Neuronal mechanisms mediating the variability of somatosensory evoked potentials during sleep oscillations in cats

Mario Rosanova[1,2] and Igor Timofeev[1]

[1] *Department of Anatomy and Physiology, School of Medicine, Laval University, Québec, Canada G1K 7P4*
[2] *Department of Clinical Science, Osp. L. Sacco, School of Medicine, University of Milan, 20157, Milan, Italy*

The slow oscillation (SO) generated within the corticothalamic system is composed of active and silent states. The studies of response variability during active *versus* silent network states within thalamocortical system of human and animals provided inconsistent results. To investigate this inconsistency, we used electrophysiological recordings from the main structures of the somatosensory system in anaesthetized cats. Stimulation of the median nerve (MN) elicited cortical responses during all phases of SO. Cortical responses to stimulation of the medial lemniscus (ML) were virtually absent during silent periods. At the ventral-posterior lateral (VPL) level, ML stimuli elicited either EPSPs in isolation or EPSPs crowned by spikes, as a function of membrane potential. Response to MN stimuli elicited compound synaptic responses and spiked at any physiological level of membrane potential. The responses of dorsal column nuclei neurones to MN stimuli were of similar latency, but the latencies of antidromic responses to ML stimuli were variable. Thus, the variable conductance velocity of ascending prethalamic axons was the most likely cause of the barrages of synaptic events in VPL neurones mediating their firing at different level of the membrane potential. We conclude that the preserved ability of the somatosensory system to transmit the peripheral stimuli to the cerebral cortex during all the phases of sleep slow oscillation is based on the functional properties of the medial lemniscus and on the intrinsic properties of the thalamocortical cells. However the reduced firing ability of the cortical neurones during the silent state may contribute to impair sensory processing during sleep.

(Received 7 July 2004; accepted after revision 2 November 2004; first published online 4 November 2004)
Corresponding author I. Timofeev: Department of Anatomy and Physiology, School of Medicine, Laval University, Québec, Canada G1K 7P4. Email: igor.timofeev@phs.ulaval.ca

Somatosensory evoked potentials (SEPs) are used in neurology to investigate the reliability of somatosensory pathways (Erwin *et al.* 1993). The traditional averaging procedure in humans and animals revealed only a slight modulation of amplitude and latency of SEPs across different states of vigilance (Cauller & Kulics, 1988; Emerson *et al.* 1988; Addy *et al.* 1989). A recent study, employing phase locked averaging in sleeping healthy subjects, demonstrated that SEPs were strongly modulated by slow oscillation (SO) (Massimini *et al.* 2003). These data contrast with the results obtained using electrical stimulation of prethalamic pathways, where early thalamic and cortical responses were absent during silent phases of SO (Steriade *et al.* 1990; Timofeev *et al.* 1996), an observation which corroborates the gating role of the thalamus during sleep.

Despite the wide clinical use of the SEP, its cellular basis and variability across the various phases of activity is not well understood. Some studies argued that the variability of cortical responses was based on the intrinsic membrane properties of cortical neurones (Istvan & Zarzecki, 1994; Zhu & Connors, 1999) and suggested that local circuit interneurones were the first cortical neurones to respond to peripheral stimuli (Yamamoto *et al.* 1988). Other data suggested that ongoing activity in the cortical network may significantly contribute to the variability of responses (Arieli *et al.* 1996; Azouz & Gray, 1999; Kisley & Gerstein, 1999; Sachdev *et al.* 2004).

SO occurs as the cyclic alternation of positive and negative EEG waves in animals and humans (Steriade *et al.* 1993*a*; Steriade *et al.* 1993*b*,*c*; Achermann & Borbely, 1997). Under this condition all cortical neurones are depolarized and fire spikes during EEG depth-negative waves while, during depth-positive EEG waves, they are hyperpolarized (Steriade *et al.* 2001; Timofeev *et al.*

© The Physiological Society 2004 DOI: 10.1113/jphysiol.2004.071381

2001*b*). In anaesthetized animals, both thalamocortical (TC) and thalamic reticular (RT) neurones are hyperpolarized during the EEG depth-positive waves. During the depth-negative EEG waves, the RT neurones fire spike bursts while TC neurones are hyperpolarized, often revealing spindle-like activity (Contreras & Steriade, 1995, 1996; Timofeev & Steriade, 1996; Timofeev *et al.* 2001*a*). Thus, both cortical and thalamic neurones are silent during depth-positive EEG waves, and reveal various forms of activity during depth-negative EEG waves. We were interested to learn how the presence of such diverse ongoing activity in the TC network would modify cortical responses to peripheral sensory stimuli. Even a moderate spontaneous hyperpolarization of TC neurones during depth-positive EEG waves is sufficient to displace them from firing threshold, thereby blocking transmission of information toward the cerebral cortex (Timofeev *et al.* 1996; Bazhenov *et al.* 1998). These findings contrast with multiple studies showing the maintenance of responses to peripheral stimuli during both sleep states and anaesthesia (Cauller & Kulics, 1988; Emerson *et al.* 1988; Istvan & Zarzecki, 1994; Azouz & Gray, 1999; Kisley & Gerstein, 1999; Zhu & Connors, 1999; Massimini *et al.* 2003).

We asked whether or not the stimulation site (prethalamic *versus* peripheral) is responsible for the difference of responses at the cortical level. We thus performed electrophysiological experiments on the somatosensory system of cats to study the cellular mechanisms underlying the modulation of SEPs during SO.

Some of these data were previously presented in abstract form (Rosanova & Timofeev, 2002).

Methods

Animal preparation

Acute experiments were carried out on 35 adult cats of both sexes that had been anaesthetized with ketamine and xylazine (10–15 and 2–3 mg kg^{-1} I.M., respectively). All pressure points and the tissues to be incised were infiltrated with lignocaine (lidocaine; 0.5%). The animals were paralysed with gallamine triethiodide and artificially ventilated, maintaining the end-tidal CO_2 concentration at 3.5–3.8%. A permanent sleep-like state, as ascertained by continuous recording of the EEG, was maintained throughout the experiments by administering additional doses of ketamine (5 mg kg^{-1}). For temporal depression of network activity, 1.0–2.0% of halothane was added to the ventilated air for 2–3 min (see Fig. 4). The body temperature was monitored by a rectal probe and maintained at 37°C via a feedback-controlled heating pad. The heart rate was continuously monitored (90–110 beats min^{-1}). The stability of intracellular recordings was ensured by cisternal drainage, bilateral pneumothorax, hip suspension and by filling the hole made for recordings with a solution of 4% agar.

Stimulation and recording procedures

For peripheral stimulation, the skin above the contralateral median forelimb nerve (MN) was carefully shaved and covered with EEG electrode paste. Tape was then used to affix a pair of silver flat electrodes over the forelimb median nerve. The intensity of stimuli was limited to values that were sufficient to elicit a minimal visible movement in the forepaws. (Similar functional criteria for the intensity of stimuli are used in diagnosis of human subjects.) After establishing the intensity of peripheral stimuli, the animal was paralysed with gallamine triethiodide (20 mg kg^{-1}), and the intensity of stimuli was not modified again. Superficial silver-ball field potential electrodes were used in each experiment to estimate the focus of neocortical response to median nerve stimuli on the contralateral side. This site was used for single, dual, triple and quadruple simultaneous intracellular recordings as well as multisite, multiunit recordings. Up to four David Kopf stepping microdrives were used to advance the intracellular micropipettes. Parallel recordings of focal field potential were obtained by means of tungsten electrodes inserted at different depths in the vicinity of recording pipettes. The same electrodes were used to collect multiunit activities. For electrical stimulation of the medial lemniscus (ML), the only pathway connecting dorsal column nuclei with ventral posterior lateral (VPL) thalamic nucleus (Steriade *et al.* 1997), a coaxial bipolar electrode was inserted ipsilaterally to the cortical or thalamic recording sites and contralaterally to the nucleus cuneatus at L 3 mm, H −1.5 mm, and A 3 mm. The intensity of ML stimuli was adjusted to elicit cortical field potential responses with an amplitude and shape similar to those evoked by contralateral MN stimuli during the active states of the network (see Fig. 2). Stimuli were applied either to the median forelimb nerve or to the ML with a frequency of one every two seconds. Between 30 and 100 stimuli were collected for analysis from each studied neurone. To obtain intracellular recordings from VPL neurones, a small portion of the suprasylvian gyrus (3–5 mm), underlying the white matter and fornix above the VPL nucleus, was removed by suction. Then, the pipettes were placed into either the anterior or middle parts of the ventrobasal complex at the expected location of the forelimb responsive neurones (Rose & Mountcastle, 1952). The neurones responding to median nerve stimuli were located at 3–5 mm ventral to the thalamic surface. Extra- and intracellular recordings from dorsal column nuclei (nucleus cuneatus) were performed at 1–2 mm lateral and 1–3 mm posterior to the obex.

Glass micropipettes for intracellular recordings were filled with 3 M potassium acetate (DC resistance, 30–70 MΩ). A high-impedance amplifier (bandpass,

© The Physiological Society 2004

10 kHz) with an active bridge circuitry was used to record and inject current into the cells. All electrical signals were sampled at 20 kHz and digitally stored on Vision (Nicolet, Wisconsin, USA). Offline computer analysis of electrographic recordings was done with IgorPro software (Lake Oswego, Oregon, USA). Statistical analysis was conducted with JMP software (Cary, North Carolina, USA). All numerical values are expressed as a mean ± standard deviation (S.D.).

At the end of experiments, the cats were given a lethal dose of intravenous sodium pentobarbital (50 mg kg^{-1}). All experimental procedures were performed according to national guidelines and were approved by the committee for animal care of Laval University.

Results

To reveal the neuronal mechanisms underlying cortical response variability to peripheral stimuli, we recorded the extra- and intracellular activities of neurones located within the main structures involved in the analysis of somatosensory information. Namely, the cortical neurones located within the focus of maximal primary somatosensory responses to electrical stimulation of the MN, the VPL thalamic nucleus, and the dorsal column nuclei (nucleus cuneatus).

Responses of cortical neurones to peripheral and prethalamic stimuli

Using single or simultaneous double, triple and quadruple intracellular recordings, we studied the responsiveness of 132 cortical neurones to forelimb median nerve and/or ML stimuli during various phases of SO. Concomitantly, we recorded the local field potentials and multiunit activities. All intracellularly recorded neurones were classified by electrophysiological criteria as regular-spiking, fast-rhythmic-bursting or intrinsically bursting (Connors & Gutnick, 1990; Gray & McCormick, 1996; Steriade *et al.* 1998). Fast-spiking neurones were not recorded in this set of experiments. Since we did not find any consistent relationship between the electrophysiological properties of neurones and their response patterns to MN or ML stimuli, we do not make further reference to the electrophysiological type of neurones in this paper.

We then compared the responses of cortical neurones as observed during different components of cortical SO (Fig. 1). All cortical neurones responded to peripheral MN stimuli during all phases of SO; however, the pattern of field potential and cellular responses varied as a function of SO phases. During active states of the network, which corresponded to the up state of cortical neurones, the initial components of field depth-negative potential were polyphasic, composed of two or three negative deflections (Fig. 1*B*). During the silent phase of network activity, which corresponds to an EEG depth-positive field potential and to the down state of cortical neurones, the responses were different. On average, latency to the peak of the field potential response increased by 4.5 ms ($P < 0.001$ (Student's paired t test) (Table 1)) and the initial phase of cortical field potential response had a tendency to be monophasic (Fig. 1*B*). Intracellularly, all the neurones located within the focus of field potential response were found to respond to forelimb MN stimulation. We found three major types of excitatory responses of cortical neurones to MN stimuli (Fig. 1*B1–3*) and one inhibitory (Fig. 1*B4*). During the active phases of network, 68.7% of neurones fired reliably and with low-latency variability (<1 ms) during the first peak of field evoked potential (Fig. 1*B*1 and Fig. 2*A*, cells 1 and 2). These neurones fired in 48.3 ± 7.2% of cases to the stimuli applied during the silent phases of cortical activity, as estimated by depth-positive EEG waves, and the latency of both the onset of synaptic response and spikes was longer and varied in a range of 2–3 ms. During active states the second group of neurones (19.7%) did not always fire spikes, but if they did, the spikes occurred during the second peak of evoked potential (Fig. 1*B2* and Fig. 2*A*, cells 3 and 4). These same neurones never fired spikes in response to stimuli applied during depth-positive EEG waves and the amplitude of depolarizing response was largely reduced during this phase of oscillation. A third group of neurones exhibiting excitatory responses (5.3%) fired throughout the initial excitatory component of field response during both the active and silent phases of SO (Fig. 1*B3*). The remaining seven neurones (5.3%) responded with primary IPSPs during active network states (Fig. 1*B4*) and their response was absent during silent network states. The response latency in a sampled group of neurones was found to have a bimodal distribution (Fig. 2*B*). Although the range of response latency during active and silent states overlapped (Table 1), the latency of responses during silent states was always longer than the latency of responses during active states ($P < 0.001$; Student's paired t test; Fig. 2*C*). All the neurones with short-latency responses (<10.5 ms) fired spikes during the active phase of SO and the variability of firing latency did not exceed 1 ms. It is likely that the firing of the VPL TC neurones directly depolarized the cortical neurones that revealed the short-latency EPSP. Once these neurones fired spikes, they excited other cortical neurones, which had longer EPSP latencies. The neurones that responded during active states with EPSP latencies of longer than 12 ms (those excited during the second peak of evoked potentials) did not fire spikes during the depth-positive phase of SO (Fig. 1*B2*) and the amplitude of their responses was reduced during the depth-positive EEG waves (Fig. 2, cell 4). Most likely, those neurones were located farther along in the intracortical functional chains and during

© The Physiological Society 2004

a depth-positive EEG wave they were not excited by intermediate neurones. Furthermore, an absence of inhibitory responses during silent network states suggests that the excitatory drive produced by MN stimulation was not strong enough to induce the firing of inhibitory interneurones during this network state. Overall, the firing probability during active states was 71.0 ± 0.5% and during silent states it decreased to 24.8 ± 0.3%.

Through our observation of 73 cortical neurones, we compared the responses to forelimb MN stimuli with responses to ML stimuli during different phases of cortical SO. Similarly to responses to MN stimuli, the firing probability of cortical neurones during active states elicited by ML stimuli was 68.3 ± 5.9%. The difference between cortical responses to MN and ML consisted of shorter response latency to ML stimuli during the active states (Table 1) and a virtual lack of response to ML stimuli during silent states (Fig. 2). Only eight tested neurones revealed some small intracellular responses to ML stimuli during silent network states (Fig. 2).

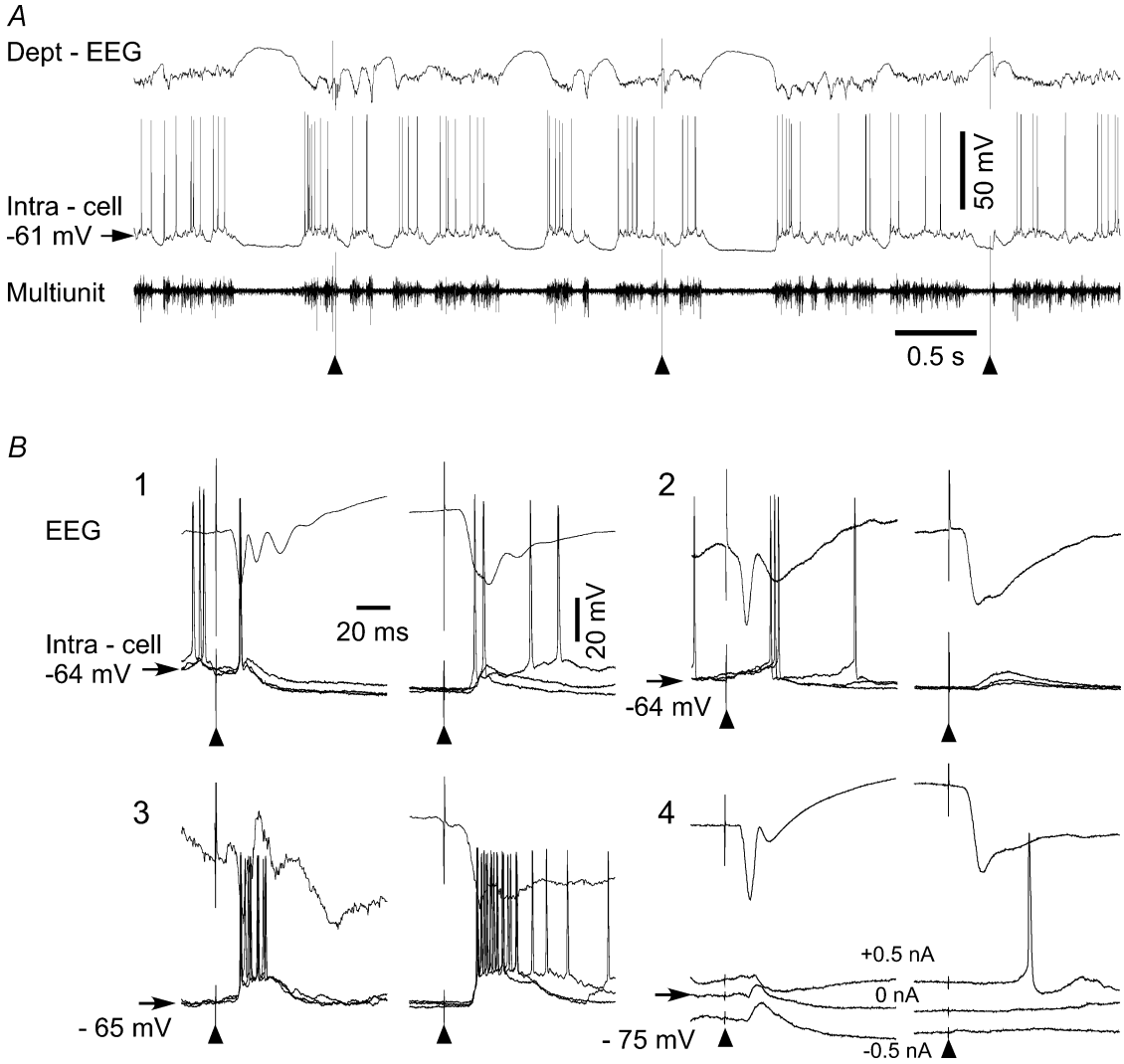

Figure 1. Modulation of cortical somatosensory evoked potential and intracellular activities during slow oscillation

All recordings were obtained from the focus of responses to the electrical stimulation of the right forelimb median nerve in the left somatosensory cortex. *A,* an example of the focal field, intracellular and multiunit recordings during a session of peripheral stimulation (▲). The first and the second stimuli were applied during active network states and the third stimulus was delivered during the silent network state. *B,* averaged field potential responses and superimposition of three intracellular single traces selected during active (left side) and silent (right side) network states. Note the multiple components of field responses during active cortical states and their modification during silent states. 1, 2 and 3 are the three types of initial excitatory responses; 4 is the initial inhibitory response. Note the increased latency of excitatory responses that occurred during EEG depth-positive wave and the absence of inhibitory response during the silent network state.

© The Physiological Society 2004

Table 1. Response latencies of cortical and VPL thalamic neurones to medial nerve and medial lemniscus stimuli during active and silent network states

	Field		Cortical neurones		Thalamic neurones	
	active ($n = 25$)	silent ($n = 25$)	active ($n = 73$)	silent ($n = 73$)	active ($n = 21$)	silent ($n = 21$)
Mean median nerve	12.8 ± 0.8	17.3 ± 0.9	11.4 ± 1.7	13.7 ± 2.5	7.6 ± 0.3	7.6 ± 0.2
Median nerve range	12.2–14.1	15.5–21.2	9.0–17.2	9.6–20.5	7.2–8.0	7.3–7.9
Medial lemniscus mean	6.7 ± 1.1	—	5.2 ± 1.8	8.3 ± 2.1 ($n = 7$)	0.71 ± 0.07	0.71 ± 0.07
Medial lemniscus range	4.5–7.7	—	−2.7–7.8	3.5–11.2 ($n = 7$)	0.6–0.9	0.6–0.9

The first maximum of depth-negativity was used to calculate the latency of field potential responses. The onset of synaptic response was used to calculate the latency of synaptic responses.

The difference in responses to ML and MN stimuli during the silent network states led to the hypothesis that the firing ability of TC neurones elicited by the two types of stimuli during these states of network was different. Thus, we obtained intracellular recordings from VPL TC neurones.

Responses of thalamocortical neurones to peripheral and prethalamic stimulation

We recorded the intracellular activities of 45 TC neurones from the thalamic VPL nucleus. Similar to the TC neurones located within ventrolateral nucleus of cats anaesthetized with ketamine–xylazine (Contreras *et al.* 1996; Timofeev *et al.* 1996), the TC neurones from the VPL nucleus revealed a slow oscillatory pattern (Fig. 3). During cortical EEG depth-positivity, the VPL neurones were hyperpolarized due to a total disfacilitation in the cortico-thalamic network. The onset of cortical depth-negativity was associated with a depolarizing potential, probably reflecting a cortical excitatory drive (Fig. 3*A*, see arrow in expanded panel), which was often followed by a rebound low-threshold spike-burst. Thereafter, the VPL neurones revealed a brief spindle sequence composed of rhythmic IPSPs, often mixed with sequences of EPSPs. Intracellular application of depolarizing current pulses during the period of disfacilitation, associated with prolonged hyperpolarizing potential, elicited a high-frequency spike burst. When current pulses with the same intensity occurred during relatively depolarized active states, they elicited tonic firing (Fig. 3*B*). All recorded VPL neurones were identified according to their ability to respond with EPSPs to the activation of skin receptive fields (Fig. 4). Seventy per cent of neurones responded to mechanical skin stimulation with tonic excitatory activities, lasting for the duration of stimulus (see upper panel in Fig. 4), while the remaining neurones responded with synaptic excitation in 'on'–'off' mode (not shown). Out of 45 neurones located within the VPL nucleus, 21 neurones responded with EPSPs to both electrical stimulation of the forelimb median nerve and electrical ML stimulation (Fig. 4). The responses of these neurones were subjected to detailed analysis. While the VPL neurones responded with a group of several spikes to MN stimuli during both active and silent phases (Fig. 3*C*), to ML stimuli they responded with an EPSP in isolation during silent network states and EPSP-spike during active network states (see Fig. 5).

All the VPL thalamic neurones that received projections from the forelimb MN invariantly fired following electrical stimulation of the skin above the MN (Fig. 4). These responses were composed of a group of 5–10 identifiable EPSPs and the total duration of these excitatory responses lasted, in sum, for ∼30 ms. The latency of the first EPSP, however, revealed slight variability (Table 1).The membrane potential of VPL neurones oscillated with amplitudes sometimes exceeding 20 mV (see above, Fig. 3). Thus, following the MN stimulation, we explored the ability of VPL neurones to trigger spikes and measured the latencies of these spikes over a wide range of membrane potentials (Fig. 5). At depolarized levels of membrane potential, the first or the second EPSP of a group of EPSPs arriving at a VPL relay neurone elicited the first spike, transmitting the peripheral signals toward the cerebral cortex. At voltages more depolarized than −65 mV, the VPL neurones usually fired between three and five action potentials. The latency of the first spike in the spike-trains was 9.28 ± 0.27 ms (Fig. 5*D*). Hyperpolarization of the neurone induced a progressive increase in the latency of the first spike (Fig. 5*B* and *D*). At hyperpolarized levels of membrane potential (i.e. below −70 mV), the first 3–5 EPSPs summated and they triggered low-threshold spikes (LTSs) crowned by fast spikes, and the variability of first spike latency increased as indicated by the large S.D. LTSs elicited by MN stimuli at hyperpolarized voltages were accompanied by 3–5 spikes. Only at voltages between −70 and −65 mV did the VPL neurones decrease their firing to 2–3 spikes (Fig. 5*C*). This suggests that, at these voltages (between −70 and −65 mV), the driving force of summated EPSPs was not strong enough to fire a greater number of spikes in the tonic mode and that LTSs could not

© The Physiological Society 2004

be fully de-inactivated to fire spike-bursts. Hence, although the VPL neurones fired spikes at de- and hyperpolarized voltages in response to peripheral stimuli, the latencied of the responses and the firing patterns were different.

The response of VPL neurones to ML stimuli was represented by a single EPSP (Fig. 5*A*, right). The latency to the onset of the EPSP was short and stable (Table 1). When the membrane potential was hyperpolarized below −60 mV, we were unable to elicit either fast action potentials or LTSs. Thus, peripheral stimulation elicited groups of EPSPs, which at more depolarized voltages fired groups of spikes in tonic firing mode and, at hyperpolarized voltages LTSs, accompanied by high-frequency spike-bursts. The latency of initial spikes varied as a function of membrane potential, providing a basis for the variability of early EPSP latencies in cortical neurones. The outright blockage of firing in TC neurones was seen only when prethalamic (ML) stimulation was used at hyperpolarized voltages and the VPL neurones did not fire spikes.

Given the dramatically different responses that VPL neurones had to ML stimulation (single EPSP) and

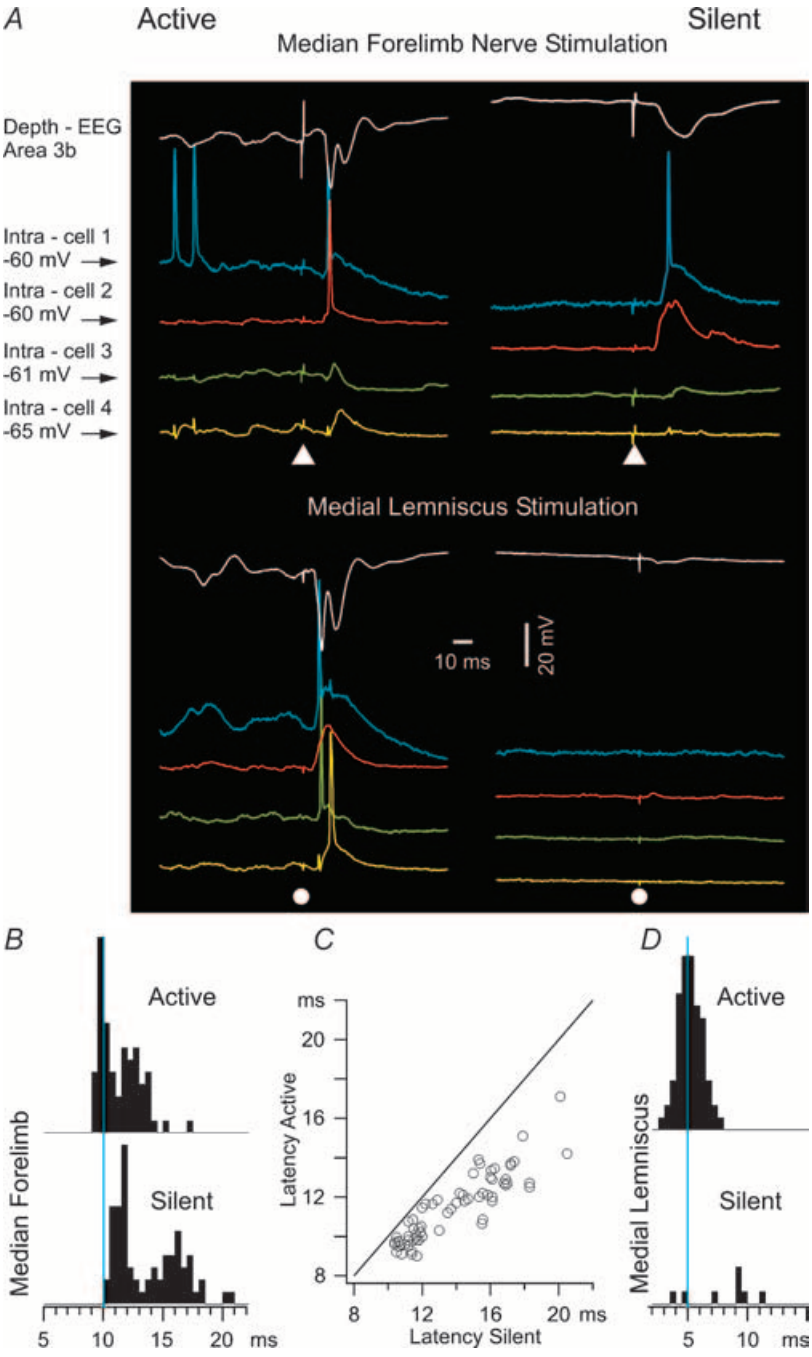

Figure 2. Differences in cortical responses to forelimb *versus* medial lemniscus stimulation during different phases of SO
A, simultaneous field and quadruple intracellular recording during responses to forelimb median nerve and medial lemniscus stimuli. Forelimb stimulation, ▲; medial lemniscus stimulation, ●. White trace: focal field potential; blue, red green and yellow: four different neurones recorded simultaneously. Left panels, responses during active network states, right panels, responses during silent network states. *B,* histograms of minimal response latency of the 73 neurones that responded to median forelimb nerve during active and silent network states. *C,* scatter plot for the response latency of cortical neurones during active *versus* silent states. *D,* histograms of minimal response latency of the 73 neurones that responded to medial lemniscus stimuli during active and silent network states.

© The Physiological Society 2004

stimulation of the MN (barrages of EPSPs), we decided to examine the firing patterns elicited by MN stimulation at the prethalamic level and thus performed recordings in the dorsal column cuneate nucleus.

Responses of dorsal column nuclei to forelimb stimulation

We intracellularly recorded the activities of 17 neurones located in the nucleus cuneatus that had responded to the MN stimuli. The pattern of cortically generated slow oscillation was not observed in these neurones. In six cases, by approaching the nucleus we were able to obtain intracellular recordings from the axons of primary afferent fibres (Fig. 6). In intra-axonal recordings, the onset of a spike was not preceded by any slow depolarizing events and the rising phase of a spike was 0.2 ± 0.07 ms. Because of such fast spiking, the electrical capacitance of the recording pipette affected the amplitude of these action potentials. The latency of spike onset from the recorded fibres was 3.8 ± 0.3 ms. The responses of peripheral fibres and the initial responses of nucleus cuneatus neurones were highly stereotyped (Fig. 6*A*). The latency of the initial EPSP recorded from the neurones of the nucleus cuneatus was 4.7 ± 0.3 ms, and each initial EPSP elicited by MN stimulation triggered a spike with a latency of 5.2 ± 0.2 ms. In response to MN stimuli, most of these neurones ($n = 10$) revealed a secondary depolarization with a latency of 6.1 ± 0.4 ms. In approximately 10% of cases, the second EPSP gave rise to a second spike with a latency of 6.6–7.8 ms (Fig. 6*A*). The latter depolarizing component could have been generated either by synaptically arriving EPSPs or by the activation of intrinsic neuronal currents. When we compared the waveform of spontaneous spikes with spikes elicited by peripheral stimuli (Fig. 6*A*, right) we found that all spikes were followed by a fast, brief and pronounced afterhyperpolarizing potential, then followed by a slight afterdepolarizing potential (ADP). In the case of the spontaneous spikes, the ADP of the spike never did reach the firing threshold, while in the case of a spike's ADP evoked by peripheral stimuli; the depolarization had a complex shape and was larger in amplitude, suggesting a contribution of synaptic components to the generation of this depolarizing potential. Therefore, the second spike occurred due to the arrival of EPSPs and not due to any strong, intrinsically generated ADP. In all cases, the first negative peak of cortical evoked potential followed the aforementioned response components of the nucleus cuneatus by 5.2 ± 6.1 ms, indicating that cortical feedback to the dorsal column nuclei (Canedo *et al.* 2000) did not contribute to the generation of initial response components. Thereafter, the neurones of the nucleus cuneatus revealed brief, multiple synaptic depolarizing potentials, lasting for 15–20 ms, which were superimposed on a steady hyperpolarizing potential (Fig. 6*A*). These activities were probably generated by intranuclear networks (Canedo & Aguilar, 2000). The second group of spikes, exhibiting a response latency of 10–15 ms, were seen in most (10 out of 17) nucleus cuneatus neurones and occurred after the second negative

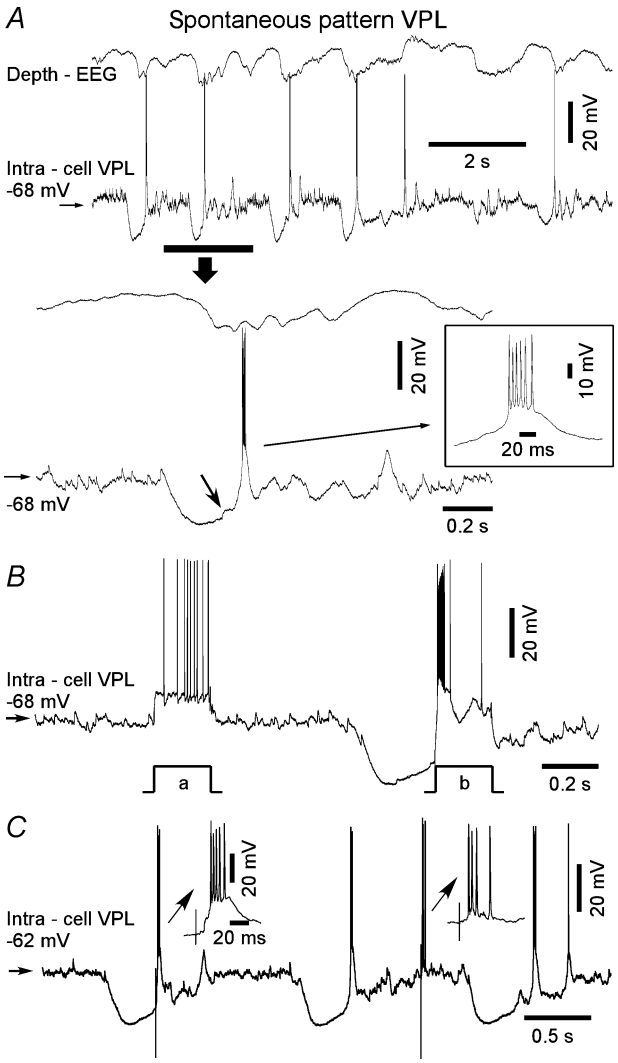

Figure 3. Spontaneous activity and response patterns of the thalamocortical neurone located in the VPL nucleus during slow oscillation

A, an example of simultaneous recordings of cortical field potential from the somatosensory cortex and intracellular recordings from a VPL neurone. The period indicated by the horizontal bar and arrow is expanded in the middle panel. The inset shows a typical spontaneous low-threshold spike burst. Note that the thalamocortical neurone was hyperpolarized during depth-positive EEG waves, displayed a rebound spike burst after the onset of cortical activities as estimated by EEG depth-negativity, and later displayed a brief sequence of spindle-related short IPSPs. *B,* depolarizing current pulses of 0.5 nA elicited burst firing of the neurone during hyperpolarizing phases of slow oscillation and the same current pulse during elicited tonic firing depolarizing phases of slow oscillation. *C,* medial nerve stimuli applied during both silent and active states elicit excitation and firing of VPL neurone.

© The Physiological Society 2004

component of the cortical evoked potential. These spikes were generated, probably, by an excitatory corticofugal drive (Canedo & Aguilar, 2000). Although the responses of dorsal column nuclei neurones to peripheral stimuli were complex, there was no variability in the initial components of their responses.

Since the responses of nucleus cuneatus neurones to peripheral stimuli were found to be stereotyped, we hypothesized that the synaptic barrages in VPL neurones could have originated from variable conductance velocities in the nucleus cuneatus axons projecting to the VPL. To test this hypothesis, we used ML stimulation and recorded anitdromic unit responses ($n = 153$) of neurones located within the nucleus cuneatus (Fig. 6*B*). Although the mean latency of the antidromic responses was 1.49 ± 0.74 ms, the range of these responses was large (from 0.6 ms to 4.5 ms). In addition, we found neurones responding with orthodromic spikes, which had a latency of 8.13 ± 2.67 ms (Fig. 6*B*). The orthodromic responses probably occurred via axon-reflex, thus, the antidromically elicited spikes propagated to the collaterals of the stimulated neurones, which, in turn, synaptically excited the other neurones of the nucleus cuneatus. The thalamic effects of the orthodromic spikes that followed ML stimulation are seen in some traces to reveal the presence of EPSPs with latencies exceeding 10 ms (Fig. 5, see arrows). In sum, the variable conductance velocity of dorsal column nuclei axons, the spike doublets of dorsal column neurones and the intranuclear network seem to be sufficient factors in the generation of EPSP trains in the VPL neurones, ensuring the constant firing of these neurones after MN stimuli.

Discussion

The major findings of this study are as follows: (a) MN electrical stimulation initiated responses within the somatosensory cortex during all phases of cortical SO, and the responses that occurred during silent network states had longer latencies than those that were elicited during active network states. (b) ML stimulation elicited cortical responses during active network states, but failed to elicit responses at the neocortical level during silent network states. (c) In the VPL nucleus, ML stimuli elicited single EPSPs at hyperpolarized voltages and EPSPs crowned by single spikes at depolarized voltages. In contrast, MN stimuli elicited barrages of EPSPs that

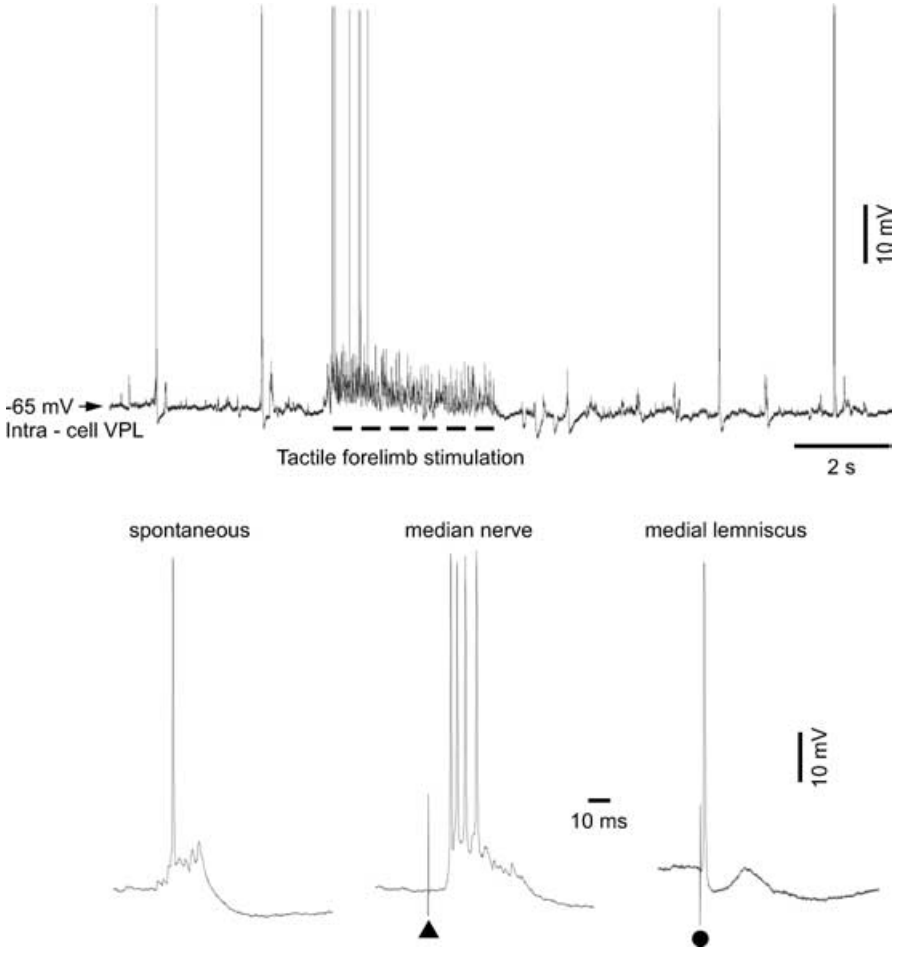

Figure 4. Response of VPL thalamocortical neurone to forelimb *versus* medial lemniscus stimulation
Upper panel, a period of intracellular activity recorded from a VPL neurone of the thalamus. The thick broken line indicates the time segment during which slight pressure was applied to the skin in the receptive field (distal forelimb) of the TC neurone. Lower panel, from left to right: Spontaneous complex synaptic events during resting membrane potential and responses to median nerve (▲) and medial lemniscus stimuli (●). Note the presence of multiple synaptic events and high-frequency spike trains elicited by forelimb stimulation and a single EPSP leading to spikes elicited by medial lemniscus stimulation.

© The Physiological Society 2004

triggered LTSs crowned by spike bursts at hyperpolarized voltages and tonic firing at depolarized voltages. Thus, the ability/inability of peripheral (MN) or prethalamic (ML) stimuli to elicit firing during silent network states was attributed to differences in the synaptic volleys produced by the two different stimuli. (d) The neurones of the nucleus cuneatus fired single spikes or spike doublets with fixed latencies to peripheral stimuli. The EPSP barrages in VPL neurones were therefore due to the difference in the conduction of the dorsal column nuclei axons projecting

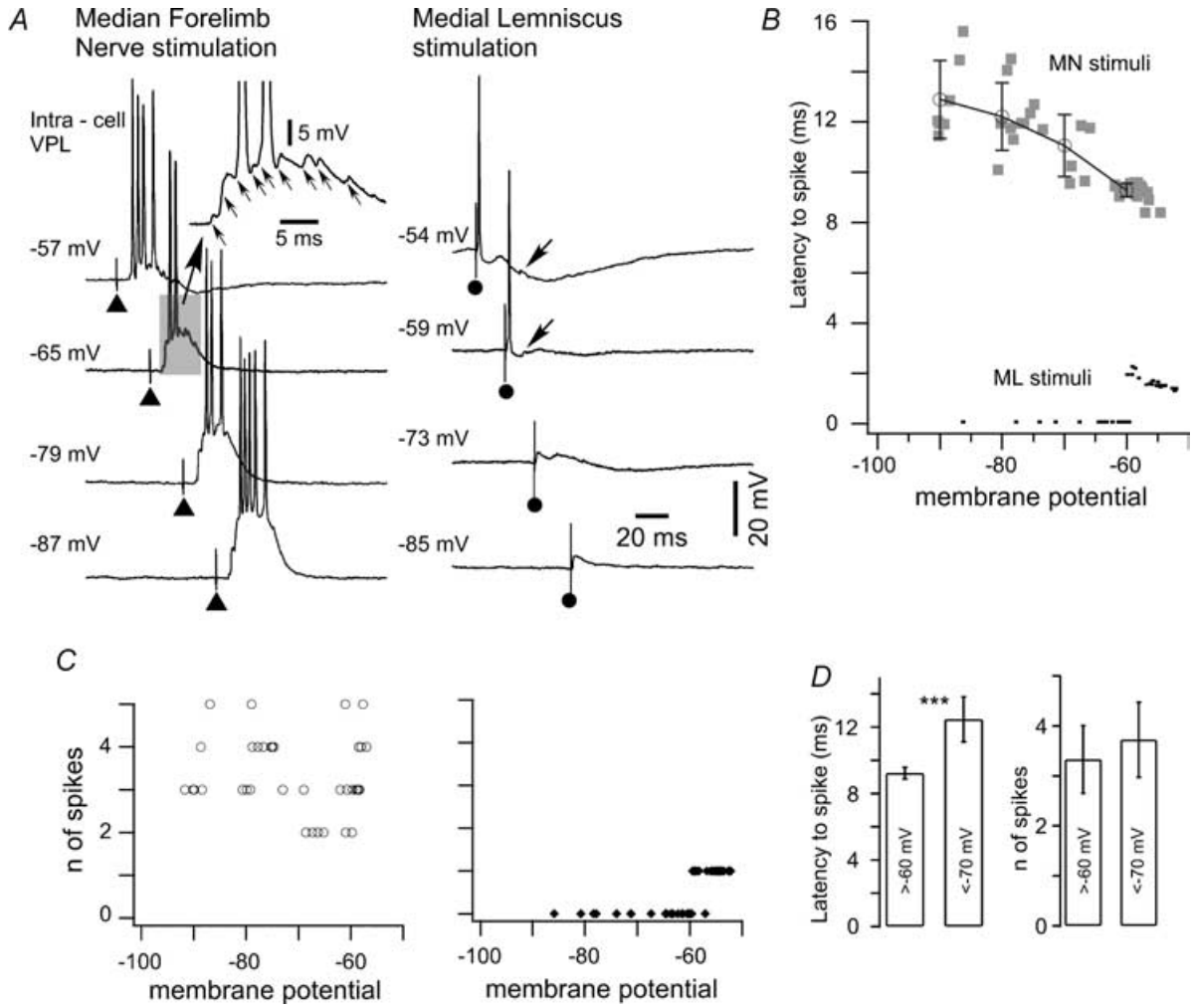

Figure 5. Voltage dependency of responses of a VPL neurone to forelimb median nerve and medial lemniscus stimuli

A, responses of a VPL neurone to MN (left) and ML (right) stimulation. In order to study the voltage dependency of responses, de- and hyperpolarizing steady DC current was injected intracellularly. At depolarized voltages the forelimb stimulation elicited high-frequency spike trains, whereas at more hyperpolarized voltages the summated multiple synaptic potentials triggered a low-threshold spike burst. Small arrows in the inset point to individual EPSPs contributing to a summated response. Note the increased latency of the first spike at more hyperpolarized voltages. In contrast, medial lemniscus stimulation at hyperpolarized voltages elicited single EPSPs that were crowned with spikes at more depolarized voltages. A small arrow points to the second EPSPs that were observed in some traces. *B,* the dependency of the first spike latency on the membrane potential. The average values for responses to MN stimuli were obtained in voltage windows from −95 mV to −85 mV, from −85 mV to −75 mV, from −75 mV to −65 mV and from −65 mV to −55 mV. There were no spikes elicited by ML stimuli (indicated as 0 ms on *y* axis) at voltages below −60 mV. *C,* the dependency of number of spikes on the membrane potential for forelimb (left) and medial lemniscus (right) electrical stimuli from the neurone shown in *A*. Note that at any given level of membrane potential, forelimb stimulation was able to trigger a high-frequency sequence of spikes (from two to five). In contrast, medial lemniscus stimulation revealed a binary behaviour characterized by the absence of spikes at hyperpolarized membrane potentials (until −60 mV) and one spike at more depolarized levels of membrane potential. *D,* mean values ($n = 7$ neurones) of first spike latency and the number of spikes at depolarized voltages of more than −60 mV and at hyperpolarized voltages of more than −70 mV as elicited by medial lemniscus stimuli. Error bars indicate standard deviation. ***significant difference ($P < 0.001$).

© The Physiological Society 2004

onto the VPL nucleus, the spike doublets of the dorsal column neurones and the involvement of the intranuclear network in information transfer.

Some remarks on terminology

SO was originally identified at the cortical level by M. Steriade and colleagues (Steriade *et al.* 1993*c*), who showed that the de- and hyperpolarizing phases of neuronal activities, respectively, give rise to EEG depth-negative and EEG depth-positive waves. Later, similar patterns were recorded in neostriatal neurones by Wilson and Kawaguchi who introduced the now widely used terms 'up state' for the depolarizing phases of SO and 'down state' for the hyperpolarizing phases of SO (Wilson & Kawaguchi, 1996). Cortically generated SO was also found to entrain the thalamus (Steriade *et al.* 1993*a*; Contreras & Steriade, 1995; Timofeev & Steriade, 1996). Similar to neocortical and neostriatal neurones, both the thalamic reticular and TC neurones are hyperpolarized during depth-positive EEG waves due to disfacilitation, i.e. an absence of spontaneous synaptic activities (Wilson *et al.* 1983; Wilson, 1986; Contreras *et al.* 1996; Steriade *et al.* 2001). During depth-negative EEG waves, cortical, neostriatal and inhibitory thalamic reticular neurones are depolarized and fire spikes, while TC neurones are hyperpolarized, reveal rhythmic IPSPs and occasionally fire rebound spike bursts (Contreras & Steriade, 1995; Timofeev & Steriade, 1996). Thus, in the dorsal thalamus, the intracellular activities occurring during depth-negative

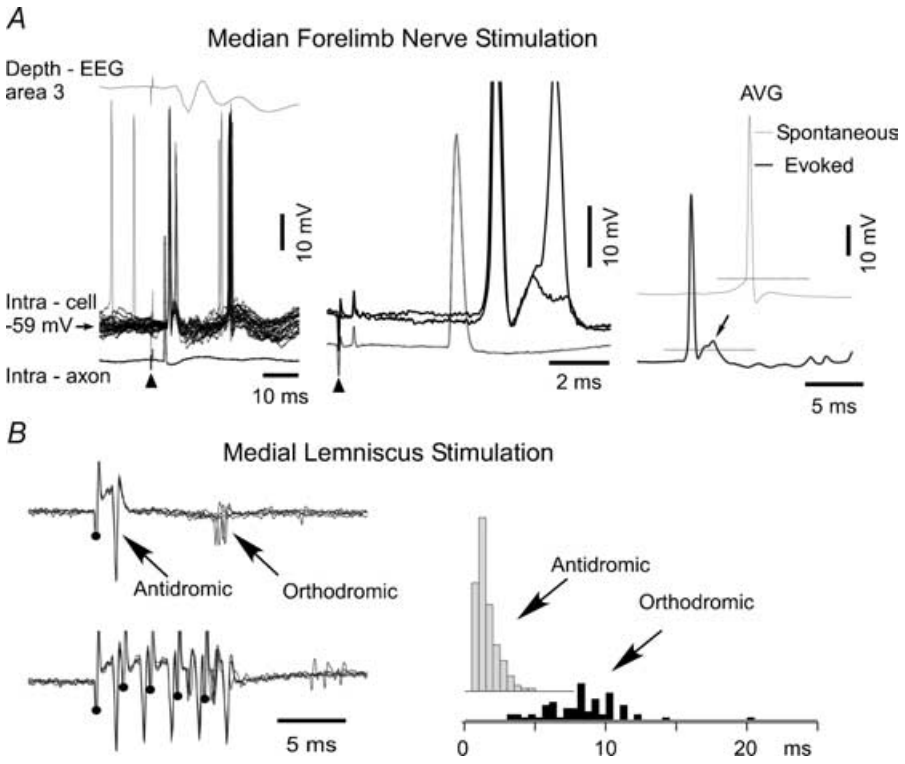

Figure 6. Stability of medullar nucleus cuneatus neuronal responses to electrical forelimb stimulation and variability of both anti- and orthodromic responses to the stimulation of the medial lemniscus
A, left panel shows an averaged somatosensory cortical evoked potential (upper trace), superimposition of responses of the nucleus cuneatus neurone (middle traces) and an averaged intra-axonal response to forelimb stimulation, presumably from dorsal root ganglion neurones (bottom dotted trace). Two single traces and intra-axonal averages are expended in the middle panel. The intracellular recording from an axon was obtained 30 μm above the intracellularly recorded neurone located in the nucleus cuneatus neurone. Primary afferent axon firing occurred prior to the onset of nucleus cuneatus neurone responses. An early component of neuronal responses was composed of one, and occasionally, two spikes. The second cluster of spikes in nucleus cuneatus neurones (upper left panel) occurred after a secondary component peak of cortical evoked potential, suggesting that cortico-medullar pathways might be responsible for the generation of secondary responses. Right panel, spike-triggered averages (AVG) were obtained from spikes elicited by forelimb stimuli (thick line) and from spontaneously occurring spikes (thin line). *B,* antidromic and orthodromic responses of nucleus cuneatus neurones to medial lemniscus stimuli. Upper traces in the left panel show the superposition of five responses to single stimuli and bottom traces show the superposition of five responses to 500 Hz pulse-trains. Right panel, histograms of antidromic ($n = 153$) and orthodromic ($n = 70$) response latencies.

© The Physiological Society 2004

EEG waves cannot be characterized as either depolarizing or up state. For the purposes of the present study, we used the terms 'active states' for processes occurring during EEG depth-negative waves and 'silent states' for processes occurring during EEG depth-positive waves. We believe that such terminology better represents the functional state of the TC network during the SO.

The latencies of somatosensory responses along the somatosensory pathway

At the medullary level, the intra-axonal recordings from prenuclear fibres revealed that peripheral stimulation produced incoming volleys at a latency of 3.8 ± 0.3 ms. Similarly to previous findings, the firing of nucleus cuneatus neurones occurred 1.1 ms later (Canedo & Aguilar, 2000). Consistent with recent studies (Coleman *et al.* 2003*a*; Coleman *et al.* 2003*b*), we found no failures or variability in latency or firing probability at the level of the cuneate nucleus, suggesting a fast and very reliable synaptic transmission of incoming signals. Occasionally, spike doublets were seen and the latency of the second spike reached 6.6–7.8 ms (Fig. 6). *In vitro*, the dorsal column nuclei neurones did not reveal bursting properties (Nuñez & Buno, 1999), although the intrinsic oscillations in cellular cultures obtained from these neurones can lead to spike clusters (Reboreda *et al.* 2003), whereas intracellular recordings *in vivo* often revealed spike doublets (Canedo & Aguilar, 2000; Canedo *et al.* 2000). Our data suggest that the second spike of a spike doublet is probably driven by synaptic events (Fig. 6) originating in intranuclear local circuits (Canedo *et al.* 2000). The second group of spikes occurred with latencies of more than 20 ms and was coincident with, or followed, the second negative component of the cortical evoked potential, suggesting that corticomedullary pathways contributed to the generation of these spikes. The multiple EPSPs that were seen between these two groups of spikes did not lead to firing because they occurred on a steady hyperpolarizing envelope, which was probably mediated by the firing of local inhibitory neurones (Canedo *et al.* 2000).

The TC neurones played a critical role in the modulation of cortical responsiveness to peripheral stimulation during SO: the latency of EPSPs in VPL–TC neurones was around 7.6 ms (Table 1) and the latency of firing of the first spike in the same neurones ranged from 8.2 to 15.8 ms (Fig. 5). Then shortest latencies were found when TC neurones were depolarized and prethalamic volleys brought the membrane potential to the firing threshold; longer latencies occurred when TC neurones were hyperpolarized and the first 3–5 EPSPs of the barrage triggered an LTS, which was covered with fast spikes. The single EPSPs that were elicited by ML stimulation were unable to trigger spikes at voltages hyperpolarized below −60 mV. Since the nucleus cuneatus neurones fired 1–2 spikes in response to peripheral stimuli and these responses were of quite similar latency, the expected response of TC neurones would consist of 1–2 EPSPs. In rats, most excitatory interactions in cuneate–VPL neuronal pairs involved intervals of 1–7 ms between successive cuneate and thalamic discharges (Alloway *et al.* 1994). Given a larger length of the dorsal column–lemniscal pathway in cats, the variability of latency of EPSPs in VPL neurones could be larger. Our data showing a variable latency of antidromic responses of cuneate neurones that followed ML stimulation suggest that different conductance velocity of axons originating in the cuneate nucleus could contribute to the origin of barrages of EPSPs in VPL neurones. Altogether, the origin of EPSP barrages in VPL neurones that followed MN stimulation was mediated by a convergence of fibres with different conductance velocities originating in the dorsal column nuclei, spike doublets of cuneate nucleus neurones and excitatory connections within the cuneate nucleus. It is unlikely that cortico-thalamic neurones contributed to the phasic excitation of VPL neurones because (a) the strength of cortico-RE connections is more than three-fold stronger than the strength of cortico-TC connections and thus cortico-TC excitation plays only a modulatory role (Golshani *et al.* 2001; Gentet & Ulrich, 2004), and (b) most cortical neurones did not fire spikes in response to MN stimuli during silent network states (Figs 1 and 2), but the responses of VPL neurones nevertheless remained strong during this network state. Also, the coticothalamic EPSPs display long-lasting rise times (Deschénes & Hu, 1990; Timofeev *et al.* 1996) contrasting the sharp rising EPSPs recorded in the present study (Fig. 5 *A*).

Consistent with other studies on cats and raccoons (Yamamoto *et al.* 1990; Istvan & Zarzecki, 1994), we found that 40% of cortical neurones responded with the shortest latencies of EPSPs being 9.5–10.5 ms. The shortest latency of a cortical response to a TC cell's firing is 1.2–1.8 ms (Steriade *et al.* 1997; Swadlow *et al.* 2002). This indicates that, theoretically, the shortest latency of EPSPs in VPL neurones to a spike should be 8.3 ms. Our recordings taken from VPL neurones, revealed that the shortest latency of firing was 8.2 ms (Fig. 5).

Different neuronal processes mediated in a different way the generation of field potential responses to MN stimuli. During active network states, the firing of the first group of neurones contributed to the generation of the first depth-negative peak (Fig. 1*B*,) and the excitation of the second group of neurones was a factor generating the second component of the depth-negative peak (Fig. 1*B*2). The histogram of latency of neuronal responses demonstrates a bimodal distribution (Fig. 2*B*), which corresponds to the two depth-negative maximums of the evoked potential. An absence of inhibitory responses during the depth-positive EEG wave (Fig. 1*B*4) transforms

© The Physiological Society 2004

the clear cut biphasic field potential response to a monophasic one (Figs 1*B* and 2*A*).

Thalamic and cortical gating during slow sleep oscillation

The TC network is composed of billions of synaptically connected neurones. The consistently integrated behaviour of such a network has been put forward as a necessary condition for conscious perception phenomena (Edelman & Tononi, 2000; Steriade, 2001). In particular, a minimal time interval of stable TC activity is required to achieve conscious perceptions (Libet *et al.* 1967), supported by continuous cortico-cortical and thalamo-cortical coherent activities (Singer & Gray, 1995; Varela *et al.* 2001). Incoming EPSP bursts to TC neurones at depolarized voltages triggered trains of spikes and, at hyperpolarized voltages, similar synaptic volleys elicited a high-frequency spike burst (Figs 3 and 5). The total number of spikes was similar under both voltage conditions, but the timing of spike occurrence was different. We would like to emphasize that outright blockage of cortical responses at the thalamic level during silent phases of activity was achieved only when prethalamic ML stimulation was used. This outcome was identical to our previous study of motor systems in which the brachium conjuctivum–VL thalamic nucleus–motocortex chain was investigated (Timofeev *et al.* 1996).

At the neocortical level, responses to peripheral stimuli varied in latency, amplitude, shape and ability to fire as a function of the phase of SO (Figs 1 and 2). The spike timing is critical in cortical information processing (Foffani *et al.* 2004). The primary source of the variability in cortical response latency was variability in the latency of TC neurones' firing, which was longer and more variable during the silent phases of slow oscillation, and shorter and less variable during active phases of slow oscillation. In the neocortex, the firing probability for neurones with the shortest latency of response was reduced by 50% during silent phases of SO, though the amplitude of synaptic responses was still high due to a stronger driving force from more hyperpolarized levels of membrane potential and higher input resistance (Fig. 2 cells 1 and 2). This last finding could explain the higher N20 amplitude during the putative silent state observed in sleeping humans (Massimini *et al.* 2003). Finally, the amplitude of EPSPs activated by oligosynaptic intracortical chains was reduced and those neurones never fired during silent phases of slow oscillation.

We conclude that: (a) sensory inputs after peripheral stimulation pass the thalamic gate during all phases of sleep slow oscillations because of the spike barrages generated in the lemniscal pathway; (b) however, due to spontaneous fluctuations in the membrane potential of both thalamic and cortical neurones, cortical responses are highly variable; (c) specifically, the reduced probability of spikes being transmitted further within cortical circuits during the silent state of the slow oscillation may contribute to impairing sensory processing during sleep.

References

Achermann P & Borbely AA (1997). Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience* **81**, 213–222.

Addy RO, Dinner DS, Luders H, Lesser RP, Morris HH & Wyllie E (1989). The effects of sleep on median nerve short latency somatosensory evoked potentials. *Electroencephalogr Clin Neurophysiol* **74**, 105–111.

Alloway KD, Wallace MB & Johnson MJ (1994). Cross-correlation analysis of cuneothalamic interactions in the rat somatosensory system: influence of receptive field topography and comparisons with thalamocortical interactions. *J Neurophysiol* **72**, 1949–1972.

Arieli A, Sterkin A, Grinvald A & Aertsen A (1996). Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science* **273**, 1868–1871.

Azouz R & Gray CM (1999). Cellular mechanisms contributing to response variability of cortical neurons in vivo. *J Neurosci* **19**, 2209–2223.

Bazhenov M, Timofeev I, Steriade M & Sejnowski TJ (1998). Computational models of thalamocortical augmenting responses. *J Neurosci* **18**, 6444–6465.

Canedo A & Aguilar J (2000). Spatial and cortical influences exerted on cuneothalamic and thalamocortical neurons of the cat. *Eur J Neurosci* **12**, 2515–2533.

Canedo A, Marino J & Aguilar J (2000). Lemniscal recurrent and transcortical influences on cuneate neurons. *Neuroscience* **97**, 317–334.

Cauller LJ & Kulics AT (1988). A comparison of awake and sleeping cortical states by analysis of the somatosensory-evoked response of postcentral area 1 in rhesus monkey. *Exp Brain Res* **72**, 584–592.

Coleman GT, Mahns DA, Zhang HQ & Rowe MJ (2003*a*). Impulse propagation over tactile and kinaesthetic sensory axons to central target neurones of the cuneate nucleus in cat. *J Physiol* **550**, 553–562.

Coleman GT, Zhang H-Q & Rowe MJ (2003*b*). Transmission security for single kinesthetic afferent fibers of joint origin and their target cuneate neurons in the cat. *J Neurosci* **23**, 2980–2992.

Connors BW & Gutnick MJ (1990). Intrinsic firing patterns of divers neocortical neurons. *Trends Neurosci* **13**, 99–104.

Contreras D & Steriade M (1995). Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci* **15**, 604–622.

Contreras D & Steriade M (1996). Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. *J Physiol* **490**, 159–179.

Contreras D, Timofeev I & Steriade M (1996). Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J Physiol* **494**, 251–264.

© The Physiological Society 2004

Deschénes M & Hu B (1990). Electrophysiology and pharmacology of the corticothalamic input to lateral thalamic nuclei: an intracellular study in the cat. *Eur J Neurosci* **2**, 140–152.

Edelman GM & Tononi G (2000). *A Universe of Consciousness*. Basic Books, New York.

Emerson RG, Sgro JA, Pedley TA & Hauser WA (1988). State-dependent changes in the N20 component of the median nerve somatosensory eveoked potential. *Neurology* **38**, 64–68.

Erwin CW, Rozear MP, Radtke RA & Erwin AC (1993). Somatosencory evoked potentials and surgical monitoring. In *Electroencephalography: basic principles, clinical applications and related fields*, ed. Niedermeyer E & Lopes da Silva FH, pp. 957–988. Williams & Wilkins, Baltimore.

Foffani G, Tutunculer B & Moxon KA (2004). Role of spike timing in the forelimb somatosensory cortex of the rat. *J Neurosci* **24**, 7266–7271.

Gentet LJ & Ulrich D (2004). Electrophysiological characterization of synaptic connections between layer VI cortical cells and neurons of the nucleus reticularis thalami in juvenile rats. *Eur J Neurosci* **19**, 625–633.

Golshani P, Liu XB & Jones EG (2001). Differences in quantal amplitude reflect GluR4- subunit number at corticothalamic synapses on two populations of thalamic neurons. *Proc Natl Acad Sci U S A* **98**, 4172–4177.

Gray CM & Mccormick DA (1996). Chattering cells: superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. *Science* **274**, 109–113.

Istvan PJ & Zarzecki P (1994). Intrinsic discharge patterns and somatosensory inputs for neurons in raccoon primary somatosensory cortex. *J Neurophysiol* **72**, 2827–2839.

Kisley MA & Gerstein GL (1999). Trial-to-trial variability and state-dependent modulation of auditory-evoked responses in cortex. *J Neurosci* **19**, 10451–10460.

Libet B, Alberts WW, WrightEW Jr & Feinstein B (1967). Responses of human somatosensory cortex to stimuli below threshold for conscious sensation. *Science* **158**, 1597–1600.

Massimini M, Rosanova M & Mariotti M (2003). EEG slow (approximately 1 Hz) waves are associated with nonstationarity of thalamo-cortical sensory processing in the sleeping human. *J Neurophysiol* **89**, 1205–1213.

Nuñez A & Buno W (1999). In vitro electrophysiological properties of rat dorsal column nuclei neurons. *Eur J Neurosci* **11**, 1865–1876.

Reboreda A, Sanchez E, Romero M & Lamas JA (2003). Intrinsic spontaneous activity and subthreshold oscillations in neurones of the rat dorsal column nuclei in culture. *J Physiol* **551**, 191–205.

Rosanova M & Timofeev I (2002). Cellular mechanisms of sleep related modulation of somatosensory evoked potentials. *Abstract Viewer and Itinerary Planner*, Program No. 840.7. Washington DC: Society for Neuro*science*, 2002. Online.

Rose JE & Mountcastle VB (1952). The thalamic tactil region in the rabbit and cat. *J Comp Neurol* **97**, 441–489.

Sachdev RNS, Ebner FF & Wilson CJ (2004). The effect of sub-threshold up and down states on the whisker evoked response in somatosensory cortex. *J Neurophysiol* **92**, 3511–3521.

Singer W & Gray CM (1995). Visual feature integration and the temporal correlation hypothesis. *Annu Rev Neurosci* **18**, 555–586.

Steriade M (2001). *The Intact and Sliced Brain*. MIT Press, Cambridge, Massachusetts.

Steriade M, Contreras D, Dossi RC & Nuñez A (1993*a*). The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci* **13**, 3284–3299.

Steriade M, Jones EG & Llinas R (1990). *Thalmic Oscillations and Signaling*. John Wiley & Sons, New York.

Steriade M, Jones EG & McCormick DA (1997). *Thalamus: Organization and Function*, vol. 1. Elsevier Science Ltd, Oxford.

Steriade M, Nuñez A & Amzica F (1993*b*). Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillations and other sleep rhythms of electroencephalogram. *J Neurosci* **13**, 3266–3283.

Steriade M, Nuñez A, Amzica F (1993*c*). A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci* **13**, 3252–3265 (1998).

Steriade M, Timofeev I, Dürmüller N & Grenier F (1998). Dynamic properties of corticothalamic neurons and local cortical interneurons generating fast rhythmic (30–40 Hz) spike bursts. *J Neurophysiol* **79**, 483–490.

Steriade M, Timofeev I & Grenier F (2001). Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol* **85**, 1969–1985.

Swadlow HA, Gusev AG & Bezdudnaya T (2002). Activation of a cortical column by a thalamocortical impulse. *J Neurosci* **22**, 7766–7773.

Timofeev I, Bazhenov M, Sejnowski T & Steriade M (2001*a*). Contribution of intrinsic and synaptic factors in the desynchronization of thalamic oscillatory activity. *Thalamus 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. *J Physiol* **494**, 265–278.

Timofeev I, Grenier F & Steriade M (2001*b*). Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: An intracellular study. *Proc Natl Acad Sci U S A* **98**, 1924–1929.

Timofeev I & Steriade M (1996). Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *J Neurophysiol* **76**, 4152–4168.

Varela F, Lachaux JP, Rodriguez E & Martinerie J (2001). The brainweb: phase synchronization and large-scale integration. *Nat Rev Neurosci* **2**, 229–239.

Wilson CJ (1986). Postsynaptic potentials evoked in spiny neostriatal projection neurons by stimulation of ipsilateral and contralateral neocortex. *Brain Res* **367**, 201–213.

© The Physiological Society 2004

Wilson C, Chang HT & Kitai ST (1983). Disfacilitation and long-lasting inhibition of neostriatal neurons in the rat. *Exp Brain Res* **51**, 227–235.

Wilson CJ & Kawaguchi Y (1996). The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J Neurosci* **16**, 2397–2410.

Yamamoto T, Samejima A & Oka H (1988). Short latency activation of local circuit neurons in the cat somatosensory cortex. *Brain Res* **461**, 199–203.

Yamamoto T, Samejima A & Oka H (1990). The mode of synaptic activation of pyramidal neurons in the cat primary somatosensory cortex: an intracellular HRP study. *Exp Brain Res* **80**, 12–22.

Zhu JJ & Connors BW (1999). Intrinsic firing patterns and whisker-evoked synaptic responses of neurons in the rat barrel cortex. *J Neurophysiol* **81**, 1171–1183.

Acknowledgements

This study was supported by The Canadian Institutes of Health Research, The Canadian Foundation for Innovation and by the University of Milan. We would also like to thank Drs S. Crochet and M. Steriade for their comments on the manuscript, P. Giguère for excellent technical assistance and S. Chauvette for taking part in one experiment.

© The Physiological Society 2004