

Short- and medium-term plasticity associated with augmenting responses in cortical slabs and spindles in intact cortex of cats *in vivo*

Igor Timofeev*, François Grenier*, Maxim Bazhenov†, Arthur R. Houweling†, Terrence J. Sejnowski†‡ and Mircea Steriade*

*Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Québec, Canada G1K 7P4, †Computational Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037, USA and ‡Department of Biology, University of California San Diego, La Jolla, CA 92093, USA

Plastic changes in the synaptic responsiveness of neocortical neurones, which occur after rhythmic stimuli within the frequency range of sleep spindles (10 Hz), were investigated in isolated neocortical slabs and intact cortex of anaesthetized cats by means of single, dual and triple simultaneous intracellular recordings in conjunction with recordings of local field potential responses. In isolated cortical slabs (10 mm long, 6 mm wide and 4–5 mm deep), augmenting responses to pulse-trains at 10 Hz (responses with growing amplitudes from the second stimulus in a train) were elicited only by relatively high-intensity stimuli. At low intensities, responses were decremental. The largest augmenting responses were evoked in neurones located close to the stimulation site. Quantitative analyses of the number of action potentials and the amplitude and area of depolarization during augmenting responses in a population of neurones recorded from slabs showed that the most dramatic increases in the number of spikes with successive stimuli, and the greatest increase in depolarization amplitude, were found in conventional fast-spiking (FS) neurones. The largest increase in the area of depolarization was found in regular-spiking (RS) neurones. Dual intracellular recordings from a pair of FS and RS neurones in the slab revealed more action potentials in the FS neurone during augmenting responses and a significant increase in the depolarization area of the RS neurone that was dependent on the firing of the FS neurone. Self-sustained seizures could occur in the slab after rhythmic stimuli at 10 Hz. In the intact cortex, repeated sequences of stimuli generating augmenting responses or spontaneous spindles could induce an increased synaptic responsiveness to single stimuli, which lasted for several minutes. A similar time course of increased responsiveness was obtained with induction of cellular plasticity. These data suggest that augmenting responses elicited by stimulation, as well as spontaneously occurring spindles, may induce short- and medium-term plasticity of neuronal responses.

(Resubmitted 2 November 2001; accepted after revision 16 April 2002)

Corresponding author I. Timofeev: Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Québec, Canada G1K 7P4. Email: igor.timofeev@phs.ulaval.ca

The state of slow-wave sleep is associated with a series of brain oscillations. While some are generated by the interplay between two of the intrinsic currents of thalamocortical (TC) neurones (McCormick & Pape, 1990; Leresche *et al.* 1991), other sleep rhythms arise through synaptic operations in thalamic and cortical networks. Among the latter, spindles (7–14 Hz) are generated by thalamic reticular (RE) neurones and their interactions with TC cells, while the slow oscillation (0.5–1 Hz) is generated within intracortical neuronal networks (reviewed in Steriade *et al.* 1993) when the number of interconnected neurones exceeds a critical minimum (Timofeev *et al.* 2000a). It was suggested that the rhythmic spike-trains or spike-bursts fired by cortical and thalamic neurones during low-frequency sleep oscillations may be involved in the consolidation of memory traces acquired during wakefulness (Steriade

et al. 1993) by massive Ca²⁺ entry in cortical pyramidal neurones. Thus, spindling may activate the molecular 'gate' mediated by protein kinase A, thus opening the door for gene expression (Sejnowski & Destexhe, 2000), a process that may allow long-term changes to subsequent inputs following sleep spindles. Indeed, the overnight improvement of visual discrimination skills depends on slow-wave sleep (Gais *et al.* 2000; Stickgold *et al.* 2000) of which spindles are a major electrical signature, and slow-wave sleep enhances plasticity in the developing visual cortex (Frank *et al.* 2001).

In the present study, we investigated the intracortical mechanisms of short- and medium-term plastic changes in responsiveness of cortical neurones exerted by stimulation in the frequency range of spindles (10 Hz). Such stimuli evoke augmenting responses (Morison &

Dempsey, 1943), defined as potentials with progressively growing amplitudes starting with the second or third stimulus in a pulse-train. Such responses have been investigated experimentally and with computational models in the thalamus of decorticated animals (Steriade & Timofeev, 1997; Bazhenov *et al.* 1998; Timofeev & Steriade, 1998) as well as in the intact cortex (Steriade *et al.* 1998) and cortical slices (Castro-Alamancos & Connors, 1996b). Here, we used the recently developed isolated cortical slab preparation *in vivo* (Timofeev *et al.* 2000a) to study the features of augmenting responses, and their consequences for synaptic plasticity, in different types of electrophysiologically identified cortical neurones. We also used normal animals with an intact cortex to follow the time course of these plastic changes following stimulus-evoked augmenting responses and naturally occurring spindles.

METHODS

Experimental procedures

Experiments were carried out on adult cats (2.5–3.5 kg) anaesthetized with ketamine and xylazine (10–15 and 2–3 mg kg⁻¹ i.m., respectively). The electroencephalogram (EEG) was monitored continuously during the experiments to maintain a sufficient level of anaesthesia. Additional doses of anaesthetic were given at the slightest tendency toward an activated EEG pattern. In addition, all pressure points and tissues to be incised were infiltrated with lidocaine (lignocaine). The neuromuscular blocker gallamine triethiodide (20 mg kg⁻¹) was given i.v. and cats were artificially ventilated to an end-tidal CO₂ of 3.5–3.8%. The heartbeat was monitored and kept constant (acceptable range, 90–110 beats min⁻¹). Body temperature was maintained at 37–39°C. Glucose saline (5% glucose, 10 ml i.p.) was given every 3–4 h during the experiments, which lasted for 8–14 h. The stability of intracellular recordings was ensured by cisternal drainage, bilateral pneumothorax, hip suspension and by filling the hole made in the skull with a solution of agar-agar (4%). All experimental procedures were performed according to national guidelines and were approved by the committee for animal care of Laval University. In 29 experiments, isolated slabs were prepared from the suprasylvian areas 5 and 7. Each slab was about 10 mm long, 6 mm wide and 4–5 mm deep. After opening a hole in the parietal bone, a small perforation was made in the dura at a place that did not contain large vessels. A crescent knife was inserted along its curve until the tip of the knife appeared ~10 mm frontally under the pia. The knife was then turned by 90 deg in both right and left directions. The pia was left intact except at the place at which the knife was inserted. The boundaries of the slab were verified in each case on 80 µm thionine-stained sections (see further technical details and histology in Timofeev *et al.* 2000a).

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

Recording and stimulation

Field potential recordings and stimulation were obtained using bipolar coaxial macroelectrodes inserted in cortical motor area 4 and suprasylvian association areas 5–7 and 21. The outer pole of the electrode was placed at the cortical surface and the inner pole at a cortical depth of 0.8–1 mm. In all monopolar recordings, the indifferent electrode was placed on neck muscles. With recordings of field potentials, the depth-negative focal waves were correlated

with excitatory phases of neuronal activity. Cortical stimuli were delivered with variable duration (0.05–0.2 ms) and intensity (0.02–0.15 mA).

Intracellular recordings were performed with sharp glass micropipettes filled with a solution of 2.5–3 M potassium acetate and DC resistance of 30–80 MΩ. A high-impedance amplifier (bandpass 0–10 kHz) with active bridge circuitry was used to record and inject current into the cell. The signals were recorded on an eight-channel tape with bandpass of 0–9 kHz and digitized at 10–20 kHz for off-line computer analysis. At least one pipette for intracellular recordings in the cortex was placed in the vicinity of one EEG electrode. In dual and triple intracellular recordings, the distance between micropipettes varied from 0.5 to 6 mm.

RESULTS

Database and neuronal identification

Data were obtained from intracellular recordings of 173 neurones in isolated slabs located in suprasylvian areas 5 and 7, which responded to 10 Hz stimulation of the neocortex. Of those neurones, simultaneous intracellular recordings were performed from 6 triplets (12 neurones) and 43 doublets (65 neurones). Neurones recorded from the slab were relatively hyperpolarized (-70.9 ± 0.98 mV, S.E.M.) and their mean input resistance was more than twice as high (49.3 ± 5.7 MΩ) as that usually recorded in the intact cortex of anaesthetized cats. Most neurones were identified by electrophysiological criteria. The relative percentage of neurones identified by electrophysiological criteria in the isolated slab was: regular-spiking (RS) 52%, intrinsically bursting (IB) 39%, fast-rhythmic-bursting (FRB) 4%, and fast-spiking (FS) 5%. In addition we recorded responses of 120 neurones to 10 Hz pulse-trains in intact cortex animals.

The experimental data are presented in following order. First, we describe the basic properties and quantification of augmenting (or incremental) responses in neocortical slabs. Next, we show the spatial and temporal alterations of augmenting responses. Finally, we demonstrate the implication of augmenting responses and spindles for mid-term plasticity.

Augmenting responses in acutely isolated neocortical slabs

The responses to pulse-trains at 10 Hz were studied in 173 neurones recorded from acutely isolated neocortical slabs. A frequency of 10 Hz was chosen on the basis of previous experiments in the thalamus and neocortex showing that, among frequencies between 5 and 15 Hz that give rise to progressive incremental responses, 10 Hz produces the greatest increase (Steriade & Timofeev, 1997; Steriade *et al.* 1998). In contrast with cortical augmenting responses to thalamic stimuli that develop from a secondary depolarizing component due to spike-bursts in thalamocortical neurones (Steriade *et al.* 1998), cortical augmenting responses to cortical stimuli applied within the isolated slab developed directly from the early excitatory postsynaptic potentials

(EPSPs) (Fig. 1). Minimal stimuli (0.05 mA) elicited only small-amplitude EPSPs of constant amplitude during the pulse-train at 10 Hz. An increase in stimulus intensity (0.08 mA) led to augmenting responses, which resulted from increased amplitude of the initial EPSP due to larger network activation (Fig. 1). This suggests that augmenting responses occur as a network-related phenomenon requiring a sufficient number of activated cortical neurones and axons.

Augmenting responses occurred as a result of interactions between excitatory and inhibitory processes (Fig. 2). (a) At threshold intensities (0.02 mA), EPSPs elicited by successive stimuli at 10 Hz displayed equal amplitudes. (b) Raising the stimulation intensity (0.04 mA) significantly increased the amplitude and the duration of the first EPSP (thus indicating that mainly excitatory connections were activated), but successive responses became decremental. (c) An increase in stimulus intensity to 0.07 mA resulted in a further increase in the amplitude of the first EPSP, but a phasic hyperpolarization was included in that depolarizing response (arrow in Fig. 2A). We ascribe this feature to the activation of inhibitory interneurons projecting to the

recorded neurone. At this intensity, only a limited part of the available network was activated and the total response remained decremental. (d) A further increase in the stimulation intensity (0.15 mA) elicited an augmenting response. The response consisted of a continuously growing depolarization, with a summation of consecutive responses and an increase from one to two action potentials. To characterize augmenting responses, we calculated the area of depolarization for the first 100 ms after each stimulus. The area of depolarization increased for consecutive stimuli only at high intensities (Fig. 2B). Clear-cut augmenting responses, involving increments in the number of spikes, similar to those depicted at higher intensities in Figs 1 and 2, were observed in 40 neurones (23%), while another 69 neurones (40%) showed a progressively growing depolarization during responses elicited by 10 Hz pulse-trains.

The largest augmenting responses in the isolated slab were found in neurones located close to the stimulating electrode. At distances greater than 2–3 mm, augmenting responses to pulse-trains at 10 Hz were not observed. The distance-dependency of augmenting responses was studied in six

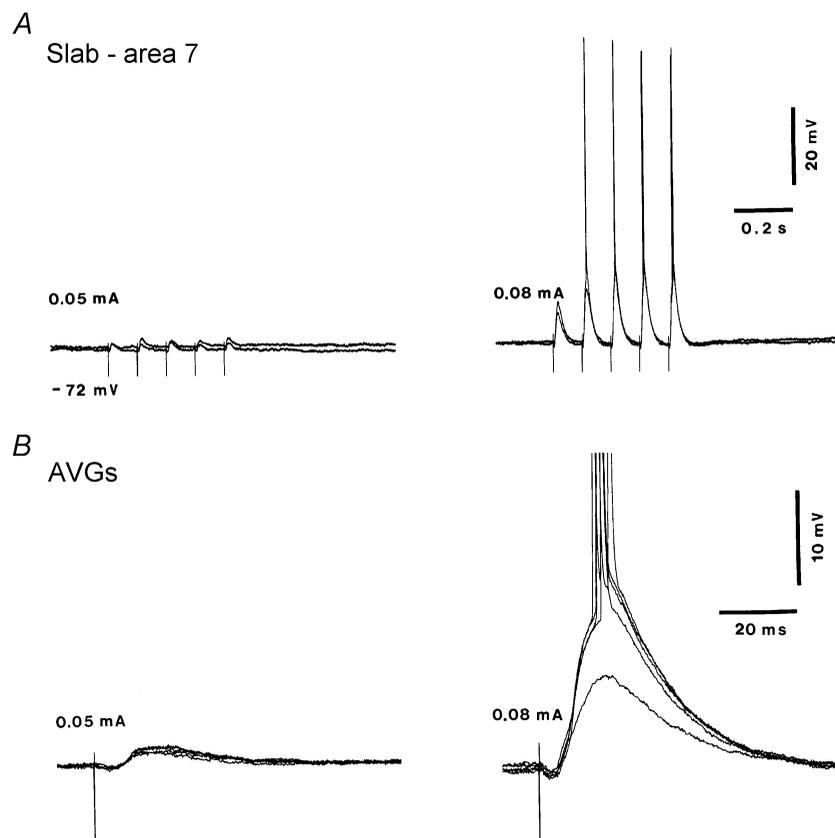


Figure 1. Augmenting responses in isolated neocortical slab from area 7

RS neurone. Responses to two intensities (0.05 and 0.08 mA) of pulse-trains at 10 Hz are shown. *A*, two superimposed traces showing that the lowest intensity elicited EPSPs, the responses to successive pulses having similar shapes and amplitudes. With a slight increase in stimulation intensity, there was an augmented response after the first EPSP. *B*, superimposition of the initial part of averaged responses (AVGs) to all 5 stimuli in the pulse-train.

triple simultaneous intracellular recordings. In two of them, two neurones were recorded close (~ 1 mm) to the stimulating electrode and the third neurone was recorded at a distance of 5–6 mm from the stimulating electrode. In the other four triple simultaneous intracellular recordings,

one pipette was close to the stimulating electrode, the second pipette was at a distance of 3 mm, and the third pipette was located at a distance of 6 mm (Fig. 3). In all these cases, augmenting responses were only found in neurones recorded close to the stimulating electrode.

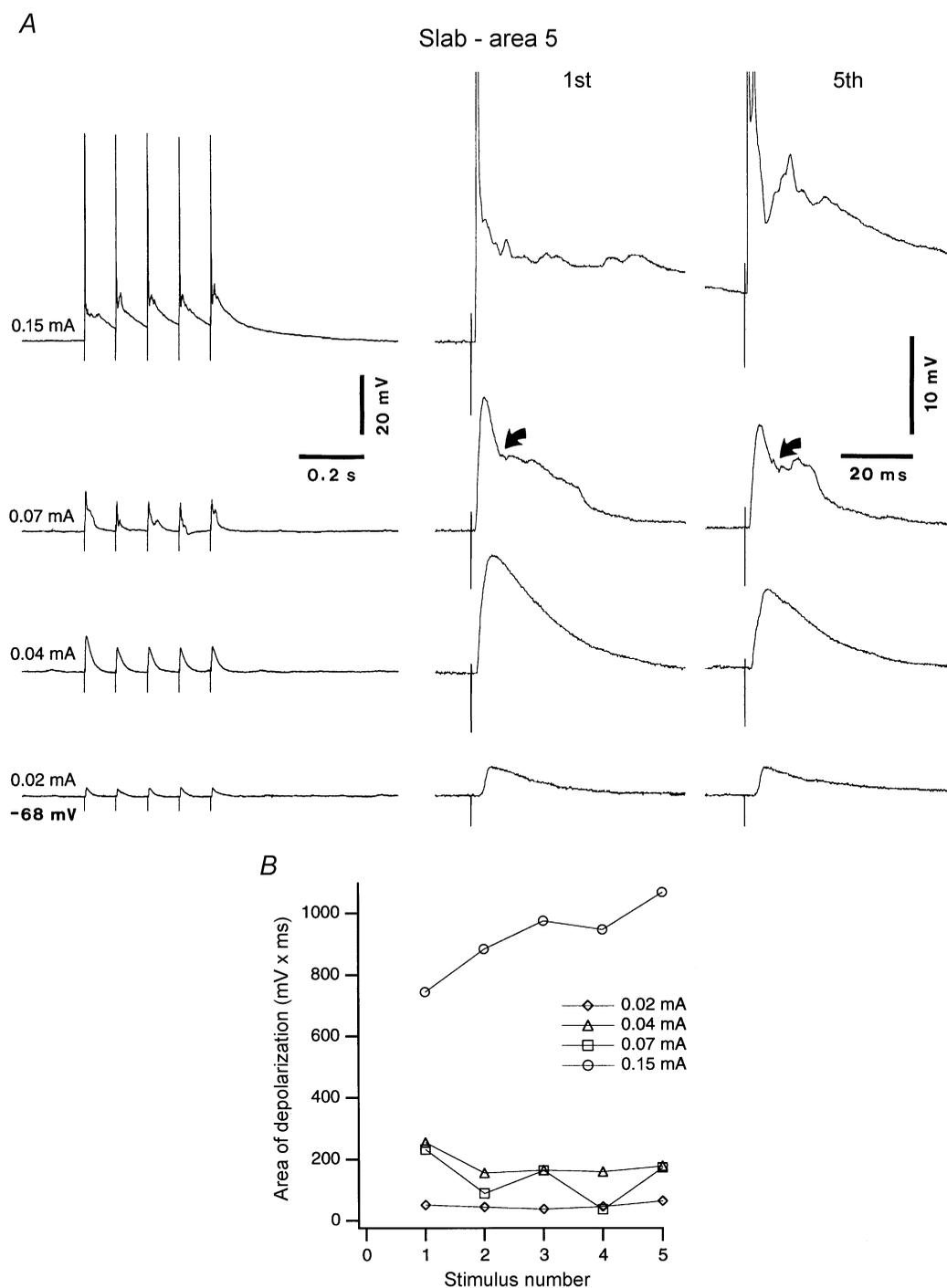


Figure 2. Decremental and incremental responses in isolated cortical slab (area 5) depend on stimulation intensity

RS neurone. *A*, left: responses to stimulation at different intensities, from 0.02 to 0.15 mA. Right, enlargements of the first and fifth responses; arrows point to IPSPs 'sculpturing' the EPSPs when stimulus intensity rose from 0.04 to 0.07 mA. *B*, depolarization area of the augmented response (ordinate) at different intensities (indicated by symbols). Depolarization area was measured during the first 100 ms after each stimulus (abscissa) with respect to pre-train membrane potential.

Thus, further intracellular recordings were performed close to the stimulating electrode.

Neurons that belonged to different electrophysiological classes were differently involved in augmenting responses. FS neurones recorded in isolated slabs usually fired many more action potentials than RS neurones during the development of augmenting responses. This is illustrated by dual simultaneous recordings of closely located FS

(cell 1) and RS (cell 2) neurones (Fig. 4) showing that, whereas the RS neurone had an enlarged depolarizing surface during augmenting responses, but fired only single action potentials, the number of action potentials in the FS neurone rose from one to five.

The main features of augmenting responses, such as the number of spikes, amplitude of depolarization, and area of depolarization, were quantitatively analysed in a population

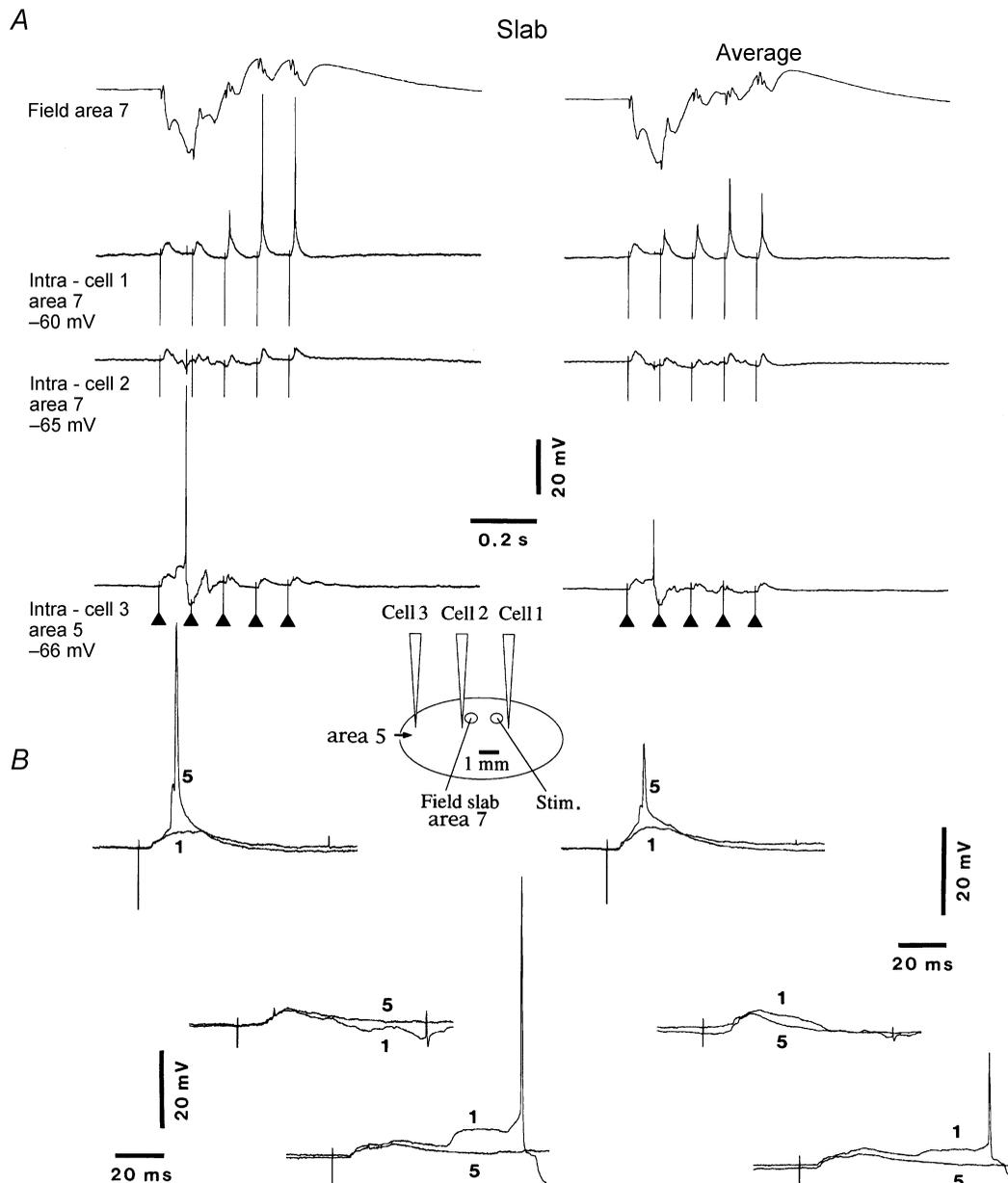


Figure 3. Triple simultaneous intracellular recordings in isolated slab from area 7 demonstrate that clear-cut augmenting responses occur in foci located close to the stimulated site

Three RS neurones (cells 1, 2 and 3) were recorded together with field potentials (see scheme of recording and stimulating electrodes in the centre). Augmenting responses in the vicinity of the stimulating electrode were only elicited at the maximal intensity used (0.75 mA). *A*, individual (left) and averaged (right) responses to pulse-trains at 10 Hz. *B* (individual and averaged responses) shows superimposed first and fifth responses in cells 1 to 3, from top to bottom, respectively.

of 24 neurones that were recorded close to the stimulating electrode. Three of them were FS, four were IB, and 17 were RS. (a) The most dramatic increase in the number of action potentials, from 0.5 to 3 (mean), was found in FS neurones (Fig. 5). Despite the intrinsic propensity of IB neurones to fire spike-bursts, during 10 Hz pulse-trains IB neurones fired at a maximum of one spike in response to the fourth and fifth stimuli. (b) The maximal amplitude of depolarization was similar in all groups of neurones, and increased to the same extent from the first to the fifth stimulus. However, the greatest increase was again found in FS neurones (212%); the amplitude increased by 80% in RS neurones and by 100% in IB neurones. (c) In the responses to all five stimuli at 10 Hz, the area of depolarization was larger in RS neurones than in the other

neuronal types. However, FS neurones revealed a greater propensity for the development of depolarization area during the process of augmenting. The area of depolarization from the first to the fifth stimulus increased by 92% in RS neurones, by only 20% in IB neurones, and by 420% in FS neurones. Paired comparison between different stimuli pairs for amplitude, area of depolarization and number of spikes in all studied neurones is shown in Table 1. Differences for all studied parameters were not statistically significant between responses to stimuli 4 and 5, where the responses approached saturation levels.

The repetition, once every second, of pulse-trains with five stimuli at 10 Hz changed the response pattern (Fig. 6). The first pulse-train elicited augmenting responses. The subsequent two or three pulse-trains resulted in a progressive

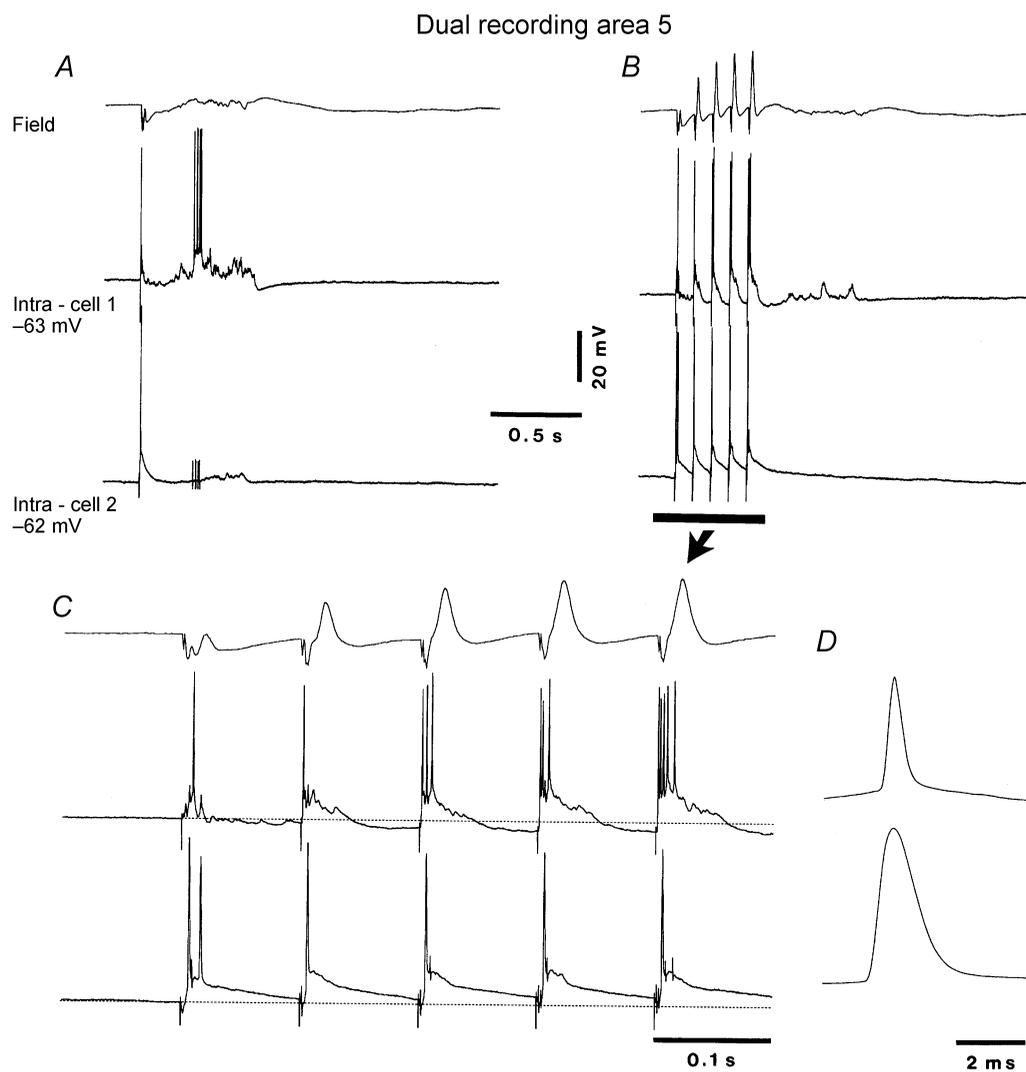


Figure 4. Differential propensities to augmentation of FS and RS neocortical neurones

Area 5 slab. Simultaneous recording of field potentials and dual intracellular recordings from FS (cell 1) and RS (cell 2) neurones. The neurones were separated by less than 0.5 mm. *A*, responses to a single stimulus at 0.06 mA (small deflections in cell 2 are due to capacitive coupling from action potentials in cell 1). *B*, responses evoked by a pulse-train at 10 Hz. *C*, responses from *B* shown on an expanded time scale (arrow). *D*, averaged ($n = 20$) action potentials of FS (top) and RS (bottom) neurones.

Table 1. The differences in responses (*P* values) to stimuli 1–5 for amplitude of depolarization, area of depolarization and number of spikes

Stimuli combination	Amplitude	Area of depolarization	Number of spikes
1–2	< 0.0001 *	0.0030 *	0.1679
1–3	< 0.0001 *	0.0017 *	0.0510
1–4	< 0.0001 *	0.0017 *	0.0187 *
1–5	< 0.0001 *	0.0011 *	0.0187 *
2–3	0.0060 *	0.0056 *	0.0368 *
2–4	0.0090 *	0.0035 *	0.0095 *
2–5	0.0080 *	0.0011 *	0.0095 *
3–4	0.0038 *	0.0444 *	0.0811 *
3–5	0.0076 *	0.0067 *	0.0811 *
4–5	0.1669	0.1232	—

*Significant difference, *P* < 0.05.

increase in the response to the first stimulus within a train with only moderate, if any, increase in responses to consecutive stimuli. The area of depolarization in the first response to the fifth pulse-train applied every second increased by 250 % in 17 studied RS neurones, and the number of spikes generated by FS neurones increased by 550 % ($n = 4$). Dual intracellular recordings obtained from a pair of FS and RS neurones (Fig. 6) showed that, in response to the first stimulus in each pulse-train, the firing of the FS neurone dramatically increased (from 1 to 14 spikes). Only a small increase in firing (from 1 to 2 spikes) was revealed in the RS neurone, but a significant increase in the area of depolarization was observed in response to the first stimulus in a pulse-train. The area of depolarization in the RS neurone was positively correlated with the number of spikes in the FS neurone, which suggests that an increase in the area of depolarization was mediated, at least

in part, by a change in the Cl^- reversal potential. In a parallel study on an intact cortex preparation, intracellular recordings with KCl-filled pipettes of responses to 10 Hz pulse-trains did not induce augmenting responses (data not shown), suggesting that Cl^- -dependent mechanisms play a role in the increased area of depolarization.

The dramatic firing of FS neurones during repeated pulse-trains at 10 Hz, accompanied by large depolarizing responses of RS neurones, resembles the basic discharge patterns of pairs of FS and RS neurones during paroxysmal depolarizing shifts (Timofeev *et al.* 2002). Indeed, a repetition of 10 Hz pulse-trains every second induced seizures in 30 % of cases that started with fast continuing runs at 10–15 Hz. Later phases of these seizures were composed of spike-wave (SW) or polyspike-wave (PSW) complexes (Fig. 7). As we have shown above, in 17 RS and four FS neurones tested decremental responses were seen

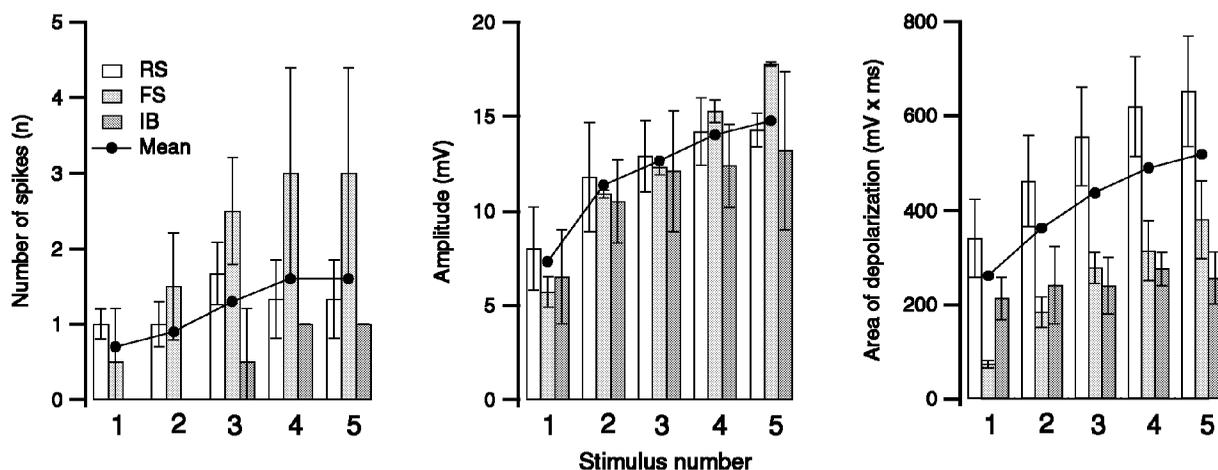


Figure 5. Number of spikes, amplitude and area of depolarization in a population of electrophysiologically identified neurones recorded from isolated slabs in areas 5 and 7

Numbers at the bottom of each histogram correspond to the stimulus number within a pulse-train at 10 Hz. Open bars represent averaged values for regular-spiking (RS) neurones, light-grey bars represent fast-spiking (FS) neurones, dark-grey bars represent intrinsically bursting (IB) neurones, and filled circles and continuous lines indicate the total mean in all included neurones. Error bars represent S.E.M.

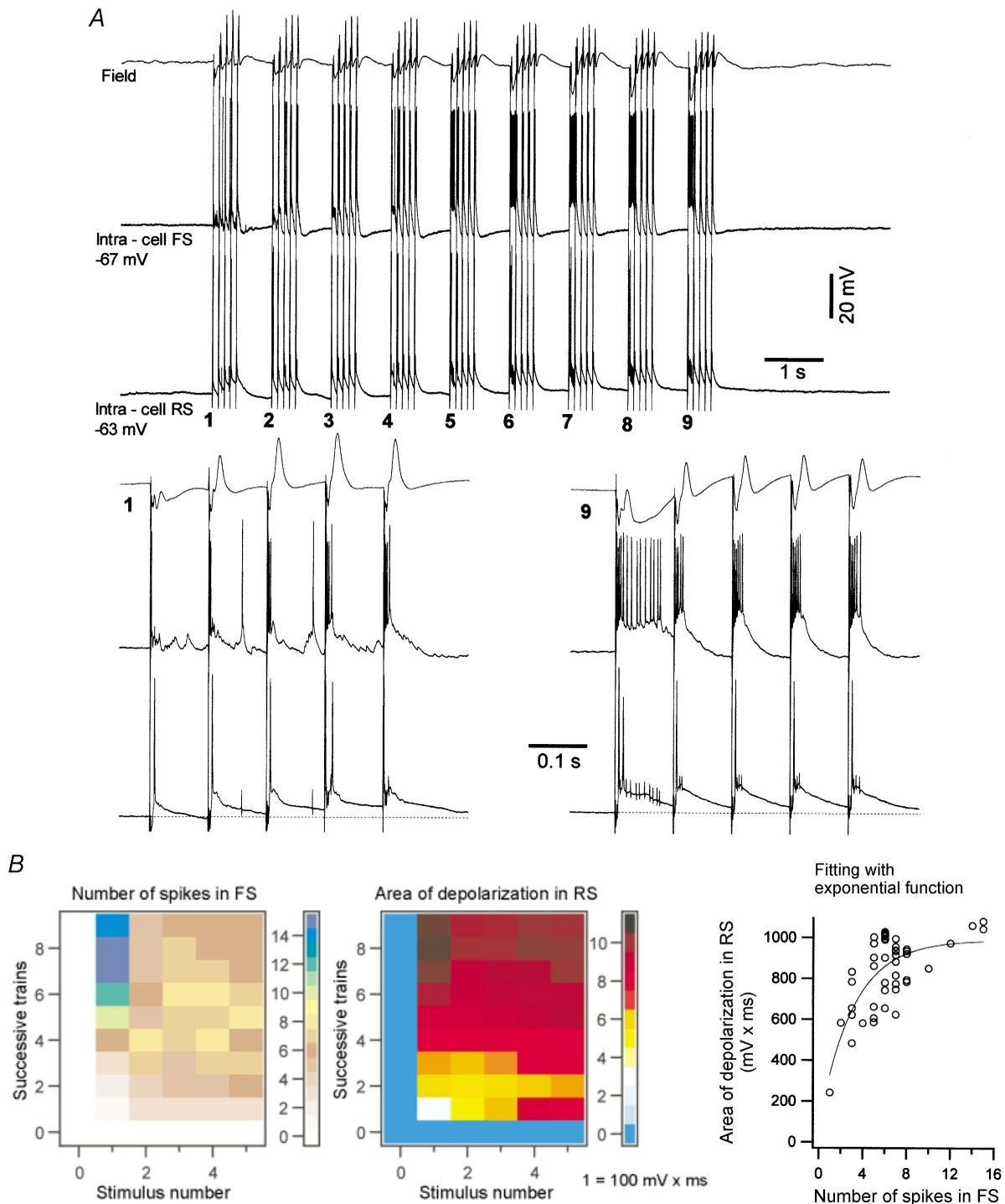


Figure 6. Repeated pulse-trains at 10 Hz modify the features of augmenting responses

Area 5 slab. *A*, simultaneous field potential and dual intracellular recording from a closely located pair of fast-spiking (FS) and regular-spiking (RS) neurones. The slab was stimulated with 9 pulse-trains of 10 Hz, repeated every second. Responses to the first and ninth stimuli are shown below on an expanded time scale. *B*, plots showing changes in the number of spikes in the FS neurone (left) and area of depolarization of the RS neurone (middle) as a function of pulse number within a pulse-train (abscissa) and within successive trains (ordinate). Right, area of depolarization in the RS neurone as a function of spike number in the FS neurone (calculated for all 5 × 9 stimuli). Data were fitted with an exponential function (continuous line). Note the major increase in the response to the first stimulus as stimulation progresses.

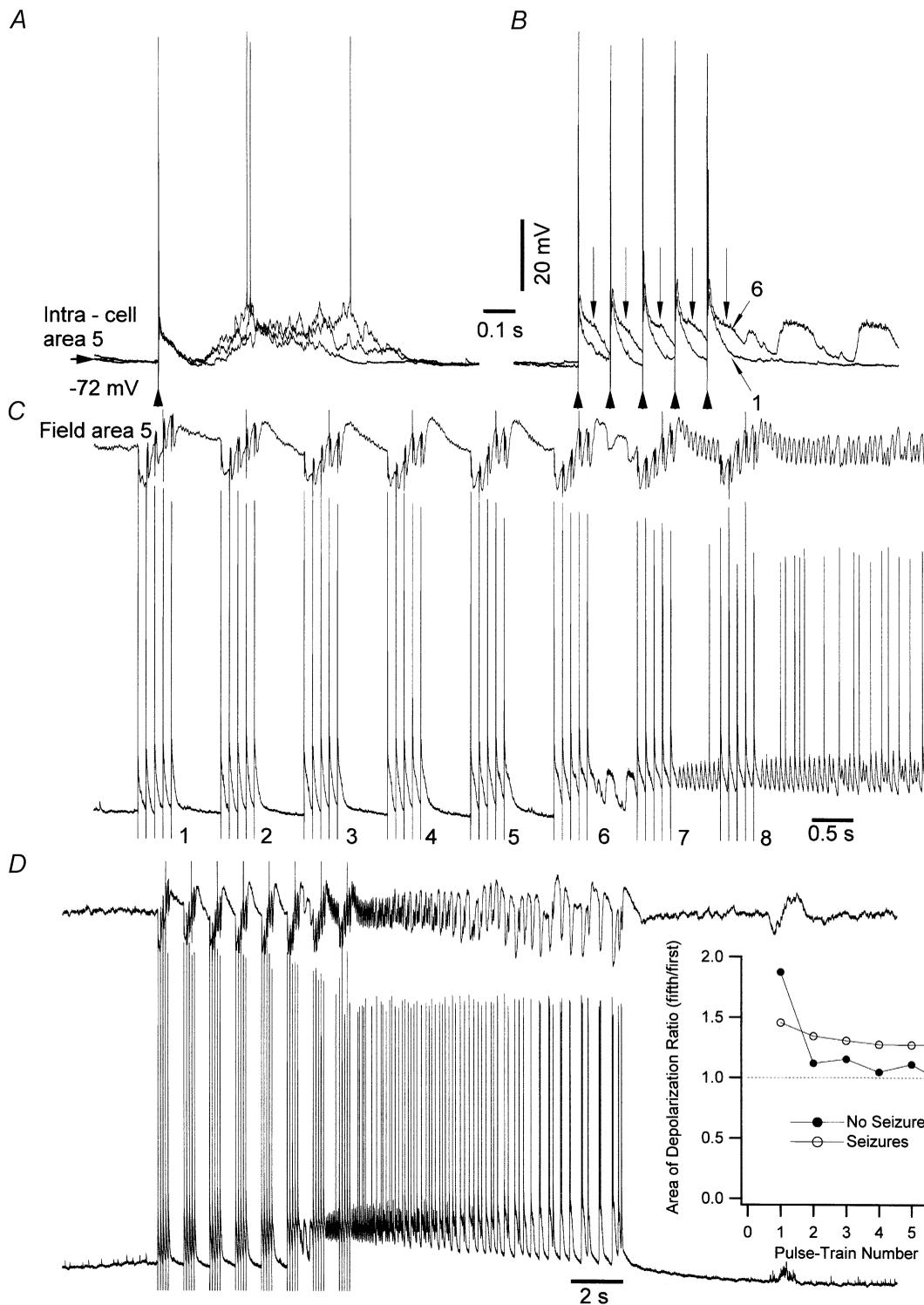


Figure 7. Repeated pulse-trains at 10 Hz may lead to self-sustained paroxysm

Intracellular recording from a RS neurone from a cortical slab in the suprasylvian gyrus. *A*, responses to single stimuli. *B*, superimposed responses to the first and sixth trains in a series of five-stimuli pulse-trains at 10 Hz, delivered every second. Note the increased responses to the sixth train, especially in the late component of the response (marked by arrows). The whole period of stimulation is shown in *C*. This increased responsiveness on repeated 10 Hz trains led to the electrical seizure shown in *D*. The graph shows how the area of depolarization ratio (fifth vs. first stimulus) progressed during five-shock trains repeated every second for cases that elicited seizures ($n = 7$, ○) and for those that did not elicit seizures ($n = 21$, ●).

after three to five pulse-trains (see example in Fig. 6) repeated every second. In these cases we did not see a development of self-sustained paroxysmal discharges. Seizures were evoked only in cases in which augmenting responses occurred even after five to eight pulse-trains ($n = 7$, see Fig. 7D). Repeated pulse-trains at other frequencies (20, 40 and 100 Hz) could also induce paroxysmal after-discharges. However, these paroxysms never started from around 10 Hz fast runs (not shown). The fact that, following 10 Hz pulse-trains, seizures started from around 10 Hz oscillations suggests that the neuronal network in the neocortical slabs reproduces the previously induced activity and oscillates with the same frequency. In experiments on neocortical slabs, we never observed spontaneously

occurring seizures, suggesting that an initial oscillation/stimulation is essential for the induction of seizures with a given frequency. By contrast, spontaneous seizures were often seen outside slabs (not shown).

Plasticity associated with cortical augmenting responses and spindles

What cellular mechanism could mediate augmenting responses? In the accompanying paper (Houweling *et al.* 2002), we describe synaptic factors that contribute to augmenting responses. Here, we provide data that associative cellular conditioning may contribute to long-lasting changes of responsiveness of cortical neurones (Baranyi *et al.* 1991). Electrical stimulation (0.06–0.15 mA) within the cortical slab elicited active periods that were characterized

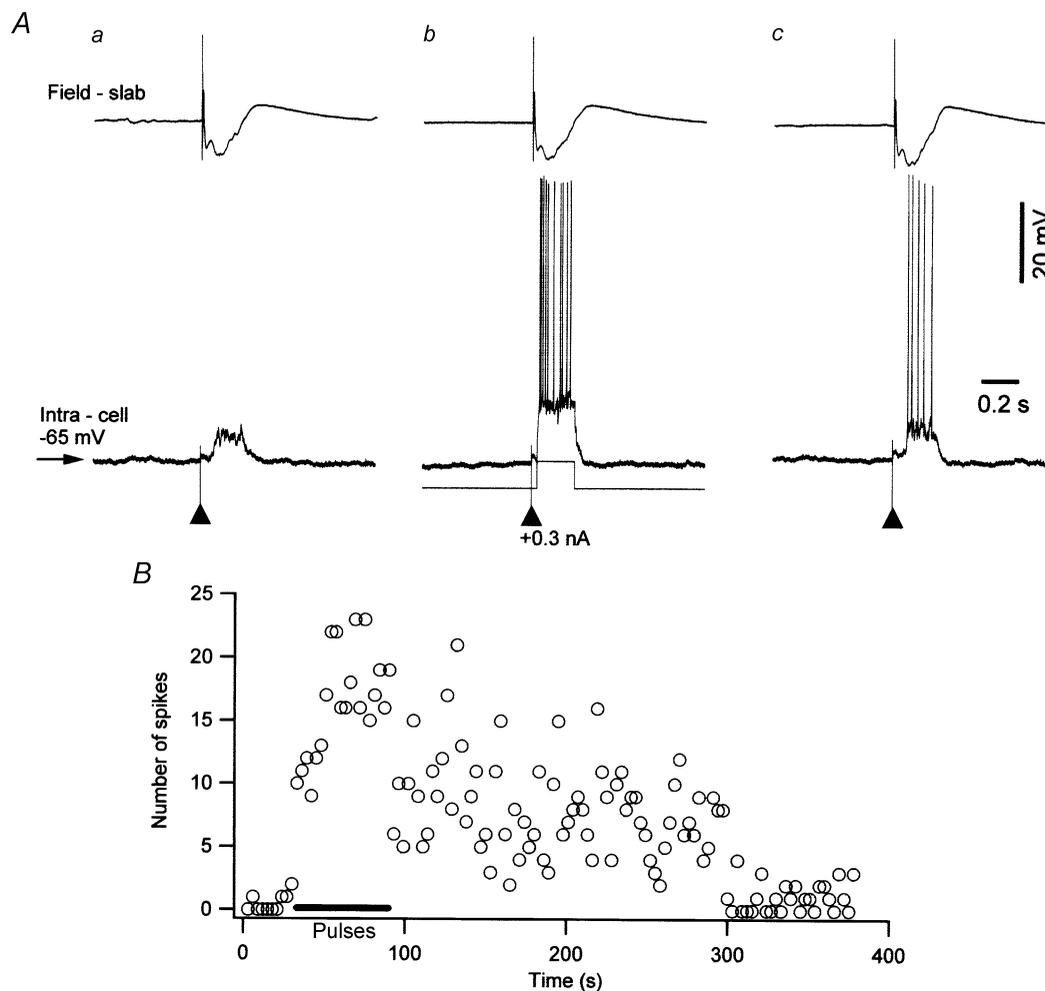


Figure 8. Intracellular plasticity persists for minutes

Slab from area 7, RS neurone. A, field potential and intracellular activities during control (a), pairing of synaptic activity with direct cellular depolarization (b) and extinction (c). In control, the slab was stimulated with low-intensity stimuli (triangle) that elicited an early EPSP followed by sustained depolarization, lasting 200–400 ms. An intracellular current pulse that elicited spikes and lasted for the duration of the active period (200 ms) was applied during the pairing. After 20 repetitions current pulses were stopped. During extinction, extracellular stimuli elicited depolarizing responses accompanied by spikes. The enhancement of responses lasted for more than 200 s. Note that the field potential was identical in all responses, indicating that the network excitability remained unchanged. B, plot showing number of spikes at each stimulus, produced by the neurone in chronological order.

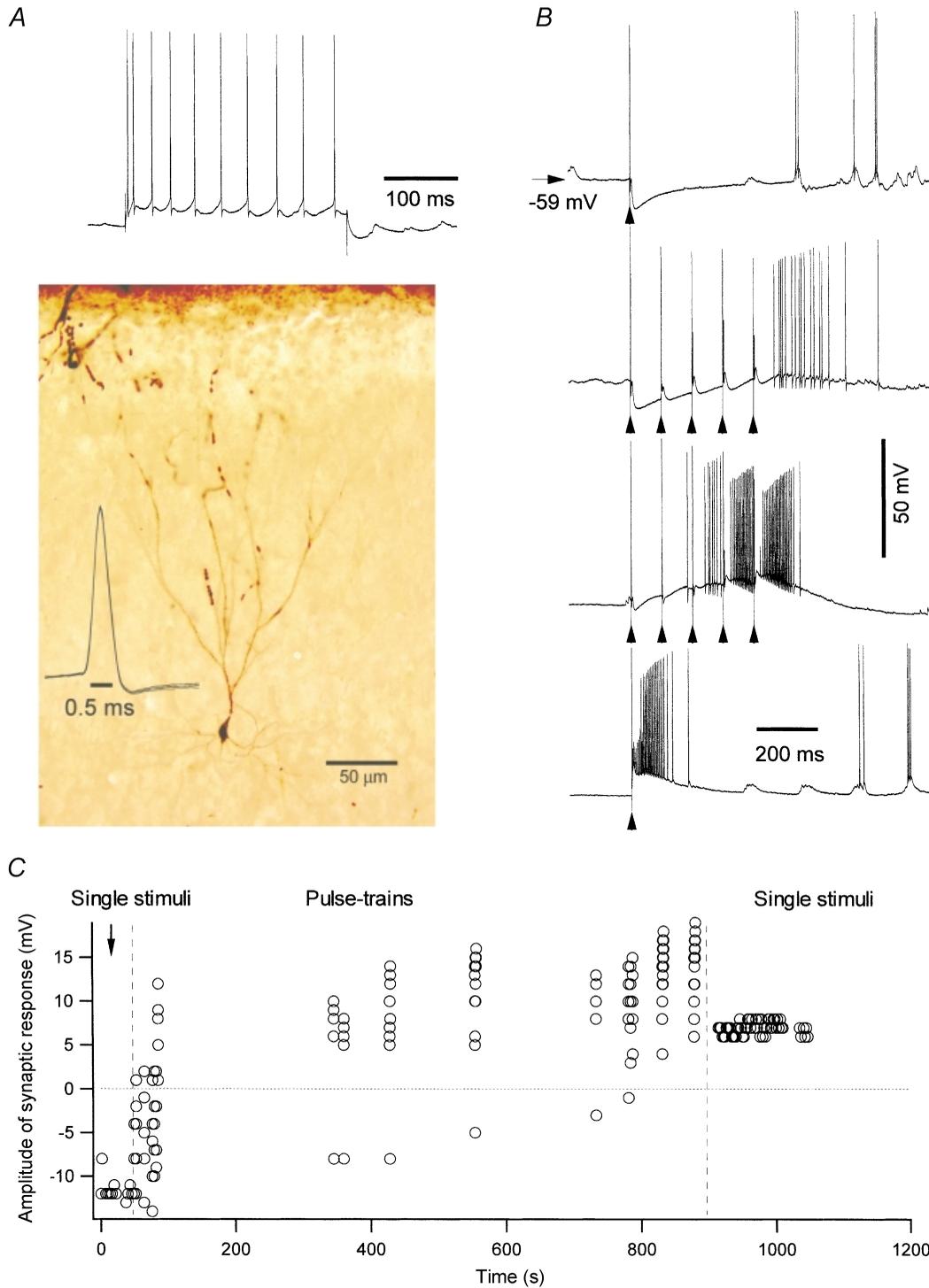


Figure 9. Cortical augmenting responses lead to long-lasting enhancement of depolarizing responses in intact cortex

A, intracellular recording from morphologically (Neurobiotine staining) and physiologically identified area 7 pyramidal RS neurone with thin spike. *B*, from top to bottom: control response to a single stimulus, early responses to pulse-trains at 10 Hz, responses to the same pulse-train applied 12 min later, and response to a single stimulus applied 16 min after the onset of stimulation session. *C*, plot showing the amplitude of stimulus-evoked response at 20 ms after the stimulus onset. Note that initially hyperpolarizing responses became depolarizing after pulse-trains at 10 Hz.

by prolonged depolarization due to the presence of synaptic activities (Figs 4A, 7A and 8Aa; see also Timofeev *et al.* 2000a). In 10% of neurones, these active periods were associated with a few or no spikes. In these neurones, we applied intracellular depolarizing current pulses that (a) lasted for the duration of active periods (usually 200–300 ms), (b) elicited spikes, and (c) had an onset that was delayed by 20–30 ms from the electrical stimuli that were applied to the slab every 3 s (Fig. 8). After a repetition (20–50 cycles) of such electrical stimuli associated with depolarizing current steps, the current pulses were stopped, and extracellular stimuli at the same intensity now elicited active periods with spikes. In three out of five tested neurones this increased excitability was powerful and lasted for 3–5 min (Fig. 8B). In 17 out of 53 neurones that revealed spiking during active periods the measurable increase in excitability was less pronounced. These data

indicate that synaptic responses associated with firing of a postsynaptic response may induce a mid-term increase in the excitability of cortical neurones.

We have shown above that the augmenting responses elicited by nearby stimulation within isolated cortical slabs were accompanied by increasing depolarization (Fig. 5) and that the rhythmic repetition of pulse-trains resulted in a progressive enhancement of the response to the first stimulus (Fig. 6). Further, we investigated 23 neurones in the intact cortex to study the enhancement of responses that outlast pulse-trains at 10 Hz. Our data show that, in barbiturate-anaesthetized cats, single cortical stimuli applied close to the recorded neurone usually elicited monophasic inhibitory postsynaptic potentials (IPSPs) (Fig. 9). These IPSPs rapidly decreased in amplitude during pulse-trains at 10 Hz. A repetition of pulse-trains every 1 to 3 s eventually transformed these IPSPs into depolarizing responses

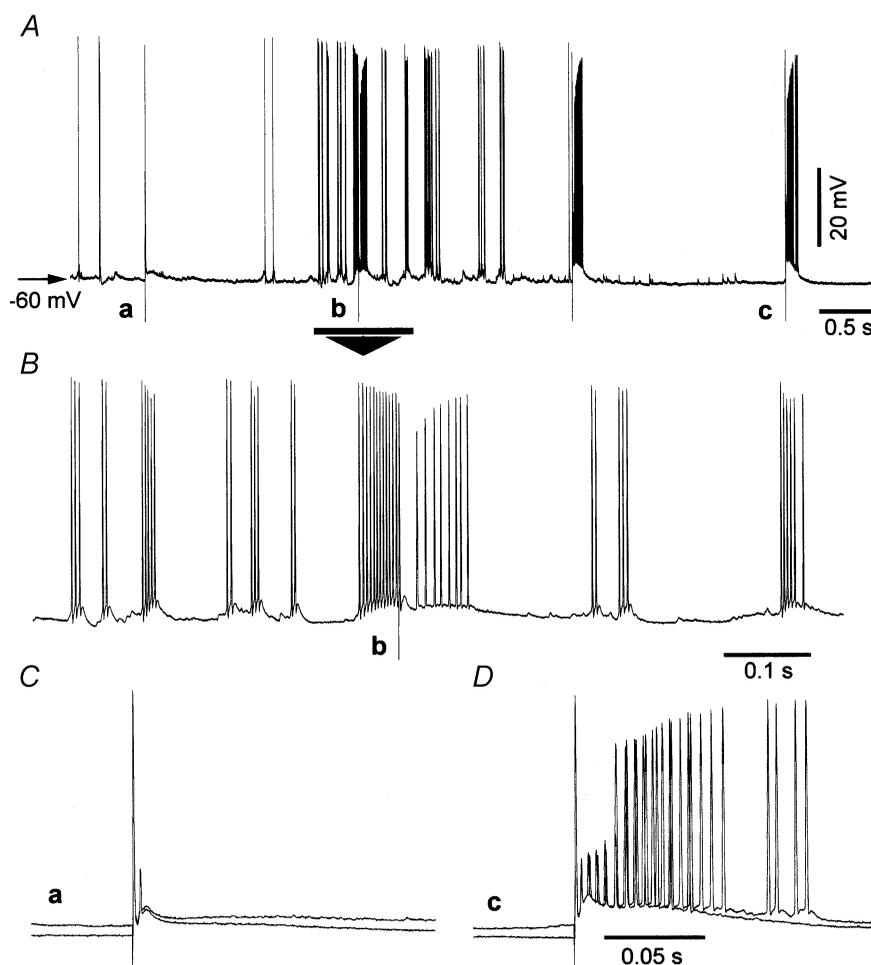


Figure 10. Spontaneously occurring spindles can lead to long-lasting changes of responsiveness in neocortical neurones

Intracellular recording from a FRB cortical neurone in area 21 of intact cortex. *A* shows an epoch with cortical stimulation of area 21 (1 stimulus every 2 s) and spontaneous spindle sequence. *B–D* show responses from *A* on an expanded time scale to stimuli applied before (*C*), during (*B*) and after (*D*) the spindle. Note the increased stimulus-evoked response applied after the spindle (*D*).

similar to those shown in Fig. 6. In seven out of 21 neurones studied, we were able not only to obtain the reversal of initial IPSPs into depolarizing responses, but also to maintain these depolarizing responses evoked by single stimuli for several minutes. Figure 9A illustrates an example of responses displayed by a pyramidal RS neurone that fired thin spikes in which a single stimulus elicited an early IPSP. Stimulation with pulse-trains at 10 Hz resulted in a diminution, down to suppression, of IPSPs. The first stimulus in the next pulse-train (3–5 s later) elicited an IPSP of smaller amplitude (Fig. 9B). Repeated 10 Hz cortical stimulation abolished the early IPSP and replaced it by an early depolarization. Thereafter, single stimuli evoked exclusively depolarizing responses. This enhancement, which remained unchanged for several minutes (Fig. 9C), suggests that electrical stimulation with a frequency of 10 Hz may induce long-lasting changes in neuronal responsiveness. We were able to obtain these responses under the following conditions: (a) a short distance (less than 2 mm) between the stimulating and recording electrodes; (b) a relatively high intensity of stimulation (0.07–0.15 mA); and (c) the use of barbiturate anaesthesia.

We wondered whether this enhanced responsiveness was an artificial phenomenon that occurs only with electrical stimuli or if it could also be induced by spontaneous rhythmic activity in the corticothalamic network. To answer this question, we studied the modulation of responses to cortical stimuli induced by spontaneously occurring spindles. We found that, occasionally (3 out of 21 neurones studied), the stimulus applied during the excitatory phase of a spindle elicited an enhancement of excitability that lasted from tens of seconds to several minutes, a time similar to the duration of associative cellular conditioning (see Fig. 8). Figure 10 illustrates this enhanced responsiveness in a FRB neurone. Electrical stimuli applied at different membrane potentials elicited depolarizing responses giving rise to single spikes (Fig. 10C). The stimulus was then delivered during one of the depolarizing phases of a spontaneously occurring spindle sequence, associated with rich neuronal discharges (Fig. 10B). Following the spindle sequence, stimuli (with the same parameters) elicited large depolarizing responses, crowned by high-frequency spike bursts (Fig. 10D; compare with control in Fig. 10C). The enhancement of responses was not voltage dependent, as it remained similar at rest and after small hyperpolarization with direct current.

DISCUSSION

Although augmenting responses can be elicited in preparations with both intact cortex and isolated neocortical slabs *in vivo* (present study), as well as in cortical slices (Castro-Alamancos & Connors, 1996b), the patterns and conditions of occurrence of responses vary under these various experimental conditions. The differences between

augmenting responses in the *in vivo* cortical slab and those obtained in animals with intact corticothalamic connections are due to the absence of thalamic connections and poor or nearly absent spontaneous activities in isolated cortical slabs *in vivo*. Augmenting responses as well as spontaneously occurring spindles may enhance the responsiveness of cortical networks for several minutes and may eventually lead to paroxysmal activities.

Interaction between synaptic and intrinsic cellular properties in the generation of augmenting responses

Broadly defined, neuronal plasticity is a change in responsiveness related to previously occurring activity. The mechanisms of plasticity include: pre- and postsynaptic modifications, such as changes in release of neurotransmitters or postsynaptic sensitivity (Buonomano & Merzenich, 1998), which may be induced by correlated activation of pre- and postsynaptic neurones (Baranyi *et al.* 1991), and conductance changes of intrinsic currents, with consequent changes in neuronal responsiveness (Marder *et al.* 1996; Marder, 1998; Turrigiano *et al.* 1998).

Rhythmic electrical stimulation of the thalamus or cortex at a frequency of 10 Hz results in responses that grow in size during the initial two to four stimuli. The mechanisms of thalamically induced augmenting responses depend on the interaction of synaptic potentials with intrinsic properties of TC neurones. Single stimuli applied to the thalamus elicit primary EPSPs that are followed by biphasic GABA_A–GABA_B IPSPs (Crunelli *et al.* 1988; Crunelli & Leresche, 1991). The duration of inhibitory responses in TC neurones elicited by a single stimulus *in vivo* is 0.15–0.25 s (Deschênes *et al.* 1984; Roy *et al.* 1984). The second, 0.1 s delayed stimulus, reaches the TC neurone at a hyperpolarized level and the initial EPSP triggers a low-threshold spike (LTS) that provides the basis of augmentation (Steriade & Timofeev, 1997; Bazhenov *et al.* 1998; Steriade *et al.* 1998). The increased excitability and self-sustained oscillations following cortical augmenting responses may be related to data described in a study in which we investigated the thalamic origin of the cortical post-inhibitory rebound (Grenier *et al.* 1998). Augmenting responses are preferentially elicited during slow-wave sleep, a state in which TC neurones are hyperpolarized (Hirsch *et al.* 1983). Indeed, the inhibitory-rebound sequences in TC neurones and, consequently, the augmented responses in cortical neurones are stronger during slow-wave sleep and are strikingly reduced upon strong arousal and REM sleep (Steriade *et al.* 1969; Castro-Alamancos & Connors, 1996a) as well as during stimulation of brainstem cholinergic nuclei (Timofeev & Steriade, 1998). During brain activation, TC neurones are relatively depolarized (Curró Dossi *et al.* 1991) and, as a consequence, the low-threshold spike-bursts are suppressed. The decremental responses of RE neurones during 10 Hz pulse-trains

(Timofeev & Steriade, 1998) may induce another type of intrathalamic augmentation (Steriade & Timofeev, 1997) that depends on activation of high-threshold Ca^{2+} current (Hernández-Cruz & Pape, 1989; Tennigkeit *et al.* 1998). The facilitation of corticothalamic EPSPs may also cause augmenting responses (Steriade & Deschênes, 1984; Deschênes & Hu, 1990; von Krosigk *et al.* 1999; Pedroarena & Llinás, 2001).

Several mechanisms could account for the intracortical mechanisms of augmenting responses. Far from being a passive reflection of thalamically generated activity, studies of neocortical activities within the frequency range of spindles indicate that complex intracortical circuits have a major influence on the incoming thalamic inputs (Kandel & Buzsáki, 1997). The mechanisms of short-term synaptic plasticity mediating intracortical augmenting responses are discussed in detail in the accompanying paper (Houweling *et al.* 2002). Other mechanisms might depend on intrinsic properties of cortical neurones. Earlier studies suggested that bursts generated by IB neurones in layer V are responsible for augmenting responses (Castro-Alamancos & Connors, 1996*b*). In our data, the averaged firing of IB neurones in response to electrical stimuli occurred several milliseconds later than the firing of other types of cortical neurones (Steriade & Timofeev, 2001). In contrast, deeply lying FRB neurones fired more spikes, at shorter latencies, than other types of cortical neurones, suggesting their leading role in the cortical amplification of thalamic inputs.

Another fact suggesting a role played by intrinsic currents of cortical neurones is based on our finding that the augmenting responses in isolated slabs were different from augmenting responses in the intact brain (compare Figs 1 and 2 with Fig. 9). Given the fact that the mean membrane potential of cortical neurones in isolated slabs is -70 mV, which is more hyperpolarized than the membrane potential of neurones in intact cortex (Timofeev *et al.* 2000*a, b*), low-threshold calcium currents (I_T) could contribute to the augmenting responses. A number of studies suggest that around 15% of pyramidal neurones are able to generate low-threshold Ca^{2+} spikes (de la Peña & Geijo-Barrientos, 1996; Paré & Lang, 1998). Results of modelling studies (Houweling *et al.* 2002) point to the role of I_T in cortical neurones in cortically generated augmenting responses.

Effects of network state and architecture on augmenting responses

The absence of augmenting responses at a relatively large distance from the stimulating electrode in isolated cortical slabs may be due to at least two different factors. Firstly, in contrast with the intact cortex, where at such distances (3–6 mm) augmenting responses do occur (not shown), neurones in the cortical slab are hyperpolarized and require high levels of excitatory input to reach firing threshold. A large synchronous excitation is produced only

around the stimulating electrode where augmentation is indeed observed. Secondly, the slab lacks corticothalamic loops. TC neurones receive cortical inputs, amplify them through the activation of rebound spike-bursts, and send an amplified response back to the cortex. Thalamic projections, especially those originating in rostral intralaminar nuclei that are a major source of inputs for area 4 as well as areas 5–7 and 21, are widespread (Steriade *et al.* 1997), thus allowing them to distribute responses of intracortical origin back to the cortex.

Possible role of sleep rhythms in memory

A number of earlier hypotheses (Buzsáki, 1989; Steriade *et al.* 1993) and recent experimental data (Gais *et al.* 2000; Stickgold *et al.* 2000; Frank *et al.* 2001) suggest that slow-wave sleep is involved in memory consolidation, although the mechanisms have not yet been fully elucidated. We have previously shown that, during slow-wave sleep, cortical responses to stimulation of afferent pathways to the thalamus are attenuated or completely obliterated (Steriade *et al.* 1969), but responses of cortical, TC and RE neurones to cortical stimuli are preserved (Timofeev *et al.* 1996). These data indicate that neurones in corticothalamic networks during slow-wave sleep do not operate with external information but are involved in the processing of internally generated signals. Our current data show that cortically generated responses that occur during the depolarizing phases of spontaneous spindles are increased and may remain enhanced for tens of seconds or minutes (Fig. 10). Thus, the interaction of cortically generated signals with sleep oscillations may result in relatively long-lasting enhancement of these signals.

What could be the mechanism of this prolonged amplification and could it be involved in memory consolidation? A likely candidate is the powerful Ca^{2+} entry in thalamic and cortical neurones, produced by spike-bursts fired during sleep oscillations (reviewed in Steriade *et al.* 1993; Sejnowski & Destexhe, 2000; Timofeev *et al.* 2000*b*). Spontaneous spindle activity is characterized by the generation of rhythmic spike bursts in TC neurones, which depolarize target cortical neurones to levels at which their intrinsic currents induce an activity that is independent of the firing of TC neurones (Timofeev *et al.* 2001). Our data demonstrate that cortical augmenting responses induced by closely located stimulating electrodes induce long-lasting steady depolarization of around 15 mV, with a large area of depolarization in RS neurones (Fig. 5). Such a significant depolarization probably induces additional Ca^{2+} entry into neurones. Thus, both cortical augmenting responses and neuronal responsiveness (Figs 9 and 10) that may be based on Ca^{2+} -dependent mechanisms.

We propose that at least two non-exclusive mechanisms may underlie increased responsiveness promoted by sleep oscillations. Firstly, the neuronal firing that occurs during the depolarizing phases of sleep oscillations would result in

a local increase in $[K^+]_o$. In addition to other effects, an increased $[K^+]_o$ would increase neuronal excitability (Traynelis & Dingledine, 1988; McNamara, 1994) and shift the reversal potential for GABA_A IPSPs to positive values (Chamberlin & Dingledine, 1988; Tasker & Dudek, 1991). Under these conditions, the synaptic volleys reaching cortical neurones during the depolarizing phase of spindles (such as in Fig. 10) or other sleep oscillations would trigger a stronger depolarization and a mixed EPSP–IPSP response, providing the basis for amplification that may remain effective for several seconds. Secondly, another possible scenario arises from the fact that association of synaptic stimuli with direct depolarization or tetanization of post-synaptic neurones with current pulses induces intracellular conditioning, reflected as an increased responsiveness of the postsynaptic neurone to the same synaptic stimuli as the conditioning stimulus (Baranyi *et al.* 1991; Volgushev *et al.* 1999). In the present experiments, both intracellular current pulses (Fig. 8) and spontaneous spindle-related depolarization associated with high-frequency firing (Fig. 10) were paired with evoked synaptic volleys of cortical origin, which in some neurones enhanced responsiveness for several minutes. Repeated depolarizing potentials associated with high-frequency firing of cortical neurones result in activation of high-threshold Ca^{2+} currents accompanied by Ca^{2+} entry into the neurone. The enhanced $[Ca^{2+}]_i$ in association with simultaneously arriving synaptic volleys might rapidly activate protein kinase A (Abel *et al.* 1997) or Ras/mitogen-activated protein kinase (Dolmetsch *et al.* 2001), which are implicated in memory consolidation. Thus, according to Hebbian rules, a spontaneous oscillation of neurones, accompanied by their firing, and paired with synaptic activation, might sculpt afferent signals and enhance the sensitivity to activity in particular synapses.

REFERENCES

- ABEL, T., NGUYEN, P. V., BARAD, M., DEUEL, T. A., KANDEL, E. R. & BOURTCHOUADZE, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell* **88**, 615–626.
- BARANYI, A., SZENTE, M. B. & WOODY, C. D. (1991). Properties of associative long-lasting potentiation induced by cellular conditioning in the motor cortex of conscious cats. *Neuroscience* **42**, 321–334.
- BAZHENOV, M., TIMOFEEV, I., STERIADE, M. & SEJNOWSKI, T. J. (1998). Cellular and network models for intrathalamic augmenting responses during 10-Hz stimulation. *Journal of Neurophysiology* **79**, 2730–2748.
- BUONOMANO, D. V. & MERZENICH, M. M. (1998). Cortical plasticity: from synapses to maps. *Annual Review of Neuroscience* **21**, 149–186.
- BUZSAKI, G. (1989). Two-stage model of memory trace formation: a role for ‘noisy’ brain states. *Neuroscience* **31**, 551–570.
- CASTRO-ALAMANCOS, M. A. & CONNORS, B. W. (1996a). Short-term plasticity of a thalamocortical pathway dynamically modulated by behavioral state. *Science* **272**, 274–277.
- CASTRO-ALAMANCOS, M. A. & CONNORS, B. W. (1996b). Cellular mechanisms of the augmenting response: short-term plasticity in a thalamocortical pathway. *Journal of Neuroscience* **16**, 7742–7756.
- CHAMBERLIN, N. L. & DINGLEDINE, R. (1988). GABAergic inhibition and the induction of spontaneous epileptiform activity by low chloride and high potassium in the hippocampal slice. *Brain Research* **445**, 12–18.
- CRUNELLI, V., HABY, M., JASSIK-GERSCHENFELD, D., LERESCHE, N. & PIRCHIO, M. (1988). Cl^- and K^+ -dependent inhibitory postsynaptic potentials evoked by interneurons of the rat lateral geniculate nucleus. *Journal of Physiology* **399**, 153–176.
- CRUNELLI, V. & LERESCHE, N. (1991). A role for GABA_B receptors in excitatory and inhibition of thalamocortical cells. *Trends in Neurosciences* **14**, 16–21.
- CURRÓ DOSSI, R., PARÉ, D. & STERIADE, M. (1991). Short-lasting nicotinic and long-lasting muscarinic depolarising responses of thalamocortical neurons to stimulation of mesopontine cholinergic nuclei. *Journal of Neurophysiology* **65**, 393–406.
- DE LA PEÑA, E. & GEIJO-BARRIENTOS, E. (1996). Laminar localization, morphology, and physiological properties of pyramidal neurons that have the low-threshold calcium current in the guinea-pig medial frontal cortex. *Journal of Neuroscience* **16**, 5301–5311.
- DESCHÈNES, M. & HU, B. (1990). Electrophysiology and pharmacology of the corticothalamic input to lateral thalamic nuclei: an intracellular study in the cat. *European Journal of Neuroscience* **2**, 140–152.
- DESCHÈNES, M., PARADIS, M., ROY, J. P. & STERIADE, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: resting properties and burst discharges. *Journal of Neurophysiology* **51**, 1196–1219.
- DOLMETSCH, R. E., PAJVANI, U., FIFE, K., SPOTTS, J. M. & GREENBERG, M. E. (2001). Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. *Science* **294**, 333–339.
- FRANK, M. G., ISSA, N. P. & STRYKER, M. P. (2001). Sleep enhances plasticity in the developing visual cortex. *Neuron* **30**, 275–287.
- GAIS, S., PLIHAL, W., WAGNER, U. & BORN, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nature Neuroscience* **3**, 1335–1339.
- GRENIER, F., TIMOFEEV, I. & STERIADE, M. (1998). Leading role of thalamic over cortical neurons during postinhibitory rebound excitation. *Proceedings of the National Academy of Sciences of the USA* **95**, 13929–13934.
- HERNÁNDEZ-CRUZ, A. & PAPE, H. C. (1989). Identification of two calcium currents in acutely dissociated neurons from the rat lateral geniculate nucleus. *Journal of Neurophysiology* **61**, 1270–1283.
- HIRSCH, J. C., FOURMENT, A. & MARC, M. E. (1983). Sleep-related variations of membrane potential in the lateral geniculate body relay neurons of the cat. *Brain Research* **259**, 308–312.
- HOUWELING, A. R., BAZHENOV, M., TIMOFEEV, I., GRENIER, F., STERIADE, M. & SEJNOWSKI, T. J. (2002). Frequency-selective augmenting responses by short-term synaptic depression in cat neocortex. *Journal of Physiology* **542**, 599–617.
- KANDEL, A. & BUZSAKI, G. (1997). Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *Journal of Neuroscience* **17**, 6783–6797.
- LERESCHE, N., LIGHTOWLER, S., SOLTESZ, I., JASSIK-GERSCHENFELD, D. & CRUNELLI, V. (1991). Low-frequency oscillatory activities intrinsic to rat and cat thalamocortical cells. *Journal of Physiology* **441**, 155–174.

- McCORMICK, D. A. & PAPE, H. C. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. *Journal of Physiology* **431**, 291–318.
- McNAMARA, J. O. (1994). Cellular and molecular basis of epilepsy. *Journal of Neuroscience* **14**, 3413–3425.
- MARDER, E. (1998). From biophysics to models of network function. *Annual Review of Neuroscience* **21**, 25–45.
- MARDER, E., ABBOTT, L. F., TURRIGIANO, G. G., LIU, Z. & GOLOWASCH, J. (1996). Memory from the dynamics of intrinsic membrane currents. *Proceedings of the National Academy of Sciences of the USA* **93**, 13481–13486.
- MORISON, R. S. & DEMPSEY, E. W. (1943). Mechanism of thalamocortical augmentation and repetition. *American Journal of Physiology* **138**, 297–308.
- PARÉ, D. & LANG, E. J. (1998). Calcium electrogenesis in neocortical pyramidal neurons *in vivo*. *European Journal of Neuroscience* **10**, 3164–3170.
- PEDROARENA, C. M. & LLINÁS, R. (2001). Interactions of synaptic and intrinsic electroresponsiveness determine corticothalamic activation dynamics. *Thalamus & Related Systems* **1**, 3–14.
- ROY, J. P., CLERCQ, M., STERIADE, M. & DESCHÉNES, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: mechanisms of long-lasting hyperpolarizations. *Journal of Neurophysiology* **51**, 1220–1235.
- SEJNOWSKI, T. J. & DESTEXHE, A. (2000). Why do we sleep? *Brain Research* **886**, 208–223.
- STERIADE, M. & DESCHÉNES, M. (1984). The thalamus as a neuronal oscillator. *Brain Research Reviews* **8**, 1–63.
- STERIADE, M., DOMICH, L., OAKSON, G. & DESCHÉNES, M. (1987). The deafferented reticularis thalami nucleus generates spindle rhythmicity. *Journal of Neurophysiology* **57**, 260–273.
- STERIADE, M., IOSIF, G. & APOSTOL, V. (1969). Responsiveness of thalamic and motor relays during arousal and various stages of sleep. *Journal of Neurophysiology* **32**, 251–265.
- STERIADE, M., JONES, E. G. & McCORMICK, D. A. (1997). *Thalamus*, vol. 1. Elsevier, Oxford.
- STERIADE, M., McCORMICK, D. A. & SEJNOWSKI, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science* **262**, 679–685.
- STERIADE, M. & TIMOFEEV, I. (1997). Short-term plasticity during intrathalamic augmenting responses in decorticated cats. *Journal of Neuroscience* **17**, 3778–3795.
- STERIADE, M. & TIMOFEEV, I. (2001). Corticothalamic operations through prevalent inhibition of thalamocortical neurons. *Thalamus and Related Systems* **1**, 225–236.
- STERIADE, M., TIMOFEEV, I., GRENIER, F. & DÜRMÜLLER, N. (1998). Role of thalamic and cortical neurons in augmenting responses and self-sustained activity: dual intracellular recordings *in vivo*. *Journal of Neuroscience* **18**, 6425–6443.
- STICKGOLD, R., JAMES, L. & HOBSON, J. A. (2000). Visual discrimination learning requires sleep after training. *Nature Neuroscience* **3**, 1237–1238.
- TASKER, G. J. & DUDEK, F. E. (1991). Electrophysiology of GABA-mediated synaptic transmission and possible roles in epilepsy. *Neurochemical Research* **16**, 251–262.
- TENNIGKEIT, F., SCHWARZ, D. W. & PUIL, E. (1998). Modulation of bursts and high-threshold calcium spikes in neurons of rat auditory thalamus. *Neuroscience* **83**, 1063–1073.
- TIMOFEEV, I., BAZHENOV, M., SEJNOWSKI, T. J. & STERIADE, M. (2001). Contribution of intrinsic and synaptic factors in the desynchronization of thalamic oscillatory activity. *Thalamus and Related Systems* **1**, 53–69.
- TIMOFEEV, I., CONTRERAS, D. & STERIADE, M. (1996). Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. *Journal of Physiology* **494**, 265–278.
- TIMOFEEV, I., GRENIER, F., BAZHENOV, M., SEJNOWSKI, T. J. & STERIADE, M. (2000a). Origin of slow cortical oscillations in deafferented cortical slabs. *Cerebral Cortex* **10**, 1185–1199.
- TIMOFEEV, I., GRENIER, F. & STERIADE, M. (2000b). Impact of intrinsic properties and synaptic factors on the activity of neocortical networks *in vivo*. *Journal of Physiology (Paris)* **94**, 343–355.
- TIMOFEEV, I., GRENIER, F. & STERIADE, M. (2002). The role of chloride-dependent inhibition and the activity of fast-spiking neurons during cortical spike-wave seizures. *Neuroscience* (in the Press).
- TIMOFEEV, I. & STERIADE, M. (1996). Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *Journal of Neurophysiology* **76**, 4152–4168.
- TIMOFEEV, I. & STERIADE, M. (1998). Cellular mechanisms underlying intrathalamic augmenting responses of reticular and relay neurons. *Journal of Neurophysiology* **79**, 2716–2729.
- TRAYNELIS, S. F. & DINGLEDINE, R. (1988). Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *Journal of Neurophysiology* **59**, 259–276.
- TURRIGIANO, G. G., LESLIE, K. R., DESAI, N. S., RUTHERFORD, L. C. & NELSON, S. B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391**, 892–896.
- VOLGUSHEV, M., MITTMANN, T., CHISTIANKOVA, M., BALABAN, P. & EYSEL, U. T. (1999). Interaction between intracellular tetanization and pairing-induced long-term synaptic plasticity in the rat visual cortex. *Neuroscience* **93**, 1227–1232.
- VON KROSIGK, M., MONCKTON, J. E., REINER, P. B. & McCORMICK, D. A. (1999). Dynamic properties of corticothalamic excitatory postsynaptic potentials and thalamic reticular inhibitory postsynaptic potentials in thalamocortical neurons of the guinea-pig dorsal lateral geniculate nucleus. *Neuroscience* **91**, 7–20.

Acknowledgements

This work was supported by the Canadian Institutes of Health Research, Human Frontier Science Program, Natural Sciences and Engineering Research Council of Canada, National Institutes of Health of the USA, Howard Hughes Medical Institute and Fonds de la Recherche en Santé du Québec. We thank P. Giguère and D. Drolet for excellent assistance.