Modulation of synaptic transmission in neocortex by network activities

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Abstract

Neocortical neurons integrate inputs from thousands of presynaptic neurons that fire in vivo with frequencies that can reach 20 Hz. An important issue in understanding cortical integration is to determine the actual impact of presynaptic firing on postsynaptic neuron in the context of an active network. We used dual intracellular recordings from synaptically connected neurons or microstimulation to study the properties of spontaneous and evoked single-axon excitatory postsynaptic potentials (EPSPs) in vivo, in barbiturate or ketamine–xylazine anaesthetized cats. We found that active states of the cortical network were associated with higher variability and decrease in amplitude and duration of the EPSPs owing to a shunting effect. Moreover, the number of apparent failures markedly increased during active states as compared with silent states. Single-axon EPSPs in vivo showed mainly paired-pulse facilitation, and the paired-pulse ratio increased during active states as compare to silent states, suggesting a decrease in release probability during active states. Raising extracellular Ca\(^{2+}\) concentration to 2.5–3.0 mm by reverse microdialysis reduced the number of apparent failures and significantly increased the mean amplitude of individual synaptic potentials. Quantitative analysis of spontaneous synaptic activity suggested that the proportion of presynaptic activity that impact at the soma of a cortical neuron in vivo was low because of a high failure rate, a shunting effect and probably dendritic filtering. We conclude that during active states of cortical network, the efficacy of synaptic transmission in individual synapses is low, thus safe transmission of information requires synchronized activity of a large population of presynaptic neurons.

Introduction

The synaptic connectivity in the neocortex is very dense. Each pyramidal cell receives 5000–60 000 synapses (Cragg, 1967; DeFelipe & Farinas, 1992). Local-circuit synapses have been estimated to account for as many as 70% of the synapses present in some areas of the cortex (Szentagothai, 1965; Gruner et al., 1974), and pyramidal cells constitute 70–80% of the total number of neocortical neurons (DeFelipe & Farinas, 1992). A given intracortical excitatory presynaptic axon forms 1–8 synaptic contacts with postsynaptic neurons (Markram et al., 1997a; Krimer & Goldman-Rakic, 2001) that elicit excitatory postsynaptic potentials from 0.1 to 10 mV, with a total mean of about 1 mV (Thomson et al., 1995; Buhl et al., 1997; Markram et al., 1997a; Feldmeyer et al., 1999; Krimer & Goldman-Rakic, 2001). The firing rate of cortical pyramidal neurons in waking and naturally sleeping animals can attain 20 Hz (Hubel, 1959; Steriade et al., 2001) and single-axon action potentials at physiological frequencies and temperature are transmitted reliably (Raastad & Shepherd, 2003). These factors should create a tremendous synaptic bombardment onto postsynaptic neurons, affecting reliability of unitary responses.

In cortical pyramidal neurons, each synapse contains one active zone with 2–20 docked vesicles (Harris & Seltan, 1995; Schikorski & Stevens, 1997, 1999). Some in vitro studies indicate that at most a single vesicle can be released in response to an action potential (Triller & Korn, 1982; Redman, 1990; Stevens & Wang, 1995; Auger & Marty, 2000; Hanse & Gustafsson, 2001), whereas others found evidence for multiple quantal release (Tong & Jahr, 1994; Auger et al., 1998; Isaac et al., 1998; Wadiche & Jahr, 2001; Oertner et al., 2002; Conti & Lisman, 2003). In any case, only one or few vesicles could activate a postsynaptic neuron when a presynaptic cell fires a spike. In these conditions, changes in release probability would have a dramatic effect on postsynaptic responses. One of the critical factors regulating the vesicle release is extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_o\)). The baseline [Ca\(^{2+}\)]\(_o\) in vivo is around 1.2 mm (Heinemann et al., 1977) and decreases with an increase in the level of neocortical activity (Heinemann et al., 1977; Massimini & Amzica, 2001). Therefore, we hypothesized that the efficacy of synaptic transmission decreases when the level of network activity increases. We found that active states in the cortical network are accompanied by increased failures of synaptic transmission, which depend on the modulation of [Ca\(^{2+}\)]\(_o\) and an important decrease in the postsynaptic response owing to a shunting effect. This provides an effective attenuation of postsynaptic excitation that controls the postsynaptic impact of presynaptic firing.

Materials and methods

Preparation

Pairs of synaptically connected cells were recorded intracellularly from the intact neocortex (areas 5 and 7) of cats under pentobarbital (30–35 mg/kg) or ketamine–xylazine (10–15 and 2–3 mg/kg i.m., respectively) anaesthesia, or from small isolated cortical slabs of cats anaeasthetized with ketamine–xylazine. Neocortical slabs were prepared as previously described (Timofeev et al., 2000). The
electroencephalogram (EEG) was monitored continuously during the experiments and additional doses of the same anaesthetic were given at the slightest tendency toward an activated EEG pattern. All pressure points and the tissues to be incised were infiltrated with lidocaine (0.5%). The animals were paralysed with gallamine triethiodide (20 mg/kg) and artificially ventilated while monitoring the end-tidal CO₂ concentration at 3.5–3.8% as well as the electrocardiogram (acceptable range, 90–110 beats/min). Body temperature was maintained at 37–38 °C. The stability of intracellular recordings was ensured by cisternal drainage, bilateral pneumothorax, and by filling the hole made for recordings with a solution of 4% agar. Experimental procedures were carried out in accordance with the Guidelines laid down by the NIH and approved by the committee for animal care of Laval University.

Stimulation, recording and microdialysis

For experiments using microstimulation (see Fig. 8A), an array of 5–7 tungsten microelectrodes (125 μm shank diameter, 9–12 MΩ impedance; FHC Bowdoinham, USA) was inserted into the cortex with an angle of 40° in the vicinity (< 0.5 mm) of the recording intracellular micropipette. The distance between the tips of neighbouring electrodes in the array was ~300 μm. Microstimuli (squared pulse, 0.03–0.1 mA, 0.01–0.02 ms) were applied every 2 s between two adjacent microelectrodes in order to produce minimal and short latency responses. The intracellular recordings were obtained using glass micropipettes filled with 3 M potassium acetate (DC resistance, 30–70 MΩ). In most cases, neurobiotin (1.5–2%; Vector Laboratories, Inc., Burlingame, CA, USA) was dissolved in the solution that filled the pipette. A high-impedance amplifier (band-pass, 10 kHz) with an active bridge circuitry was used to record and to inject currents into the cells. Immediately after impairment a small negative current was injected into the neurons. In the vast majority of the cases, the holding current was removed after stabilization of the recording. All electrical signals were digitized online with a sample rate of 20 kHz. Changes in [Ca²⁺], in the cortex in vivo was achieved using a reverse microdialysis method. The membrane of the microdialysis probe (2 mm length, 0.22 mm diameter; EICOM, Kyoto, Japan) was inserted into the cortex and the recording micropipettes were placed 0.2–0.3 mm from the membrane. The microdialysis probe was perfused with the following solutions (containing in mM): control (NaCl, 124; KCl, 2.5; NaHCO₃, 26; NaH₂PO₄, 1.25; MgSO₄, 2; MgCl₂, 1; CaCl₂, 1); high calcium (NaCl, 124; KCl, 2.5, NaHCO₃, 26, NaH₂PO₄, 1.25, MgSO₄, 2, MgCl₂, 0, CaCl₂, 5), Calcium free (NaCl 125, KCl, 2.5, NaHCO₃, 26, NaH₂PO₄, 0, MgSO₄, 2, MgCl₂ 1, CaCl₂ 0, MnCl₂ 1). The perfusion velocity was 5 μL/min and the total volume of tubing from the liquid switch to the probe was 12 μL. In previous studies the tissue concentration and the diffusion of different drugs have been estimated, respectively, at 10% of concentration of the perfused solution at 0.5–1 mm around the probe membrane (Juhasz et al., 1989; Quan & Blattein, 1989; Crochet & Sakai, 1999). However, to the best of our knowledge, this study is the first attempt ever to modify the concentration of an endogenous ion in vivo. To validate the method, we measured the actual [Ca²⁺], at the recording site by means of Ca²⁺ sensitive microelectrodes (Diamond General, Ann Arbor, MI, USA). To avoid a thermocoupling effect, the calibration of electrodes was carried out in artificial cerebrospinal fluid (ACSF) at 37–38 °C. The tip of the Ca²⁺-sensitive electrode was quite large (2–3 μm in diameter) and thus did not make possible the measurement of the actual [Ca²⁺] at synaptic cleft. The change in [Ca²⁺], levels in the cortex and its physiological effects started to occur at 8–10 min after switching the microdialysis probe perfusion from one solution to another. The dialysed solutions were applied for 30–40 min, a time that was probably sufficient for diffusion to the synaptic clefts.

Analysis

An initial screening of simultaneously recorded neuronal pairs was done during the experiment to assess possible synaptic connection. About one-third of simultaneously impaled neuronal couples, the ones in which we assumed the presence of a synaptic connection were recorded for off-line analysis. To establish definitely synaptic connection between simultaneously recorded neurons, the spike-triggered averages (STA) were computed off-line. We considered the pair of neurons to be linked via an excitatory connection when the STA, computed from the action potentials (APs) of the presynaptic neuron, revealed a depolarizing response in the postsynaptic neuron that followed the presynaptic spike. In experiments with microstimulation, stimulus-triggered averages were used to identify the responses. Averaged responses obtained from 100 to 1000 segments were used to determine the latency, time to peak and duration at half amplitude of excitatory postsynaptic potentials (EPSPs). Thereafter, the amplitude of individual responses was measured as the difference in voltages between the beginning and the peak of the response taken at the same times, as in averaged response (see dotted lines in Fig. 3B). To compare the distribution of the amplitude of the response with that of the background synaptic noise, we also measured in the same way the amplitude for the period immediately preceding the presynaptic spike or microstimulus.

In order to better characterize the effects of network activities on the effectiveness of the synaptic transmission we distinguished presynaptic stimuli that induced a response detectable at the soma level (successful responses) from those that did not (apparent failures). For the detection of apparent failures, we first estimated the jitter of the latency of the responses during silent periods, where the distinction between responses and failures was clear. We then determined a time window after the presynaptic spike or microstimulus during which the response is expected to occur. Then we screened and classified each individual segment as a ‘response’ or ‘failure’ as following: we obtained the first derivative of each segment after smoothing by averaging each pair of successive values. The responses were characterized by a positive peak in the differentiated trace in the established time window (see Fig. 3B). The smoothing of original trace was an essential procedure, which enabled us to reduce a high frequency electronic noise originating from amplifiers and acquisition system. To be sure that only failures were selected, we compared the averaged failures with the average obtained from random reference times (compare Random average in Fig. 2C with Failure average in Fig. 3B). We also checked that the distribution of amplitude measured for the failures match that of background synaptic noise (see Fig. 3C).

Because the detection of failures in individual trials can be problematic during periods of intense background synaptic activity, we also used a statistical approach to estimate the failure rates from the distribution of amplitude of spike- or stimulus-triggered events. We assumed that triggered events are composed of failures and responses (i.e. EPSPs) and that the distribution of failures amplitude matches that of the background noise. Both triggered event and background noise amplitudes were measured in the same way as previously described. We also assumed that the amplitude of responses, as measured here, should be mostly positive, thus the contribution of responses in the negative range of the histogram of amplitude should be negligible and the negative range of the histogram should be mainly composed of
failures. We therefore fitted the noise amplitude histogram with a Gaussian curve and the amplitude of the resulting curve was adjusted automatically so that it matches the negative part of the triggered event (total) amplitude histogram. The final Gaussian curve then represents the distribution of amplitude of the failures (see Fig. 3D, fitting failures). The total amplitude histogram was then smoothed to extract the envelope of the histogram (see Fig. 3D, fitting total) and we subtracted the failures fitting to the total smoothed histogram to obtain the response amplitude distribution (see Fig. 3D, fitting responses). The failure rate was calculated as the ratio between the area of the Gaussian fitting of the failures and the total smoothed histogram (see Fig. 3D). As a control, we applied this method to responses to short intracellular current pulses that mimic an EPSP and never failed (see Fig. 5), as well as randomized reference time triggered events (100% failures; see Fig. 2C). The failure rates calculated from the amplitude histograms were less than 3% and more than 98%, respectively.

For the analysis of spontaneous synaptic activity, intracellular recordings were filtered off-line between 0.1 Hz and 600 Hz to eliminate high-frequency noise. All spontaneous depolarizing events with amplitude higher than 0.2 mV were then extracted automatically using a custom-written routine under IgorPro software. Briefly, the events were detected as a positive peak in the first derivative of the intracellular signal; the depolarizing slope of each event was then fitted with a sigmoid function. The detection of nonmonotony in the rising phase allowed the discrimination of compound events seen as single peak (see Fig. 9). The amplitude was calculated as the difference in voltage between the onset and the maximal depolarization of the detected event. The maximal slope was determined as the peak of the first derivative of the sigmoid function that best fitted the event.

All numerical data are expressed as mean ± SD.

Results

To study the properties of responses elicited by a single presynaptic axon we performed simultaneous intracellular recordings from pairs of synaptically coupled cortical neurons in vivo. Out of more than 1000 simultaneously recorded pairs of neurons, we obtained 35 recordings from pairs linked by excitatory connections. Using STA, the synaptic connectivity was determined by the ability of APs in presynaptic neurons to induce EPSPs in postsynaptic neurons. All recorded neurons were identified by electrophysiological criteria (McCormick et al., 1985; Gray & McCormick, 1996; Steriade et al., 1998), and some of them (n = 7 pairs) were also intracellularly stained with neurobiotin (Figs 1 and 2). In barbiturate-anaesthetized cats the mean membrane potential was −73.7 ± 0.8 mV and the input resistance during interspindle lulls was 28.4 ± 1.3 MΩ; in ketamine-xyazine anaesthetized cats the mean membrane potential was −71.2 ± 0.7 mV (input resistance 25.5 ± 1.3 MΩ) during silent phases of the slow oscillation and −63.5 ± 0.5 mV (input resistance 15.4 ± 2.4 MΩ) during active phases.

Morphological characteristics of recorded cell pairs

A majority of paired recordings from synaptically connected neurons (33/35) were performed within the first 600 μm from the cortical surface suggesting that these recordings were from layer II–III neurons (Hassler & Muhs-Clement, 1964). Of seven stained neuronal pairs, we made recordings from one pyramid to double-bouquet pair, one pyramid to large basket cell pair, one pyramid to small basket cell pair, and one large basket cell to large basket cell pair, and the

Fig. 1. Postsynaptic impact of spontaneous presynaptic firing. (A) Two synaptically connected neurons were aspiny nonpyramidal fast-spiking interneurons (red) and pyramidal regular-spiking (blue) from area 5 of the cat suprasylvian cortex. Distance between neurons corresponds to the scale. The Neurolucida reconstruction was made from three successive 80-μm sections and the bodies of two neurons were located on two consecutive sections. (B) A fragment of spontaneous activity of these neurons during an active period of the cortical network. The first presynaptic spike of pyramidal neuron elicited an EPSP in the postsynaptic interneuron, whereas the second failed to elicit a response. (C) Spike-triggered average (STA) of postsynaptic response (n = 160). (D) Histograms of synaptic noise (grey) and amplitude of postsynaptic spike-triggered events (responses and failures, red) amplitudes (bin 0.1 mV). Note the presence of a significant number of failures in responses of postsynaptic neuron to presynaptic spikes. (E) Histogram of mean amplitude and (F), histogram of mean duration at half amplitude for 35 synaptically connected neurons.

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Fig. 2. Modulation of single-axon EPSPs by network activities. A period of spontaneous network activity in which the presynaptic neuron was stimulated by intracellularly applied current pulses to elicit firing during active and silent states of network. (B) Microphotograph of a fragment of the axon of the presynaptic neuron that passes through the dendritic tree of the postsynaptic neuron and three-dimensional Neurolucida reconstruction of these two neurons from five successive sections. Distance between neurons corresponds to the scale. The body of one neuron and a part of the body of the second neuron were located on the same section and the estimated space between them in three dimensions was 80 µm (C). Examples of individual responses during active (left) and silent (middle) periods. Below, histograms of amplitude of spike-triggered events (red) and noise (grey) (bin 0.1 mV) during active and silent states. The right panel shows the random time average and the spike-triggered averages (STA) of all, and only successful, responses of postsynaptic neuron to spikes elicited in the presynaptic neuron. Note, much higher variability of responses during active states (CV = 2.19) as compared to silent states (CV = 1.15).
remaining three connections were pyramid to pyramid pairs. In one case, there was a reciprocal excitatory-inhibitory connection (pyramid to small basket cell). The inhibitory responses are not analysed in the present study. The increase in failure rates during active network states reported below was found in all types of excitatory connections. In two of the stained pairs we were able to trace in detail the local arborizations of presynaptic axon. In both cases the bright field images showed a close proximity of presynaptic axon and postsynaptic dendrites, which formed three putative synapses. Thus, we assume that most, if not all, single-axon EPSPs recorded in this study were generated by several synapses.

Properties of spontaneous single-axon EPSPs

To characterize the properties of single-axon EPSPs, we dissociated for each connection the successful responses from apparent failures (see Materials and methods). Figure 3 illustrates an example of the dissociation between successful responses and apparent failures in a pair of neurons recorded under ketamine–xylazine anaesthesia. The accuracy of the distinction between responses and failures was confirmed by the absence of response in the failure average and the distribution of failure amplitude that matched that of synaptic noise, whereas the distribution of response amplitude was clearly distinct.

Spontaneous APs occurred in presynaptic neurons exclusively during active network states. Single-axon EPSPs elicited by spontaneous APs were characterized by a small amplitude and a short duration. Overall the postsynaptic impact of presynaptic firing was small with a mean amplitude for successful responses of $0.71 \pm 0.31$ mV and a mean duration at half amplitude of $10.7 \pm 3.7$ ms (Fig. 1). However, the characteristics of single-axon EPSPs were highly variable from connection to connection, with a mean amplitude that varied in the range $0.2$–$1.5$ mV and a duration at half amplitude that varied from $6.5$ to $16.8$ ms ($n = 33$; Fig. 1E and F). In addition, single-axon EPSPs also showed a strong trial to trial variability for a given connection. The same neuron responded to spikes of the same presynaptic neuron with EPSPs that ranged from $6.5$ to $16.8$ ms ($n = 33$; Fig. 1E and F). In both cases the bright field images showed a close proximity of presynaptic axon and postsynaptic dendrites, which formed three putative synapses. Thus, we assume that most, if not all, single-axon EPSPs recorded in this study were generated by several synapses.

Properties of evoked single-axon EPSPs

We hypothesized that the high activity in the cortical network in vivo creates conditions responsible for the variability and failures of cortico-cortical synaptic transmission. To compare postsynaptic responses during silent network states with those during active network states, we stimulated presynaptic neurons with depolarizing current pulses (1 per second) to elicit their firing during hyperpolarizing (silent states) and depolarizing (active states) phases of the cortical slow oscillation (Steriade et al., 1993) under ketamine–xylazine anaesthesia (Fig. 2) or during spindles (active states) and interspindle lulls (silent states) under barbiturate (pentobarbital) anaesthesia (not shown). In eight pairs of neurons of cats anaesthetized with ketamine–xylazine, we were able to stimulate intracellularly the presynaptic neuron and elicit its firing during both silent (mean duration 200 ms) and active network states (mean duration 900 ms). We found that the EPSPs elicited during the silent periods were characterized by a lesser variability than those elicited during active periods, as shown by the narrower amplitude distribution (Fig. 2C) and by approximately 50% smaller CV (Table 1). The increase in the CV of single-axon EPSP during active network states was found in all connected pairs. In addition, the mean evoked EPSPs were reduced in amplitude and duration during active states as compared with silent states. The mean amplitude of successful single-axon EPSPs elicited by intracellular stimulation of the presynaptic neuron was $0.96 \pm 0.26$ mV during silent states and decreased to $0.73 \pm 0.35$ mV during active states, which did not differ statistically from the single-axon EPSPs elicited by spontaneous presynaptic firing (see above). Thus, the mean amplitude of successful responses (failures excluded) during active phases was reduced by 32% (range 20–50%, $n = 8$) as compared with responses elicited during silent periods (see STAs in Fig. 2). The EPSPs elicited during active periods also showed a significant (Student’s paired $t$-test; $P < 0.001$) decrease in their duration as compared with those elicited during silent periods, from $10.3 \pm 3.9$ to $5.9 \pm 2.2$ ms (Fig. 2C). Interestingly, the number of apparent failures was also strongly affected by network states. The mean failure rate estimated by statistical analysis during the silent
periods was 32 ± 7% (range 10–50%) and markedly increased during the active periods to 63 ± 11% (range 20–79%).

To study how the duration of silent periods affects the properties of single-axon EPSPs, we performed experiments in isolated neocortical slabs, in which silent periods last for tens of seconds (Timofeev et al., 2000). Four out of 126 pairs of neurons were synaptically connected. During silent states of the network, firing was elicited in the presynaptic neuron by intracellular injection of steady depolarizing current (Fig. 4). Responses during silent periods in isolated cortical slabs appeared to be more reliable than in the intact cortex. The CV of

Properties of single-axon EPSPs in vivo

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Results given as mean ± SD (range given in parentheses).

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<th>Single-axon responses</th>
<th>Microstimulation responses</th>
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<td>Slab (silent)</td>
<td>Intact cortex (silent)</td>
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<tr>
<td>( n = 4 )</td>
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<tr>
<td>( 0.32 ± 0.05 )</td>
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<tr>
<td>Slab (active)</td>
<td>Intact cortex (active)</td>
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<td>( n = 4 )</td>
<td>( n = 33 )</td>
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<tr>
<td>( 1.15 ± 0.31 )</td>
<td>( 1.57 ± 0.40 )</td>
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<td>( 0.80–1.51 )</td>
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<td>Intact cortex (silent)</td>
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<td>( 0.45 ± 0.13 )</td>
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Results given as mean ± SD (range given in parentheses).

Single-axon EPSPs was similar to the higher values reported for excitatory connections in vitro (Thomson, 1997) and it was lower than the CV during silent periods of slow oscillation (Table 1). The amplitude of responses showed a lesser trial to trial variability for a given connection (from failure to 2.5 mV) and the failure rate was significantly lower than in the intact cortex (3–20%). The mean amplitude of successful single-axon EPSPs in the four pairs was between 0.6 and 1.4 mV (total mean 0.9 ± 0.1 mV). In these experiments, active network states were elicited by low-intensity electrical stimulation applied to the cortex. The STAs revealed that during active states the amplitude of the EPSP evoked by the same axon was 0.6 ± 0.2 mV. The duration at half amplitude of the single-axon EPSP was also markedly reduced from 17.4 ± 3.5 ms during silent periods to 5.0 ± 0.9 ms during active states (Fig. 4). As observed in the intact cortex, the decrease in amplitude of averaged EPSPs during active states was mainly due to the presence of a high number of failures, as revealed by the peak around 0 mV in the histogram of EPSP amplitude, and to a minor extent to the decrease in amplitude of successful EPSPs, as disclosed by the left shift of the peak of the amplitude histogram (Fig. 4).

To characterize further the network effects on synaptic responses we used microstimulation \((n = 31)\) in cats under barbiturate anaesthesia. The intensity of microstimulation was adjusted to obtain minimal responses. A decrease in the intensity of stimulation by 1–2% resulted in a complete abolition of the response. This criterion indicates a high likelihood, but does not guarantee that only a single presynaptic axon was activated. Monosynaptic responses were established on the basis of their short (< 2 ms, mean 1.39 ± 0.39 ms) and relatively fixed latency (jitter < 0.3 ms). Similarly to responses on single-axon stimuli, the responses to microstimuli were highly variable in amplitude (from failures to 4.1 mV). The amplitude of the responses and their duration during active states varied to a greater extent than during silent periods (Table 1) and the averaged response during active periods was of much smaller amplitude and duration than during silent network state (Fig. 5).

Two factors could contribute to the diminution of averaged responses during active states. The first factor is a shunting inhibition induced by the important increase in conductance during the active network states (Borg-Graham et al., 1998; Hirsch et al., 1998). Indeed, the input resistance of cortical neurons significantly decreases during active network states (Contreras et al., 1996; Paré et al., 1998) and this would be a sufficient factor accounting for the decrease in the amplitude and duration of the EPSPs. The second factor is an increase in the number of failures. To assess the relative effects of shunting and increased failure rate on the response during active states, we compared microstimuli-evoked responses \((n = 15)\) with responses to short depolarizing current pulses that mimic an EPSP \((n = 6)\). We then compared the averaged synaptic responses excluding failures during active and silent periods with the averaged responses to current pulses (Fig. 5B and C). We found that the major decrease in amplitude of the total mean response during active states was due to an increased number of failures (Fig. 5C). In fact, the failure rate during active states \((37.8 ± 21\%); range 21–72\%\) was significantly higher than during silent states \((13.3 ± 13\%); range 0–33\%) \((n = 15, P < 0.001; \text{Student’s paired } t\)-test), whereas the decrease in amplitude of successful responses was not significant: \(1.74 ± 1.05 \text{ mV} (\text{range } 0.73–3.96 \text{ mV})\) during silent periods vs. \(1.38 ± 0.63 \text{ mV} (\text{range } 0.68–2.47 \text{ mV})\) during active periods \((P > 0.05; \text{Fig. 5D})\). In good agreement with these observations, the response to short current pulses was only slightly decreased during active states. Averaging all synaptic responses (successful and failures) the decrease in the mean response during active periods was significant \([1.71 ± 0.11 \text{ (silent states) vs. } 1.14 ± 0.62 \text{ (active states); } P < 0.01, \text{Student’s paired } t\)-test\]. Overall, the decrease in amplitude of successful EPSPs during active network states was 20.7 ± 32.0% and the decrease in amplitude of the responses to current pulses was 13.1 ± 8.4%. The shunting effect produced by network activities mainly affected the duration of the responses to both synaptic activation and current pulse. The duration at half amplitude of successful synaptic responses decreased significantly from 19.4 ± 9.3 ms during silent states, to 11.4 ± 6.2 ms during active states (Student’s paired \(t\)-test, \(P < 0.001; \text{Fig. 5D})\). The decrease in duration of responses to both synaptic volleys and current pulses measured at half-amplitude was similar \((41.1 ± 15.4\% \text{ and } 34.4 ± 14.2\%, \text{respectively})\).

An increase in apparent failures during active states may reflect a decrease in release probability at the presynaptic site as well as a decrease in dendritic excitability that would circumvent the propagation of the EPSPs to the soma. To investigate the change in release probability during active states, we used a paired-pulse protocol \((n = 14)\). The paired-pulse ratio reflects the release probability at the synapse (Stevens & Wang, 1995; Debanne et al., 1996). Paired stimuli were thus given every 2 s, with an interstimulus interval of 20 ms. During silent states, paired-pulse facilitation was found at most of the connections tested \((9/14)\) while the other connections showed depression \((4/14)\) or no change \((1/14)\). During active states, the average EPSPs were markedly decreased as compared with silent states and almost all the connections \((13/14)\) showed paired-pulse facilitation. Overall, the paired-pulse ratio increased for all connections from silent to active states. The mean paired-pulse ratio was 1.27 ± 0.38 during silent states and significantly increased to 2.08 ± 0.85 during active network states (Student’s paired \(t\)-test, \(P < 0.001; \text{Fig. 6})\), thus indicating a decrease in release probability during active states.

\([\text{Ca}^{2+}]_o\) modulates synaptic activity

What caused a higher failure rate during active states of the network?

As previously demonstrated (Massimini & Amzica, 2001) and confirmed in the present study (see Fig. 7B), \([\text{Ca}^{2+}]_o\) in vivo decreases by approximately 20% during active states of the network.
effects of \([\text{Ca}^{2+}]_o\) fluctuations on synaptic transmission, we per-
network states might contribute to a high failure rate. To study the
active state of the network (red). The bin of the histogram is 0.2 mV.

We thus hypothesized that a relatively low \([\text{Ca}^{2+}]_o\) during active
network states might contribute to a high failure rate. To study the
effects of \([\text{Ca}^{2+}]_o\) fluctuations on synaptic transmission, we per-
formed two types of experiments. First, in ketamine
\([\text{Ca}^{2+}]_o\) (Fig. 7). Similarly to experiments performed under
barbiturate anaesthetized cats we measured the modulation of response ampli-
tude elicited by microstimulation in parallel to the fluctuations of
\([\text{Ca}^{2+}]_o\) (n = 9, Fig. 7). Similarly to experiments performed under
barbiturate anaesthesia, the amplitude and duration of responses
during active network states were reduced as compared with silent
network states (Fig. 7A). Measurements of individual responses demonstrated: (i) a progressive increase of their amplitude starting from the onset of the silent state, as estimated from the onset of the depth-positive field potential and neuronal hyperpolarization; (ii) an abrupt decrease in EPSPs' amplitude at the onset of the active state; and (iii), a recovery of the response amplitude at later states. The progressive increase in EPSPs' amplitude during silent state occurred in parallel with an increase in the \([\text{Ca}^{2+}]_o\) (Fig. 7B and C). The decrease in the EPSPs' amplitude at the onset of active state was probably owing to both a decrease in the input resistance of the neuron and a decrease in the \([\text{Ca}^{2+}]_o\). As the time constant of \(\text{Ca}^{2+}\) electrodes was long (up to hundreds of milliseconds) the exact time-
course of the changes in \([\text{Ca}^{2+}]_o\) was certainly underestimated and the decrease in the \([\text{Ca}^{2+}]_o\) might have occurred immediately after the onset of the active state.

In order to provide further evidences of the involvement of
\([\text{Ca}^{2+}]_o\) fluctuations in the higher failure rate during active network states, we combined microstimulation with microdialysis of ACSF containing different \([\text{Ca}^{2+}]_o\), and direct \([\text{Ca}^{2+}]_o\) measurements with
\(\text{Ca}^{2+}\) sensitive electrodes (Fig. 8, see methods for the composition of ACSF) in barbiturate anæsthetized cats. Perfusion of the control solution (1.0 mM \(\text{Ca}^{2+}\)) yielded a free \([\text{Ca}^{2+}]_o\) of 1.1–1.2 mM (Fig. 8D); the high \(\text{Ca}^{2+}\) solution (5.0 mM \(\text{Ca}^{2+}\)) raised the \([\text{Ca}^{2+}]_o\), to 2.5–3.0 mM and the \(\text{Ca}^{2+}\) free solution lowered the \([\text{Ca}^{2+}]_o\), to 0.7–
0.8 mM. Microstimulation-evoked responses (n = 7) were compared for the three conditions when \([\text{Ca}^{2+}]_o\) reached steady levels (Fig. 8D). The total averaged response was increased in high
calcium condition and decreased in low calcium condition (Fig. 8B).

As shown by the histograms of response amplitude (Fig. 8C), both the amplitude of successful responses and the failure rate were affected by changes in \([\text{Ca}^{2+}]_o\). In control condition, the mean amplitude of successful EPSPs was 0.83 ± 0.85 mV and the overall failure rate was 42 ± 16%. Raising \([\text{Ca}^{2+}]_o\) increased the amplitude of EPSPs to 1.23 ± 1.06 mV, and decreased failure rates to 23 ± 14%. Lowering \([\text{Ca}^{2+}]_o\) reduced the amplitude of EPSPs to 0.49 ± 0.36 mV and increased failure rate to 67 ± 17%. Paired t-test revealed that these differences were significant at \(P < 0.05\). Additionally, in some occasions we observed a bimodal distribution of the amplitude of successful responses (see control in Fig. 8C). Invariantly, the second peak of histogram increased in high \([\text{Ca}^{2+}]_o\), condition and was abolished in low \([\text{Ca}^{2+}]_o\), condition. These data suggest that presynaptic stimuli in control condition could activated one or several release sites; increasing \([\text{Ca}^{2+}]_o\) increased the probability of simultaneous activation of two or several release sites and lowering \([\text{Ca}^{2+}]_o\) allowed activation of maximum one release site per stimulus.

To evaluate further the postsynaptic effects of presynaptic firing, we analysed the spontaneous synaptic activities under various \([\text{Ca}^{2+}]_o\) conditions in barbiturate anaesthetized cats (Fig. 9). From intracellular recordings we automatically extracted all spontaneous depolarizing events (presumably EPSPs) that were more than 0.2 mV in amplitude (n = 7, Fig. 9). We found that the mean frequency of spontaneous depolarizing events in control conditions was 177.60 ± 41.1 Hz, their mean amplitude was 0.43 ± 0.09 mV and their maximal rising slope was 0.68 ± 0.24 V/s (Fig. 9). An increase in \([\text{Ca}^{2+}]_o\), raised the frequency of detected events to 208.2 ± 37.7 Hz, increased their amplitude to 0.60 ± 0.14 mV and their maximal rising slope to 0.85 ± 0.23 V/s (Fig. 9). A decrease in \([\text{Ca}^{2+}]_o\), had the opposite effects; the mean frequency of detected events decreased to 145.2 ± 26.5 Hz, the mean amplitude and the mean maximal slope decreased, respectively, to 0.35 ± 0.09 mV and 0.59 ± 0.20 V/s (Fig. 9). The histograms of amplitude and maximal slope distribution in the three \(\text{Ca}^{2+}\) conditions (n = 7) show that the changes in \([\text{Ca}^{2+}]_o\), shifted the distribution of amplitude and maximal slope of detected events (Fig. 9D and E). We found that increasing \([\text{Ca}^{2+}]_o\), led to a significant decrease in the number of low amplitude and slow events and a significant increase in number of high amplitude and fast events, whereas lowering \([\text{Ca}^{2+}]_o\), had the opposite effect (Fig. 9D and E). The presence of faster rising events in high \([\text{Ca}^{2+}]_o\), conditions implies a higher probability of simultaneous activation of different release sites (Berger & Luscher, 2003). In our experimental conditions the major part of this phenomenon was probably due to a higher release probability at different release sites activated by the same presynaptic axon. This suggests that in high \(\text{Ca}^{2+}\) conditions, the postsynaptic neuron sensed inputs from almost the same number of presynaptic neurons, but the postsynaptic impact of individual presynaptic spikes was higher.
The major finding of this study is that spontaneous network activities in vivo significantly modulate both the synaptic transmission and the postsynaptic sensitivity to presynaptic volleys. The active network states, during which neurons usually fire spontaneously, were associated with an increased failure rate. The postsynaptic impact of single-axon EPSPs was additionally decreased via shunting effects, creating a higher variability of responses as opposed to silent network states. Thus, variability and failures of synaptic transmission in individual synapses in cortical networks constitute an essential feature of normal cortical functions including cognitive processes, persistent activities and memory.

The variability of synaptic transmission has been shown in vitro in the hippocampus and the neocortex, and has been attributed to modulation in release probability, variable amounts of neurotransmitters filling each vesicle and ability of postsynaptic neurons to respond (Allen & Stevens, 1994; Thomson et al., 1995; Markram et al., 1997a; Petersen & Sakmann, 2000; Conti & Lisman, 2003). In addition to this variability due to inherent properties of synaptic transmission, we found that, in vivo, the degree of variability of single-axon EPSPs at the soma is high and depends on the activity of the cortical network.
Two factors appeared particularly important to account for this high variability. First, the intense synaptic bombardment received by cortical neurons during active states of the network results in membrane potential fluctuations and changing passive membrane properties (input resistance and time constant; Contreras et al., 1996; Paré et al., 1999; Rhodes & Llinas, 2001; Rudolph & Destexhe, 2003) that may account, partly, for the variability of the EPSPs at the soma; as shown by the higher variability of responses to short current pulses during active periods. Second, the failure rate of single-axon EPSPs was higher during active states than during silent states, increasing further the variability of the postsynaptic response. The CV of single-axon responses during silent periods in the neocortical slab was similar to the one in vitro; whereas during active states the important synaptic bombardment and the increase in failure rate raises the variability of single-axon EPSPs.

In addition to an increased variability, the high conductance states generated by the intense synaptic bombardment during active states also reduced markedly the duration of successful EPSPs, whereas their amplitude was only affected slightly. These effects can be attributed principally to a decrease in the input resistance and time constant in the postsynaptic neuron, and shunting inhibition (Borg-Graham et al., 1998; Hirsch et al., 1998; Destexhe et al., 2003). Changes in passive membrane properties of the postsynaptic neuron (primarily decrease in input resistance and time constant) was responsible for the decrease in the duration of EPSPs, as demonstrated by the similar effect on the responses to short current pulses, which will make the decay of the
EPSP responses to current pulses faster. In addition, inhibitory postsynaptic potentials could curtail the EPSPs during active states. The slight decrease in the amplitude of EPSPs during active states might depend also partially on the changes in membrane properties: a decrease in input resistance is expected to decrease the amplitude of the postsynaptic response. Another factor that may account for the decrease in amplitude is a decrease in the driving force at more depolarized voltages. However, we have tested the effect of depolarization on the EPSPs by injecting DC current into the cell, and we found that during silent periods, depolarizing the cell had little or no effect on the amplitude of the EPSPs and, in most of the cases, increased the duration of EPSPs (I. Timofeev and S. Crochet, unpublished data). This observation is in good agreement with similar results in cortical neurons in vitro, in which an increased duration and amplitude of EPSPs when the cell is depolarized as been ascribed to the activation of persistent Na+ current (Thomson et al., 1988; Gonzalez-Burgos & Barrionuevo, 2001). The somatic impact of remotely located synapses could be significantly facilitated by a variety of dendritic intrinsic currents at depolarized voltage (Spencer & Kandel, 1961; Benardo et al., 1982; Turner et al., 1991; Amitai et al., 1993; Magee & Johnston, 1995; Schwindt & Crill, 1995) and simultaneous or close time-related activation of several synapses (Markram et al., 1997b; Wang et al., 2000; Stuart & Hauser, 2001). Thus, the surprising finding that the amplitude of successive EPSPs was not significantly decreased during active states could results from the summation of different phenomena, which tend either to reduce their amplitude (decreased input resistance, decreased driving force) or to increased their amplitude (activation of voltage-dependent currents, dendritic action potentials).

Another important finding of the present study is that active states were associated with a marked increase in apparent failures. Mainly, two factors can be responsible for the absence of response at the soma when a presynaptic neuron fires: a failure in the synaptic transmission or a failure in the propagation of the EPSP to the soma. A decrease in input resistance and shunting inhibition during active states could limit the propagation of EPSPs to the soma, but, by contrast, the more depolarized level of membrane potential of cortical neurons during active states would facilitate an active propagation of distal EPSPs to the soma due to regenerative dendritic potentials (Destexhe et al., 2003). Thus the increased number of apparent failure of synaptic transmission during active states can not be ascribed exclusively to changes in membrane properties in the postsynaptic neuron. Furthermore, our results demonstrate that the increase in apparent failures was at least partially owing to a decrease in release probability. First, a direct influence of inhibitory activities on synaptic transmission was unlikely, because the high [Ca2+]o increased the effectiveness of both, excitatory and inhibitory activities and, in our experiments, was associated with a decrease in failure rates. Second, consistent with the residual calcium hypothesis (Katz & Miledi, 1968; Zucker & Regehr, 2002), the paired-pulse ratio increased during active states. Previous studies indirectly suggested that active network states would be associated with increased failures of synaptic transmission from a depletion of extracellular Ca2+ (Egelman & Montague, 1998; Rusakov & Fine, 2003). The modulation of synaptic transmission in active vs. silent network states greatly depended on the spontaneous fluctuations in [Ca2+]o. In vitro data show that a slight decrease in [Ca2+]o results in a significant increase in failures of synaptic transmission (Thomson et al., 1993; Thomson, 1997; Markram et al., 1998). In the connections between layer 4 spiny neurons and layer 2–3 pyramidal cells in slices from rat barrel cortex, a bath solution containing 1.0 mM [Ca2+]o, instead of 2.0 mM, led to a mean failure rate of 82% (Silver et al., 2003).

An important observation in our study is the high heterogeneity between different connections, as can be seen in the differences in amplitude, apparent failure rate and paired-pulse ratio. This indicate that some connections between cortical neurons present a much greater
Our data showing a modulation of spontaneous and evoked single-axon EPSPs by $[\text{Ca}^{2+}]_o$, suggest that usually more than one vesicle can be released by each presynaptic spike. This occurs either from multiple vesicle release from one synapse (Tong & Jahr, 1994; Auger et al., 1998; Oertner et al., 2002; Conti & Lisman, 2003) or simultaneous activation of several synapses by the same presynaptic axon and single vesicle release from each synapse (Triller & Korn, 1982; Redman, 1990; Stevens & Wang, 1995; Auger & Marty, 2000; Hanse & Gustafsson, 2001). Despite the fact that several release sites might be activated by a single presynaptic spike, the effectiveness of synaptic transmission between two connected neurons was usually low, possibly because of low release probability. Our results thus demonstrate that determination of cortical neuron output by a single presynaptic input is unlikely, but rather that only particular temporal presynaptic code (Zador, 1998) or simultaneous activity of multiple neurons would reliably transmit information to a target neuron (Allen & Stevens, 1994).

Taking into account the mean number of presynaptic axons terminating on a cortical pyramidal neurons (5 000–60 000, see Introduction), the ratio of excitatory : inhibitory synapses (70–80 : 30–20), the mean number of synapses formed by one presynaptic axon (3–5) and the mean firing rate in our experimental condition (1.8 Hz, barbiturate anaesthetized cats), each cortical neuron should be driven each second by as many as 1260–25 200 EPSPs [between (5000 × 70%/5) × 1.8 = 1260 and (60 000 × 70%/3) × 1.8 = 25 200]. Given a mean failure rate of 70%, the number of events detected at somatic level should be between 822 and 17 640 per second. We found that the number of depolarizing events (presumably EPSPs recorded from soma) of a cortical neuron was only about 180 per second (Fig. 9). The actual number of EPSPs reaching neuronal soma in our experiments was probably underestimated because: (i) the events of amplitude lower than 0.2 mV where not detected; and (ii) simultaneously occurring EPSPs probably appeared as single events. However, the strong difference between the number of expected EPSP and the number of depolarizing events actually detected at the somatic level indicates that, in vivo, only a minor part of the presynaptic firing influence the membrane potential at the soma and thus participate in the output spike train of the neuron. Thus a low proportion of presynaptic spikes impacting at the soma is certainly a result of failures in the synaptic transmission as well as dendritic filtering. Raising $[\text{Ca}^{2+}]_o$ increased the absolute number of spontaneous synaptic events only moderately ($P > 0.05$), but significantly increased the amplitude and the slope of both evoked and spontaneous EPSPs (Figs 8 and 9). This suggests an increased release probability in high $[\text{Ca}^{2+}]_o$ conditions, leading to an increased number of released vesicles in response to a presynaptic AP, and thus generating EPSPs of higher amplitude and faster rising slope. In the mean time, dendritic filtering might prevent the propagation of smallest EPSPs, thus limiting the number of EPSPs that reaches the soma.

In conclusion, our study shows that only a small amount of the presynaptic information participates in determining the output of a cortical neuron in the intact brain. Thus, safe transmission of information in cortical networks occurs only when the presynaptic population activity is engaged, or in particular connections that show high reliability (Silver et al., 2003). Finally, a full set of intrinsic, synaptic and extracellular factors accompanying intact cortical networks during states of vigilance (Steriade, 2001) might further affect the expression of single-axon EPSPs and then regulate the transfer of presynaptic information to the soma. For example, distally arriving EPSPs might be amplified by active dendritic conductances at levels of membrane potential more depolarized than those recorded during barbiturate anaesthesia (Stuart & Sakmann, 1995; Stuart &
synchronous inputs can activate regenerative dendritic potentials that boost the postsynaptic potential (Rudolph & Destexhe, 2003; Crochet et al., 2004); and neuromodulators would alter the effectiveness of single-axon connections (Gil et al., 1997).

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Abbreviation

ACF, artificial cerebrospinal fluid; AP, action potential; Ca$^{2+}$, calcium; [Ca$^{2+}$]o, extracellular calcium concentration; EEG, electroencephalogram; EPSP, excitatory postsynaptic potential; STA, spike triggered average.

Fig. 9. Influence of [Ca$^{2+}$]o on the spontaneous EPSPs. (A) Depth EEG and intracellular recording of a neuron during control conditions. (B) Expanded intracellular recordings (black lines) and automatically selected spontaneous events (coloured lines) for high Ca$^{2+}$ (red), normal Ca$^{2+}$ (blue) and low Ca$^{2+}$ (green). A dotted line indicates ~80 mV. Lower, a superimposition of averaged spontaneous EPSPs of this neuron in three Ca$^{2+}$ conditions. Number segments used for averages is: 12473, high Ca$^{2+}$ 10632, normal Ca$^{2+}$ and 8712, low Ca$^{2+}$. A small hyperpolarizing deflection occurring before EPSP onset might reflect the extracellular field due to presynaptic firing. (C) Histogram of total mean number of spontaneous depolarizing events ($n = 7$ neurons, *P < 0.05) in three Ca$^{2+}$ conditions. (D and E) Averaged histograms of amplitude and maximal slope distribution of spontaneous EPSPs for seven neurons; each histogram was obtained by averaging the number of events for each amplitude or maximum slope class (bin 0.05 mV for amplitude and 0.05 V/s for maximal slope) from seven cells in each condition (control, low Ca$^{2+}$, and high Ca$^{2+}$). The statistical difference between the low and high Ca$^{2+}$ conditions and the control condition was calculated using a two-tailed t-test for each amplitude or maximum slope class. The plots below the histograms show the P-values for each class. Values < 0.05 were considered statistically significant.


