Journal of Physiology (1993), **470**, *pp.* 273–294
With 12 *figures*
Printed in Great Britain

ELECTROPHYSIOLOGICAL PROPERTIES OF CAT RETICULAR THALAMIC NEURONES *IN VIVO*

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(*Received 8 October 1992*)

SUMMARY

1. The electrophysiological properties of neurones of the reticular thalamic (RE) nucleus were studied in acutely prepared cats under urethane anaesthesia.

2. Two main types of neuronal firing were recorded. At the resting membrane potential (−60 to −65 mV) tonic repetitive firing was elicited when the cell was activated synaptically or by current injection. From membrane potentials more negative than −75 mV, synaptic or direct stimulation generated a burst of action potentials.

3. The burst of RE cells consisted of a discharge of four to eight spikes riding on a slowly growing and decaying depolarization. The discharge rate during the burst showed a characteristic increase, followed by a decrease in frequency.

4. The burst response behaved as a graded phenomenon, as its magnitude was modulated by changing the intensity of the synaptic volley or the intensity of the injected current.

5. Spike-like small potentials presumably of dendritic origin occurred spontaneously and were triggered by synaptic or direct stimulation. They were all-or-none, voltage-dependent events. We postulate that these spikes originate in several hot spots in the dendritic arbor, with no reciprocal refractoriness and may generate multi-component depolarizations at the somatic level.

6. Excitatory postsynaptic potentials (EPSPs) evoked by internal capsule stimulation consisted of two components, the late one being blocked by hyperpolarization. Such compound EPSPs were followed by a period of decreased excitability during which a second response was diminished in amplitude.

7. A series of depolarizing waves at the frequency range of spindle oscillations was triggered by internal capsule stimulation. The individual depolarizing waves constituting the spindle oscillation gradually decreased in amplitude when decreasing the intensity of the stimulation.

8. These results, showing that RE cells are endowed with an excitable dendritic tree and a graded bursting behaviour, support the proposed role of RE nucleus as the generator and synchronizer of spindle rhythmicity.

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INTRODUCTION

The reticular (RE) thalamic nucleus consists of inhibitory neurones covering the rostral, lateral and partly the ventral surfaces of the thalamus. Its constituent neurones use γ-aminobutyric acid (GABA) as a neurotransmitter. The RE nucleus is the target of cortical and dorsal thalamic neurones and, in turn, has widespread projections to virtually all cortically projecting thalamic nuclei, thus essentially contributing to the feedforward and feedback inhibitory circuits of the thalamus (Steriade, Jones & Llinás, 1990).

The pacemaking properties of RE neurones in the genesis of spindle (7–14 Hz) rhythmicity of the electroencephalogram (EEG) are demonstrated by a series of experimental data. Spindles are absent in dorsal thalamic territories either disconnected from RE nucleus (Steriade, Deschênes, Domich & Mulle, 1985) or naturally devoid of RE inputs (Paré, Steriade, Deschênes & Oakson, 1987). Moreover, spindles are preserved in the RE nucleus deafferented from the dorsal thalamus and cerebral cortex (Steriade, Domich, Oakson & Deschênes, 1987). The current hypothesis proposes that, in species with RE dendrodendritic inhibitory synaptic junctions, such as the cat and primates (Deschênes, Madariaga–Domich & Steriade, 1985; Yen, Conley, Hendry & Jones, 1985; Ohara, 1988), spindles are both generated and synchronized through an avalanche process within the RE nucleus, while some inputs may be decisive in starting this process (Steriade *et al.* 1987). The point here is that the RE nucleus is the only factor accounting for the synchronization of spindling activity throughout the thalamus, because there is little, if any, cross-talk between various dorsal thalamic nuclei (see Steriade & Deschênes, 1984; Jones, 1985).

The stigmatic activity of RE neurones during spindle oscillations consists of spike bursts significantly longer than those fired by thalamocortical cells (Domich, Oakson & Steriade, 1986; Steriade, Domich & Oakson, 1986). The spike bursts of RE cells are generated by a special type of Ca^{2+}-dependent T-current (I_t), with different kinetics from those of the I_t in thalamocortical cells (Huguenard & Prince, 1992). The higher somatic hyperpolarization required to de-inactivate this current (Mulle, Madariaga & Deschênes, 1986; Llinás & Geijo-Barrientos, 1988; Avanzini, De Curtis, Panzica & Spreafico, 1989) suggests a dendritic localization of the low-threshold rebound spike (LTS) underlying the high-frequency spike bursts. The Ca^{2+}-dependent LTS is a fundamental intrinsic property of thalamic cells, implicated in their propensity to oscillation (Llinás, 1988; Steriade *et al.* 1990).

The aim of this study was to explore further bursting characteristics of RE cells and their dendritic excitability in order to shed light on the mechanisms of spindle generation.

METHODS

Experiments were performed on forty-seven adult cats (2·5–3·5 kg) under urethane anaesthesia (1·8 g/kg I.P.). Animals were paralysed with gallamine triethiodide and artificially ventilated with control of the end-tidal CO_2 concentration at $3·7 \pm 0·2$%. The body temperature was maintained at 37–39 °C. All wounds and pressure points were infiltrated once with lidocaine. The depth of anaesthesia was continuously monitored by the presence of high-amplitude slow waves in the EEG recording and maintained by additional doses of urethane (0·3–0·5 g/kg I.V. or I.P.) at any sign of tendency to EEG desynchronization. The EEG was recorded by means of screws placed on the bone

over the pericruciate region and the suprasylvian gyrus. Experiments lasted 8–10 h. Saline glucose was supplemented as a fluid therapy during the experiment. Blood pressure was not monitored.

Coaxial stimulating electrodes were placed in the internal capsule (approximately 2 mm in front of the region where RE cells were recorded) and brachium conjunctivum, both according to conventional stereotaxic co-ordinates.

The stability of the recordings was ensured by cisternal drainage, bilateral pneumothorax and hip suspension. The cortex and the white matter overlying the head of the caudate nucleus were removed by suction to facilitate the passage of the recording micropipettes. The pipettes were then lowered 3 mm through the caudate nucleus to reach the rostral pole and rostrolateral sector of the RE nucleus. After positioning the micropipette, the hole over the thalamus was filled with a 4% agar solution to further control vascular pulsations.

Intracellular recordings were performed with glass micropipettes filled with a 3 M solution of potassium acetate, having DC resistances of 25–35 MΩ. A high impedance amplifier with active bridge circuitry was used to record and inject current into the cells. The signals were recorded on a four-channel tape with bandpass of 0–5 kHz, and thereafter digitized at 20 kHz for off-line computer analysis. The bandwidth of the DC amplifier used was 0–5 kHZ.

Only intracellular recordings lasting at least 15 min (but up to 50 min) of neurones having a membrane potential (V_m) more negative than −55 mV and overshooting action potentials were selected for analysis. Due to the spontaneous oscillatory activity present in RE cells, the responses to current pulses or internal capsule activation that were used for the analysis were taken from periods of quiescent membrane potential.

Penetrations were assumed to be intrasomatic since the diameter of the tip of an electrode of 30 MΩ is of the order of 1–2 μm, the soma is 25 × 45 μm, and the bases of dendritic shafts around 2–4 μm.

At the end of the experiments the cats were given a lethal dose of pentobarbitone.

RESULTS

Recordings were obtained from fifty-one cells of the rostral pole and anterolateral sector of the RE nucleus. The neurones were identified by their characteristic response to internal capsule stimulation, consisting of an initial high-frequency burst of four to eight spikes, followed by a sequence of depolarizing waves within the frequency range of spindle oscillation (Fig. 1*B*). The RE cells were also activated, probably bisynaptically, by stimulation of the brachium conjunctivum (Fig. 1*A*). Spontaneous spindle sequences (Fig. 1*C*) occurred with the same characteristics as those of rhythmic depolarizing events evoked by internal capsule stimulation.

The resting V_m of RE neurones was $-63{\cdot}8 \pm 1{\cdot}2$ mV (mean ± S.E.M.). Action potentials had an amplitude of 68 ± 1 mV and a duration at the base of $0{\cdot}9 \pm 0{\cdot}3$ ms. The spikes were followed by an after-hyperpolarization (AHP) that had an amplitude of $16{\cdot}6 \pm 0{\cdot}8$ mV and a duration of $5{\cdot}7 \pm 0{\cdot}5$ ms. Input resistance was estimated by applying square hyperpolarizing current pulses at the resting V_m and was $37{\cdot}6 \pm 1{\cdot}6$ MΩ. When hyperpolarizing pulses were applied at different levels of membrane polarization most neurones displayed anomalous rectification. In Fig. 2 data from four neurones are plotted showing different degrees of V_m dependency of the apparent input resistance. Longer lasting hyperpolarizing pulses did not reveal signs of the slow inward rectifier I_h described in dorsal thalamic relay neurones (McCormick & Pape, 1990).

Burst generation in reticular thalamic cells

RE cells displayed two distinct modes of action potential generation (Fig. 3*A*). At rest, or slightly depolarized V_m values, the response to a depolarizing current pulse consisted of a train of action potentials, each followed by an AHP. The firing rate was

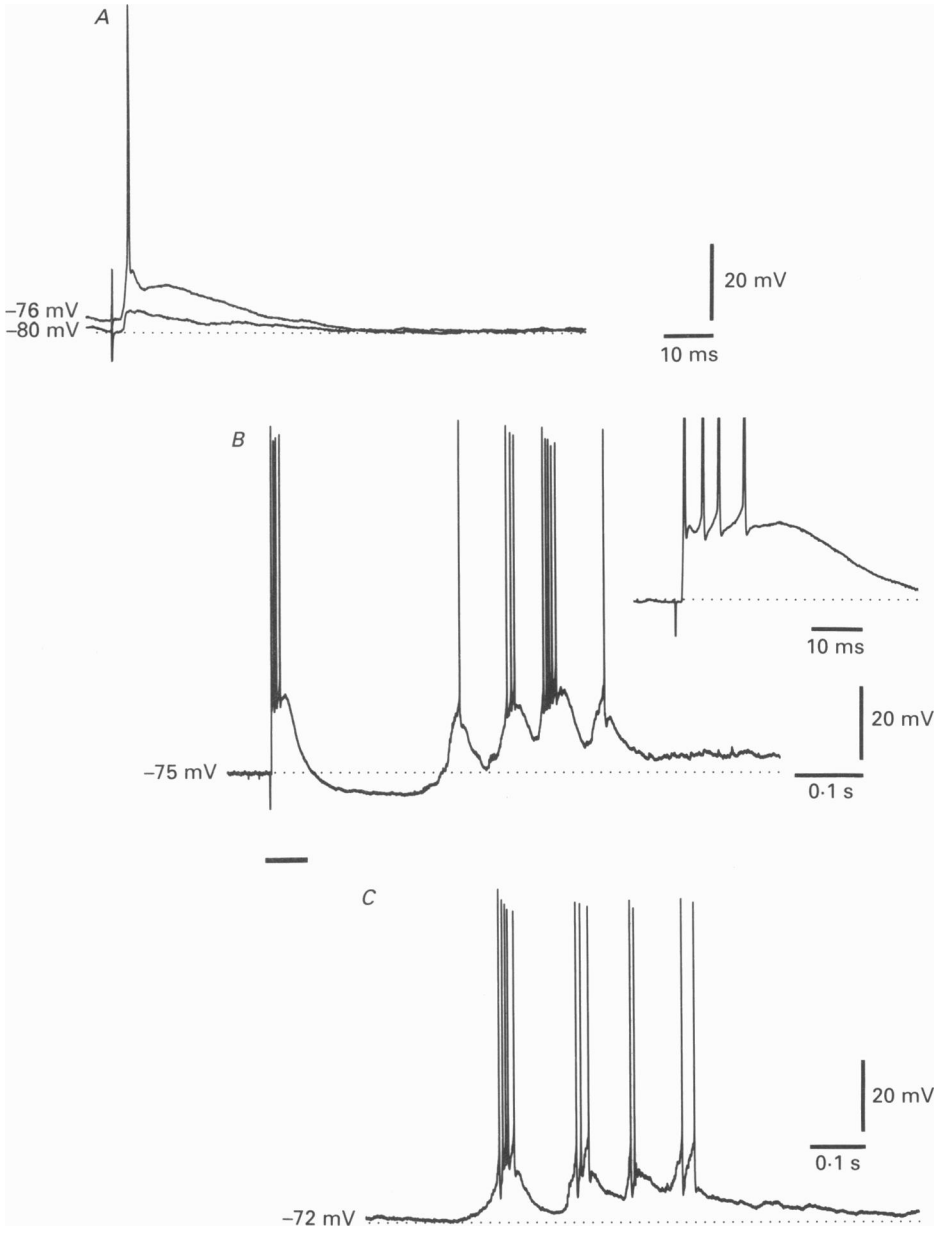

Fig. 1. Electrophysiological identification of RE cells. *A*, orthodromic responses to brachium conjunctivum stimulation at resting V_m (−74 mV) and under slight DC hyperpolarization. *B*, orthodromic response to stimulation of the internal capsule in the same cell as in *A*. The inset represents the initial burst of the response at a faster time scale (spikes truncated). *C*, spontaneous spindle sequence at the resting V_m in the same cell. In this and following figures the V_m is indicated.

related to the amount of current injected and showed a slight early frequency adaptation. If the V_m was hyperpolarized beyond −75 mV, a burst of somatic action potentials was generated upon depolarization (Fig. 3*A*). The burst response was

composed of a slowly rising and falling potential, crowned by five to eight fast spikes occurring at frequencies between 120 and 300 Hz. The spike discharge during the burst showed a characteristic increase, followed by a decrease, in firing rate (Domich *et al.* 1986). This pattern is the key feature that distinguishes the bursts of RE cells

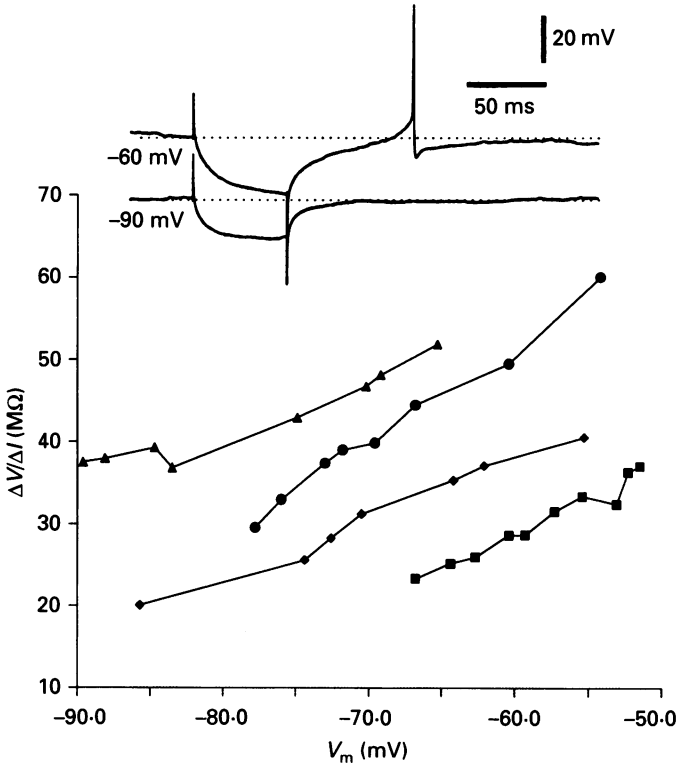

Fig. 2. Current–voltage relationships of RE neurones. Upper traces: a small constant hyperpolarizing pulse was delivered as the V_m (−60 mV) was changed with DC hyperpolarizing current. Examples of current–voltage relationships are plotted below from four different neurones.

from those of thalamocortical neurones which display continuously increasing durations of interspike intervals. Therefore, we termed burst discharges as those having frequencies above 100 Hz and an acceleration–deceleration pattern.

Figure 3*A* also shows that the development of the burst response upon hyperpolarization was paralleled by the appearance of a silent period between the burst discharge and the tonic firing that followed. This pattern of burst discharge has already been described extracellularly in RE cells (Domich *et al.* 1986).

The characteristic sequence of intervals during the spike burst in RE cells and the transition from tonic to burst firing upon hyperpolarization, can be evaluated better in the plots shown in Fig. 3*B* and *C*. The reciprocal of successive intervals (instantaneous frequency) was plotted against time for increasing levels of DC hyperpolarization while the amplitude of a depolarizing pulse was kept constant. The

transition from tonic to burst firing occurred smoothly as the cell was progressively hyperpolarized, until a maximum burst response was obtained. The burst response was variable from cell to cell, but its discharge pattern consistently showed the acceleration–deceleration sequence described above.

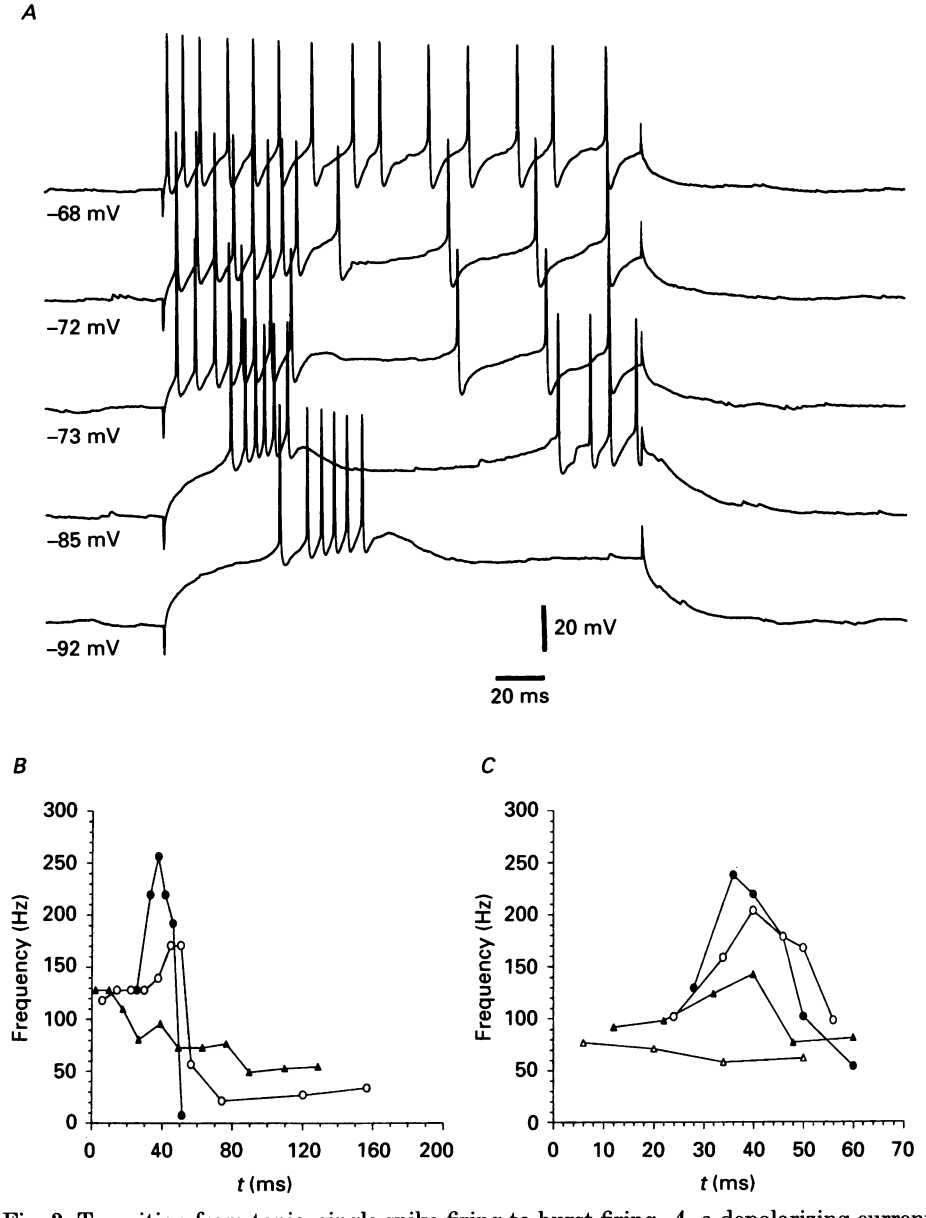

Fig. 3. Transition from tonic, single spike firing to burst firing. *A*, a depolarizing current pulse of constant amplitude (0·8 nA) was applied while the V_m was hyperpolarized by DC current injection. *B* and *C*, instantaneous frequency plots of two different neurones tested with the same protocol as in *A*. △, −60 mV; ▲, −67 mV; ○, −78 mV; ●, −85 mV. In the left panel, values for a V_m of −60 mV (△) are not represented.

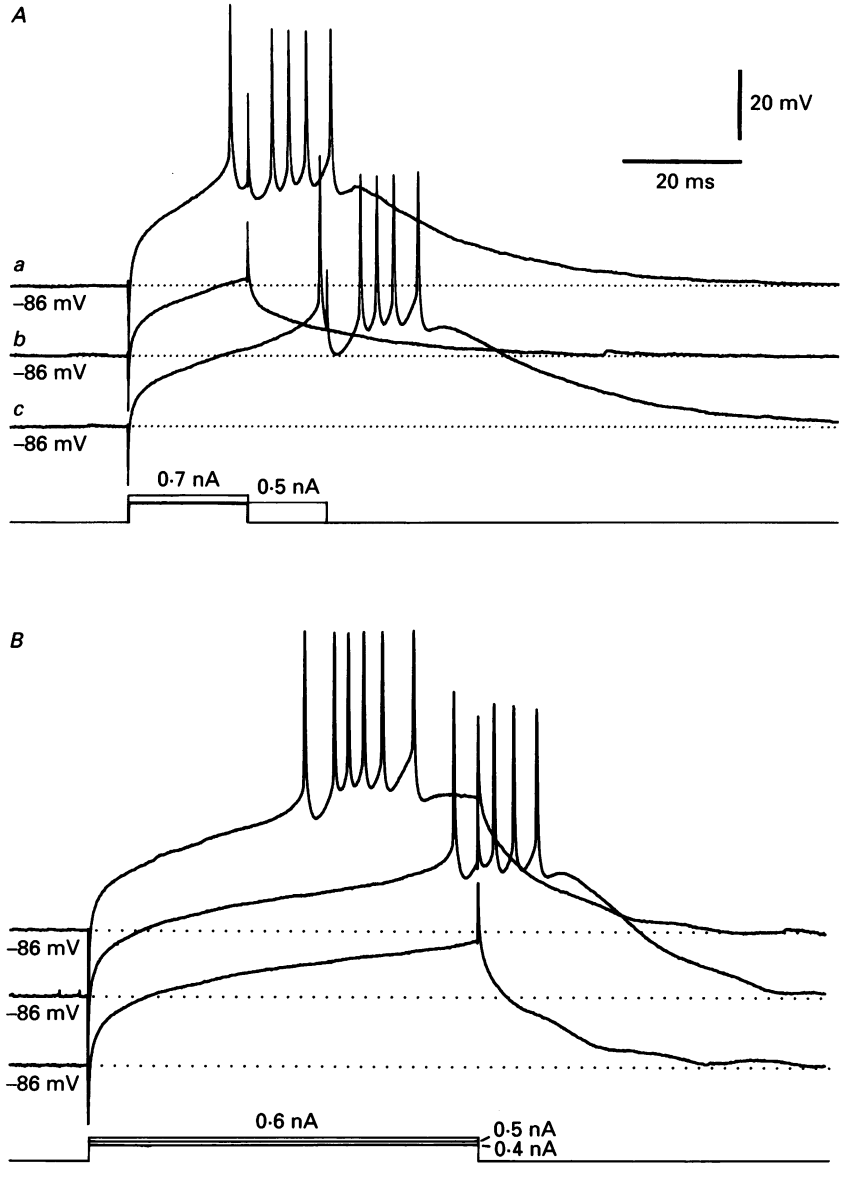

Fig. 4. Stereotyped burst responses of RE cells. In *A*, a RE cell was held hyperpolarized by DC current injection. A pulse of 0·5 nA and 20 ms duration elicited a passive response (trace *b*). A step increase in the amplitude or the duration of the pulse elicited a fully developed burst response that outlasted the pulse (traces *a* and *c*). All sweeps displaced artificially for clarity. In *B*, in the same cell, the amplitude of the injected current was increased from 0·4 to 0·6 nA.

At the hyperpolarized V_m values at which the LTS was presumably de-inactivated, adequate stimulus parameters gave rise to an all-or-none burst response. As shown in Fig. 4*A*, a step increase in either the amplitude or the duration of a depolarizing pulse triggered a fully developed burst response that outlasted the

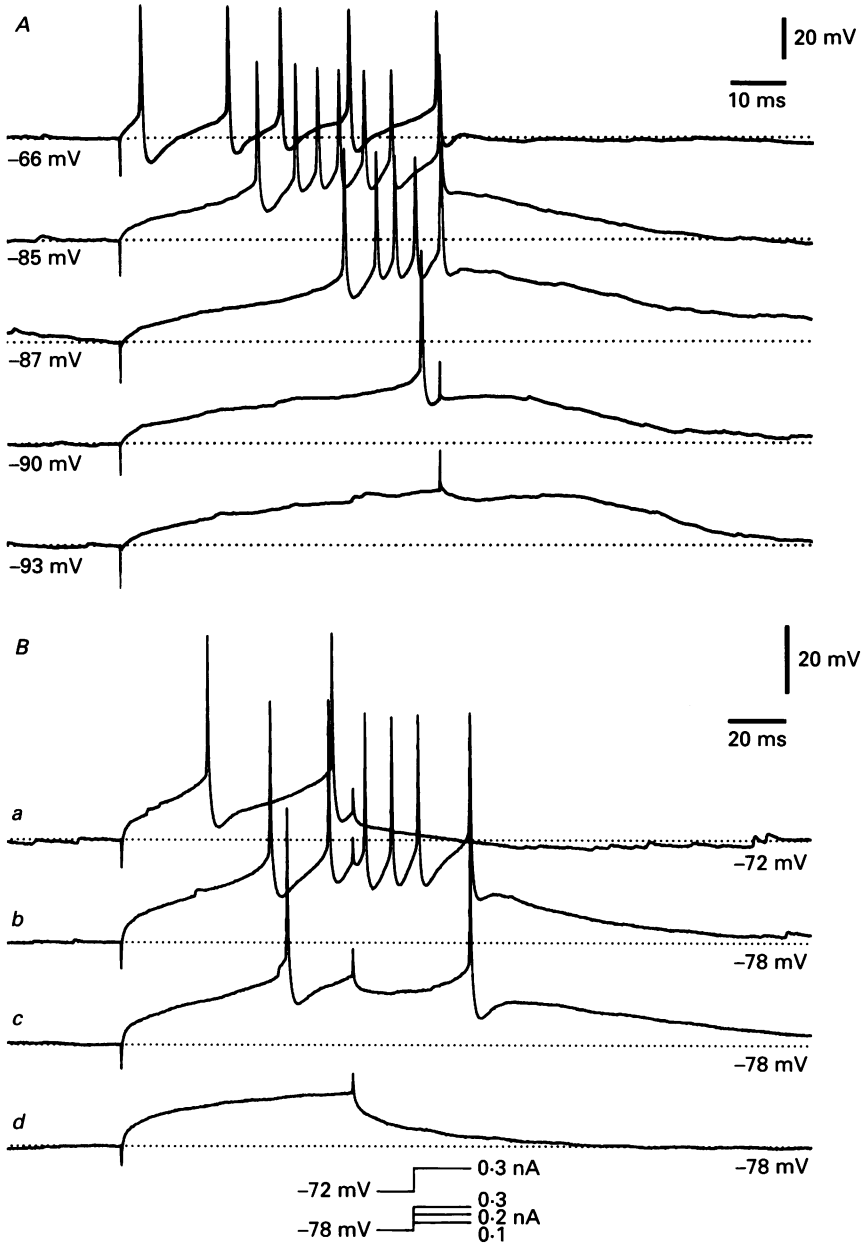

Fig. 5. Gradual nature of the burst response. In *A*, a depolarizing pulse of constant amplitude was applied while the cell was progressively hyperpolarized by DC current. In *B*, a depolarizing pulse of 0·3 nA was applied at rest (trace *a*) and at a hyperpolarized V_m (trace *b*). The V_m was then kept constant and the pulse was reduced in amplitude. The burst response diminished in parallel.

pulse. In this condition, small increases in the amplitude of the triggering pulse did not affect the pattern of spike generation, but did shorten the latency to burst triggering (Fig. 4*B*).

However, burst generation appeared to be graded. In the example shown in Fig. 5*A*, a cell stimulated by depolarizing current pulses developed from tonic to burst firing mode when hyperpolarized by DC current injection from −66 mV to −85 mV. The burst response had a slowly decaying phase that outlasted the pulse and originated a prolonged after-depolarization. The same amplitude pulse, applied at a slightly more hyperpolarized V_m (−87 mV), elicited a burst response with a longer latency, shorter duration and decreased number of spikes. When the cell was further hyperpolarized (−90 mV), the same testing pulse triggered a slowly rising, slowly decaying response that had a similar apparent threshold (−68 mV, as seen from the soma) and a similar duration as the slow events underlying the fully developed burst. This response, however, triggered only a single spike. At −93 mV, the same depolarizing pulse elicited a response with similar threshold and time course as before, but which did not reach the threshold for somatic spike firing.

The after-depolarization could reach the threshold for spike generation. As shown in Fig. 5*B*, a depolarizing pulse of 0·3 nA induced single-spike firing at −72 mV. When a pulse with the same amplitude was delivered at −78 mV, a spike burst was triggered, outlasting the pulse. At the same membrane potential (−78 mV), a smaller amplitude pulse (0·2 nA) triggered a single spike and a plateau response which was effective in triggering an action potential well after the end of the pulse. Further decreasing the amplitude of the depolarizing current injection gave rise to only a passive response (Fig. 5*Bd*).

The responses shown in Fig. 5, had a similar apparent threshold and duration to the slow events underlying burst responses, suggesting similar underlying ionic mechanisms.

Thus, RE cells demonstrate a long-lasting burst response whose amplitude can be modulated both by the level of membrane polarization and by the intensity of depolarizing inputs. This indicates that, contrary to the stereotyped all-or-none burst responses of relay thalamic cells, RE cells exhibit a bursting behaviour with a broader range of integrative properties.

Dendritic spikes in RE cells

Spike-like potentials of small amplitude (3–7 mV) were recorded in 10% of the cells studied ($n = 5$). They had a fast rising phase, a duration of 3–6 ms (measured at the base) and a fast falling phase indicating an active repolarization. They could be consistently triggered by depolarization, whether by means of current injection (Fig. 6*A*), or by cortically elicited EPSPs (Fig. 6*B*), or by the depolarizing waves constituting a spindle sequence (Fig. 6*C*). The apparent firing level was consistently lower when triggered by a cortically elicited EPSP (−74 mV in Fig. 6*B*) than when triggered by current injection (−65 mV in Fig. 6*A*).

In view of the dendritic location of the incoming cortical axons (Jones, 1985) and assuming a somatic placement of the recording electrode, we presume that these spike-like responses are of a dendritic origin. That they are all-or-none is strongly suggested by the example of Fig. 7*A* in which stimuli with equal amplitudes were applied to the internal capsule while the cell was progressively hyperpolarized. At rest (−76 mV), two full action potentials were triggered by the cortically induced EPSP. By hyperpolarizing the cell, the synaptic response became subthreshold for

somatic spike firing and dendritic spikes were revealed in isolation. When the cell was further hyperpolarized, the EPSP became subthreshold for dendritic spikes, without any transition in the amplitude or duration of the dendritic spike. The same phenomenon occurred when the dendritic spikes were triggered by the depolarizing

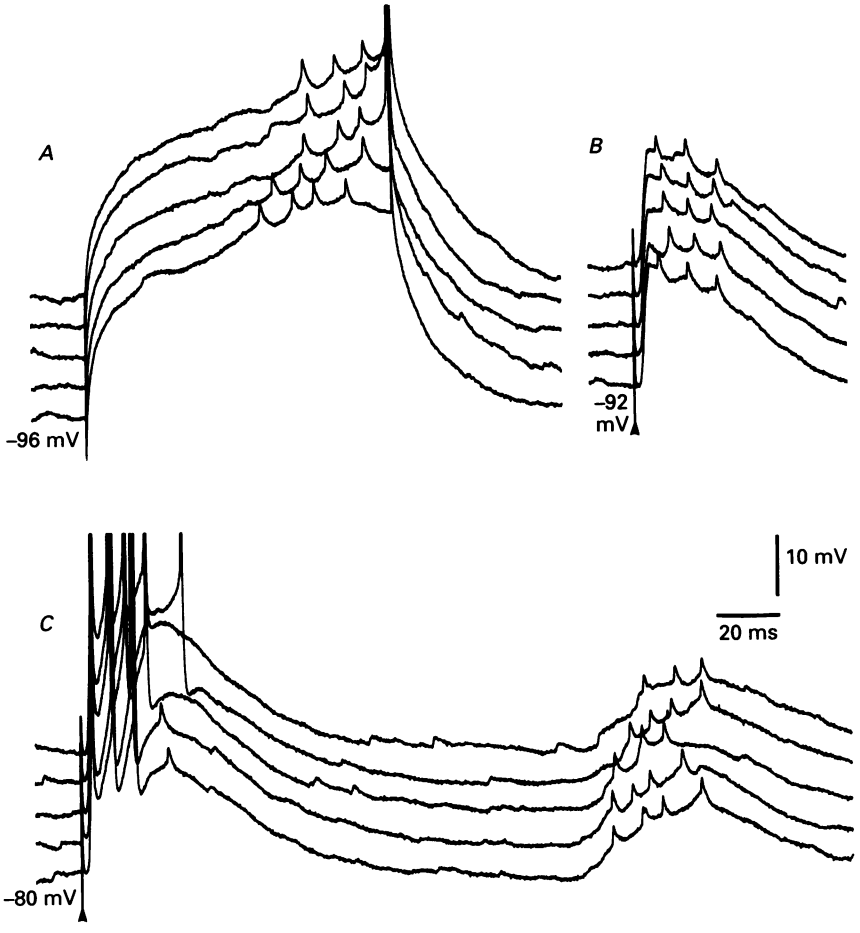

Fig. 6. Dendritic spikes elicited by depolarization of RE cells. In *A*, constant amplitude depolarizing pulses were applied at the same V_m. In *B*, the EPSP evoked by internal capsule stimulation consistently triggered a discharge of dendritic spikes. The depolarizing waves of cortically evoked spindle sequence were also capable of triggering the discharge of dendritic spikes, as shown in *C*. Traces are displaced vertically for clarity. The same cell is shown in *A*, *B* and *C*.

waves constituting a spindle sequence. In Fig. 7*B* the intensity of internal capsule stimulation was diminished and the amplitude of both the first EPSP and the evoked spindle oscillation decreased, until the latter was no longer capable of triggering dendritic spikes. Again, there was no transition in the amplitude and duration of dendritic spikes.

Figure 8*A* further supports the voltage dependency and the all-or-none nature of

dendritic spikes. A low-intensity depolarizing pulse elicited only a passive response; with increased current, the threshold for generation of dendritic spikes was reached and further increase in the injected current increased the number of dendritic spikes, without changing their stereotyped amplitude and duration. The apparent single

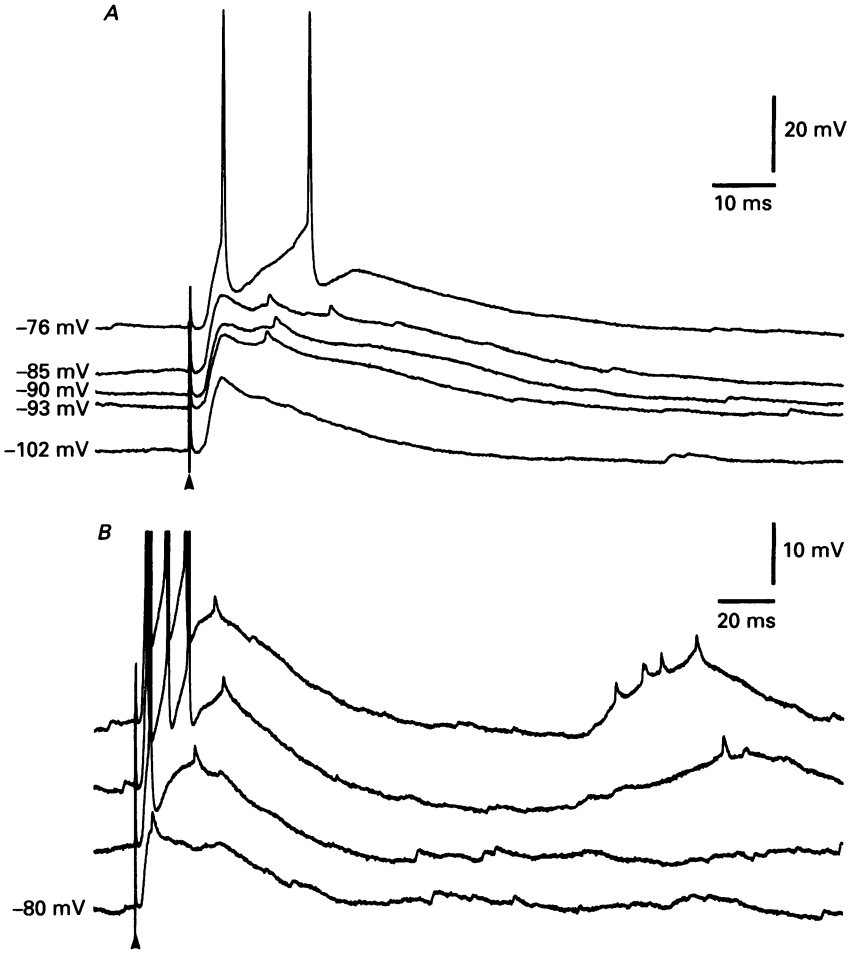

Fig. 7. Dendritic spikes are all-or-none, voltage-dependent events. In *A*, the cell was progressively hyperpolarized with current injection while applying internal capsule stimulation of constant intensity. In a different cell in *B*, the membrane potential was maintained constant and the intensity of the internal capsule stimulation was diminished. Action potentials truncated. Traces are displaced vertically in *B*, for clarity.

component nature of the evoked dendritic spikes also suggests that they are originating in a single discrete area of the dendrites.

The generation of dendritic spikes may influence somatic activity and the eventual output of RE cells. In Fig. 8*B* a depolarizing current pulse could trigger dendritic spikes in isolation, but when the amplitude of the pulse was increased and dendritic spikes occurred on a background of a more depolarized somatic membrane, they were

able to trigger plateau potentials that eventually led to firing of full action potentials (see 0·9 and 1 nA pulses in Fig. 8*B*).

Dendritic spikes also occurred spontaneously, without being associated or triggered by any visible depolarizing event. Figure 9*A* shows such dendritic spikes

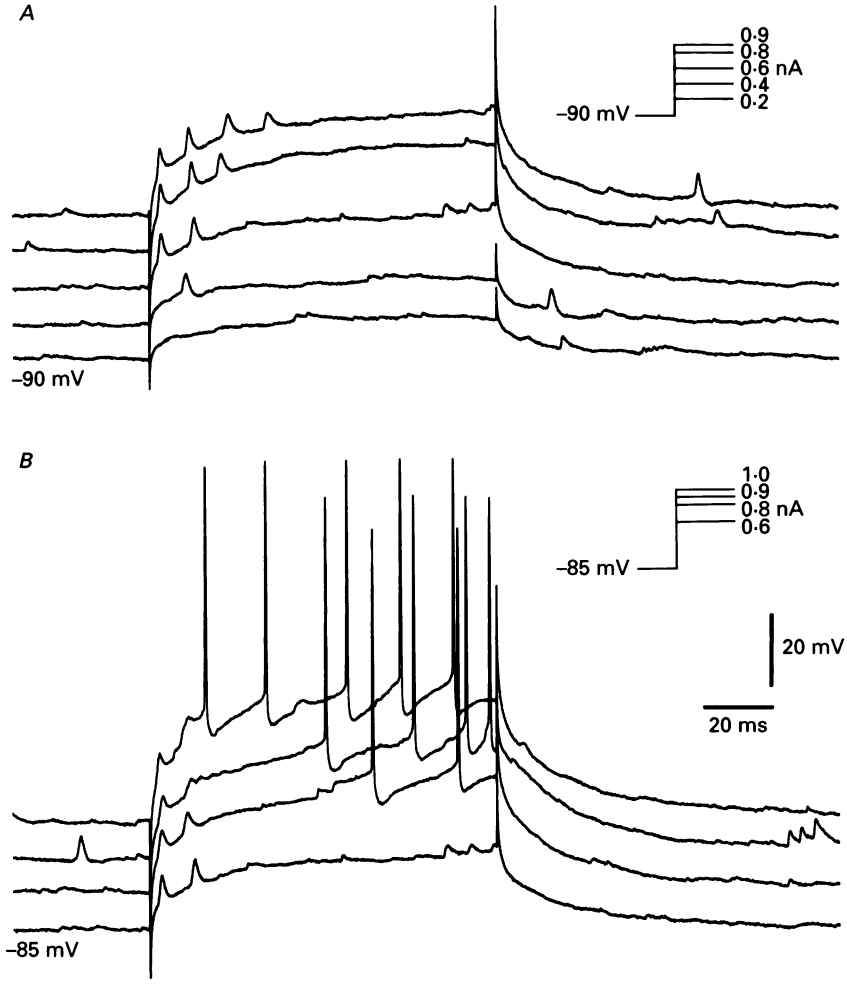

Fig. 8. Dendritic spikes modify the output of RE cells. In *A*, depolarizing pulses of increasing amplitude (indicated) triggered an increasing number of all-or-none dendritic spikes. The same stimulation protocol was applied in *B*.

firing tonically, without apparent temporal relation to the ongoing synaptic noise. The apparent single component nature of the dendritic spikes depicted in Fig. 9*A* suggests that they were originating in a single hot spot. That dendritic spikes may originate in many different foci in one single cell is shown in Fig. 9*C*. Dendritic spikes of differing amplitudes and durations could be seen at the somatic level, either individually or building up complex depolarizing events.

Another property of dendritic spikes was their ability to fire in bursts. Bursts of 2–5 dendritic spikes with a frequency of 110–150 Hz could be elicited when

depolarizing the cell by means of current injection (see again Fig. 6*A*) or by orthodromically evoked EPSPs (Fig. 6*B*) or during the depolarizing phases of a spindle sequence (Fig. 6*C*). Bursts of up to 400 Hz occurred spontaneously, as shown in Fig. 9*B* and *C*.

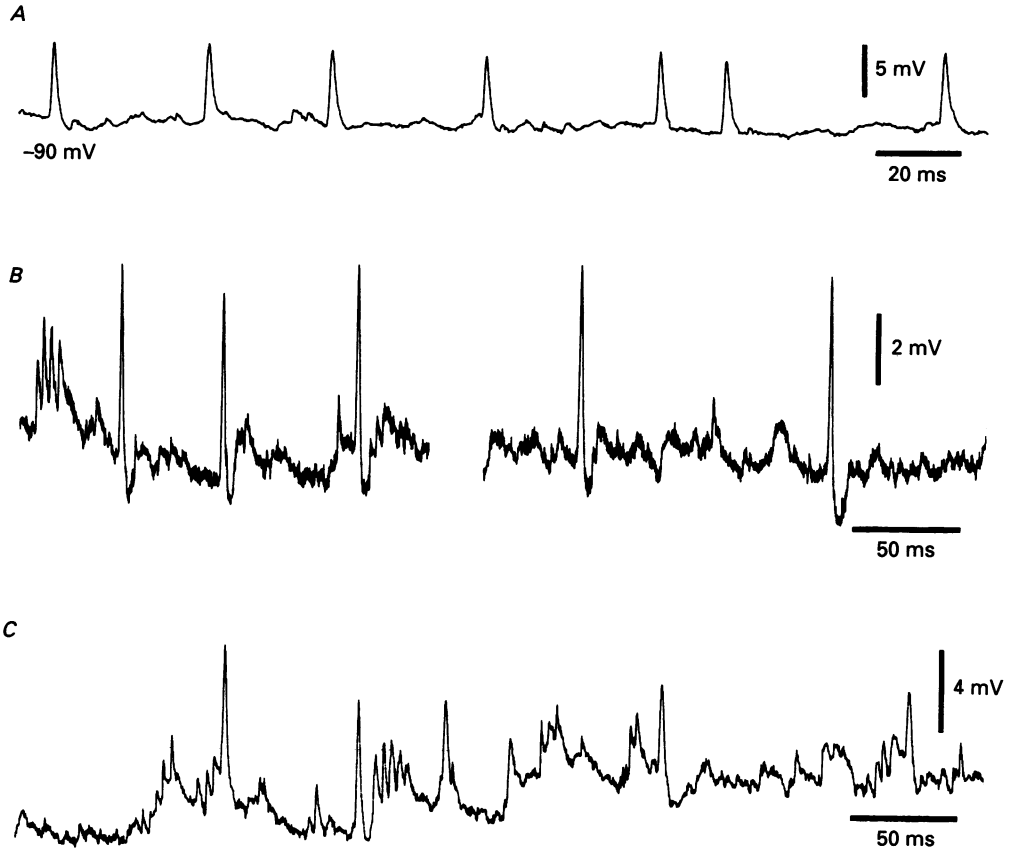

Fig. 9. Dendritic spikes may fire tonically, as in *A*. In *B*, some dendritic spikes were even followed by a clear AHP. *C*, dendritic spikes with different amplitudes and durations. In the example shown, the firing of dendritic spikes was associated with a sustained depolarization in the V_m. All traces from the same cell.

In some of the somatically recorded dendritic spikes a pronounced AHP was present (Fig. 9*B*). This was not a frequent finding, but it may indicate that Ca^{2+}-dependent K^+ conductances are present in the dendrites of RE cells.

Synaptic responses

The major synaptic input of RE cells is provided by the collaterals of corticothalamic axons when they cross the RE nucleus to reach the dorsal thalamus (Jones, 1985).

Among the fifty-one cells included in the data base, seventeen were tested for the dependency of the cortically evoked response on the V_m and the intensity of the stimulation. The results were the same for all tested cells.

Figure 10*A* shows the response of an RE cell to a stimulation of the internal capsule. When the cell was progressively released from hyperpolarization, the postsynaptic potential reached the threshold for activation of the LTS and a fully developed burst response was triggered at −77 mV. Closer to the resting V_m the LTS

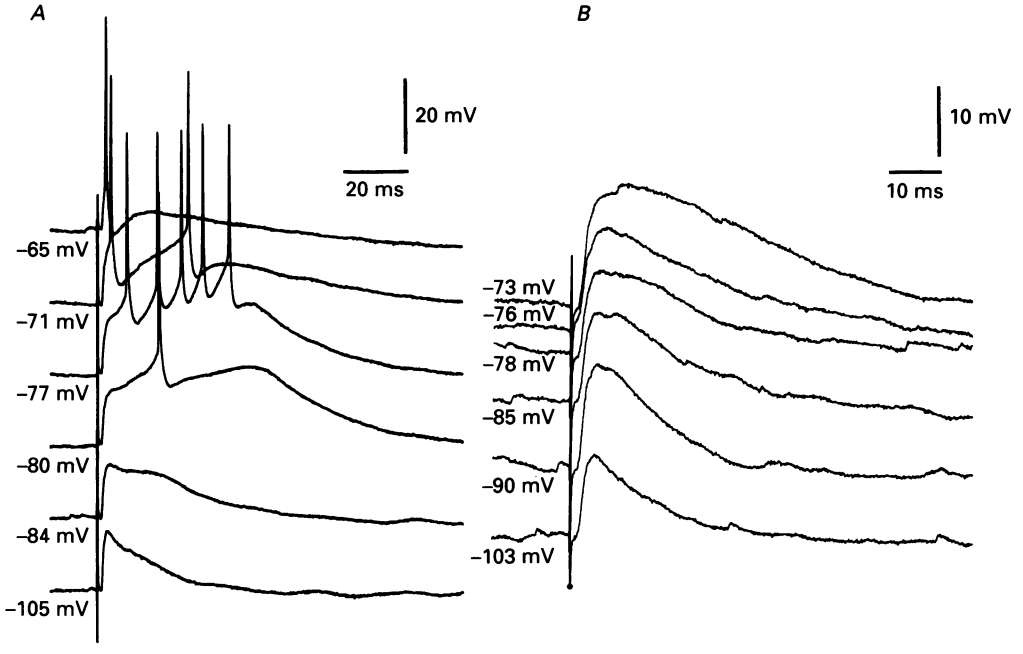

Fig. 10. The discharge pattern in response to synaptic stimulation depends critically on the V_m. The cell depicted in *A* was held at progressively hyperpolarized V_m values and stimulated orthodromically with a constant intensity. The cell depicted in *B*, was progressively hyperpolarized by means of DC current injection and shocks of constant intensity were applied to the internal capsule. Hyperpolarization decreased the amplitude and duration of the EPSP.

progressively inactivated and at the resting V_m (−65 mV) only a single spike was fired. Here again it can be seen that the burst response does not behave as an all-or-none response. This is the case at −80 mV, in which the elicited response had a similar time course to the burst response, but triggered only a single spike. Figure 10*B* illustrates in a different cell and in more detail the behaviour of the elicited EPSP for a range of potentials below spike firing. At −73 mV a compound EPSP was elicited with a latency of 1·3 ms, an amplitude of 17 mV and a duration of around 75 ms. Upon hyperpolarization up to −103 mV, the peak amplitude of the EPSP was reduced to 11 mV and its duration to 26 ms.

The bursting behaviour in response to internal capsule stimulation was also dependent on the intensity of the stimulation. In the cell illustrated in Fig. 11*A*, the V_m was held constant at values at which a full burst response was elicited by the orthodromic volley and the intensity of the shock delivered to the internal capsule was progressively diminished. It can be seen that the burst response diminished gradually, in contrast to an all-or-none response, until the EPSP became

subthreshold for firing. More interestingly, the whole oscillatory spindle response that followed the cortically evoked EPSP critically depended on the intensity of internal capsule stimulation. In Fig. 11*C*, the spindle sequence elicited by the maximal intensity volley (*a* and *b*) diminished gradually with the decrease in the

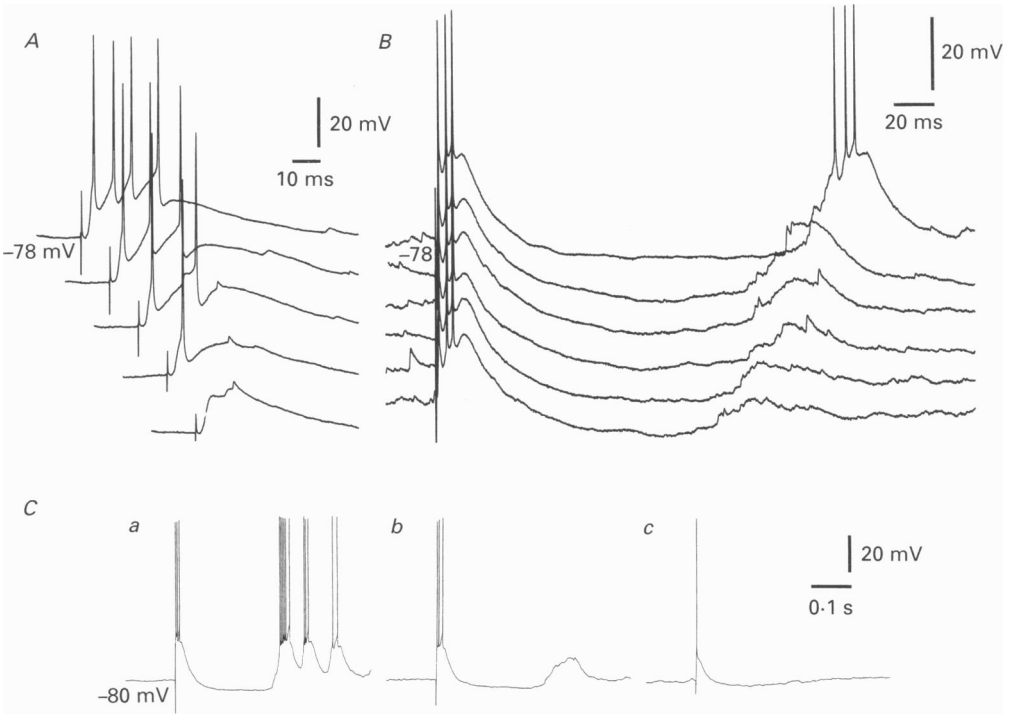

Fig. 11. The burst response decreases gradually in amplitude with the decrease in the intensity of the orthodromic stimulation. In the cell depicted the V_m was maintained constant and the intensity of the stimulation applied at the internal capsule progressively diminished. Traces are displaced vertically and horizontally for clarity. *B*, in this cell the intensity of stimulation was progressively reduced in a range that did not affect the early bursting response but did gradually decrease the rebound response that initiated the spindle sequence. All sweeps at the same membrane potential. Traces displaced artificially for clarity. *C*, the amplitude of the spindle sequence evoked by internal capsule stimulation depends on the intensity of the stimulation. A cell in which the V_m was held constant and the intensity of the internal capsule stimulation was progressively reduced (from traces *a* to *c*) is shown.

stimulus intensity, until no oscillation at all was evoked by the incoming input (*c*). Furthermore, the occurrence of an evoked spindle oscillation depended solely on the intensity of the stimulation, and not on the amplitude of the early burst response. This is demonstrated in Fig. 11*B*, in which a slight decrease in the intensity of the stimulation drastically affected the postinhibitory rebound initiating the spindle sequence, but was still in the range to evoke an early fully developed spike burst.

Following the first EPSP in response to internal capsule stimulation a period of decreased excitability preceded the beginning of the evoked spindle sequence. While in most cells this period was only associated with a simple repolarization of the

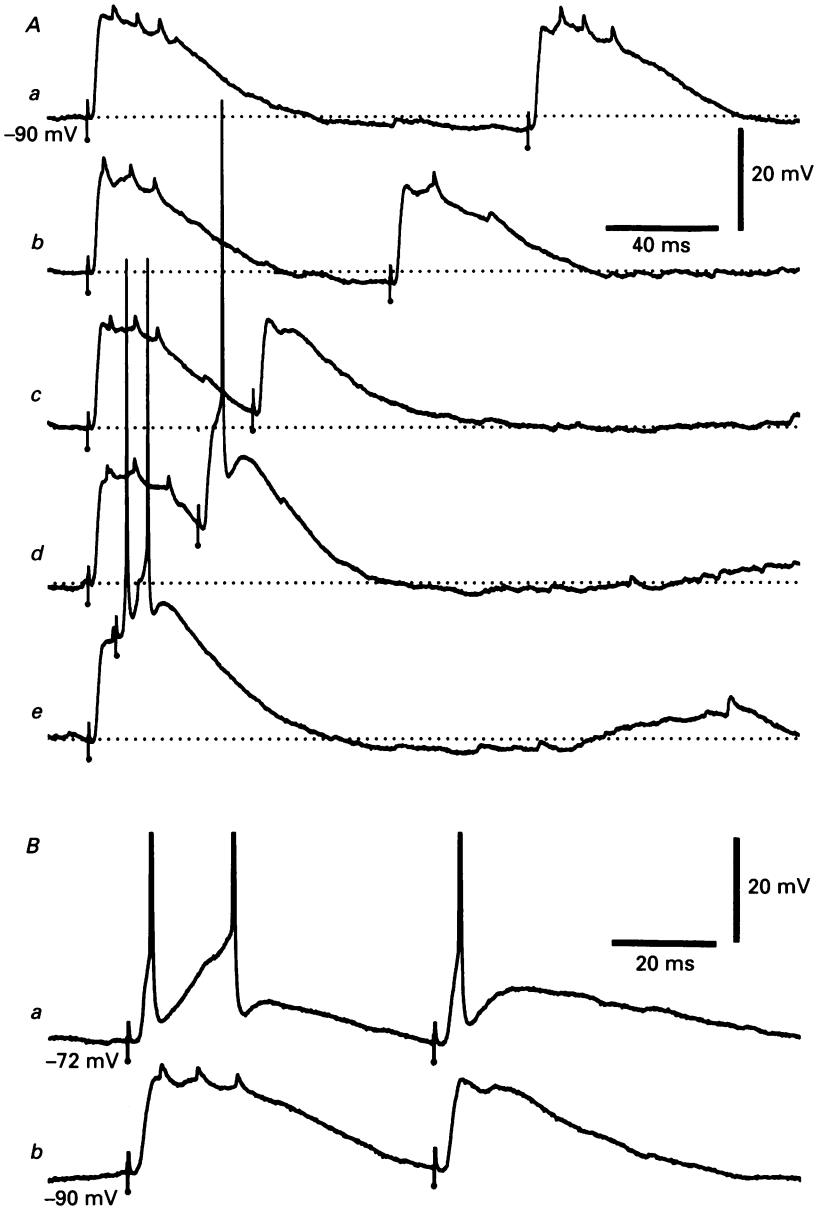

Fig. 12. Following the EPSP elicited by cortical stimulation there is a period of reduced excitability. In *A*, a pair of shocks given in the internal capsule was repeated at progressively shorter intervals (from trace *a* to *e*). The V_m was held constant at -90 mV by DC current injection. In the same cell in *B*, a pair of shocks was given with the same interval but at different V_m. In both cases a decrease of excitability during the second was evident.

membrane, in a few ($n = 7$) neurones a clear hyperpolarization was seen. This hyperpolarization had a duration ranging between 60 and 210 ms and an amplitude with a maximum of 13 mV. Hyperpolarizing square current pulses applied during the

inhibitory period revealed no change in input resistance (not shown), but the inhibition could be detected by its shunting effect over a second orthodromic stimulation (Fig. 12). When the second shock was delivered 70 ms after the return to baseline of the first EPSP, a control response was obtained (Fig. 12*A a*). When the second shock was administered at progressively shorter intervals, a shunting effect became visible as the number of dendritic spikes triggered by the EPSP decreased to zero and the EPSP itself shortened (Fig. 12*A b* and *c*). Then, for shorter intervals, temporal summation of the EPSPs occurred, and the second response reached the threshold for firing somatic spikes (Fig. 12*A d* and *e*). During the inhibitory period the amplitude and the rising phase of the EPSP were, however, only slightly modified and DC hyperpolarization almost did not alter the V_m trajectory. This suggests that the inhibitory effect was located in the dendrites in a more restricted area than the cortical excitation. This phenomenon was also seen at the resting V_m, by a decrease in the number of triggered spikes (Fig. 12*B*).

DISCUSSION

Our results reveal new electrophysiological features characterizing the behaviour of RE cells and indicate possible consequences for the overall output of these neurones when immersed into the corticothalamocortical network.

Three main findings are presented in this paper. (a) The burst response of RE cells may be expressed partially not only according to their V_m, as in dorsal thalamic relay cells, but also depending on the magnitude of the depolarizing influences. (b) The dendrites of RE cells are capable of generating spikes in many different active sites along the dendritic branches; these dendritic spikes may fire either tonically or in bursts. (c) The amplitude of the oscillatory spindle response evoked by stimulation of the internal capsule depends on the stimulus intensity.

Burst responses in RE cells

The burst of Na^+ somatic spikes displayed by RE cells is generated by the activation of the low-threshold transient Ca^{2+} conductance, I_t (Avanzini *et al.* 1989; Huguenard & Prince, 1992). The biophysical properties of this T-current (Huguenard & Prince, 1992), i.e. its positive reversal potential, its steep activation curve and its voltage-dependent rate of activation, determine the generation of an all-or-none LTS and a stereotyped burst response. The burst response presented by RE cells, however, could be modulated in our experiments by modifying the amplitude of the testing pulse or the intensity of the orthodromic stimulation. A possible explanation may be the activation of other voltage-dependent ionic conductances that reduce the burst response by hyperpolarizing influences.

The transient potassium current, I_A, has been suggested to be present in RE neurones (Llinás & Geijo–Barrientos, 1988; Contreras, Curró Dossi & Steriade, 1992). In other systems, such as pedunculopontine neurones (Kang & Kitai, 1990; Leonard & Llinás, 1990) or cortical Betz cells (Schwindt, Spain, Foehring, Stafstrom, Chubb & Crill, 1988), I_A has been demonstrated to prevent burst firing. In dorsal thalamic relay cells, which have a prominent I_A (Jahnsen & Llinás, 1984*b*), biophysical studies have demonstrated that the steeper voltage dependence of activation and the

slightly more negative activation threshold of I_t (Coulter, Huguenard & Prince, 1989) compared with I_A (Huguenard, Coulter & Prince, 1991) will ensure the regenerative depolarization that is the LTS. Instead, in RE cells, the T-current has a less steep voltage dependency of activation and a higher activation threshold in comparison to relay cells (Huguenard & Prince, 1992), but voltage-clamp data on I_A are lacking. Another source for the attenuation of the burst response in RE cells may be anomalous rectification. Thus, in the range of somatic V_m values at which the burst response is fully activated, anomalous rectification decreases the input resistance of the cell (see Fig. 2), thus dampening the regenerative mechanism of the LTS.

A different hypothesis to explain the graded expression of spike-bursts in RE cells would be that, as in substantia nigra pars compacta neurones (Llinás, Greenfield & Jahnsen, 1984), this conductance is distributed in the dendritic tree of RE cells. In this way, the LTS observed in the soma would be the summation of many levels of low-threshold spiking and a fully developed burst would represent a massive and synchronous invasion of the RE cells somata by the T-current originating in different sites along the dendritic tree.

It has been suggested that the T-conductance is located in the dendrites on the basis of the long duration of the burst and the more hyperpolarized potentials required to remove its inactivation in comparison to thalamic cells (Mulle *et al.* 1986). The first argument may rather be explained by the biophysical properties of the T-current in comparison to thalamic relay cells, namely, because the rate of I_t inactivation in RE cells is slower and voltage-independent (Huguenard & Prince, 1992), the resulting LTS is not self-limiting and, consequently, the burst response will be prolonged in RE cells. As to the second argument, since the steady-state inactivation curves of the T-current in RE (Huguenard & Prince, 1992) and dorsal thalamic cells (Coulter *et al.* 1989) are superimposable, a dendritic location should account for the difference in the levels of hyperpolarization required for its de-inactivation. In addition, the degree of somatic hyperpolarization required for the de-inactivation of the LTS would be difficult to be reached in physiological conditions. Further support for the dendritic location of the I_t in RE cells is given by our data showing that the apparent threshold of activation of the burst response is around -64 to -70 mV, while voltage-clamp studies (Huguenard & Prince, 1992) show that the voltage activation range begins at -60 mV and the current is half-activated at -49 mV. Thus, the readings of membrane potential in the soma are most probably around 10 mV more hyperpolarized than in the site of origin of the LTS. Data on the electrotonic properties of RE cells are lacking for further precision.

Thus, a combination of the above factors may be responsible for the particular bursting behaviour presented by RE cells. In the case of burst responses evoked by current injection, the somatic hyperpolarization necessary for the de-inactivation of the LTS activates anomalous rectification and reduces the electrical coupling between the soma and the dendrites. As a consequence, more current becomes necessary to activate synchronously the distributed dendritic region responsible for the generation of the LTS and the possibility is then opened for the activation of partial burst responses.

In terms of RE cell output, the above hypothesis implies that a distributed and synchronous depolarizing input should reach a hyperpolarized dendritic tree to elicit

a burst response. Two inputs seem to be well suited to meet these requirements: the dendrodendritic GABAergic synapses (Deschênes *et al.* 1985; Yen *et al.* 1985) and the input from collaterals of corticothalamic and thalamo-RE pathways (see Jones, 1985).

Dendritic spikes

The direct demonstration that dendrites could generate active responses in the form of dendritic spikes was obtained from intradendritic recordings in the Purkinje cells of the alligator cerebellum (Llinás & Nicholson, 1971). Direct recordings *in vitro* confirmed the capacity of dendritic membranes to generate Na^+ and Ca^{2+}-mediated spikes (Wong, Prince & Basbaum, 1979; Llinás & Sugimori, 1980; Regehr, Konnerth & Armstrong, 1992). Dendritic spikes have also been found in relay thalamic cells (Maekawa & Purpura, 1967; Deschênes, Paradis, Roy & Steriade, 1984).

In our study, unitary dendritic spikes were clearly recorded in only 10% of the cells, but complex depolarizing phenomena composed by several all-or-none events resembling dendritic spikes were a common finding. The observation that dendritic spikes were summing at the somatic level with no apparent refractoriness indicates that they are generated in many different foci and that certain functional independence among dendritic branches exists (Kuno & Llinás, 1970; Llinás & Nicholson, 1971).

Different functions for dendritic spikes in RE cells may be proposed. The most straightforward is to ascribe to dendritic spikes the role of boosting distal synaptic potentials and transforming into axonal spike trains distant EPSPs that would otherwise be ineffective. A second important consequence of dendritic spikes would be to promote soma–dendritic propagation of excitation and the consequent activation of other dendritic voltage dependent conductances. This role for dendritic excitability has been proposed as the basis for the Ca^{2+}-dependent dendritic release of dopamine and acetylcholinesterase from substantia nigra pars compacta neurones (Llinás *et al.* 1984). This mechanism would be of great relevance in RE cells since they are known to possess reciprocal GABAergic dendrodendritic synapses which are believed to be the basis for the synchronization of RE cells into the spindling rhythmicity (Steriade *et al.* 1987). The occasional observation of an AHP following dendritic spikes (see Fig. 9*B*) is an indication that Ca^{2+} would enter during the spike enough to activate a K^+ conductance.

Thus, the finding of dendritic spikes in RE cells is in conformity with other central neurones and further supports the idea that spike generation may be a common property of dendrites.

We propose that dendritic spikes are crucial in spindling generation and synchronization by playing the triple role of depolarizing the soma, triggering dendritic LTSs and rapidly conducting depolarizing influences to distal dendrites to assure a correct timing of GABA release.

Synaptic responses

The stimulation of the internal capsule induced a multiunitary EPSP that, depending on the V_m, led to tonic spike firing at rest, burst firing at hyperpolarized levels, or was subthreshold for firing action potentials. Corticothalamic axons synapse on the dendrites of RE cells (Jones, 1985) and there is evidence that they

release glutamate as neurotransmitter (Baughman & Gilbert, 1980; Giuffrida & Rustioni, 1988; Montero & Wenthold, 1989). Moreover, the reduction in amplitude and duration of the cortically evoked EPSP by hyperpolarization may represent the voltage-dependent blockage of the NMDA receptor (De Curtis, Spreafico & Avanzini, 1989).

Most often, the period of decreased excitability that followed the first EPSP elicited by the internal capsule stimulation was a repolarization to the resting V_m and no change in input resistance was detected in the soma. During this period, however, the response to a second cortical shock was diminished and we suggest that this inhibition is mainly due to the GABAergic action of axonal recurrent collaterals that other RE cells give within the nucleus and also to the GABAergic action that takes place in the dendrodendritic synapses of RE cells. The activation of GABA receptors would then produce an increase in conductance and a shunting effect mainly over postsynaptic potentials produced in the dendrites.

The stimulation of the internal capsule evoked a spindle sequence that was almost identical to a spontaneous one (compare Fig. 1*B* with *C*). The same similarity between evoked and spontaneous spindle sequences was demonstrated for relay thalamic cells (Roy, Clercq, Steriade & Deschênes, 1984). The most noticeable characteristic of the evoked response to internal capsule stimulation was the dependency of the amplitude of the individual depolarizing waves, constituting the spindle sequence, upon the intensity of stimulation, indicating that a spindle oscillation requires a certain degree of input synchronization to be generated.

Spindling may result from (a) rebound responses to GABAergic IPSPs imposed by neighbouring RE cells through dendrodendritic synapses, (b) EPSPs from burst discharges in the thalamocortical cells, either alone or triggering dendritic spikes and (c) intrinsic oscillatory properties. Dendrodendritic interaction would also serve the purpose of synchronizing RE cells into the spindling rhythm. A model based on this idea (Wang & Rinzel, 1993) has recently shown the viability of this type of sychronization mechanism (by mutual inhibition and postinhibitory rebounds), provided that the synaptic conductance possesses a slow decay, i.e. a $GABA_B$-like response. The role of EPSPs in spindle generation has been suggested on the basis of small deflections that are visible at the onset of individual waves constituting the spindle oscillation (Mulle *et al.* 1986; Shosaku, Kayama, Sumitomo, Sugitani & Iwama, 1989). We believe that those deflections also consist of dendritic spikes. Finally, it has been demonstrated that RE cells of guinea-pig may oscillate intrinsically at around 10 Hz (Avanzini *et al.* 1989; Bal & McCormick, 1993).

The above mechanisms may not be exclusive especially when considering that RE cells are immersed within the thalamocortical network during natural sleep. In this sense, our results, indicating a highly excitable dendritic tree and suggesting that the LTS of RE cells originates in multiple foci along the dendrites, reinforces the hypothesis of a dendritically mediated origin and synchronization of spindle waves.

This work was supported by grant MT-3689 from the Medical Research Council of Canada. D.C. and R.C.D. are graduate students in this laboratory. We thank P. Giguère and D. Drolet for technical assistance.

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