Prolonged hyperpolarizing potentials precede spindle oscillations in the thalamic reticular nucleus

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The thalamic reticular (RE) nucleus is a key structure in the generation of spindles, a hallmark bioelectrical oscillation during early stages of sleep. Intracellular recordings of RE neurons *in vivo* revealed the presence of prolonged hyperpolarizing potentials preceding spindles in a subgroup (30%) of neurons. These hyperpolarizations (6–10 mV) lasted for 200–300 ms and were present just before the onset of spontaneously occurring spindle waves. Corticothalamic volleys also were effective in generating such hyperpolarizations followed by spindles in RE neurons. A drop of up to 40% in the apparent input resistance (R_{in}) was associated with these hyperpolarizing potentials, suggesting an active process rather than disfacilitation. Accordingly, the reversal potential was approximately −100 mV for both spontaneous and cortically elicited hyperpolarizations, consistent with the activation of slow K^+ conductances. QX-314 in the recording pipettes decreased both the amplitude and incidence of prolonged hyperpolarizations, suggesting the participation of G protein-dependent K^+ currents in the generation of hyperpolarizations. Simultaneous extracellular and intracellular recordings in the RE nucleus demonstrated that some RE neurons discharged during the hyperpolarizations and, thus, may be implicated in their generation. The prolonged hyperpolarizations preceding spindles may play a role in the transition from tonic to bursting firing of RE neurons within a range of membrane potential (−60 to −65 mV) at which they set favorable conditions for the generation of low-threshold spike bursts that initiate spindle sequences. These data are further arguments for the generation of spindles within the thalamic RE nucleus.

Spindles (7–15 Hz) are a hallmark oscillation during early sleep states. During the past two decades, a series of studies have demonstrated that spindles are generated within the thalamic reticular (RE) nucleus, which is uniquely composed of neurons using γ-aminobutyric acid (GABA) as neurotransmitter. Spindles are transferred to the cerebral cortex through the interactions between RE and thalamocortical neurons. Thus, experimental evidence has shown that spindles are abolished in thalamocortical systems after lesions of RE neurons or transections separating them from thalamocortical neurons (1) but survive in the RE nucleus deafferented from the dorsal thalamus and cerebral cortex (2). Computational studies agreed with the initiation of spindle rhythmicity in the isolated RE nucleus (3–5) and suggested that, at relatively hyperpolarized levels of membrane potential (V_m), as is the case during slow-wave sleep, the inhibitory postsynaptic potentials (IPSPs) between RE neurons can be reversed, and $GABA_A$-mediated depolarizing potentials can generate persistent spatio-temporal patterns in the RE nucleus (6). The transfer of spindles from the RE nucleus, which is devoid of cortical projections, to the cerebral cortex is caused by RE cells' interactions with thalamocortical neurons (7–9).

The mechanisms underlying spindle initiation in the RE nucleus are not completely elucidated. Our hypothesis (2) predicted that hyperpolarization of RE cells' dendrites through dendrodendritic synapses of GABAergic RE neurons (10, 11) would deinactivate a low-threshold Ca^{2+} conductance, triggering a Ca^{2+} spike followed by GABA exocytosis and hyperpolarization in postsynaptic RE cells' dendrites. The hyperpolarization in synaptically coupled dendrites was thought to initiate spindle oscillation and to spread it to adjacent elements. In essence, any idea aiming at explaining the genesis of spindles in the isolated network of RE neurons implicates the possibility that active RE neurons would hyperpolarize adjacent neurons and create an avalanche spread of the oscillation within the nucleus, with ultimate implication of target thalamocortical neurons. The source for activating a set of RE neurons that would hyperpolarize adjacent and/or more distant RE neurons may be any excitatory synaptic drive acting on these cells but primarily the neocortex, which is known for being particularly potent in triggering (12) and synchronizing (13) spindles.

In the present experiments, by using intracellular recordings *in vivo* in the rostrolateral sector of the cat RE nucleus, we show the presence of spontaneous as well as cortically elicited prolonged hyperpolarizing potentials (PHPs) leading to the onset of spindle oscillations in a sample (30%) of RE neurons. These hyperpolarizations were associated with a decrease in the apparent input resistance (R_{in}), thus suggesting an active inhibition rather than disfacilitation. Extracellularly recorded RE neurons in the vicinity of the intracellularly recorded neurons showed that their firing was temporally related with the hyperpolarization leading to spindles. We propose that the prolonged hyperpolarizations are part of the mechanisms that initiate spindles within the RE nucleus.

Methods

Preparation. Experiments were performed on adult cats (2.5–3.5 kg), anesthetized with pentobarbital (25 mg/kg, i.p.). When the cats showed ocular and electroencephalogram (EEG) signs of deep anesthesia, the animals were paralyzed with gallamine triethiodide and artificially ventilated with control of the end-tidal CO_2 concentration at ≈3.5%. Body temperature was maintained at 36–38°C. The depth of anesthesia was monitored continuously by EEG, and additional doses of anesthetics were administered at the slightest tendency toward activated (low-voltage and fast EEG) rhythms. At the end of experiments, animals were given a lethal dose of pentobarbital (50 mg/kg).

Recordings and Stimulation. Current–clamp intracellular recordings from thalamic RE and relay neurons were performed with glass micropipettes [direct current (DC) resistance, 30–60 MΩ]. To avoid breaking of recording micropipettes, the cortex and white matter overlying the head of the caudate nucleus were removed by suction. The pipettes entered ≈3 mm through the caudate nucleus to reach the rostral pole or the rostrolateral sector of the thalamic RE nucleus. Pipettes generally were filled with 3 M solution of K-acetate and, in some experiments, with 50 mM of QX-314 (Sigma). The stability of intracellular recordings was ensured by cisternal drainage, bilateral pneumothorax, hip suspension, and filling the hole over the thalamus with 4%

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Abbreviations: RE, thalamic reticular; R_{in}, apparent input resistance; GABA, γ-aminobutyric acid; V_m, membrane potential; EEG, electroencephalogram; LTS, low-threshold spike; EPSP, excitatory postsynaptic potential; V_{rev}, reversal potential; PHP, prolonged hyperpolarizing potential; DC, direct current.

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agar solution. A high-impedance amplifier with active bridge circuitry was used to record and inject current inside the cells. Most intracellular recordings included in the database lasted for periods longer than 30 min.

For simultaneous extracellular unit recordings, tungsten electrodes (10–15 MΩ; Frederick Haer, Bowdoinham, WA) were inserted through the caudate nucleus, 0.5–1 mm rostrolateral to the intracellular recording micropipette. RE neurons were recognized by their long (>50-ms) and typical accelerando–decelerando spike bursts in both intracellular and extracellular recordings (see Figs. 1*A* and 5*B*).

Electrical stimulation of corticothalamic fibers was performed by descending one or two bipolar stimulating electrodes to the internal capsule and applying current pulses (200 μs, 500–1,000 μA, 0.5–1 Hz).

The R_{in} was estimated as follows. Synaptic responses evoked by stimulation of cortical areas or corticothalamic fibers were recorded under different levels of membrane polarization obtained by current injection through the pipette (from −1 to + 1 nA steady current). *I–V* curves were built by plotting the holding V_m against different levels of DC injected. Plots were made for multiple time intervals, generally in increments of 10 ms, before and after stimulation onset. For each plot, a linear function was fitted whose slope was considered to be the apparent R_{in} for that particular time interval (see Fig. 1*C*).

Data Analysis. All data analysis was performed with IGOR PRO 4.0 (WaveMetrics, Lake Oswego, OR). Values are expressed as mean ± SD, and *t* tests were used to assess statistical differences, when necessary. Differences were considered as significant at $P < 0.05$.

Results

Spindle Oscillations May Be Preceded by Prolonged Hyperpolarizations in RE Cells. The intracellularly recorded RE neurons ($n = 31$) were able to generate spontaneously low-threshold spikes (LTSs) without current injection. The neuron depicted in Fig. 1*A* was recorded at a V_m of −79 mV and generated prolonged spike bursts (>50 ms; Fig. 1*A Inset*) that occurred during spindle waves.

A subgroup of the recorded RE neurons (32%; 10 of 31) displayed PHPs preceding spontaneously occurring spindle oscillations. One of those neurons is illustrated in Fig. 1*A* and shows simultaneous occurrence of spindles in the EEG and intracellular recording. The neuron displayed prolonged hyperpolarizations before every single spindle sequence. Generally, however, hyperpolarizations preceded spindles in only ≈60% of cases (see below, Fig. 3*D*). The initial phase of the hyperpolarization was rather constant, but the late part was variable, because rebound activity in the network was evident as depolarizing events with different amplitudes in the intracellular recording (Fig. 1*A*, arrowhead in superimposition). The activation of the hyperpolarization could be well fitted with a single exponential function (time constant, $\tau = 36$ ms) and its duration was ≈300 ms (Fig. 1*A*). The average τ for the activation of the hyperpolarization was 42 ± 4.8 ms ($n = 5$) and the duration was 200–300 ms ($n = 10$).

The hyperpolarizations favored the occurrence of bursting behavior in RE cells. The neuron in Fig. 1*B* was slightly depolarized with positive current injection through the pipette to induce continuous, tonic firing. This discharge pattern was present until the onset of spindle waves, when spontaneous membrane hyperpolarization was followed by robust bursting discharges. Therefore, hyperpolarization was able to switch the neuronal firing pattern from tonic to bursting, because it likely deinactivated the Ca^{2+}-dependent T-current (14–17) so that incoming inputs, probably excitatory postsynaptic potentials (EPSPs) of cortical or dorsal thalamic origin, could elicit LTSs. Then, the hyperpolarization is a mechanism for switching discharge patterns in RE neurons from tonic to bursting (Fig. 1*B*), thus contributing to generation of spindling. Plotting the amount of hyperpolarization necessary to reach the threshold of postinhibitory rebound spike bursts indicated that hyperpolarization is effective in controlling the transition from tonic to bursting discharges within a narrow band of V_m (−60 to −65 mV; data not shown).

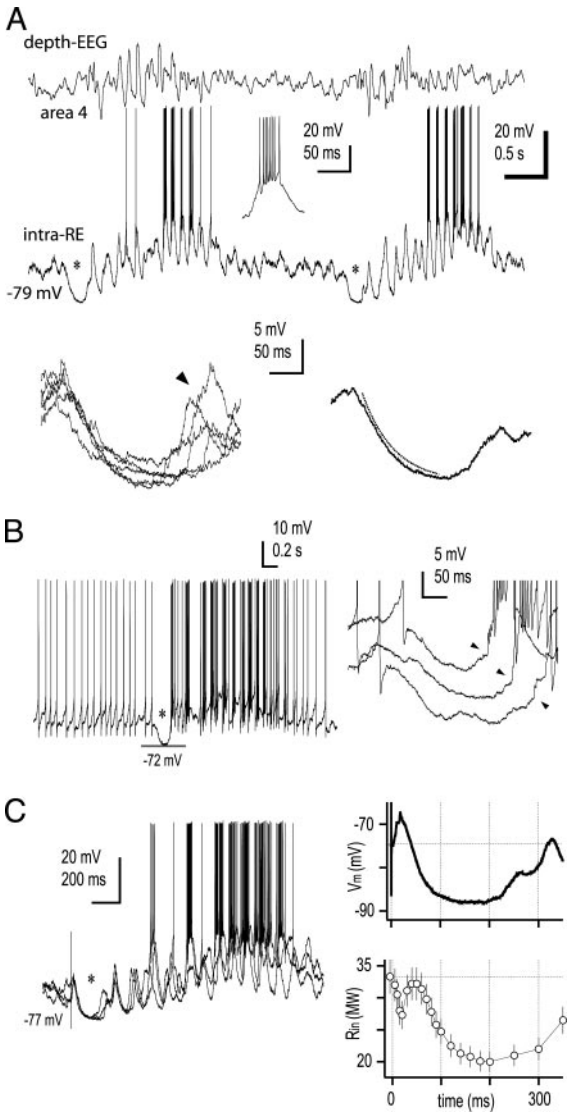

Fig. 1. PHPs precede spontaneous and evoked spindle oscillations. Barbiturate anesthesia is depicted in this and all subsequent figures. (*A Upper*) EEG from motor cortex (area 4) and intracellular recording of RE neuron from the rostral pole of the nucleus. (*Inset*) Typical LTS burst of RE cell is expanded. (*Lower Left*) Superimposition of PHPs ($n = 5$) shows constant activation phase followed by irregular late phase, frequently leading to rebound activities (arrowhead). (*Lower Right*) Averaged PHPs ($n = 20$). Activation phase (first 100 ms) was fitted with a monoexponential function ($\tau = 36$ ms). (*B Left*) Another RE neuron depolarized by steady current injection (+0.5 nA) and displaying spontaneous PHPs. PHPs hyperpolarized V_m to −72 mV, where LTSs could be generated and spindles initiated. (*Right*) Expanded epochs showing PHPs. Arrowheads point to the onset of LTS responses. Traces are displaced for clarity. (*C Left*) Superimposed traces ($n = 3$) showing responses to stimulation of cortical area 4 (same neuron as in *A*). In all cases a sequence of EPSP-PHP-spindle was generated. (*Upper Right*) Average ($n = 10$) of early responses to cortical stimuli applied at resting V_m. (*Lower Right*) Time evolution of R_{in} during PHP. Each point represents the slope of linear fitting to the V_m–DC relation calculated for each time point in the graphic. In this and subsequent figures, asterisks mark PHPs.

Previous studies have shown that electrical stimulation of appropriate neocortical areas or corticothalamic fibers is effec-

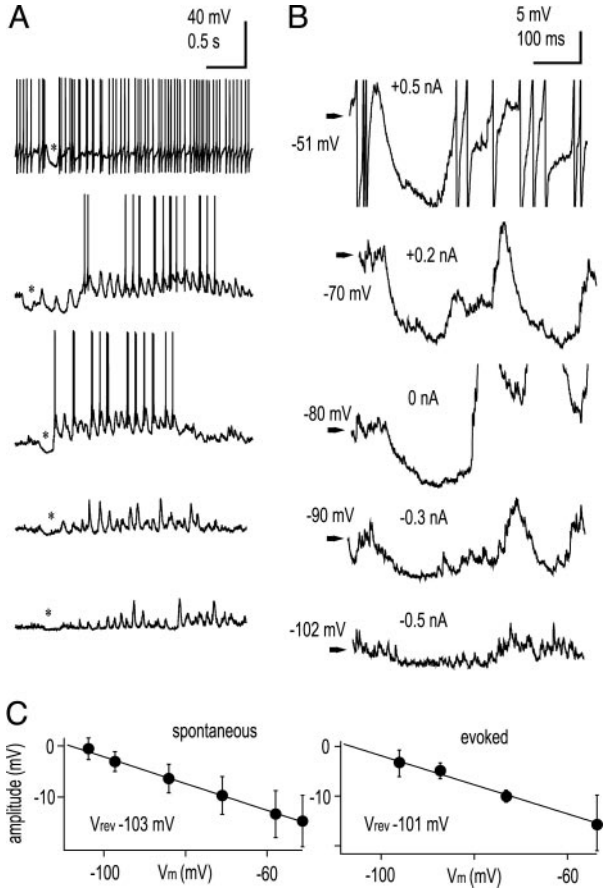

Fig. 2. Voltage dependency of spontaneous PHPs is consistent with a K^+ conductance. RE neuron displaying PHP and held at different V_m values by current injection in the pipette is shown. (*A* and *B*) *A* shows spindles and *B* shows expanded PHPs for the same periods. Values of injected current are indicated in *B*. (*C*) Estimation of the V_{rev} for spontaneous (*Left*) and evoked (*Right*) PHP for various neurons ($n = 5$ in each plot). Solid line represents a linear fitting that predicts V_{rev}s of −103 mV and −101 mV for spontaneous and evoked PHPs, respectively.

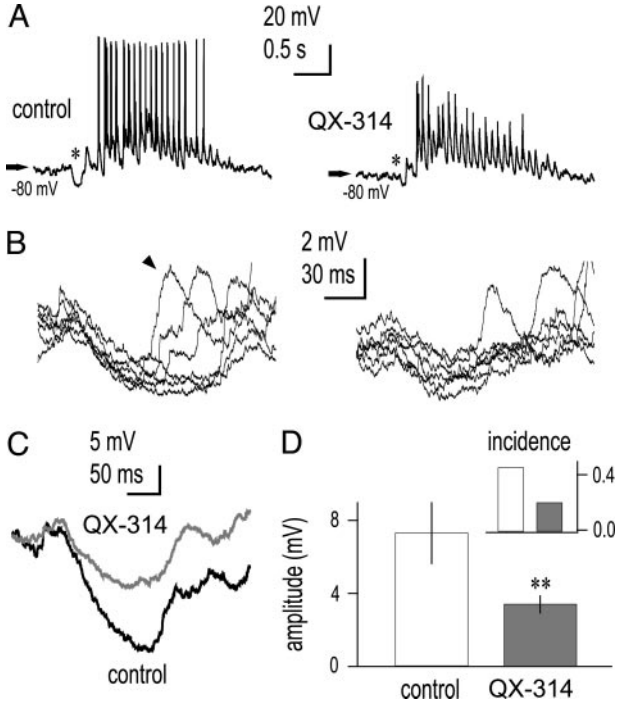

Fig. 3. QX-314 decreased both the amplitude and incidence of PHPs. (*A*) RE neuron recorded under QX-314 (50 mM). During the first 5 min (control), spikes and PHP displayed normal amplitudes, which decreased after 45 min (QX-314). (*B*) Superimposed PHPs ($n = 6$) from the neuron illustrated in *A*, in both conditions. (*C*) Averages ($n = 10$) from PHPs at the beginning (control) and toward the end (QX-314) of the recording. (*D*) Amplitude and incidence of PHPs for both conditions. **, $P < 0.05$.

tive in generating spindle oscillations (12, 18). The same type of electrical stimulation also was able to induce hyperpolarizations preceding spindles in all tested neurons ($n = 5$). In such cases, an initial EPSP preceded a prolonged hyperpolarization, which was followed by spindles (Fig. 1*C*). Averaged responses to corticothalamic stimulation demonstrated similar durations and amplitudes for spontaneous and evoked hyperpolarizations (Fig. 1, compare panels *A* and *C*). Recent *in vitro* data also indicate that focal stimulation of cortical layer VI produces di- or polysynaptic inhibitory currents in RE neurons (19).

During the prolonged hyperpolarizations, the apparent R_{in} of RE neurons could drop by 40% (32 ± 12%, $n = 5$; Fig. 1*C Lower Right*), suggesting it to be the result of active inhibition processes rather than disfacilitation in the network.

Voltage Dependency of Prolonged Hyperpolarizations. To determine the voltage dependence of hyperpolarizations, different levels of V_m were attained in intracellular recordings by injecting steady current through the recording pipette. For each of such levels, the amplitude of hyperpolarizations was measured at the holding V_m. Fig. 2 shows a representative example where a RE neuron was studied by this procedure. Fig. 2*A* displays the spindles that follow the spontaneous hyperpolarizations, marked by asterisks, whereas Fig. 2*B* shows expanded traces for hyperpolarizations at different V_ms. The hyperpolarization displayed higher amplitude as V_m was depolarized, which is consistent with the activation of inhibitory conductances. At more negative values of the V_m, the hyperpolarization became progressively smaller until it was virtually abolished around −100 mV to −105 mV (Fig. 2 *A–C*). The reversal potential (V_{rev}) was estimated by the extrapolation of a linear fitting to the *I–V* curve (Fig. 2*C*). Even though there was large variability in the voltage dependency of hyperpolarizations among different RE neurons, the V_{rev} remained similar in most cases ($V_{rev} = -103 \pm 4.6$ mV; $n = 5$), a value similar to the expected V_{rev} for K^+ in these cells (20, 21). Similar to the case of spontaneously occurring hyperpolarizations, the voltage dependency of evoked ones proved to be consistent with the activation of K^+ conductances: such hyperpolarizations reversed at similar V_m values compared with spontaneously occurring ones ($V_{rev} = -101 \pm 2.7$ mV; $n = 5$) (Fig. 2*C*).

Ionic Basis of Prolonged Hyperpolarizations. To elucidate some aspects of the ionic bases of prolonged hyperpolarizations, intracellular recordings were performed with the inclusion of QX-314 (50 mM) in the pipette. QX-314 is a blocker of multiple membrane conductances, including some K^+ currents (see *Discussion*). In the three neurons recorded under QX-314, in which hyperpolarizations preceding spindles could be detected, both the amplitude and incidence of hyperpolarizations were decreased. During the initial period of recording (2–5 min), RE neurons displayed full action potentials coincident with spindle waves preceded by hyperpolarizations (Fig. 3*A*, control). As time elapsed (15–60 min), QX-314 blocked Na^+ currents and, thus, decreased the spikes' amplitudes during spindles (Fig. 3*A*, QX-314). Not only the action potentials but also the preceding hyperpolarizations were affected, showing decreased amplitudes

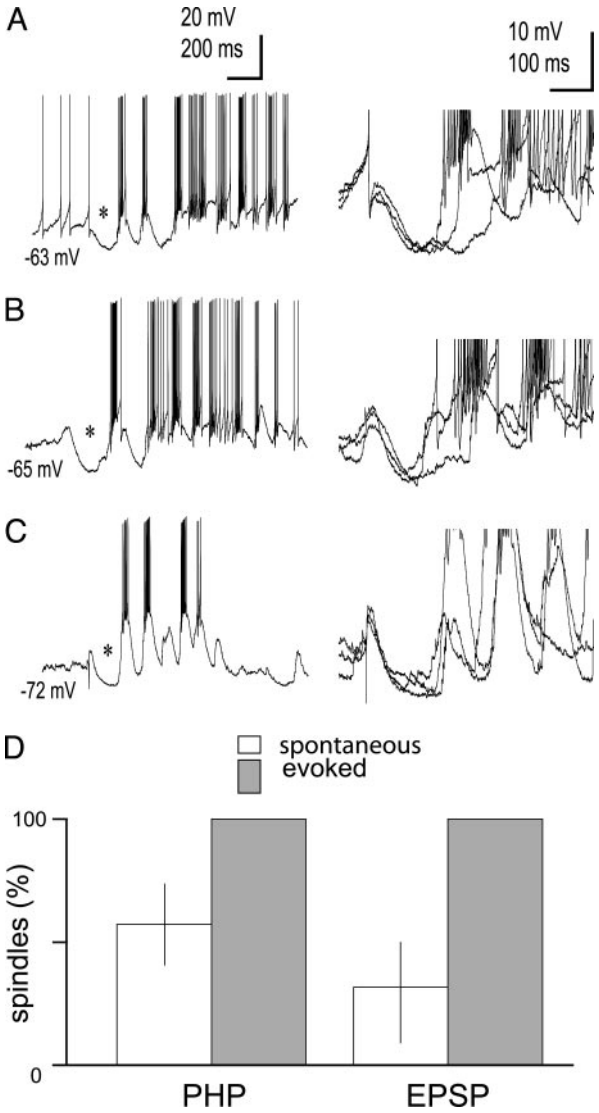

Fig. 4. Cortically evoked EPSP may precede PHPs. (*A*) RE neuron depolarized by steady current injection (+0.3 nA) displayed single, tonic spikes preceding PHPs. (*Left*) Intracellular spindle. (*Right*) Three superimposed PHPs expanded. (*B*) Spontaneous fluctuations in V_m showed an EPSP (underlying single spike, as in *A*) when the cell was slightly hyperpolarized. (*C*) Corticothalamic stimulation could mimic the spontaneous sequence EPSP-PHP-spindle. (*D*) Summary for the recorded RE neurons displaying PHPs ($n = 10$). Bars on the left indicate the proportion of spindles in each neuron that displayed PHPs (PHP; 57.2 ± 16.7%), and bars on the right depict the fraction of those PHPs preceded by putative EPSPs (EPSP; 31.6 ± 18.4%). For each cell, the fraction was calculated from 30 to 50 spindle oscillations. Note that evoked spindles are always preceded by PHPs and EPSPs.

(Fig. 3*A*, QX-314). Rebound activity occurring during the late part of hyperpolarizations also was affected by QX-314, displaying decreased incidence (Fig. 3*B*). Averages ($n = 10$) of hyperpolarizations selected from initial (Fig. 3*C*, control) and late (Fig. 3*C*, QX-314) periods of recordings with QX-314 showed a 2-fold decrease in amplitude of hyperpolarizations preceding spontaneous spindles (Fig. 3*D*).

Prolonged Hyperpolarizations May Be Preceded by Cortically Elicited EPSPs. RE neurons occasionally fired single spikes just before the onset of prolonged hyperpolarizations. In Fig. 4*A*, a RE neuron was depolarized by current injection (+0.3 nA) and discharged tonic spikes just before the onset of the PHP preceding spindles. When the same neuron was slightly hyperpolarized, because of membrane fluctuations, it displayed depolarizing potentials, likely EPSPs (Fig. 4*B*) that occasionally led to the action potentials that preceded the hyperpolarization (see Fig. 4*A*). Electrical stimulation of corticothalamic fibers was effective in mimicking the spontaneous behavior as, on stimulation, EPSPs were evoked, followed by hyperpolarization and subsequent spindling (Fig. 4*C*).

The occurrence of spontaneous hyperpolarizations heralded ≈60% of spindles recorded in RE neurons (Fig. 4*D*). However, in two RE neurons, such hyperpolarizations could be detected preceding every single spindle (see Fig. 1). On the other hand, evoked spindles always were preceded by hyperpolarizations (Fig. 4*D*) when corticothalamic fibers (Fig. 4*C*) or cortical areas (Fig. 1*C*) were stimulated. Only half of hyperpolarizations preceding spindles were initiated by an EPSP (Fig. 4*D*), suggesting that corticothalamic inputs are important for the generation of such hyperpolarizations but not obligatory.

Network Operations in the RE Nucleus During Hyperpolarizations Preceding Spindles. Although the present evidence pointed to the cerebral cortex as a probable source for the generation of hyperpolarizations (see Figs. 1*C* and 4*C*), their *direct* origin within the RE nucleus remained to be determined because both thalamic and cortical inputs to RE neurons are glutamatergic, therefore excitatory in nature. To support the hypothesis of intra-RE origin of these PHPs, three pairs of simultaneous extracellular and intracellular recordings could be performed in the RE nucleus, to study local activities within the RE network during the hyperpolarizations preceding spindle oscillations. One of such paired recordings is depicted in Fig. 5, showing the intracellular activity of one RE neuron and extracellular activities of at least two RE units. In most cases, at least one extracellularly recorded unit fired before the onset of the intracellular spindles, the discharge being temporally related with the prolonged hyperpolarization heralding the spindle sequence in the intracellularly recorded neuron (Fig. 5 *A* and *B*). The hyperpolarizations preceding the first three spindle sequences in Fig. 5*A* were temporally associated with firing of one extracellularly recorded unit (*a*), whereas the last hyperpolarization was accompanied by firing of the second extracellularly recorded unit (*b*). These events are expanded in Fig. 5*B*.

Autocorrelograms for action potentials showed that intracellular and both extracellular units presented oscillatory activity in the range of spindle activities (7–14 Hz), as seen by clear presence of secondary peaks at ± 110 ms (≈9 Hz, Fig. 5*C*). The crosscorrelation for spikes showed that both extracellularly recorded units fired in phase, usually one of them (*b*) preceding the firing of the other (*a*) (Fig. 5*D*). However, both units generally preceded the activity in the intracellular recording (Fig. 5*C*). Consistent with extracellular discharges preceding those in the intracellular recording, the extracellular recordings showed spiking activity at the beginning and throughout the prolonged hyperpolarizations (Fig. 5*D*). Therefore, the latter were correlated with action potential discharges in nearby and/or distant locations in the RE nucleus, suggesting that these potentials may be locally generated within the RE network.

Discussion

We demonstrated the presence of a prolonged hyperpolarization that precedes spindle oscillations in one third of RE neurons. These hyperpolarizations (*i*) were detected in spontaneous spindle waves but also were induced by activation of corticothalamic projections, in which case they were preceded by EPSPs; (*ii*) were associated with decreased neuronal R_{in}, displayed V_{rev} around −100 mV, and were sensitive to intracellular QX-314, suggesting the implication of G protein-dependent K^+ currents; (*iii*) may act as a switch between tonic and bursting discharge modes in RE neurons; and (*iv*) could have a local direct presynaptic origin, because of the discharges of other RE neurons.

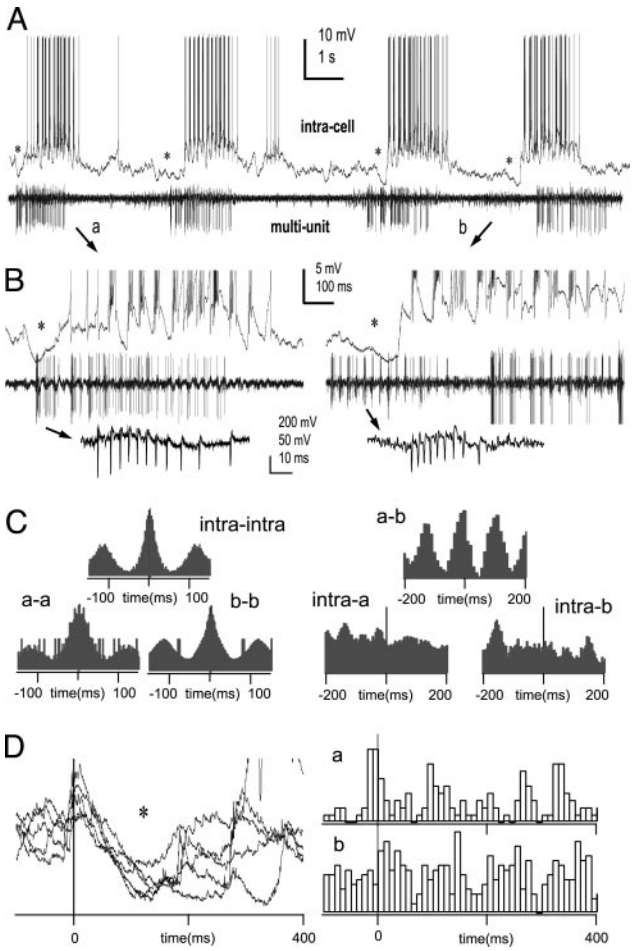

Fig. 5. Distant RE neurons discharge during PHPs. (*A*) Simultaneous recording of intracellular (intra-cell) and extracellular (multi-unit) activities in the RE nucleus. Extracellular electrode was located ≈1 mm anterior to the intracellular electrode. Note the presence of two units in the extracellular recording (*a* and *b*). (*B*) Expanded traces from epochs in *A*, indicated by arrows. (*Left*) The first spindle sequence in *A* for both intra- and extracellular recordings. Note discharge of one unit (*a*) during PHP, preceding spindles. (*Right*) The last spindle sequence in *A*. Note the second unit (*b*) firing during PHP. Bottom traces depict typical accelerando–decelerando bursts for both units, which identify them as RE neurons. (*C*) Autocorrelograms and crosscorrelograms of action potentials for intracellular and extracellular recordings. Bin size is 1 ms and 5 ms for autocorrelograms and crosscorrelograms, respectively. (*D*) Five superimposed traces showing PHPs (*Left*) and correlated discharge in both units (*Right*) for the same period. Time 0 was set at the peak of depolarizing potentials preceding PHPs. Bin size is 10 ms.

Both spontaneously occurring and evoked hyperpolarizations produced a significant drop in the R_{in} of RE neurons, suggesting the involvement of active conductances. Therefore, disfacilitation, like that occurring in cortical (22) and thalamic (23) neurons during the slow oscillation in natural sleep or anesthesia, can be discarded as a possible mechanism underlying the prolonged hyperpolarizations leading to spindles in RE neurons. The V_{rev} (around −100 mV) of RE neurons' hyperpolarization suggests the activation of K^+ conductances (20, 21). The V_{rev} for $GABA_A$-mediated inhibitory postsynaptic potentials in RE cells was estimated at −70 mV (6, 24, 25). At that range of V_m, the hyperpolarization preceding spindles displayed significant amplitude, thus discarding the role of $GABA_A$-mediated inhibitory postsynaptic potentials in their generation. Moreover, the *I–V* relation for this hyperpolarization was linear for a large range of V_m values (−100 mV to −50 mV), implying a very small contribution, if any, of $GABA_A$-mediated potentials at V_m more depolarized than −70 mV. QX-314 in the pipette decreased both their amplitude and incidence, consistent with the activation of K^+ conductances during the prolonged hyperpolarizations. QX-314 affects a wide range of membrane conductances, including the blockade of fast and persistent Na^+ currents (26), low- and high-voltage activated Ca^{2+} currents (27), K^+ currents (28, 29), and hyperpolarization-activated currents (30). It is unlikely that depolarizing (Ca^{2+} and Na^+) currents are implicated in the genesis of the prolonged hyperpolarizations. On the other hand, it has been shown that QX-314 also blocks G protein-activated, inwardly rectifying K^+ channels (31). This opens the possibility that the prolonged hyperpolarization in RE neurons is an inhibitory potential activated by G protein coupled to second messenger cascades (32). Candidates for such an effect are $GABA_B$ receptors (21) as well as peptidergic receptors for somatostatin (33) and neuropeptide Y (34), which are expressed by RE neurons (35, 36) and may initiate second-messenger cascades that end up in the activation of G protein-activated, inwardly rectifying K^+ channels (32, 37, 38). The case of $GABA_B$ receptors might be controversial, because *in vitro* experiments have shown that young rodents express very little $GABA_B$ postsynaptic responses in RE neurons (21). However, those experiments did not assess a critical point, that is, how much $GABA_B$ conductance is activated during thalamic oscillations when large populations of neurons fire in synchrony (13, 18). And experiments in ferret slices have shown that $GABA_B$ responses to glutamate applied in the perigeniculate sector of the RE nucleus are not so small, as the rebound spike bursts were blocked by an antagonist of $GABA_B$ receptors (39). Therefore, the possibility that the RE nucleus expresses a significant $GABA_B$ response during spindle oscillations cannot be discarded. Because we cannot determine the precise ionic bases of prolonged hyperpolarizations preceding spindles in RE neurons, we suggest that at least some of the above-mentioned receptors for GABA, somatostatin, and neuropeptide Y may trigger these hyperpolarizations.

Experimental evidence and computational models have shown that soma and dendrites of RE neurons are electrically compartmentalized (40) and, because the RE cells' soma must be strongly hyperpolarized to generate LTSs (41), the T-current is located in dendrites (16, 40). A similar reasoning suggests that the hyperpolarizations preceding spindles may have a dendritic origin because, in some cases, strong soma depolarization did not increase their amplitude but abolished them, leaving instead a low-frequency discharge period (data not shown). Indeed, G protein-activated, inwardly rectifying K^+ channels (31) may mediate dendritic hyperpolarizations (34).

The simultaneous intra- and extracellular recordings in the present experiments demonstrated that some RE neurons fire spike bursts that were temporally related to the hyperpolarizations in adjacent neurons. These data suggest that some hyperpolarizations leading to spindles are generated within the RE nucleus. Because corticothalamic excitatory volleys also could elicit such hyperpolarizations and knowing the propensity of cortex to evoke spindling (12, 13, 18), we propose that firing in some cortical neuronal pools may excite RE neurons, generating EPSPs and, in some cases, LTSs crowned by spike bursts, which would generate prolonged hyperpolarizations in adjacent and/or distant RE neurons. There is indeed evidence for both dendrodendritic (10, 11) and axoaxonic synapses (42), which mediate inhibitory interactions in the RE nucleus (25, 39).

In sum, the present demonstration of prolonged hyperpolarizations leading to spindles and generated locally in the RE nucleus is in keeping with the proposal that spindles are initiated in the pacemaking RE nucleus (2, 7, 43).

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