery, 1967; Jones and Powell, 1969). Corticothalamic volleys induce strong augmentation and synchronization responses in thalamic cells, much more so than the ascending inputs along specific afferent pathways to the thalamus (Steriade et al., 1972; Steriade, 1984). Corticothalamic projections do, therefore, underlie the performance of specific functional tasks during alert states, but they are also able to spread diffusely within the thalamus cortically generated sleep rhythms and to unite into ensembles singly oscillating cells within the boundaries of various dorsal thalamic nuclei.

In the two preceding articles we have analyzed a newly discovered slow neocortical rhythm (≈0.3 Hz) and its relations, at the intracellular and EEG levels, with intrinsic and synaptic oscillations known to originate in the thalamus (Steriade et al., 1993a,b). The following questions then arose: how is the slow cortical oscillation reflected in RE and thalamocortical cells, and what is the role of the thalamus in projecting back to the cortex the synchronized thalamic rhythms? Some evidence for cortically induced potentiation and synchronization of thalamic sleep rhythms was provided in earlier studies. Morison and

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Dempsey (1943) have described the reinforcement of thalamic spindle waves by local application of prostigmine and ACh to the appropriate cortical area, and the reduction of thalamic rhythmicity after section of internal capsular fibers. In a study on intrinsically generated delta oscillation in thalamic cells, we have shown that the critically required hyperpolarization is induced by corticothalamic volleys, and suggested that the facilitatory and synchronizing actions on delta rhythmicity are mediated through GABAergic RE and local-circuit thalamic neurons (Steriade et al., 1991). The aim of the present article, the logical continuation of the preceding two articles, was to provide direct intracellular evidence for the involvement of thalamic cells in the association between spindles (7–14 Hz) and delta (1–4 Hz) and slow (< 1 Hz) oscillations occurring during the state of sleep with EEG synchronization. Our investigations led us to propose a unified concept of sleep rhythmicity.

Materials and Methods

Experiments were performed on 78 adult cats, 38 for RE cell recordings and 40 to investigate thalamocortical neurons. All animals were anesthetized with urethane (1.8 gm/kg, i.p.) or with ketamine (40 mg/kg, i.m.) supplemented with nitrous oxide or with xylazine (2–2.5 mg/kg). In order to maintain a permanent pattern of bioelectrical synchronization, typical for an anesthetized brain, the EEG was continuously recorded from the pericruciate cortical surface and additional doses of urethane or nitrous oxide were administered at the most precocious signs of decreased amplitudes and/or increased frequencies of EEG rhythms. Barbiturates were not used because they prevent the appearance of delta waves in dorsal thalamic nuclei due to a decrease in the membrane input resistance (Curró Dossi et al., 1992) and create an overwhelming spindling picture in thalamocortical systems, obscuring the slow cortical oscillation. Thus, slow, delta, and spindle rhythms illustrated in this article were all obtained under urethane or under ketamine and nitrous oxide or xylazine. However, as reported in the companion articles, the slow cortical rhythm prevails over spindle oscillations under urethane anesthesia. In addition to general anesthetics, pressure points and tissues to be incised were infiltrated before surgery with lidocaine. The animals were paralyzed with gallamine triethiodide and artificially ventilated. The end-tidal CO_2 concentration was kept around 3.7–3.8%. Internal temperature (37–38°C) and heartbeat were monitored. Parts of the cortex and fornix overlying the thalamic nuclei where recordings were planned were removed to facilitate the passage of the micropipettes. The stability of intracellular recordings was ensured by cisternal drainage, bilateral pneumothorax, hip suspension, and by filling the hole over the thalamus with 4% agar.

Intracellular recordings were performed with micropipettes (tip diameter, 0.5 μm) filled with 3 M solution of K-acetate (DC resistance, 25–40 MΩ). A high-impedance amplifier with active bridge circuitry was used to record and inject current into neurons. Signals were stored on a magnetic tape with bandpass of 0–9 kHz and thereafter digitized at 20 kHz for off-line analysis. Occasionally, RE cells were recorded extracellularly to assess the similarities with intracellularly recorded oscillations (see Fig. 5). Coaxial stimulating electrodes were inserted in prethalamic pathways, internal capsule, and various cortical areas for identification procedures and for inducing thalamic oscillations. At the end of the experiments, a lethal dose of sodium pentobarbital (50 mg/kg) was administered.

Results

Data base, general properties, and identification of RE and thalamocortical neurons

The results are based on 291 thalamic cells. Fifty-one neurons were impaled in the rostrolateral part of the RE nucleus and 240 cells were recorded from CL, lateroposterior (LP), and ventroanterior–ventrolateral (VA-VL) thalamic nuclei. Only neurons with resting membrane potential (V_m) more negative than −55 mV and overshooting spikes were analyzed. The resting V_m of RE neurons averaged −63.8 ± 1.2 mV (mean ± SE), their action potentials had an amplitude of 68.2 ± 1.1 mV, and their input resistance (estimated by applying short hyperpolarizing pulses at the resting V_m) was 37.6 ± 1.6 MΩ. In thalamocortical cells, the V_m was −61.5 ± 0.7 mV, the action potential amplitude was 64.1 ± 0.9 mV, and input resistance averaged 18.4 ± 0.9 MΩ.

RE neurons were activated bisynaptically by stimulation of prethalamic pathways (Fig. $1A_1$). They were identified by a series of electrophysiological characteristics. (1) Their typical response to internal capsule or cortical stimulation consisted of a high-frequency burst, followed by a sequence of spindle-like depolarizing oscillations with superimposed spike bursts (Fig. $1A_2$). It is known that, by contrast, thalamocortical cells respond to corticothalamic volleys by a sequence of hyperpolarizing oscillations, leading to low-threshold rebound spikes (LTSs), occasionally crowned by high-frequency bursts of somatic action potentials (see Steriade and Llinás, 1988). (2) RE neurons displayed two different firing modes, tonic and bursting, in response to depolarizing current pulses applied at different V_m (Fig. $1B,C$). This is similar to the two functional states of thalamocortical cells. However, at variance with the progressive increase in duration of successive interspike intervals within the bursts of thalamocortical cells, the bursts of RE neurons showed an acceleration in frequency (Fig. $1B$), often followed by deceleration (see such bursts in Figs. 5, 6). This accelerating–decelerating burst pattern of RE neurons, distinct from that of relay neurons recorded from the dorsal thalamus, was quantitatively studied in our previous study of naturally sleeping animals (Domich et al., 1986). (3) In 10% of the RE cell sample ($n = 5$), we revealed small (3–7 mV), fast (3–6 msec), all-or-none depolarizing potentials that could be triggered by either depolarizing current pulses or spindle depolarizing waves (dots in Fig. $1C_1,C_2$). These presumed dendritic spikes (see also Llinás and Geijo-Barrientos, 1988) are further described elsewhere (Contreras et al., 1993).

Thalamic relay cells were formally identified by their antidromic invasion from internal capsule or cortex stimulation and monosynaptic response to stimulation of prethalamic axons (data not shown). Other electrophysiological features of dorsal thalamic cells included (1) their hyperpolarizing spindle sequences (see Fig. 9 and compare it with Fig. $1A_2$ depicting an RE neuron); (2) the large-amplitude, biphasic IPSPs in response to cortical stimulation (see Fig. 11), mediated by $GABA_{A\text{-}B}$ receptors (Hirsch and Burnod, 1987; Crunelli et al., 1988; Paré et al., 1991); and (3) the hyperpolarization-activated delta oscillation (see Figs. 10–12), resulting from the interplay of I_h and I_t (McCormick and Pape, 1990a); by contrast, depolarizing sags, leading to rhythmic LTSs in the delta frequency, were not triggered by hyperpolarizing current pulses in rostrolateral RE cells.

The slow rhythm in RE thalamic neurons

We recorded the slow rhythm (mostly 0.3–0.5 Hz) in 33 out of 51 recorded cells (65%). They belonged to either of the two RE cell classes, defined electrophysiologically as having predominantly bursting or tonic discharge patterns (Contreras et al., 1992). In the overwhelming majority of slowly rhythmic cells, the oscillation consisted of depolarizing envelopes (Figs. 2, 3), while in four neurons a clear-cut, long-lasting (0.4–0.7 sec) inhibition periodically sculptured the spontaneous firing of RE neurons (Fig. 4).

At the resting V_m, the slow depolarizations gave rise to trains of single spikes, with frequencies varying from 5 to 30 Hz in different neurons. DC hyperpolarizations reduced and eventu-

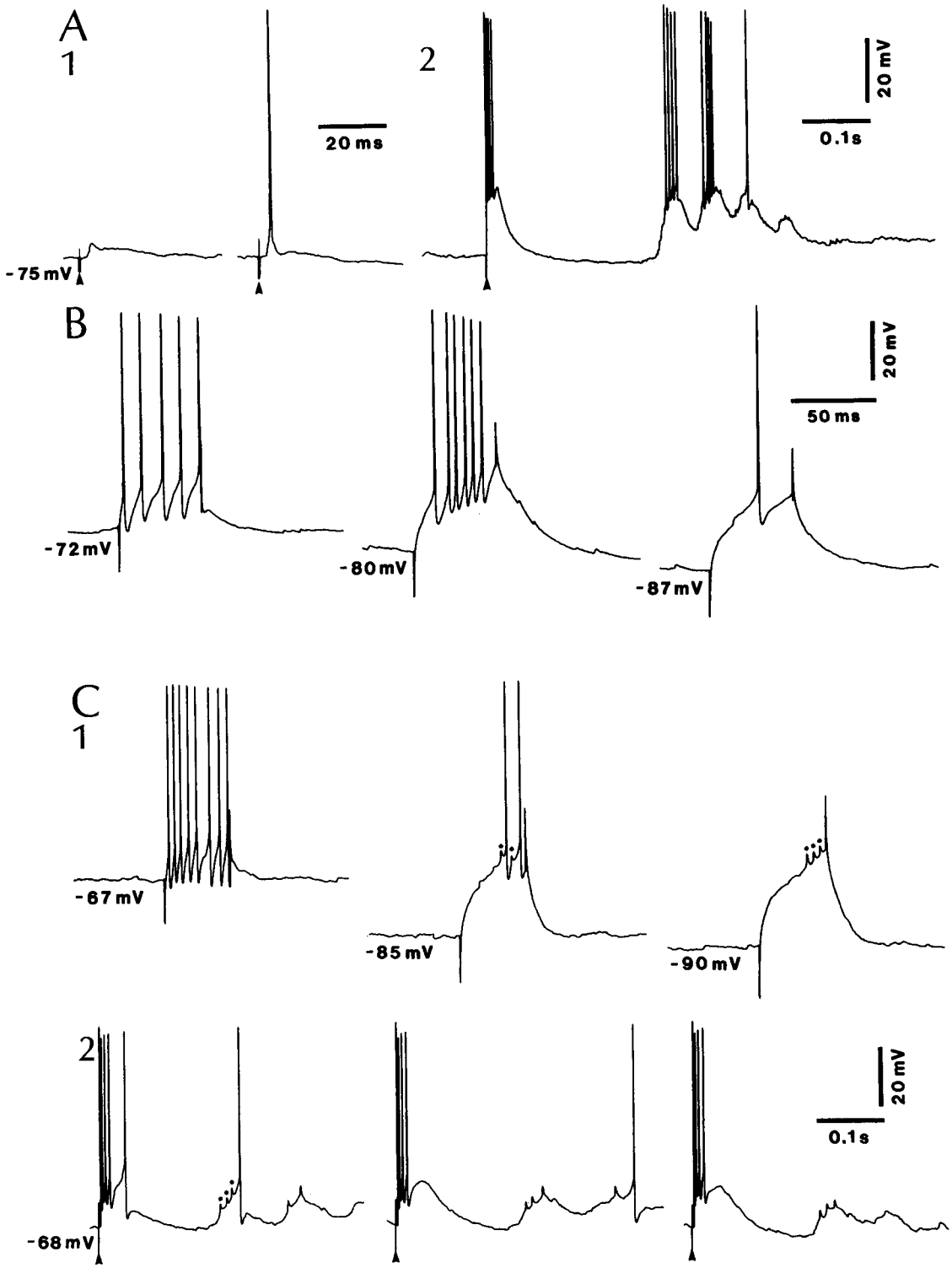


Figure 1. Electrophysiological properties identifying RE cells. *A–C* are from three different cells. *A*, Bisynaptic response to brachium conjunctivum stimulation (*1*) and spike burst followed by depolarizing spindle sequence evoked by internal capsule stimulation (*2*). Stimuli are marked by *arrowheads*. *B*, Tonic and burst firing modes are elicited by applying constant depolarizing current pulses (+0.6 nA) at different membrane potential (V_m). *C*, Presumed dendritic spikes (*dots*) elicited by constant depolarizing current pulses (+0.8 nA) after blockage of somatic action potentials by DC hyperpolarization (*1*) and appearing on the spindle-like depolarizing waves (*2*) evoked by internal capsule stimuli (*arrowheads*); three evoked spindles. In this and following figures, V_m is indicated (during oscillations, the V_m is taken at the trough of the hyperpolarizing phase).

ally blocked the action potentials, while the amplitude of the slow depolarization and the superimposed synaptic noise was increased (Fig. 2*A*). The same phenomenon was observed when hyperpolarizing current pulses were injected during the expected occurrence of a periodic depolarizing envelope (see *a* in Fig. 2*B*).

In 27 RE cells, we could not suppress or even reduce the slowly rhythmic depolarizations by DC hyperpolarization.

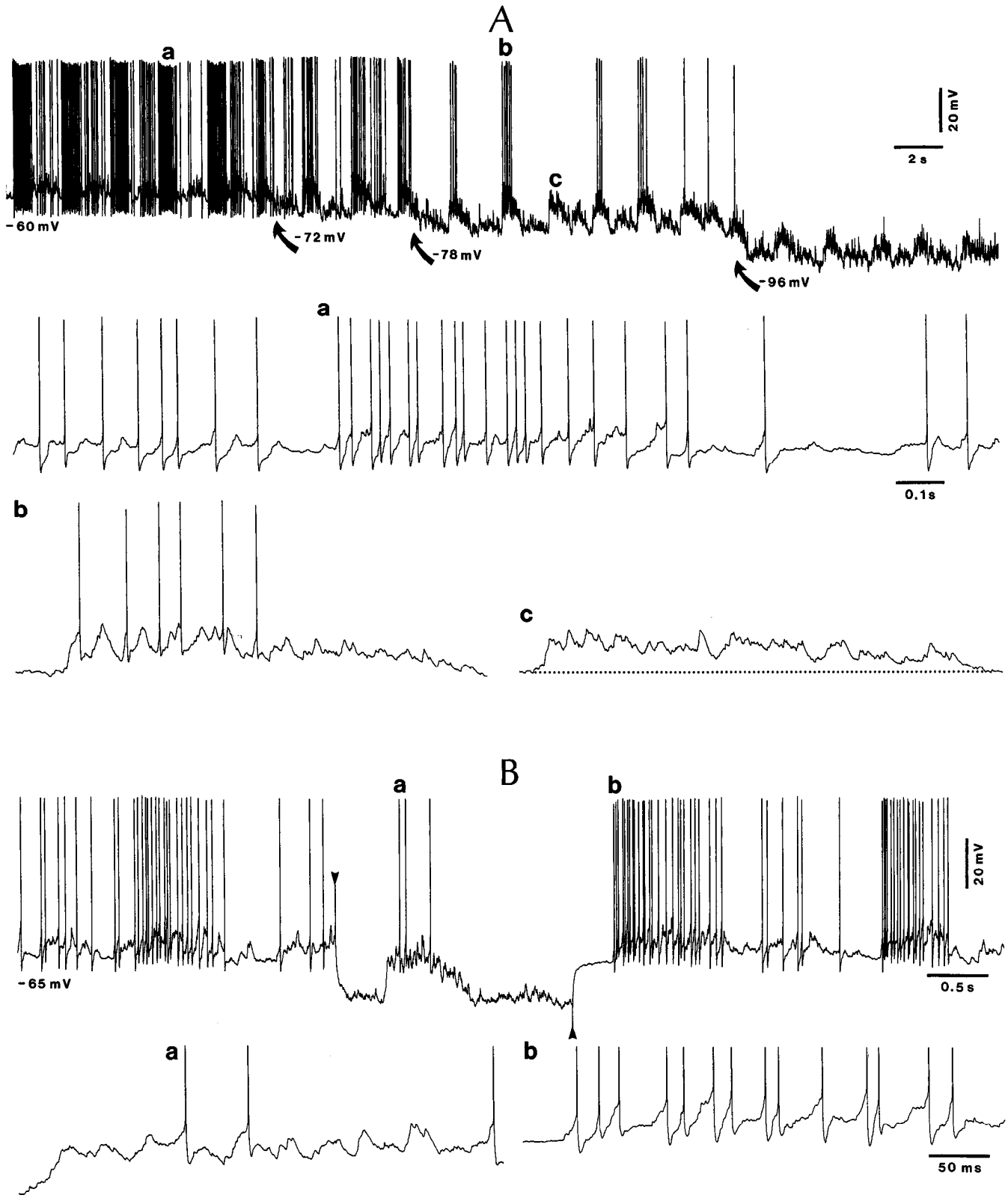


Figure 2. The slow rhythm in RE cells. *A*, Slow (0.5 Hz) depolarizing oscillation with superimposed trains of action potentials (20–30 Hz) at the resting V_m (−60 mV), and the effects of DC hyperpolarization (−0.7 nA, −1 nA, and −1.8 nA, *oblique arrows*) bringing the V_m to −72, −78, and −96 mV, respectively. Three parts of slow oscillation at −60 mV and −78 mV, marked by *a–c*, are expanded *below* (*dotted line* tentatively indicates the baseline). *B*, Another RE cell displaying an increased amplitude of the depolarizing envelope during a hyperpolarizing current pulse (−1.1 nA) applied between *arrowheads*. *a* and *b* are expanded *below*.

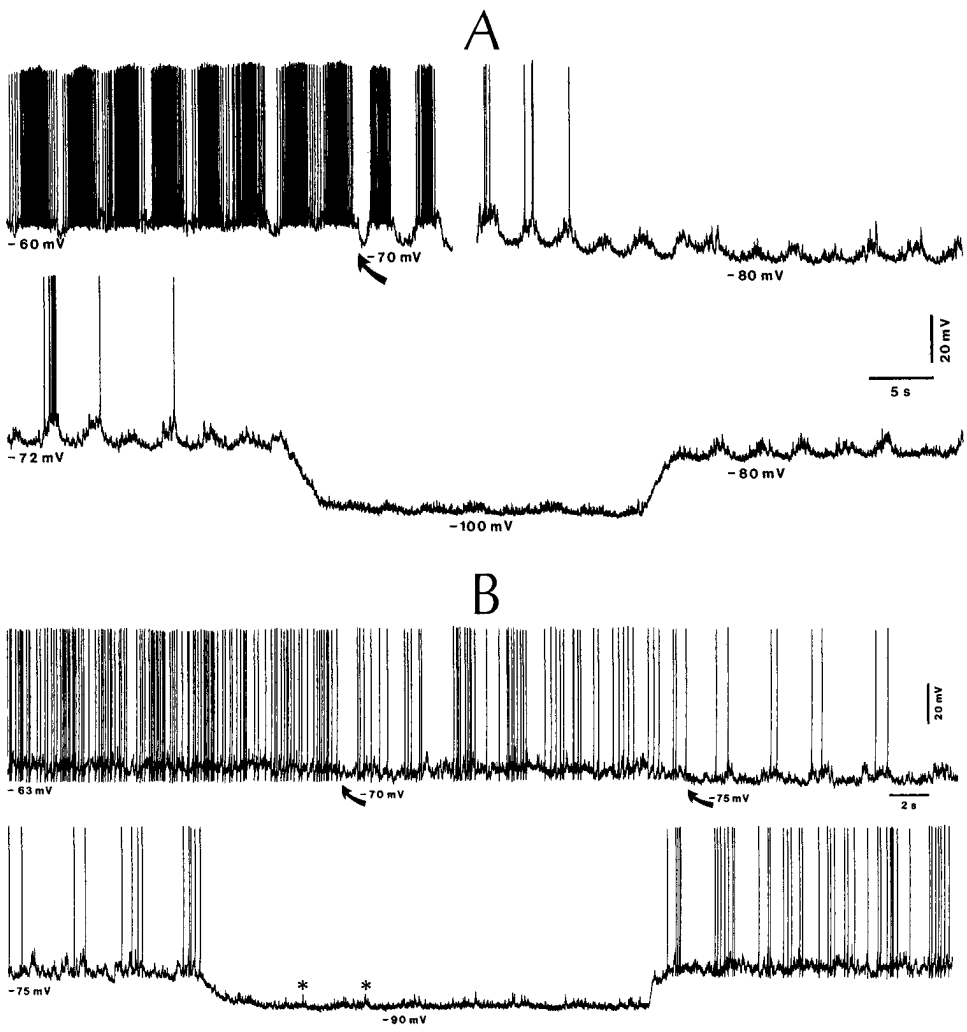


Figure 3. Strong DC hyperpolarization may drastically reduce or completely block the prolonged, slowly recurring depolarizing envelopes in RE cells. *A*, Slow rhythm (0.3 Hz). *Top trace*, At the resting V_m (−60 mV), under slight DC hyperpolarization (−0.2 nA at *oblique arrow*) bringing the V_m to −70 mV, and under further DC hyperpolarization (−0.5 nA) to −80 mV (after interruption of trace). *Bottom trace*, Slow depolarizing rhythm at −72 mV, its drastic reduction in amplitude (while the synaptic noise was left intact) by further DC hyperpolarization (−1 nA) to −100 mV, and recovery of depolarizing envelopes at −80 mV. *B*, Another RE cell with a slow rhythm at 0.4 Hz. *Top trace*, Slightly removing the DC depolarizing current, from +0.05 nA and +0.03 nA (V_m at −63 and −70 mV, respectively), to the resting V_m (−75 mV). *Bottom trace*, Suppression of rhythmic depolarizing envelopes by DC hyperpolarization (−0.3 nA) bringing the V_m to −90 mV; the initial phasic events of the slowly rhythmic depolarizations remained intact, however (*asterisks*).

However, we were able to block the slow depolarization in six RE neurons. Figure 3 depicts two cells in which DC hyperpolarization to −90 or −100 mV drastically diminished (*A*) or suppressed (*B*) the prolonged depolarizing envelopes. However, the phasic EPSPs were still visible and they recurred periodically, with the same frequency as the rhythmic slow depolarization at more positive V_m (asterisks in Fig. 3*B*).

One of the four slowly oscillating cells in which rhythmic inhibitions were the most prominent event is illustrated in Figure 4. At the resting V_m, a biphasic hyperpolarization could occasionally be seen in its full duration of ≈0.5 sec (first oscillation in Fig. $4A_1$). At V_m more negative than −70 mV, the hyperpolarizations had a smooth, late start, as if they exclusively consisted of a secondary IPSP (Fig. $4A_2$ after oblique arrow; see Discussion). During the hyperpolarization the synaptic noise was strongly reduced or suppressed (Fig. 4*B*). A similar hyperpolarization was elicited in the same RE neurons by stimuli applied to corticothalamic axons, occurring immediately after the monosynaptic excitatory response and preceding the typical depolarizing spindle sequence composed by rhythmic spike bursts (right part in Fig. $4B_2$).

The preceding figures illustrated various patterns of slow oscillations in intracellularly recorded RE neurons at various levels of membrane polarization. As expected, the number of spikes during the depolarizing envelopes was a function of the V_m and the interdepolarization lulls had many or very few action potentials depending on the same factor. Similar aspects are depicted in Figure 5, with extracellular recordings, in which one RE cell tonically fired single spikes during the periods between the grouped oscillatory activity (*A*), another RE cell discharged less spikes between the slow rhythm (*B*), and the third RE unit displayed interoscillatory lulls completely free of action potentials (*C*). All these neurons had the features distinguishing RE cells, namely, spike bursts evoked by internal capsule stimulation (Fig. 5*A*), and high-frequency (200–250 Hz) spontaneous bursts with a first long interval, several shorter intervals, and a last longer interval (Fig. 5, asterisk in *A*; C_2).

Association between the slow oscillation and faster rhythms in RE neurons

It is known that barbiturate anesthesia produces a stereotyped and sustained spindling activity in thalamic neurons. Under this condition, RE cells display spike bursts within the frequency range of spindles (7–14 Hz), superimposed upon slowly growing and decaying depolarizations that recur with a rhythm of 0.1–0.2 Hz (Mulle et al., 1986; Hu et al., 1989). While urethane produces an EEG pattern more similar to that observed during natural sleep, including electrographic characteristics of late sleep stages, spindling occurs less often during this type of anesthesia and, when it appears, the frequency of this oscillation is more

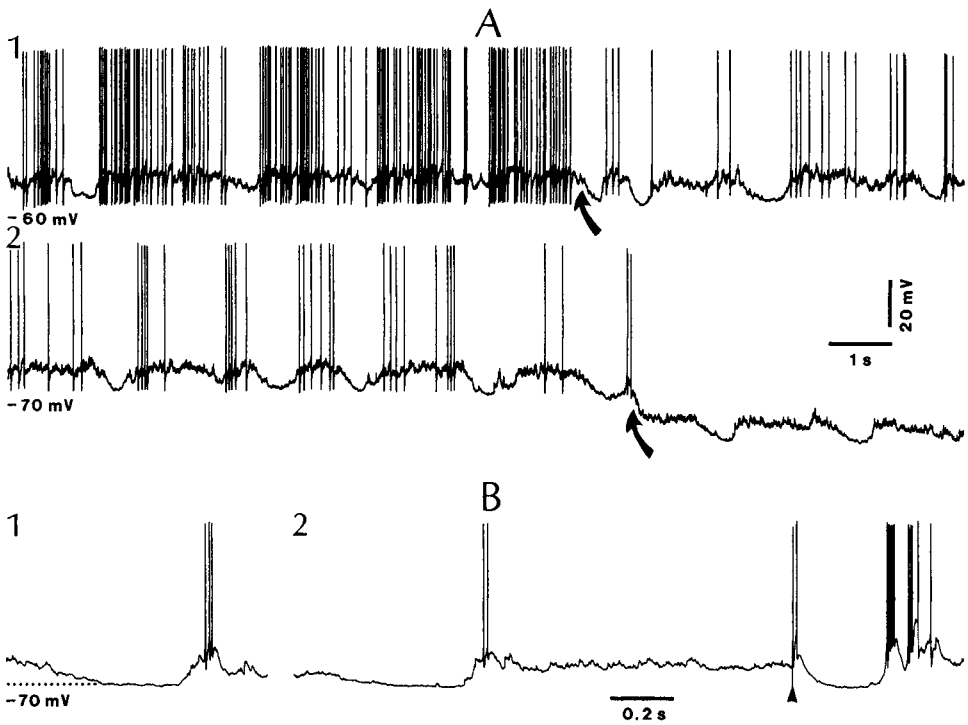


Figure 4. Rhythmic hyperpolarizations (≈0.5 Hz) in RE cell. *A*, Oscillation at the resting V_m (−60 mV), under DC hyperpolarization with −0.4 nA (*oblique arrow in 1*), and under DC hyperpolarization with −0.9 nA (*oblique arrow in 2*). *B*, Expanded periods with spontaneously occurring rhythmic hyperpolarizations (in *1* and *left part in 2*), for comparison with inhibition triggered by internal capsule stimulation (*arrowhead, right part in 2*). *Dotted line in 1* tentatively indicates the trough of the hyperpolarization.

variable. This is the case of the two cells illustrated in Figure 6. One of them (in Fig. 6*A*) exhibited sequences of four or five depolarizing waves at 8 Hz, repeated with a slow rhythm around 0.9 Hz. At a V_m more negative than −68 mV, the depolarizations could trigger spike bursts similar to those in all other RE neurons. However, these sequences of spindles did not last for more than 0.5 sec (while barbiturate-induced spindle sequences have a duration of 1–2 sec) and they recurred with much faster frequencies. In the other RE cell (Fig. 6*B*), rhythmic sequences of depolarizations recurred with a slow rhythm of 0.2 Hz, but the frequency of "spindles" within such sequences was quite low, ≈4–5 Hz. However, their aspect was that of bona fide spindles in RE cells, that is, cyclic depolarizations with a waxing and waning pattern. At a V_m more negative than −75 mV, the rhythmic depolarizations gave rise to LTSs crowned by spike bursts of the RE type, with a first long interval (see expanded record in Fig. 6*B*).

Relations between the slow rhythm of RE cells and cortical EEG, and state dependency of the RE slow rhythm

In all oscillating neurons, the slow (≈0.3 Hz) rhythm of intracellularly recorded RE cells occurred in close time relation with wave complexes recorded from the cortical surface and recurring at a similar frequency (n = 33). The groups of EEG waves were initiated by a surface-positive or, less often, surface-negative sharp deflection, roughly coincident (Fig. 7*A*) or preceding the slow depolarization of RE neurons by ≈0.3–0.4 sec (see Fig. 7*B*), followed by delta waves and eventually leading to spindles, much the same as described in the preceding article (see Figs. 1, 2 in Steriade et al., 1993b).

The dependency of the slow rhythm in RE cells on the general EEG state could be studied in those neurons that were recorded during repeated transitions in the patterns of EEG synchronization (n = 13). Without exception, the slow cellular rhythm tended to disappear at decreased levels of EEG synchronization and signs of decreased slow neuronal oscillation preceded overt EEG desynchronization. A typical example is illustrated in Figure 8. This neuron exhibited the slow rhythm (0.3 Hz) under urethane anesthesia, but a slight reduction in the synchronized state of the EEG required additional anesthesia. In this case, nitrous oxide was administered to keep the animal in a deep anesthetic state. The combination of two anesthetics (urethane plus nitrous oxide or ketamine) may induce a highly synchronized EEG state called "burst suppression" and consisting of EEG wave complexes separated by relatively long (3–10 sec) periods of electrical silence. A precursor pattern of "burst suppression" is seen in Figure 8*A*, with a slow oscillation of RE cell closely related to EEG wave complexes starting ≈0.3–0.4 sec before the depolarization of the neuron. At this point, the nitrous oxide was removed and, after ≈2 min, the neuron continued to exhibit the slowly recurring depolarizations while the EEG displayed the pattern seen in Figure 8*B*, namely, EEG complexes that recurred with the same rhythm as above, but distinctly consisting of grouped fast (≈20 Hz) waves. One minute later, the EEG still displayed signs of synchronization, but the slow oscillation of the cell virtually disappeared (Fig. 8*C*). This was the indication that nitrous oxide or another anesthetic should again be administered.

Slow, delta, and spindle oscillations in thalamocortical cells

In 105 out of 240 recorded thalamic relay cells (44%), the slow rhythm was found in isolation, or was associated with spindle or delta oscillations, or was reflected as a grouping of delta potentials within the frequency of 0.2–0.4 Hz.

The association between the slow rhythm and spindle oscillations was detected in the same thalamocortical neuron (n = 18). This relatively small number reflects the rarity of clear-cut EEG spindle rhythms in urethane-anesthetized animals. In the relay neuron illustrated in Figure 9, the slow rhythm had a frequency of 0.8 Hz and consisted of depolarizing envelopes

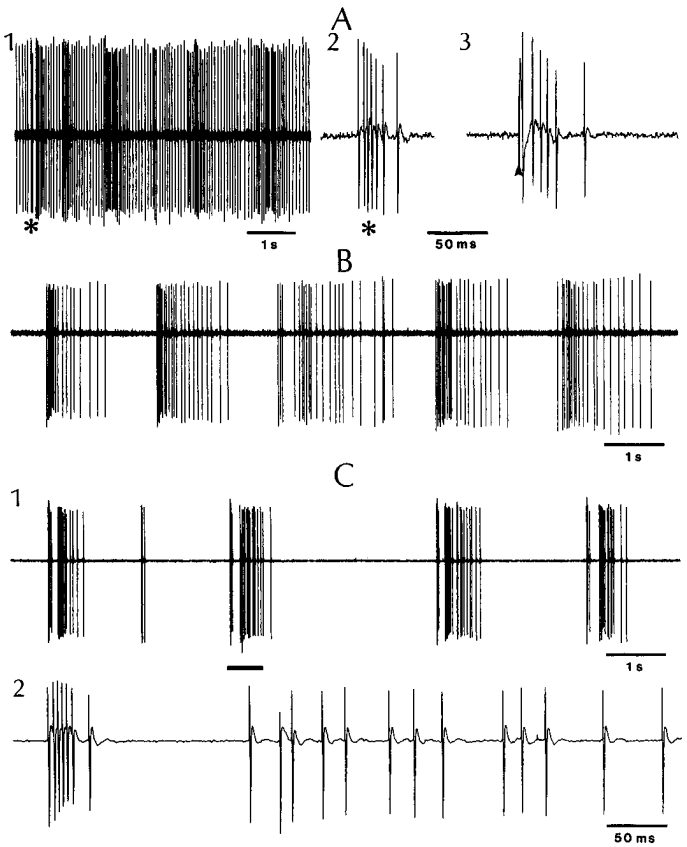


Figure 5. Various aspects of slow rhythms in extracellularly recorded RE neurons. *A–C* are from three different cells. *A*, Slow rhythm (0.7–0.8 Hz) consisting of spike bursts with a frequency of ≈250 Hz, separated by tonic firing at ≈15 Hz. Burst indicated by *asterisk in 1* is expanded in *2*. In *3*, burst response to internal capsule stimulation (*arrowhead*) is shown. *B*, Slow rhythm at 0.4 Hz consisting of spike bursts followed by prolonged (1 sec) tonic tails. *C*, Slow rhythm (0.4 Hz) consisting of high-frequency bursts (250 Hz) followed by tonic tail after ≈0.1 sec. Part marked by *horizontal line in 1* is expanded in *2*.

lasting for ≈0.5 sec, with superimposed fast excitatory events at 20–25 Hz. The spindle sequences had the typical aspect of waxing and waning IPSPs, recurring with a frequency of 9 Hz and occasionally leading to a rebound LTS. Similarly to the effect induced by the slow rhythm on intrinsic delta oscillation (see below, Fig. 10), the appearance of the slow rhythm was prevented by spindles and its reappearance was delayed after a spindle sequence (Fig. 9).

The hyperpolarization-activated delta oscillation was also conjointly seen with the slow rhythm in the same thalamocortical neuron ($n = 47$). At a V_m more negative than −75 mV, the delta oscillation occurred spontaneously. It consisted of self-sustained sequences of LTSs, crowned by action potentials and followed by pronounced afterhyperpolarizations (AHPs). In Figure 10*A* the frequency of this intrinsic LTS-AHP delta oscillation was 1.5–2 Hz. The same cell reflected the cortically generated slow rhythm (0.3–0.4 Hz), consisting of sequences of fast EPSPs and IPSPs recurring periodically, every 2.5–3 sec. The slow rhythm was seen throughout the recording period and it reset the delta rhythm, delaying by ≈0.3–0.4 sec the appearance of the following LTS-AHP sequence. The slow rhythm in another thalamocortical neuron, depicted in Figure 10*B*, was able to cut off quickly the series of delta waves triggered by a hyperpolarizing current pulse. In that cell, the slow rhythm mainly consisted of fast IPSPs, as revealed by injection of depolarizing current (see right part in Fig. 10B_2).

Delta oscillation was triggered not only by direct injection of hyperpolarizing currents, but also by corticothalamic synaptic volleys. Indeed, cortically triggered IPSPs could initiate a series of potentials with a frequency of 2–4 Hz ($n = 36$). This frequency was different from that of delta oscillation evoked in the same neurons by hyperpolarizing current pulses, suggesting a different underlying mechanism. In the CL thalamocortical neuron illustrated in Figure 11, the intrinsic delta rhythm (after oblique arrow pointing to the injection of current) had a frequency of ≈1 Hz, whereas the few self-sustained oscillatory cycles following the cortically evoked IPSP had a frequency of ≈3–4 Hz (see also below, Fig. 12). The IPSP had a duration of 250 msec and two components. The peak of the first IPSP appeared at 30 msec after the cortical stimulus, while the late IPSP had a much longer duration (parts 1, 2). When cortical volleys were delivered during the hyperpolarization-activated delta oscillation, at a V_m of −75 to −80 mV, the early IPSP was reversed while the late IPSP remained hyperpolarizing (see part 3). This differential behavior suggests that the early IPSP is Cl^- dependent, whereas the late one is K^+ dependent. The biphasic IPSP led to a postinhibitory rebound and one or more successive LTSs within the delta frequency. The biphasic IPSP evoked by corticothalamic stimulation is generated by RE and/or local-circuit GABAergic neurons. Both these inhibitory cell types are directly driven by corticofugal axons (see Steriade et al., 1990b) and have access to $GABA_A$ and $GABA_B$ receptors mediating the early and late IPSPs, respectively (Hirsch and Burnod, 1987; Crunelli et al., 1988; Paré et al., 1991).

Finally, the effect of the cortical slow rhythm on thalamocortical cells took the form of grouping the intrinsically generated delta potentials within the frequency of slow oscillation ($n = 22$). The delta potentials triggered by a hyperpolarizing current pulse in Figure 12*A* had a frequency of 2.5 Hz. They tended to dampen and another group of delta potentials was reinitiated after 3–4 sec, only to dampen again after a few cycles. In this way, the delta potentials were grouped periodically every 3–4 sec, that is the frequency of the slow cortical rhythm (0.2–0.3 Hz). In the same VL cell, corticothalamic volleys produced an EPSP–IPSP sequence, followed by a sequence of delta oscillation consisting of cyclic (3 Hz) LTSs crowned by spike bursts, that dampened and were again revived (Fig. 12*B*). This was similar to the events induced in Figure 12*A* by a hyperpolarizing current pulse. These findings suggest that the cortical spike trains composing the slow oscillations are reflected in thalamocortical cells as rhythmic (0.3 Hz) depolarizations and hyperpolarizations resulting, respectively, from the direct excitatory actions of corticothalamic fibers and indirect IPSPs mediated by RE neurons (see Discussion).

Discussion

Surprisingly, cells with rather different intrinsic electrophysiological properties, such as RE thalamic and neocortical neurons, displayed very similar patterns of slow oscillation. We were accustomed for many years to considering RE neurons as essentially bursting at the spindle frequency. This remains valid for the experimental condition of barbiturate anesthesia. So, when we first observed the slow oscillation of RE cells under other anesthetics (but also during natural sleep; M. Steriade and D. Contreras, unpublished observations), it was difficult to distinguish it from the same rhythm in cortical cells. And, comparing Figure 3 in the first article (Steriade et al., 1993a), as well

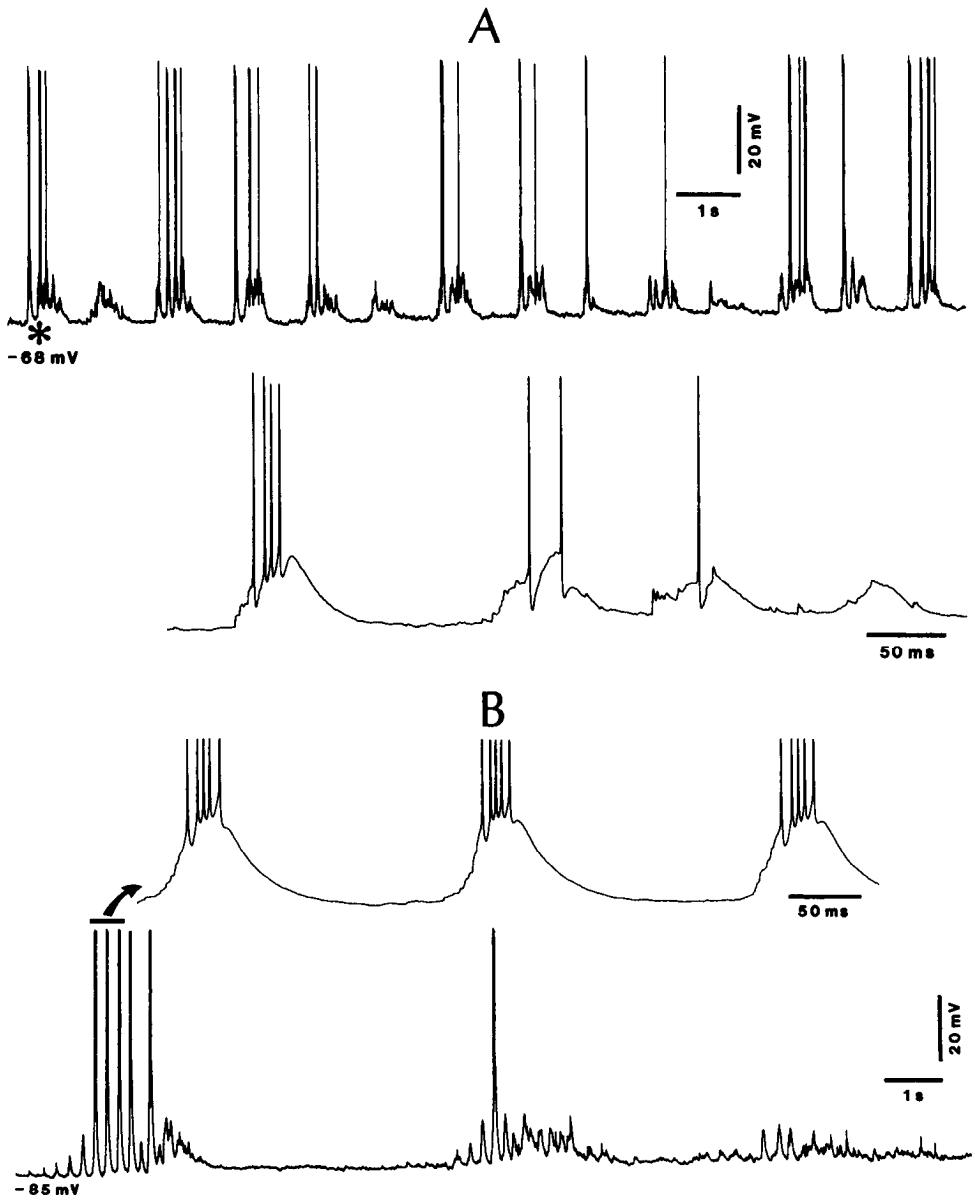


Figure 6. Slow rhythm associated with spindle-like oscillations in RE neurons. *A*, Neuron displaying slowly recurring (0.9 Hz) depolarizations consisting of phasic components at 8 Hz. Depolarizing envelope marked by *asterisk* is expanded *below*. *B*, Another neuron oscillating at 0.2 Hz with waxing and waning depolarizing events at ≈5 Hz. Part marked by *horizontal bar* is expanded *above* (spikes truncated).

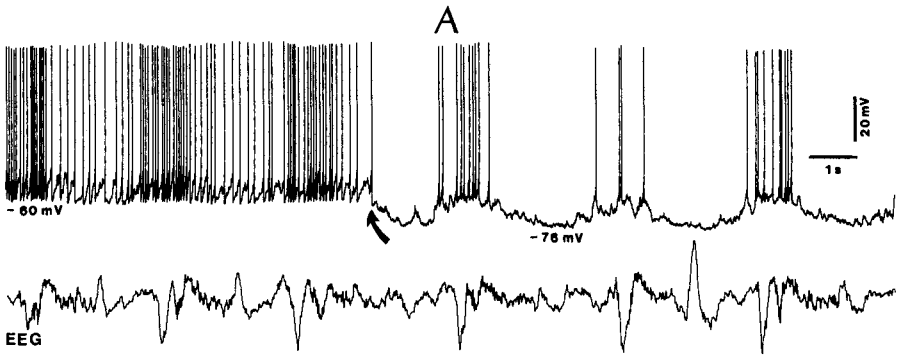
as other similar figures depicting cortical neurons, to Figure 2 and following figures in this article defies the conventional wisdom of sharp distinctions between cortical and RE thalamic cells. This emphasizes that we are dealing with an oscillation uniting different neuronal types of the corticothalamic network into common activity patterns, regardless of their intrinsic properties.

Below, we discuss the aspects of the slow rhythm in RE and thalamocortical cells, propose a scenario for the genesis of various oscillations generated by cortical and thalamic networks and underlying EEG synchronization, and comment on the diversity of rhythms and their chronology during the behavioral state of quiescent sleep.

The slow rhythm in thalamic cells

The slow rhythm was observed in the majority (65%) of cells recorded from the rostrolateral parts of the RE nucleus and in almost half (44%) of our thalamocortical cell sample.

We observed similar slow oscillations (0.3–0.5 Hz) in cells that were extracellularly recorded from the perigeniculate (PG) sector of the RE nucleus (M. Steriade, F. Amzica, and A. Nuñez, unpublished observations). Thus, the whole RE nucleus reflects the slow cortical oscillation, corroborating the presence of the ≈0.3 Hz rhythm in all investigated (associational, visual, and motor) cortical areas (Steriade et al., 1993a). The cortical slow oscillation spreads over the rostrocaudal extent of the RE nucleus despite the fact that there are some differences in the intrinsic properties between cells in the rostrolateral sectors of the RE nucleus and PG neurons. Indeed, a third of PG neurons fired intrinsic spike bursts within the delta frequency, at 1–4 Hz (Amzica et al., 1992), whereas we only occasionally could drive neurons in the rostrolateral RE sectors to fire a few cycles of spike bursts at delta frequency by applying depolarizing current pulses at a holding V_m of −90 mV (data not shown). Differences between the intrinsic properties of two classes of rostrolateral RE neurons have also been observed by studying their prevalently bursting or tonic discharge patterns (Contreras et al., 1992). These heterogeneities within a nucleus commonly considered as consisting of an unique neuronal type should be further examined. Morphological and immunohistochemical investiga-



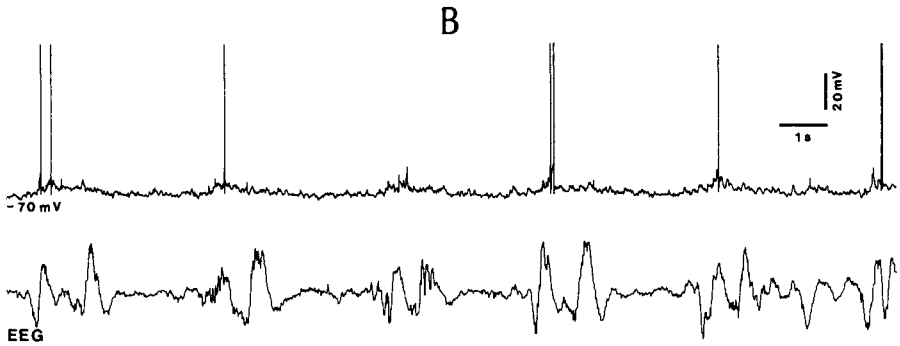
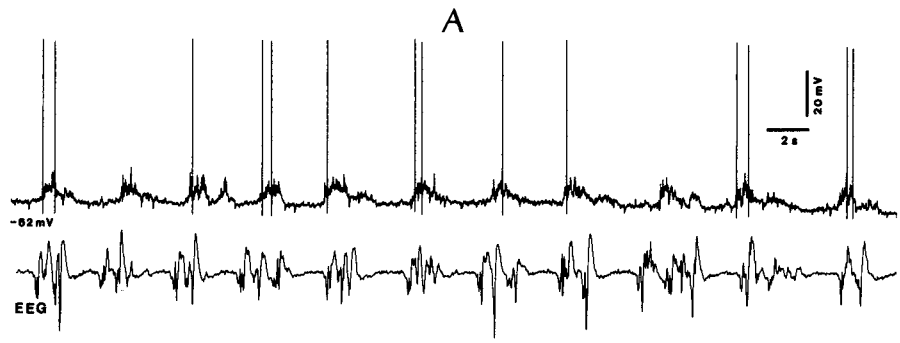
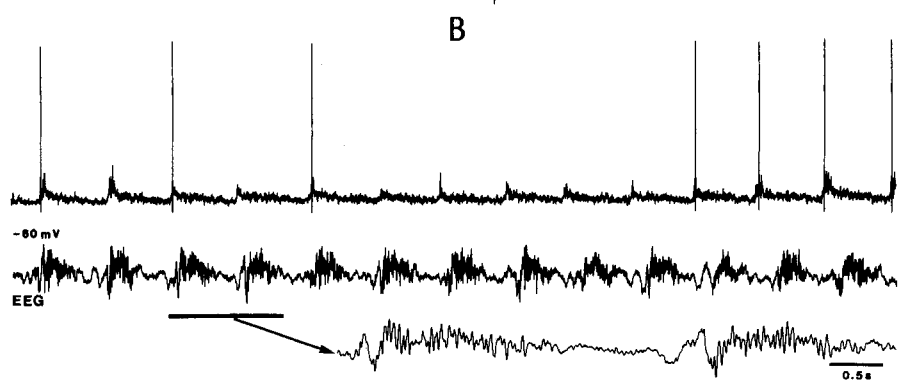


Figure 7. The slow (≈0.3 Hz) oscillation of RE cells and its relation with cortical EEG. *A*, Neuron discharging trains of action potentials (10–15 Hz) every ≈3 sec at the resting V_m (−60 mV), the effect of DC hyperpolarization (−0.4 nA), and close time relation of rhythmic depolarizations–spikes with EEG wave complexes recurring at the same frequency (0.3 Hz). *B*, Similar cellular rhythm and relations with cortical EEG in another neuron.





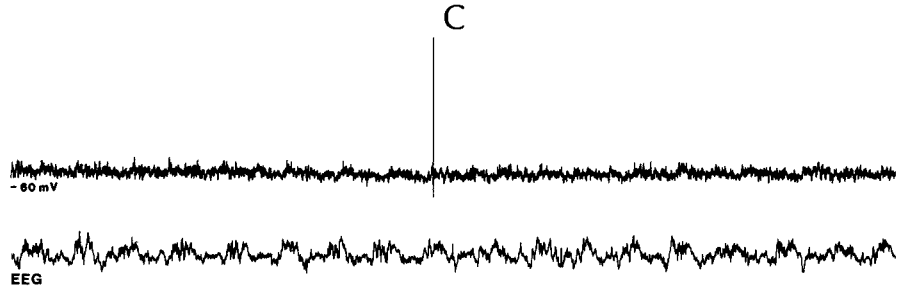


Figure 8. Dependency of slow (0.3 Hz) RE rhythm upon the state of EEG synchronization. *A*, Under urethane anesthesia, supplemented with nitrous oxide. *B*, After partial removal of nitrous oxide. Part marked by *horizontal line* is expanded *below* to illustrate the sequences of fast (≈20 Hz) EEG waves, recurring with the slow (0.3 Hz) rhythm. *C*, Late stage of diminished EEG synchronization during which, however, the slow rhythm of cortical EEG was still visible. RE cell's slow oscillation virtually disappeared. Thereafter, nitrous oxide was again administered.

tions of the cat RE nucleus have identified several cellular groups (Clemence and Mitrofanis, 1992) whose inner connectivity and differential projections remain to be studied.

Our data, then, suggest that the slow rhythm of RE cells is synaptically driven from the cortex. This relation is supported by the appearance of RE cell depolarizing events 0.3–0.5 sec after the initial sharp deflection in the cortical EEG (see Figs. 7*B*, 8*A*). The dependency of the slow RE rhythm upon the general state of cortical EEG (Fig. 8) is also consistent with the corticothalamic control of this oscillation. However, some intrinsic properties of RE neurons contribute to the similarities between cortical and RE slow oscillation. Among them, we mention the persistent Na^+ conductance, $g_{Na(p)}$, present in both neocortical (Stafstrom et al., 1982, 1985; Huguenard et al., 1988) and RE (Mulle et al., 1986; Avanzini et al., 1989) neurons. The involvement of this conductance probably accounts for the voltage dependency of the slow depolarizing envelope. The suppression of the slow depolarizing envelope at −90 or −100 mV (see Fig. 3 in the present article) suggests that a hyperpolarization-activated current is also involved in the reduction of apparent input resistance and may contribute to the disappearance of the oscillation.

Whereas the inhibitory sculpturing generating the slow oscillation was observed in 22% of neocortical cells (Steriade et al., 1993a), the slow rhythm mainly consisted of rhythmic inhibitions in only <8% of RE neurons. The cyclic inhibitory events were quite similar to cortically evoked IPSPs in the same cells (Fig. 4). Such rare events stand in contrast with the usual observation that the inhibitory phase of RE cells, following their early synaptic excitation, corresponds to a simple repolarization. The difficulty in obtaining overt IPSPs in somatic impalements of RE neurons is emphasized by a parallel study in this laboratory, showing that, in general, the inhibition of RE neurons can only detected by its shunting effect over the responses to a second orthodromic volley delivered at intervals of 50–100 msec (Contreras et al., 1993). The rarity of clear-cut IPSPs in RE cells suggests that at least some of them originate far away from the soma. Less often, clear-cut, long-lasting (≈0.4 sec) IPSPs with amplitudes of ≈10 mV (Fig. 4) are generated by corticothalamic volleys reaching the membrane close to the somatic impalement and are effective in modifying the background firing so as to generate the slow rhythm. These long-lasting IPSPs are presumably mediated by both $GABA_A$ and $GABA_B$ receptors of the RE nucleus (Bowery et al., 1987; Chu et al., 1990). The reciprocal inhibitions between GABAergic RE cells are produced by their recurrent axonal collaterals as well as, in cats and primates, by their dendrodendritic synaptic contacts (Deschênes et al., 1985; Yen et al., 1985; Ohara, 1988). Modeling of reciprocal inhibitions between RE neurons showed that oscillations in these cells can be synchronized to an average of zero phase difference, provided that the kinetic properties of $GABA_B$ receptors, with long onset latency and large decay time constant, are introduced in the model of mutually inhibitory elements (Wang and Rinzel, 1993).

Compared to neocortical and RE thalamic neurons, thalamocortical cells fired fewer spikes during their slow depolarizations. This is probably due to the shunting of Na^+ electrogenesis by the inhibitory inputs from RE and local-circuit thalamic cells that are concomitantly driven during the cortically generated slow rhythm. The scarcity of action potentials associated with the depolarizing envelopes of thalamic relay cells suggests that the slow cortical oscillation is reflected in dorsal thalamic neurons, but is not necessarily reinforced by feedback projections to the neocortex.

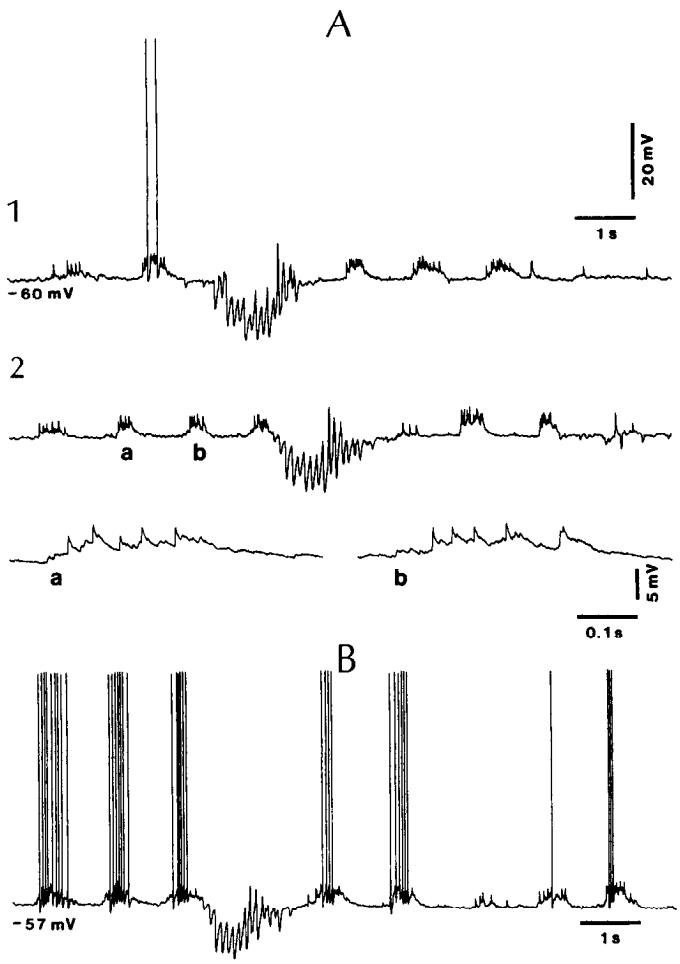


Figure 9. Slow rhythm and spindle oscillation in LP thalamocortical cell. *A*, Fast depolarizing events were grouped in sequences recurring with the slow rhythm (0.8 Hz). Sequences of spindles at 9 Hz are visible in *1* and *2*. Two slowly recurring depolarizations (*a* and *b*) are expanded *below*. *B*, Same events at a resting V_m a few millivolts more positive (spontaneous depolarization).

Proposed scenario for genesis of sleep rhythms in interconnected cortical and thalamic networks

First, we briefly review the evidence of thalamic genesis of spindle and delta oscillations, and the development from one EEG rhythm to the other during the state of natural sleep as a function of changes in the V_m of thalamic cells. Then, we consider the newly described cortical slow oscillation and its possible effects on spindle and delta rhythmicities in RE thalamic and thalamocortical neurons. Finally, we address general questions regarding the diversity of sleep oscillations and their possible significance.

(1) Spindle oscillation. The EEG spindles (7–14 Hz) are the landmark of sleep onset. This oscillation is synaptically generated in thalamic networks. The spindle-related spike bursts of RE cells impose rhythmic IPSPs in target thalamic relay neurons, leading to postinhibitory rebound bursts that are transferred to the cortex (Steriade et al., 1990b). The pacemaking role of RE neurons in the genesis of this EEG rhythm was demonstrated by abolition of spindles in thalamic territories

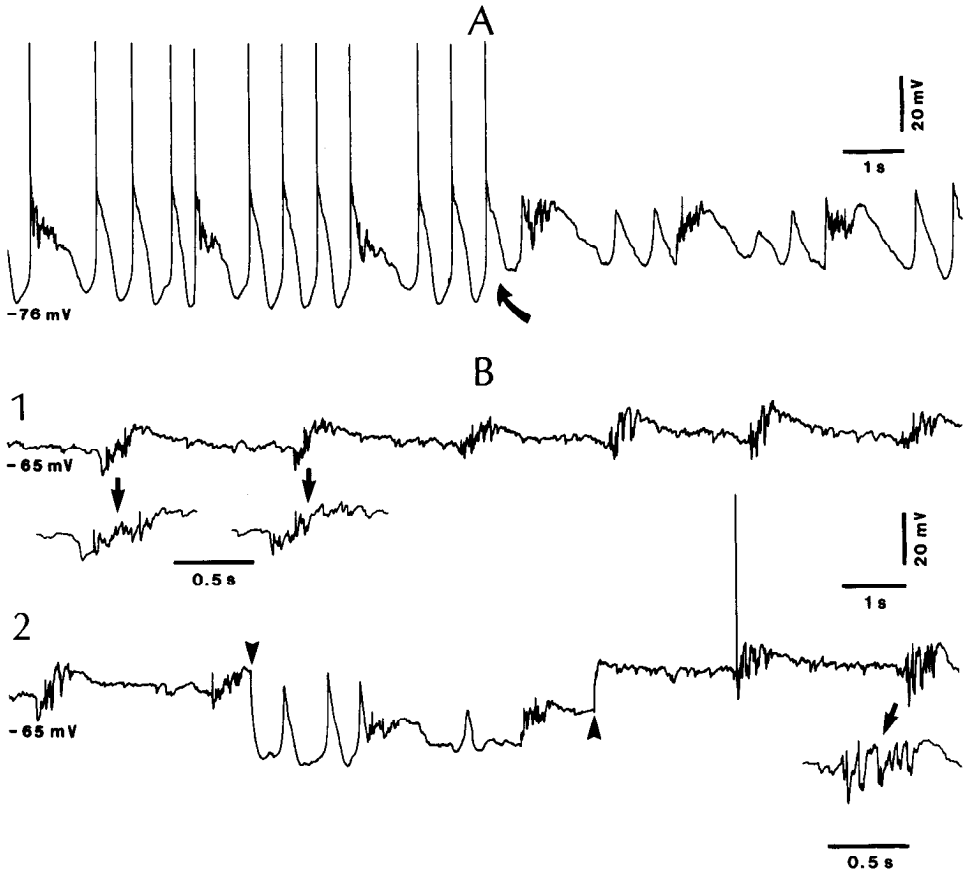


Figure 10. Slow rhythm (0.4 Hz) and delta oscillation in LP thalamocortical cells. *A*, Delta oscillation (sequences of LTSs and AHPs recurring at 1.5–2 Hz) occurred when the V_m was more negative than −75 mV (cell held under DC hyperpolarization of −0.4 nA) and was diminished by bringing the cell at its resting V_m (*oblique arrow*). The same neuron also displayed a slow oscillation (0.35 Hz) of PSPs sequences. *B*, Another neuron showing the slow rhythm of fast PSPs at the resting V_m (*1*). In *2*, a hyperpolarizing current pulse (−0.3 nA, between *arrowheads*) induced delta oscillation, quickly prevented by the appearance of sequences of fast PSPs. At the *extreme right in 2*, slight DC depolarization (+0.1 nA) revealed that the majority of fast synaptic potentials of slowly recurring sequences were IPSPs. Three *downward arrows* point to expanded sequences of fast PSPs at the resting V_m (in *1*) and at a slightly depolarized level (*right part in 2*).

deprived from RE inputs (Steriade et al., 1985; Buzsáki et al., 1988), elicitation of spindles in thalamocortical systems by chemical stimulation of RE perikarya (Marini et al., 1992), and preservation of spindles in the RE nucleus disconnected from its dorsal thalamic and cortical inputs (Steriade et al., 1987).

These congruent results point to the role of RE cells in the induction of spindles, even in the absence of the dorsal thalamus and cerebral cortex. Nonetheless, we have emphasized that the RE nucleus is a conditional spindle pacemaker that may start the rhythm as a direct consequence of activity changes in afferent brainstem modulatory systems, but may also be indirectly set into action by the effects of such changes at the level of thalamocortical neurons (Steriade et al., 1987). Indeed, hyperpolarization in isolated dorsal thalamic foci may result from either diminished firing rates of activating brainstem–thalamic neurons with transition from wakefulness to sleep (Steriade et al., 1990a) or, hypothetically, from active inhibitory actions of GABAergic neurons increasing their activity at sleep onset (see Steriade and McCarley, 1990). Both ACh and noradrenaline (NA) depolarize thalamocortical cells recorded from dorsal thalamic slices (McCormick and Prince, 1987, 1988) and stimulation of mesopontine cholinergic nuclei induces a long-lasting muscarinic depolarizing response of thalamocortical cells, closely associated with a prolonged activation of EEG rhythms (Curró Dossi et al., 1991). Then, the hyperpolarization of thalamocortical cells at sleep onset would be followed by postinhibitory rebound bursts that in turn would drive RE neurons, eventually resulting in a avalanche oscillatory process within the RE nucleus through axonal recurrent collaterals and dendrodendritic synapses in RE nucleus. The issue here is that spindling is a global EEG event and that the only synchronizing device of the whole thalamus, accounting for the simultaneity of spindle sequences over the cerebral cortex, is the RE nucleus with its widespread projections to virtually all cortically projecting thalamic nuclei (Steriade et al., 1984).

The slow (0.1–0.3 Hz) rhythm of periodic spindle sequences was observed in the deafferented RE nucleus, isolated from its major thalamic and cortical inputs (Steriade et al., 1987). The ionic and/or synaptic mechanisms of this slow rhythmicity remain unknown. The cortical potentiating role on this interspindle rhythm will be discussed below (see below, point 3), in the context of effects produced by the slow oscillation on thalamically generated oscillations.

(2) Delta oscillation. By contrast to spindles, delta oscillation (1–4 Hz) of thalamocortical cells is an intrinsic rhythm, resulting from the interplay between two currents (I_h and I_t) occurring at hyperpolarized levels in these neurons (McCormick and Pape, 1990a,b; Leresche et al., 1991; Soltesz et al., 1991; Steriade et al., 1991; Curró Dossi et al., 1992). Delta oscillation is generated at a V_m more negative than spindles (Steriade et al., 1991; Nuñez et al., 1992). The transformation of spindles to delta rhythms was also obtained by means of microinjections of NMDA blockers into the thalamus (Buzsáki, 1991), likely producing a hyperpolarization of thalamocortical neurons. As delta appears during later stages of sleep than spindles, the occurrence of these two sleep oscillations at different V_m led us to postulate a progressive hyperpolarization of thalamocortical neurons with deepening of EEG-synchronized sleep (Steriade et al., 1991),

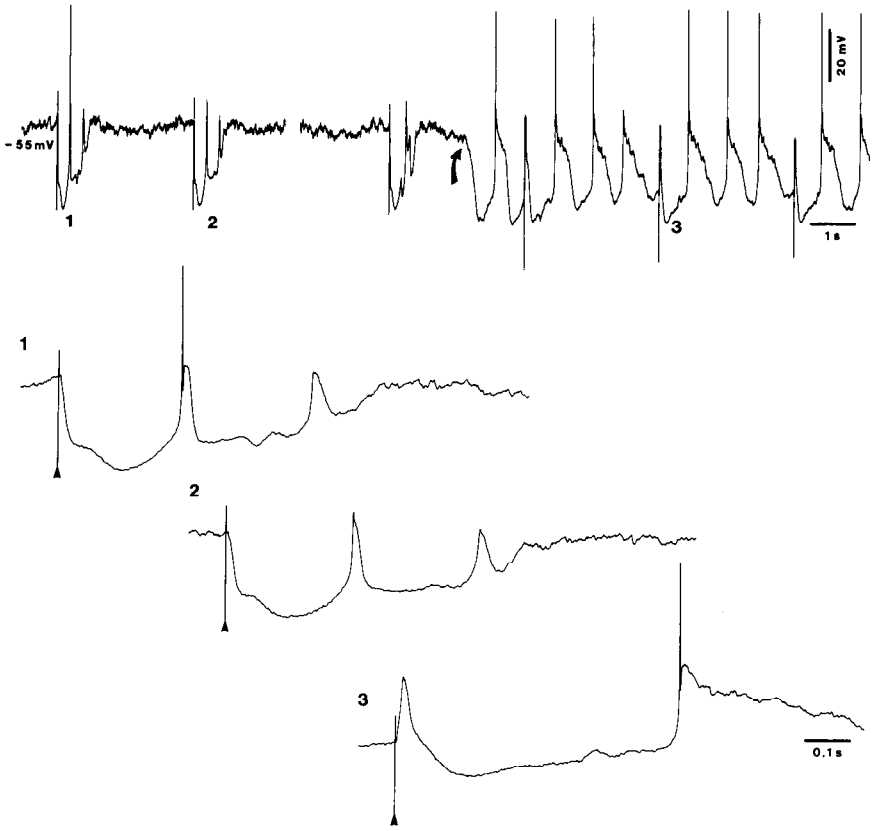


Figure 11. Hyperpolarization-activated delta oscillation in CL thalamocortical cell. *Top trace,* Stimuli to the precruciate area 6 (*1* and *2*). After interruption of trace, another cortical stimulus. Thereafter, DC hyperpolarization (−1.5 nA at *oblique arrow*) induced delta oscillation while cortical stimuli continued every 3 sec. *1–3* from *top traces* are expanded *below.*

due to the progressive reduction in the firing rates of brainstem cholinergic and monoaminergic cells (see Steriade and McCarley, 1990).

Similarly, a progressive hyperpolarization takes place in RE neurons during the transition from arousal to quiet wakefulness, drowsiness and, thereafter, to deeper stages of EEG-synchronized sleep. Several data support this assumption. The study of discharge rates and patterns of RE neurons during these behavioral transitions from natural states of waking to sleep indicated a shift from tonic firing (20–40 Hz) during arousal, to lower

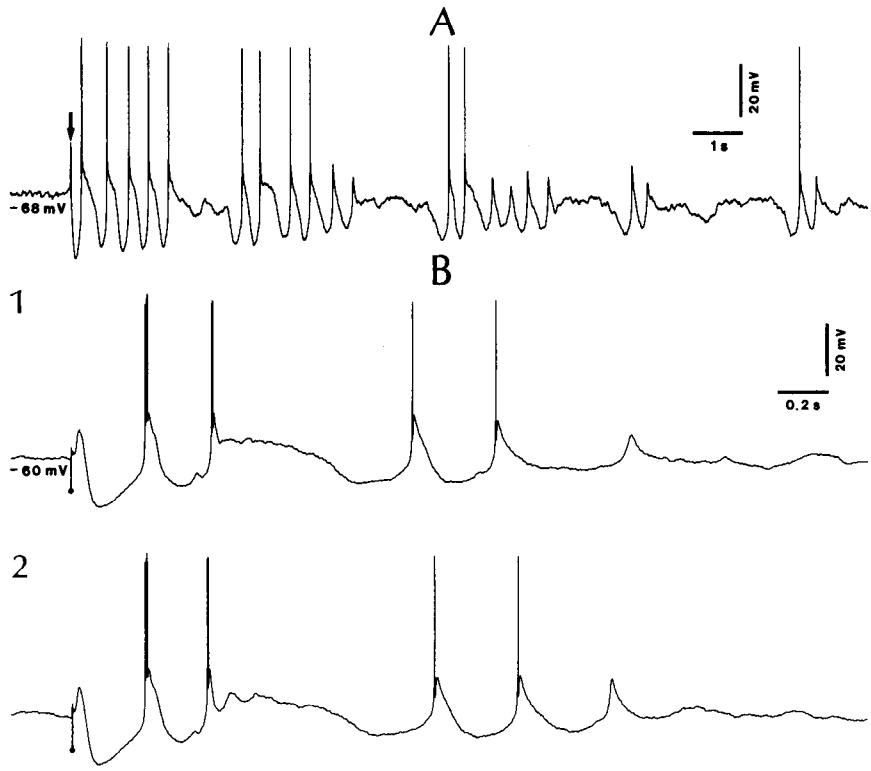


Figure 12. Grouping of delta oscillation within the frequency of slow rhythm in VL thalamocortical cell. *A*, Hyperpolarizing current pulse (−0.6 mV; *arrow*) from resting V_m induced delta oscillation (−2.5 Hz) that tended to dampen and periodically revived within the frequency of the slow rhythm (0.2–0.3 Hz). *B*, Same cell. Stimulation of postcruciate cortical area 4 induced delta oscillation (3–4 Hz), dampening and reappearing periodically.

frequencies (10–20 Hz) toward the end of wakefulness and drowsiness, and to rhythmic spike bursts during full-blown EEG-synchronized sleep (see Fig. 4*A* in Steriade et al., 1986). These changes betray a progressive hyperpolarization of RE neurons, as also indicated by intracellular recordings from RE slices (Bal and McCormick, 1993), and they are probably due to the diminished firing rates of some brainstem modulatory neurons, NAergic and serotonergic cells, projecting to GABAergic RE neurons (Asanuma, 1992) and exciting them (McCormick and Wang, 1991). That spindles and delta oscillations in RE cells occur at different V_m, like in thalamocortical neurons, is also suggested by our intracellular recordings *in vivo* showing that spindles are triggered in rostrolateral sectors of RE nucleus at the resting V_m (≈ -60 mV), while rhythmic spike bursts at delta frequency require depolarizing pulses applied at -90 mV (see above).

These data support the idea that spindle and delta oscillations progressively develop during EEG-synchronized sleep as a consequence of V_m changes in thalamocortical and RE thalamic cells. Our prediction of a reciprocal relation between spindles and delta oscillations, due to their dependency upon different levels of V_m in thalamic cells (Steriade et al., 1991), found support in studies of EEG patterns recorded from the CL intralaminar thalamic nucleus in naturally sleeping cats, with spindles being maximal at sleep onset and decreasing thereafter whereas delta waves increasing gradually during EEG-synchronized sleep (Lancel et al., 1992). Reciprocity between EEG spindles and delta oscillations was also found during sleep in humans (Uchida et al., 1991).

We stress that the thalamically generated delta oscillation has, in view of its intrinsic nature, a stereotyped, clocklike appearance that can hardly account for the rather irregular aspect of EEG delta waves during natural sleep or anesthesia. Two points have to be made here: (1) the cerebral cortex is not a passive receiver of synchronized delta potentials of thalamic origin, and these inputs are reorganized by the intrinsic properties and synaptic events in cortical circuits; and (2) the possibility remains that polymorphous waves within the delta frequency range are generated in the cortex, in the absence of the thalamus (see Fig. 12 in Steriade et al., 1993b).

(3) Slow oscillation. The cortical slow ($\approx$0.3 Hz) oscillation is generated in the network involving regular-spiking and intrinsically bursting cells identified as pyramidal-shaped elements by intracellular staining (Steriade et al., 1993a,b) as well as local-circuit GABAergic neurons, by inference from the repetitive IPSPs grouped within the frequency of the slow rhythm (see Fig. 6 in Steriade et al., 1993a). The various patterns of slow oscillation in cortical neurons, with prevalent depolarizing envelopes or prominent hyperpolarizations sculpturing the background firing, are probably due to different input organization of recorded pyramidal neurons, some of them being the preferential targets of excitatory thalamic and cortical neurons, some others being prevalently driven by local-circuit inhibitory cells. This assumption corroborates circuitry data from intracellular recordings of cat visual cortex neurons indicating prevalent EPSPs or prevalent IPSPs, depending on their location in different cortical layers (see Fig. 2 in Douglas and Martin, 1991). Thus, the newly described slow rhythm is a synaptic oscillation involving all types of cortical neurons through activity that excites simultaneously pyramidal cells and inhibitory interneurons that interact among themselves and shape the oscillation according to the position of various neurons in the columnar microcircuitry. The subdivisions in the first article of this series (referring to mainly depolarizing or hyperpolarizing oscillations, with predominant EPSPs/dendritic spikes or IPSPs; Steriade et al., 1993a) were used to simplify a complex reality in which all these phenomena may be present and intermingled.

Although thalamic volleys were found to be able to modulate the frequency of the cortical rhythm, the genesis of this slow oscillation within neocortical networks was demonstrated by its survival, with all defining characteristics, after virtually total destruction of perikarya in various dorsal thalamic nuclei projecting to the recorded cortical neurons (Steriade et al., 1993b). Experiments in progress by F. Amzica and M. Steriade (unpublished observations), by means of multisite simultaneous extra- and intracellular recordings of neurons in cat motor areas 4 and 6, somatosensory areas 3b and 1 and 2, associational areas 5 and 7, and visual areas 17 and 18, revealed the presence of strongly time-correlated cellular activities, grouped in sequences recurring within the frequency of the slow rhythm (mainly 0.3–0.5 Hz). This study provided evidence that any of the above-mentioned cortical areas contain neurons whose discharges may precede those of neurons recorded from neighboring or distant areas. The leading times in cross-correlograms ranged from <10 msec ($\approx$15%) to 10–100 msec ($\approx$60%) and to >100 msec ($\approx$25%). There was no sign of a preferential origin of the slow cortical rhythm in a sort of "grandmother" neuron(s) or area(s) that would be the earliest in the line of successively developing activities. Then, the slow rhythm probably arises in one or several cortical areas and is distributed through short- or long-range synaptic linkages to a great part or the whole extent of the neocortex. The origin of this slow oscillation is likely to be the diminished firing rates, during sleep, of discharges in activating modulatory neurons projecting to the cortex either directly or after a synaptic relay in the thalamus. Indeed, stimulation of mesopontine cholinergic neurons or NAergic locus coeruleus neurons effectively blocks the slow cortical oscillation, mainly by suppressing the long-lasting interdepolarizing lulls (M. Steriade, F. Amzica, and A. Nuñez, unpublished observations).

The circuitry involved in the genesis of the slow cortical rhythm comprises pyramidal-shaped neurons recorded from layers II–VI as well as local-circuit inhibitory cells (Steriade et al., 1993a). In the absence of the thalamus, this oscillation spreads to the other hemisphere over callosal neurons, as shown in the first two companion articles. The presence of the slow rhythm after thalamectomy and callosal cuts (Steriade et al., 1993b) points to the ipsilateral distribution of this oscillation by corticocortical neurons. Morphological studies have shown that both corticocortical and callosal neurons are the only types of efferent neurons located in the supragranular layers of various sensory and motor areas (e.g., Gilbert and Kelly, 1975; Gilbert and Wiesel, 1983; Code and Winer, 1985; DeFelipe et al., 1986; Schwark and Jones, 1989). Our recordings revealed the presence of slowly oscillatory neurons in these superficial layers, in preparations with extensive thalamic lesions and callosal transections. Within the vertical dimension of a functional column, the major targets of layer II–III cells are neurons in layers I–III and layer V; besides, the axons of layer II–III cells give rise to long-range collaterals that run parallel to the cortical surface for several millimeters (Ojima et al., 1991). These intracortical connections are likely implicated in the spread of oscillation throughout the ipsilateral cortex. However, the study of postsynaptic elements to local axon collaterals in the neocortex is only at its beginnings. For example, most corticocortical axon terminals form asymmetric synapses onto dendritic spines in layers III and V, whereas terminals belonging to the local axon collaterals of corti-

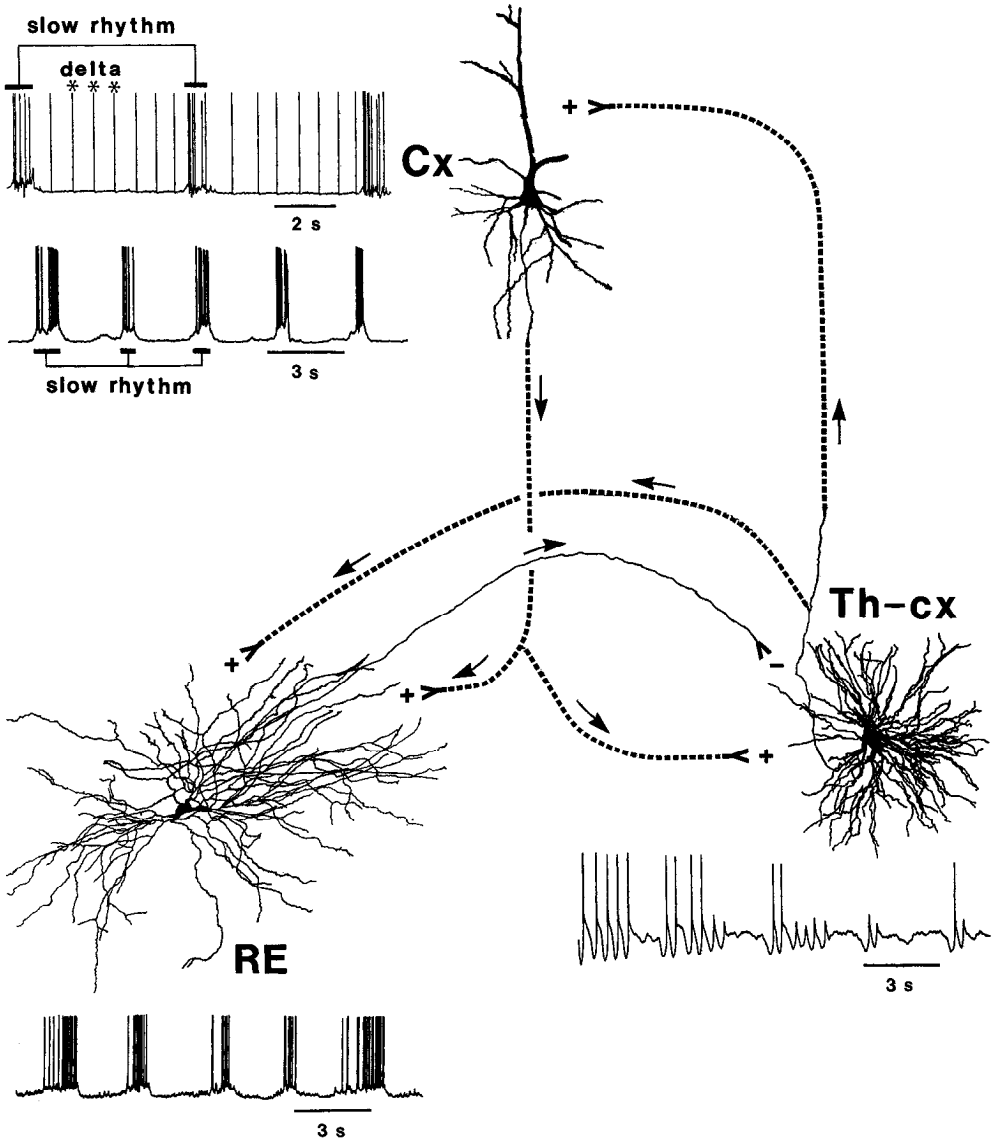


Figure 13. Summary diagram of slow (≈0.3 Hz) cortical (*Cx*) rhythm and its effects upon RE thalamic and thalamocortical (*Th-cx*) neurons: intracellularly stained neurons, *Cx* with Lucifer yellow (modified from Steriade et al., 1993a), and *RE* (rostral part) and *Th-cx* (ventrolateral nucleus) with HRP (modified from Steriade and Deschênes, 1984). Direction of axons is indicated by *arrows* and excitatory or inhibitory signs are indicated by + or − Explanations of sleep rhythm scenario in Discussion.

cothalamic cells mainly contact the dendritic shafts of nonspiny local neurons (White and Keller, 1987; Elhanany and White, 1990). The complexity of intracortical synaptic linkages defies further comments.

(4) Interactions between cortical networks generating the slow rhythm and thalamic networks generating spindle and delta oscillations. We propose that the following sequence of events take place in reciprocal corticothalamocortical networks, involving the slow cortical oscillation and its reflection in thalamic neurons (Fig. 13).

(a) The slowly recurring depolarizing envelopes associated with repetitive action potentials in cortical neurons drive thalamic cells.

(b) RE neurons display slow rhythmic depolarizations within the same frequency range. The cortical inputs are also effective in triggering spindle oscillations in RE cells (see Fig. 6). The idea of simultaneously ongoing activities of two EEG rhythms, one being determined by inputs from a connected brain structure, the other generated autonomously by the intrinsic and synaptic properties of neurons in the target region, has also been envisaged in a model of hippocampal oscillations (Pedley and Traub, 1990). While both spindles (7–14 Hz) and their periodic recurrence with a slow rhythm (0.1–0.2) can be generated by RE neurons, even after disconnection from the dorsal thalamus and cerebral cortex (Steriade et al., 1987), the cortex may potently potentiate the slow rhythm of spindle sequences.

(c) The reflection of the slow cortical rhythm at the level of thalamocortical cells is expressed by depolarizing envelopes with a similar time course. However, these prolonged depolarizations trigger much less action potentials in thalamic relay neurons because of the shunting effect due to the discharges arising from the concomitant excitation of GABAergic RE neurons. The simultaneous occurrence of the slow rhythm and spindle oscillations in thalamocortical cells (see Fig. 9) points to the independence of these rhythms and to the converging inputs of cortical and RE origin. At the single-cell level, the increased membrane conductance of thalamocortical neurons outlasting spindles (Nuñez et al., 1992) accounts for the diminished probability of cortically induced excitatory events immediately after a spindle sequence (see Fig. 9*B*). The background activity of thalamocortical cells during late stages of EEG-synchronized sleep consists of delta oscillation (1–4 Hz), largely due to the V_m hyperpolarization resulting from the deafferentation produced by diminished activity in cholinergic and monoaminergic neurons

as well as the reduced firing rates of corticothalamic neurons. The rebound spike bursts of delta oscillation are transmitted to cortical neurons (see Figs. 1–4 in Steriade et al., 1993b). Delta oscillation is periodically interrupted by the slow rhythm (≈0.3 Hz) consisting of depolarizing inputs of cortical origin and inhibitory inputs of RE origin. These rhythmic inputs produce an increase in membrane conductance, with the consequence of grouping delta potentials every ≈3 sec.

General conclusions

Why such a diversity of sleep rhythms? To answer this question, we should first consider the intrinsic currents of different cellular classes and the variety of synaptic circuits in the thalamus and cerebral cortex. The electrophysiological properties, dendritic meshwork, and widespread projections of RE neurons are ideally suited to organize spindling activity and to spread this oscillation throughout thalamocortical systems. Two intrinsic currents of thalamocortical neurons generate delta oscillation at a more hyperpolarized V_m during late sleep stages. The slow rhythm is postulated to result from synaptic activities and long-lasting ionic conductances in neocortical cells. These three major sleep rhythms do not, however, appear in isolation. This is one of the major results of the present studies. The association between the three types of oscillatory activities at the single-cell and global EEG levels, as they occur during natural sleep, emphasizes the need of a preparation with preserved circuitry when analyzing the interactions between thalamic and cortical networks.

While the variety of sleep rhythms points to the differential propensity of the RE thalamus, dorsal thalamus, and cerebral cortex in generating diverse oscillations, all these sleep rhythms may lead to a convergent outcome during a behavioral state in which the brain is closed from the outside world. It is known that the thalamus is the first cerebral station where incoming signals are inhibited from the very onset of sleep and that negligible, if any, changes occur at the level of prethalamic relays with transition from waking to quiescent sleep (Steriade et al., 1990b). Both spindle and delta oscillations are associated with blockage of synaptic transmission through the thalamus, and this prevents the cortex from receiving the inputs required to elaborate a response. We submit that, besides this deafferentation process that is a prerequisite for falling deeply asleep, the state of sleep with EEG synchronization also provides cyclic trains of single spikes or rhythmic spike bursts that may reinforce/specify the circuitry and stimulate dendrites to grow more spines, thereby leading to consolidation of memory traces acquired during wakefulness. Moruzzi (1966) suggested that sleep function may concern those neurons that during wakefulness are related to conscious behavior. We propose that the rhythmic spike bursts in thalamocortical axons resulting from spindle and delta oscillations, as well as the continuous synaptic bombardment from intrinsic cortical networks generating the slow rhythm, prevailingly affect certain neuronal groups for which plasticity is important, as is the case of cortical association neurons that provided the main bulk of the present data.

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